An-Najah National University
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Synthesis of zinc oxide and cobalt oxide nanoparticles in surfactant / antibiotics shell and investigating their anti-bacterial activities

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Dedication

To my Prophet Mohammad

(peace be upon him)

Then,,

To my beloved

mother, father and family,

dear sisters and

all who gave me

help and support

throughout my life.

Acknowledgment

At the beginning, thanks Allah for what I have now…

I would like to express my special thanks to Dr. Amjad Hussien and Dr. Mohammad Suleiman for their supervision. Without their endless support and help this work could not be achieved.

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And I can't forget my beloved friends, their encouragement was my motivation.

الإقرار

أنا الموقع أدناه موقعة الرسالة التي تحمل العنوان:

Synthesis of zinc oxide and cobalt oxide nanoparticles in surfactant / antibiotics shell and investigating their anti-bacterial activities

تحضير أكسيد الزنك وأكسيد الكوبالت بأحجام النانو وتغليفها بالمواد الفعالة سطحيا / المضادات الحيوية ودراسة تأثيرها كمضادات بكتيرية

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجـــة أو لقـــب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Students name: **اسم الطالبة:**

Signature: **التوقيع:**

Date: **التاريخ:**

List of Abbreviation

Symbol	Abbreviation
A °	Angstrom
AFM	Atomic Force Microscopy
B. subtilis	*Bacillus subtilis* bacteria
CoO	Cobalt oxide
DMSO	Dimethylsulphoxide
E. coli	*Escherichia coli* bacteria
EDX	Energy Dispersive X-ray
FWHM	Full width at half maximum
GRAS	Generally Recognized As Safe
M	Molarity
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
NA	Nutrient Agar
NB	Nutrient Broth
nm	Nanometer
NP	Nanoparticles
QAS	Quaternary Ammonium Surfactants
S. aureus	*Staphylococcus aureus* bacteria
SEM	Scanning Electron Microscopy
TOA	Tetra octyl ammonium salts
TOAB	Tetraoctyl ammonium bromide
XRD	X-ray diffraction
ZnO	Zinc oxide

Table of Contents

List of Tables

List of Figures

Synthesis of zinc oxide and cobalt oxide nanoparticles in surfactant / antibiotics shell and investigating their anti-bacterial activities

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Abstract

With the increasing bacterial resistance for antibiotics, the demand for alternative agents is increased. Scientists go on for nanoparticles, a nanoscale material ranging from 1-100 nanometers. One of the famous nanoparticles is zinc oxide nanoparticle which is one of metal oxide nanoparticles that possess many properties such as semiconducting properties, antibacterial activity. Cobalt oxide nanoparticles, on the other hand, have less antibacterial activity research concerns.

In this study, the two kinds of the nanoparticles, ZnO and CoO nanoparticles, were synthesized using chemical reduction method in different forms: the nanoparticle alone, the nanoparticle stabilized in tetra octyl ammonium bromide (TOAB) and the nanoparticle stabilized in surfactants and mixed with three diffrent types of antibiotic; Amoxicillin, Cephalexin and Streptomycin. Then, the antibacterial activity of prepared nanoparticles were studied on three bacterial strains, *S. aureus*, *B. subtilis* and *E. coli*. Antibacterial activity of each preparation was tested separately. Antibacterial activity of the synthesized nanoparticles showed a noticeable increase when stabilized in TOAB surfactant. Interestingly, the antibacterial activity is several times increased by the addition of the

antibiotics to the NPs stabilized in TOAB; it seems that the nanoparticles stabilized in TOAB and mixed with antibiotics have synergestic effect in inhibition of bacterial growth.

Interestingly, the results indicated that CoO NPs stabilized in surfactants and mixed with antibiotics have more significant increase in the antibacterial activity against the three types of bacteria in comparison to the comparable ZnO nanoparticles preparation. Therefore, this study opened the new door to find the magic cure against the multidrug resistant bacterial strains with the lowest toxic effect of the usage of high doses of the different antibiotics and nanoparticles.

Key words: Zinc oxide nanoparticles, Cobalt oxide nanoparticles, Tetraoctyl ammonium bromide, *S. aureus*, *B. subtilis*, *E. coli,* Amoxicillin, Cephalexin, Streotomycin.

Chapter One

Literature review

Chapter one
Literature review

Nanotechnology is the area of science that deals with nanoscale materials and structures that are ranging from 1 to 100 nanometers (nm). This science provided an innovative solutions in scientific areas [32] in diagnostic techniques, drug delivery, sunscreens, antimicrobial bandages, disinfectant, a friendly manufacturing process that reduce waste products, as catalyst for greater efficiency in current manufacturing process by minimizing or eliminating the use of toxic materials, to reduce pollution (e.g. water and air filters) and an alternative energy production (e.g. solar and fuel cells) [27]. Nanotechnology gave the solution to medicine because it has the ability to find materials in nanoscale diameter that have an enhanced bioactivity [10].

Nanoparticles are a serious member of nanotechnology that obtain a special interest from scientists and researchers in different fields, the main reason for this importance is the increased specific surface area of these nanoparticles in comparison to their volume, which enables their interaction with bio-organics present on the viable cell surface [20]. Nanoparticles provided many applications in high-density magnetic recording media and biomedical, such as magnetic resonance imaging (MRI), cell and DNA separation, drug delivery, gene cloning, and hyperthermia for cancer therapy, etc. [39]

Nanomedicine is one of the main branches that affected tremendously by the nanoparticle applications that may be defined partially

in the following areas: the monitoring, repair, construction and control of human biological systems at the molecular level, using engineered nanodevices and nanostructures. These days nanomedicine has a vital role as bacteria are gaining resistance to traditionally used antibiotics at an alarming rate [26]. Three of these pathogenic bacteria are: *Enterococcus, S. aureus* and *Streptococcus*, common closely related species that increase mortality and morbidity [17].

Today for the increased demand of antimicrobial products that can solve the problem of resistant strains instead of existing antimicrobial drugs, metallic nanoparticles, which have a great attention by many researchers to study these NPs, and obtained many important results in this field [20]. Metal oxides NPs such as: ZnO, MgO, TiO_2, SiO_2, CuO and CoO, play a vital role as antimicrobial agents, in other words, these metal nanoparticles can be used as antimicrobial activity because of their effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance. In addition, they provide mineral elements essential to human cells [18].

The most important and famous metallic NP is silver, Ag has antimicrobial effects from the last decades and now and after the introduction of NPs, silver nanoparticles have a critical role in diverse applications: dental work, catheters, and burn wounds [20]. In the second order, zinc oxide nanoparticle occupy a high importance as it has the ability to accumulate in bacterial membrane and cytoplasm regions of bacterial

cells after increasing the membrane permeability of *E. coli* cells and so slow down the *E. coli* growth rate as Jones *et al*. said in his research after using plate assays and TEM analysis to know the toxicological effect of 12 nm particle size ZnO nanoparticles [17]. In the same research Jones *et al*. added that ZnO nanoparticle has a significant antibacterial effect against *S. aureus* more than other five metal oxide nanoparticles [17]. Cobalt oxide nanoparticles have an important structural, magnetic, electronic, and catalytic properties [39]. In addition it has been found that oxidized cobalt, zinc and nickel nanoparticles produce higher death rate of *E. coli* using electrospray technique [15].

The method by which the NPs are synthesized has a special interest, this is because it has many factors that cooperate to adjust the size, morphology, stability and properties (chemical and physical) of the metal nanoparticles, some of these factors are: the experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of stabilizing agent with metal nanoparticles [32]. There are many ways to produce nanoparticles, which are: electrochemical method, thermal decomposition, laser ablation, microwave irradiation and sonochemical synthesis [13]. One of these methods is chemical reduction method, this method has many advantages, such as: simple equipment, short process and easy industrial production [29]. In this method, ionic salt is reduced in an appropriate medium using reducing agent [13] which is in this research sodium hydroxide (NaOH).

This is not the whole, but in addition, these metallic nanoparticles evolved to be coated with cationic material to increase their activity, which called “surfactants”, these material provided a powerful tool to produce stabilized nanoparticles [10]. The most important and famous family of this surfactant is the quaternary ammonium salts (QAS) that possessing at least one alkyl substituent are able to kill microorganisms such as bacteria and fungi by interacting with the cell membrane [19] and the antibacterial activity depends on the length of hydrophobic chains, the size of the dendrimer and its concentration. This biocides may have bromide or chloride anions but bromide anions are more potent [9]. QAS with low molecular mass has cationic disinfectants or biocidal coating which are widely used to prevent the growth of microorganisms on the surface of materials, and it was indicated that polycationic biocides possess high positive charge density and excellent process ability and have found remarkable utility in hygiene and in biomedical applications [19]. From this point many researchers reported that long chain polycations coated the nanoparticles are able to kill both gram negative and gram positive bacteria [17], scientists did not stop here, they also added a professional addition to the nanoparticles, which is the antibiotic itself.

Banoee *et al*. studied the effect of combination of silver nanoparticles with many antibiotics which are pencillin G, amoxicillin, erythromycin, clindamycin and vancomycin against *S. aureus* and *E. coli,* the results showed that the antibacterial activity of the above antibiotics increased in the presence of the nanoparticles [5]. Banoee *et al*. also

applied the above idea on ZnO nanoparticles against two types of bacteria: *S. aureus* and *E. coli*, ZnO antibacterial activity is measured in the presence of major classes of antibiotics, the results showed ZnO decreased the antibacterial activity of amoxicillin, penicillin G and nitrofurantoin in *S. aureus*, whereas the antibacterial activity of ciprofloxacin increased in the presence of ZnO. The other antibiotics were almost indifferent to the presence of ZnO.

Chapter Two

Introduction

Chapter two
Introduction

2.1 Nanoparticles

Nanotechnology these days plays a vital role, that is, science is moving towards it in many branches: information, energy, environmental, medical technologies. Because of the quantam size effect of nanoparticles that is different from the bulk, nanoparticles' physical and chemical properties qualified them to be used in many applications in the electronic, chemical and mechanical industries, drug carriers, sensors, magnatic and electronic materials [28].

Our field in this research is the application of nanoparticles in medicine, due to the increased number of deaths and hospitalizations because of increased bacterial resistance to multiple antibiotics within both gram positive and gram negative microorganisms, and the continuing emphasis on health-care costs [20], health concern moving towards metal oxide nanoparticles as a an effective and efficient antibacterial therapeutic and diagnostic methodologies and techniques [26].

Metallic nanoparticles which have unique physicochemical characteristics due to their high specific surface area [31] and also a unique adsorption properties because of different distributions of reactive surface sites that can be functionalized with various chemical groups to increase their affinity towards target compounds [35]. Metallic oxide nanoparticles are prepared and stabilized by physical and chemical methods; the

chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction [32] and these days via green chemistry route [31].

2.2 Stabilizers

Stabilizers such as surfactants play a critical role to prepare a stable nanoparticles that are composed of a wide range of metals and compounds. In the process of nanoparticles formation, agglomeration of particles has high percentage to take place, and so to inhibit this agglomeration and also to control particles growth, protective agent as surfactant is added during the process of nanoparticles formation, the surfactant form a layer of molecular membrane around the nanoparticles and polymers that provide steric hindrance between nanoparticles [17].

Another action that surfactants play is to attack the charged macromolecules found in the cell wall of bacteria, the cell wall of pathogenic bacteria is the main target for the control and prevention of bacterial infection because it composed of surface proteins for adhesion and colonization and components such as polysaccharides and teichoic acid, these macromolecules protect bacteria from host defense and environmental conditions, and so the long chains polycations surfactant that coated the nanoparticles can effectively killed both gram positive and gram negative bacteria [17]. One cationic surfactants are tetra-octyl ammonium salts that show two significant effects: first, they ensure the morphological and chemical stabilization of metallic clusters; secondly,

TOA salts have significant antimicrobial activity because they belong to QAS disinfectants class. Thus, TOA salts give a synergestic disinfecting effect in combination with nanometals and so a significance increase in the TOA salts efficacy as a bioactive coatings [10].

2.3 Zinc and Cobalt oxide nanoparticles

Two of the metallic nanoparticles are studied in this research; zinc oxide nanoparticles and cobalt oxide nanoparticles:

2.3.1 Zinc oxide nanoparticles

Zinc oxide has received a great attention since past times due to its various properties as: antibacterial, semiconducting properties, growth promoter [38], catalytic efficiency, chemical stability and strong adsorption ability. Because of these activities it has been used as an active ingredient for dermatological applications in creams, lotions and ointments and also ZnO which is widely used as food additive and food supplement [5], that was Jalal *et al*. who added that ZnO is one of five zinc compounds that are listed as generally recognized as safe (GRAS) by the U.S. Food and Drug administration [16].

Either two antimicrobial mechanisms of ZnO were supposed:

i. Hydrogen peroxide, which is generated from the surface of zinc oxide, can penetrate through the cell membrane, produce some type of injury, and inhibit the growth of the cells.

ii. The affinity between zinc oxide and bacterial cells is an important factor for antibacterial activity [38].

In addition zinc oxide is an interesting semiconductor material this is seen through its application on solar cells, gas sensors, ceramics, and varistors [27].

In nanoscience, ZnO nanoparticles show a significant growth inhibition under normal laboratory lighting conditions and in the same time they have selective toxicity and are regarded as a safe reagent to humans and animals, this action can be understood because ZnO inhibiting the adhesion and internalization of bacteria and so ZnO can protect against intestinal diseases caused by *E. coli* [17], and Wang *et al*. proved through the atomic force microscopy (AFM) and scanning electron microscopy (SEM), the morphological changes of *E. coli* K88 treated with 0.8μg/mL zinc oxide nanoparticles, which showed that ZnO nanoparticles could damage the membrane of this bacteria and so led to the leakage of cytosolic components and finally killed the bacterial cell [38]. By this result, Wang *et al*. also proved that zinc oxide nanoparticles have stronger antibacterial activity than zinc oxide, due to largely increased surface or enhanced the affinity [38]. In comparison to other 5 NPs (MgO, TiO_2, Al_2O_3, CuO and CeO_2), ZnO appeared that it has a significant antibacterial activity against a wide range of bacterial species and in particular against *S. aureus* [26]. In addition, another feature that made ZnO nanoparticles compete other metal oxide nanoparticles is its antibacterial activity against a broad range of bacteria: gram positive and gram negative [17].

2.3.2 Zinc oxide stabilized in surfactant

As mentioned above, ZnO nanoparticle is an active ingredient in many dermatological applications, since it inhibits the adhesion of bacteria to the host cells, this action is developed by adding various chemical groups that can disrupt the action and location of the main bacterial target, which is the cell wall structure which is composed of surface proteins for adhesion and colonization and components such as polysaccharides and teichoic acid that protect bacteria against host defenses and environmental conditions. The long chain polycations of stabilizer coated the metallic NP surfaces can efficiently kill on contact both gram-positive and gram-negative bacteria [17].

2.3.3 Zinc oxide stabilized in surfactant and mixed with antibiotics

The most recent progress in studying the antibacterial activity is the mixtures of different nanoparticles with antibiotics, this new idea were evolved to face the incidence of high bacterial resistance to different antibiotic classes.

Tiwari *et al*. in his study supposed mechanisms of action for the synergestic effect of antibiotics, especially ß-lactam, and silver nanoparticles (without surfactants), some of these mechanisms are that the active groups of amoxicillin such as hydroxyl and amido groups reacted with nanosilver by chaletion, amoxicillin molecules bind with each other by weak bonds, and so antimicrobial groups of nanosilver core and the

surrounding amoxicillin come into contact with surface of bacterial cells, which means an increasing in the antimicrobial agents concentration and so more destruction for the bacterial cells [35]. Another supposed mechanism Tiwari *et al*. added is that, the cell membrane of bacterial cells are composed of glycoprotein and phosphplipids, which means hydrophobic barrier, and because nanosilver is hydrophobic not the amoxicillin which is hydrophilic, nanosilver is able to approach the membrane of the target cells and so the antimicrobial groups easily transfer the amoxicillin to the cell surface [35].

2.3.4 Cobalt oxide nanoparticles, cobalt oxide nanoparticles stabilized in surfactant, cobalt oxide nanoparticles stabilized in surfactant and mixed with antibiotics

Most of previous studies concentrated on the antibacterial activity of the famous nanoparticles represented by silver followed by ZnO and CuO. However, there is less concerns of the other nanoparticles like CoO. Cobalt oxide nanoparticle displays structural, magnetic, electronic and catalytic properties [39] but in last few decades CoO is used as bactericides for water disinfection [35] so this property enables cobalt oxide nanoparticles to be used as antibacterial activity. Horst *et al*., examined the antibacterial activity of cobalt oxide nanoparticles, nickel oxide NPs, zinc oxide NPs, copper oxide NPs, iron oxide NPs and titanium dioxide NPs against *E. coli* using two methods: culturing in liquid media containing one of these nanoparticles and electrospraying the NPs directly onto bacterial surface.

The results indicate a significant cell death when *E. coli* was exposed directly using electrospray exposure method to oxidized nickel, zinc and cobalt species; but no antibacterial properties from titanium, iron and copper oxide [15].

Magnetic nanoparticles have both cohesive forces and magnetic dipolar interactions so surfactants is important to coat these NPs types during chemical synthesis to prepare well-dispersed nanoparticle colloid. Oleic acid is used as a surfactant and provides the stability for cobalt oxide nanopartcles colloid [39].

The most recent progress in studying the antibacterial activity is the mixtures of different nanoparticles with antibiotics, this new idea were evolved to face the incidence of high bacterial resistance to different antibiotic classes, for example Matthews *et al*., said in his nanomedicine article that the combination of silver nanoparticles with antibiotic as: penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin increases the effectiveness of antibiotic [26].

2.4 Bacterial strains

2.4.1 *Staphylococcus aureus* (*S. aureus*)

Staphylococci are gram-positive bacteria, characterized by individual cocci, which divide in more than one plane to form grape-like clusters, also this bacteria are non-motile, non-spore forming [14]. *S. aureus* are facultative anaerobes that grow by aerobic respiration or by fermentation

and can grow at a temperature range of 15-45 °C and at NaCl concentrations as high as 15 percent [36].

S. aureus antibiotic resistance is increased due to the firm protective coat, Adhesions or MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) expressed on the surface of the *S. aureus* and promoted the adhesion of the bacteria to the host proteins such as fibronectin and fibrinogen. *S. aureus* is considered to be a major pathogen that colonizes and infects both hospitalized patients with decreased immunity, and healthy immuno-competent people in the community. In normal situation, these bacterial types are found naturally on the skin and in the nasopharynx of the human body but this minor infection are not life threatening, however, if *S. aureus* violates the underlying tissue due to trauma or surgery, it will create its characteristic local abscess lesion but if it reaches the lymphatic channels or blood it can cause septicaemia. Enterotoxin A-E, toxic shock syndrome toxin-1 (TSST-1) and exfoliative toxins A and B are extracellular toxins. Ingestion of enterotoxin produced by *S. aureus* in contaminated food can cause food poisoning. [14].

2.4.2 *Bacillus subtilis* (*B. subtilis*)

Bacillus subtilis is a gram positive bacteria characterized by aerobic, spore-forming, rod-shaped bacteria that are motile by peritrichous flagella. These bacteria are extensively spread throughout the environment, particularly in soil, air, and decomposing plant residue [7]. *B.*

subtilis bacteria has an optimal temperature 25-35 °C and the most optimal activity of *B. subtilis* occurs at 37 °C and a basic pH of 8 [4].

The distinction of these bacteria are their capability of producing endospores that are highly resistant to unfavorable environmental conditions and that also have capacity to grow over a wide range of temperatures including that of the human body. *B. subtilis*, among the studied bacterial strains in this study, the following specific features were noticed:

i. They do not have virulence factor genes, they may acquire such genes from other bacteria, particularly from closely related bacteria within the genus.

ii. Lecithinase; an enzyme which disrupts membranes of mammalian cells, is one of *B. subtilis* product, however this enzyme has no correlation between it and human disease in *B. subtilis*.

iii. Subtilisin; is an extracellular toxin that is produced by *B. subtilis*, this proteinaceous compound is capable of causing allergic reactions in individuals who are repeatedly exposed to it [7].

2.4.3 Escherichia coli (E. coli)

It's one of the most popular gram negative bacteria. *E. coli* is considered as a facultative anaerobic bacteria that can live in the presence or absence of oxygen. It characterized by a non-sporeforming, motile, rode-shaped bacteria that ferments lactose [22]. *E. coli* is one of the most

common inhabitants of the human intestinal tract, its optimal growth occurs at 37°C [11], and the optimum pH growing in a culture at 37°C is 6.0-7.0 also it has a minimum pH level of 4.4 and a maximum level of 9.0 required for growth [12].

2.5 Antibiotics

2.5.1 Amoxicillin

Amoxicillin is a semisynthetic antibiotic, an analog of ampicillin, with a broad spectrum of bactericidal activity against many gram-positive bacteria and a limited range of gram-negative bacteria [1].

It's mechanism of action by inhibiting bacterial cell wall synthesis by binding to one or more of penicillin binding proteins (PBP) which in turn inhibits the final transcription step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested [21]

2.5.2 Cephalexin

Cephalexin is the first generation of cephalosporins, this medication is active against gram–positive cocci, including: staphylococci and streptococci. Also this drug has minimal activity against gram-negative cocci, enterococci, methicillin-resistant *S. aureus*, and most gram-negative rods [34].

It's mechanism of action by preventing bacteria from forming their cell wall, and so bacteria can not able to survive and stop the spread of infection in the body [8].

2.5.3 Streptomycin

Streptomycin is one of the aminoglycoside antibiotic that has a bactericidal action against many gram-negative aerobes and against some strains of Staphylococci.

In the cell aminoglycosides bind to the 30S, and to some extent to the 50S, subunits of the bacterial ribosome, inhibiting protein synthesis and generating errors in the transcription of the genetic code [25].

2.6 The toxicological effect of metal oxide nanoparticles on human

Ready *et al*., showed that, by using flow cytometry based assays, zinc oxide nanoparticles (~ 13 nm) had minimal effects on primary human T-cell viability at concentrations toxic to both gram negative and positive bacteria [30]. Also by the same study, Reddy *et al*., demonstrated the following results: on prokaryotic system; the ZnO-NPs killed *E. coli* (gram negative bacteria) at concentration ≥ 3.4 mM, whereas the gram positive bacteria, *S. aureus* was inhibited at lower concentration (≥ 1mM), in contrast, micron sized bulk ZnO powder has no effect on the T-cell viability, whereas using ZnO in the nanoscale range has limited cytotoxicity to T-cell [30]. Collectively, after the comparison of the toxic nature of metal oxide NPs, selectivity of these NPs for both prokaryote and

eukaryote was shown, also zinc oxide NPs proved that, it can be used as nanomedicine based antimicrobial agents at selective therapeutic dosing regimens [30].

2.7 Objectives of this study

In this study, zinc oxide and cobalt oxide nanoparticles are in the circle of our interest. Zinc oxide nonparticles had tremendous of concern previously. Cobalt oxide nanoparticles were chosen for their rareness in previous studies. Moreover, different forms of NPs were investigated in this study including: the NPs alone, NPs stabilized in surfactant and the stabilized NPs were mixed with antibiotics (Fig 2.1).

This study has the following specific objectives:

1. Synthesis of zinc oxide (ZnO) & cobalt oxide (CoO) nanoparticles.
2. Stabilization of ZnO & CoO NPs with Tetra-Octyl Ammonium Bromide surfactant (TOAB).
3. Studying the antibacterial activities of ZnO & CoO NPs with and without TOAB.
4. Studying the antibacterial activity for ZnO & CoO NPs stabilized in TOAB mixed with Amoxicillin, Cephalexin and Streptomycin antibiotics.

To study the synergistic effect obviously, three bacterial isolates were used: *E. coli, S. aureus and B. subtilis,* in each step.

Nanoparticles (with TOAB and without TOAB) were characterized using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) and X-ray diffracriton (X-ray). In each step of experiments the MICs were determined using tube dilution method by measuring the absorbance of the prepared test tube of sterile bacterial with the specific NPs preparation.

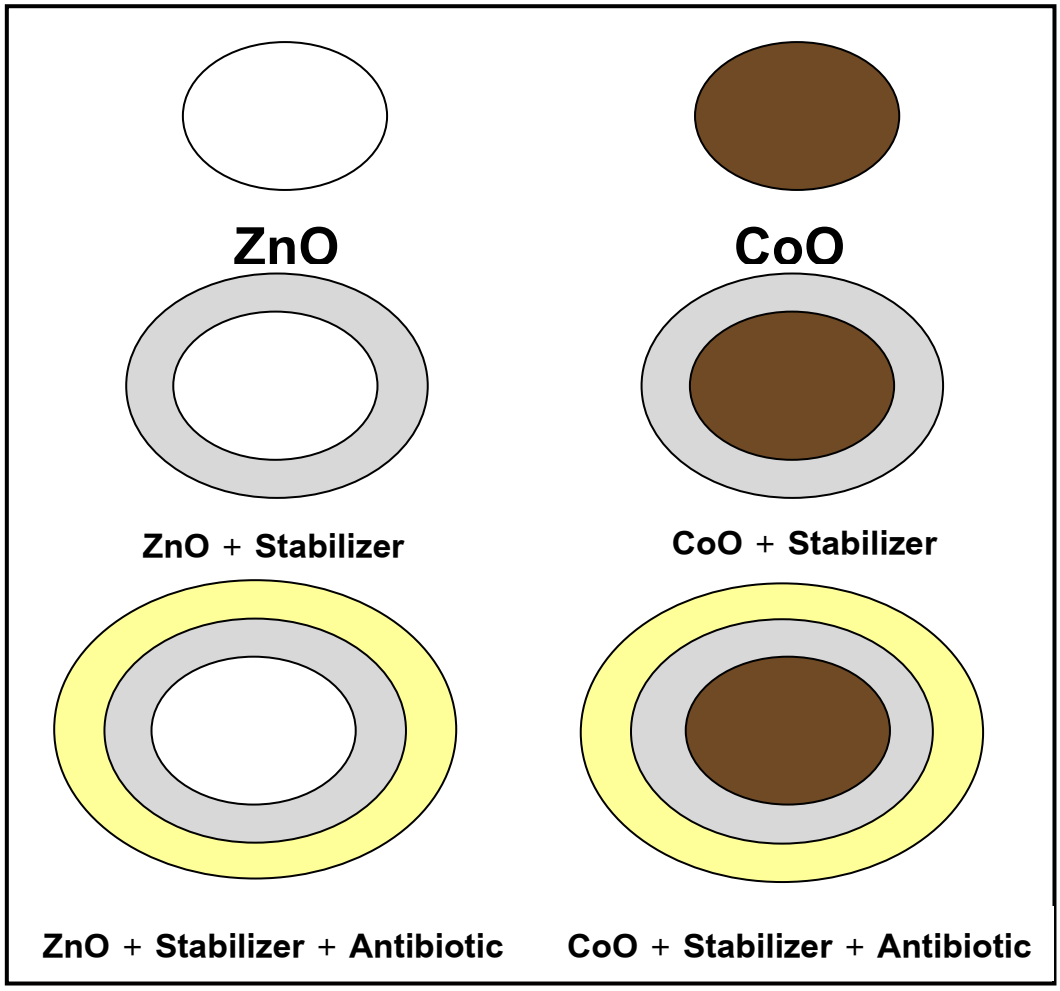

Figure (2.1): The three forms of nanoparticles that have been synthesized by this research: nanoparticle then nanoparticle stabilized in surfactant shell and finally nanoparticle stabilized in surfactant shell and mixed with antibiotic.

Chapter Three

Methodology

Chapter three
Methodology

3.1 Chemicals & materials

All materials used in this study were of analytical grade. Zinc sulphate ($ZnSO_4$) was purchased from the Riedel Company (catalogue # 14455), cobalt sulphate ($CoSO_4$) was purchased from the Sigma-Aldrich Company (catalogue # 544167). Tetraoctylammonium bromide (TOAB): ($[CH_3(CH_2)7]_4N$ Br) is purchased from Aldrich Company with purity 98% (catalogue # 294136).

Antibiotics raw materials, which are: amoxicillin, cephalexin and streptomycin were purchased from OMEGA company, Nablus. The certificates of analysis for each antibiotic are attached in the appendix.

Nutrient broth (catalogue # Moo1_500G) was purchased from Hi media, Dimethylsulphoxide (DMSO) (catalogue # 34943) was purchased from Riedel-dehaen, Barium chloride ($BaCl_2.2H_2O$) (catalogue # 1.01719) was purchased from Merck, H_2SO_4 (catalogue # 30743) was purchased from Riedel –dehaen.

Glycerol was purchased from the Riedel Company with purity of 86-88% (catalogue # 33224), NaOH was purchased from the Frutarom Company (catalogue # 2355535200), NH_4OH was purchased from the Frutarom Company (catalogue # 2355502600).

3.2 Sample preparation

The nanoparticle sample preparation methods will be described in the following sections.

3.2.1 Zinc oxide nanoparticles preparation

Zinc oxide was prepared by dissolving 1.001 gm of $ZnSO_4$ in 50 mL of distilled water, then the solution was thermostated at 80 °C and stirred using 120 rpm. The pH during that must be > 9 so it is monitored each time interval (10 min) here no need to add NH_4OH. 80 mL of 0.25 M NaOH was added to thermostated solution. The addition was stepwise, and under inert gas atmosphere (N_2). The reaction mixture was allowed to be completed and the pH during that was 13-14.

After the completion of reaction, a precipitate was observed and allowed to stand for 12 hours, the precipitate was filtered from the reaction mixture, and dried.

3.2.2 Zinc oxide nanoparticles stabilized in TOAB preparation

1.000 g of $ZnSO_4$ was dissolved in 50 mL of 0.002 M TOAB solution, the TOAB solution was prepared by dissolving 0.059 g in 70 mL H_2O, then the reaction was processed as in section (3.2.1).

3.2.3 Cobalt oxide nanoparticles preparation

80.0 mL of 1 M NaOH was prepared then it was thermostated at 80 °C and stirred using 160 rpm. Solution of 0.7955 gm of $CoCl_2$ in 100 mL of

distilled water was added to thermostated solution, pH during that must be > 9 so it is monitored each time interval (10 min), here 0.2000 gm of $NaBH_4$ was added to complete the reaction. The addition was stepwise, and under inert gas atmosphere (N_2). The reaction solution was allowed to be completed and the pH during that was 13-14.

After the completion of reaction, a precipitate was observed and allowed to stand for 12 hours, the precipitate was filtered from the reaction mixture, and dried.

3.2.4 Cobalt oxide nanoparticles stabilized in TOAB preparation

0.7955 gm of $CoCl_2$ was dissolved in 50 mL of 0.002 M of TOAB solution, the TOAB solution was prepared by dissolving 0.0500 g in 50 mL H_2O, then the reaction was processed as in section (3.2.3), but addition was conversed, NaOH was added to $CoCl_2$ and TOAB solution.

3.3 Samples characterization

Characterization of the nanoparticles with TOAB and without TOAB was done using XRD and SEM.

3.3.1 X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis was performed on solid dispersion powders, using a Philips PW1710 diffractometer, with Bragg–Brentano geometry (2θ) and Ni-filtered CuK radiation.

3.3.2 Scanning Electron Microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX)

The morphology of the prepared solid dispersions as well as the initial materials was examined in a scanning electron microscopy (SEM) type Jeol (JMS-840).

The films were covered with a carbon coating to have good conductivity of the electron beam. Operating conditions were as follows: accelerating voltage 20 KV, probe current 45 nA, and counting time 60 s.

3.4 Antibacterial activity

3.4.1 Bacterial isolates

E. coli, S. aureus & B. subtilis bacterial isolates were used for antibacterial activity testing of the different nanopoarticles, surfactants and antibiotics combinations. These bacterial isolates were isolated from clinical specimens and diagnoised in medical laboratory sciences department / An-Najah National University according to the standard diagnostic methods [24, 41].

The bacterial isolates stock culture were manipulated under the same conditions as following: at the beginning the isolates were cultured in nutrient broth then incubated overnight at 37 $^{\circ}$C to reach the log phase. Stock cultures for the three bacterial isolates were prepared by adding a loopfull bacterial isolates to 15% glycerol then saved in freezer (-40 C) and used as needed.

3.4.2 Bacterial cultures preparation

Bacterial growth were determined using the UV-spectrophotometer, model no. UVs – 2700 from Labomed inc. company, California, United states country. UV-spectrophotometer was used to determine the appropriate λ of measuring the bacterial growth absorbance.

3.4.2.1 McFarland preparation

Bacterial culture preparation was adjusted using the turbidity of bacterial suspensions according the 0.5 McFarland standard solution that represent $1.5X10^8$ bacteria/mL. McFarland solution is prepared from 0.50 mL of 1.175% (wt/vol) $BaCl_2.2H_2O$ and 99.5 mL of 1.00% (vol/vol) H_2SO_4, they were mixed together to be sure that it is suspended. Then the absorbance was measured on spectrophotometer at wavelength = 625nm, distilled water were used as the standard blank, to obtain turbidity within 0.08 – 0.1 that reflect bacterial concentration of about $1.5X10^8$ bacteria/mL. The McFarland solution was sealed tightly to prevent evaporation and foiled with aluminum foil to protect from light [2]. Then prepared bacterial solution diluted to each experiment to obtain the required final concentration of about $1.0X10^6$ bacteria/mL.

3.4.2.2 Nutrient broth preparation

Nutrient broth was used to prepare the serial dilutions to detect the antibacterial activity. According to the manufacturer, NP was prepared by weighting 13.0 g of nutrient broth powder in 1.00 L distilled water, then

dissolved by heating on bunsen burner with shaking. NB were sterilized at 121 °C for 15 min, sterilization was confirmed by the control blanks and the usage of the sterilization indicator tapes. Sterilized NB was used as required for making the dilutions and preparing the bacterial cultures.

3.4.3 pH examination

As pH is one of the important factors in effecting the antibacterial activity; all nanoparticles effect on pH were determined in the used nutrient broth. The pH of the NB before and after the addition of the nanoparticles at a concentration of 5 mg/mL were measured using a calibrated pH meter. The dissolving process were aided by sonicater.

3.4.4 Serial dilution preparation and MIC determination

This is the basic technique for this research to determine the minimum inhibitory concentration (MIC) for each chemical compound preparation:

I. Tetraoctylammonium bromide (TOAB)

II. Dimethylsulphoxide (DMSO)

III. Antibiotics: Amoxicillin, Cephalxin and Streptomycin.

IV. Zinc oxide nanoparticles & Cobalt oxide nanoparticles

V. Zinc oxide nanoparticles stabilized in TOAB & Cobalt oxide nanoparticles stabilized in TOAB.

This method is achieved by:

For each NPs preparation of materials mentioned above, serial dilution was prepared by dissolving the material in 15% DMSO in NB, (except TOAB were dissolved in distilled water that were added by heating). Nanoparticles stock solutions were prepared by weighing the needed quantity, for nanoparticles, and dissolving in 15% DMSO in NB that were aided by stirring or sonication. All test tubes and glassware used in serial dilution preparation were autoclaved at 121 $^{\circ}$C for 15 min to make sure no contamination. Serial dilution is achieved by transferring specific amount from one test tube to another, until the tube before the last one, here the transferred amount was discarded before the last tube, so the last tube hadn't the active ingredient that will be used as control. Each test tube was labeled according to its actual concentration, during serial dilution the sterilized conditions were achieved and also shaking each test tube. After serial dilution of the above mentioned materials in each tube, the bacterial strain was added to each tube without exception according to McFarland theory, as mentioned in part (3.4.2.1), to have a final concentration of about $1.0X10^6$ bacteria/mL in each tube. The above mentioned material serial dilution tubes that contain bacterial culture of a final concentration $1.0X10^6$ CFU/mL were incubated overnight and the next day were read for MIC. Even MIC can be read visually, absorbance of each tube was taken at the lambda max of all strains at 625 nm, using NB as the blank.

3.4.5 The ratios of nanoparticles stabilized in TOAB to antibiotics

Specific ratio of the two components were mixed and different concentrations of mixtures were applied to get the MIC of the new mixture. Previously, nanoparticles were mixed with antibiotics without the usage of surfactant agent that was investigated for their antibacterial activity [35].

Amoxicillin and Cephalexin were mixed with nanoparticles stabilized in TOAB in a fixed ratio which was 1 (antibiotics) : 5 (nanoparticles stabilized in TOAB), and for Streptomycin, it mixed with nanoparticles stabilized in TOAB in a fixed ratio which was 1 (Streptomycin): 25 (nanoparticles stabilized in TOAB).

To measure the MIC as explained in the serial dilution technique, absorbance of all tubes was measured after overnight incubation for all test tubes in incubator, each tube absorbance was measured at $\lambda = 625$ nm for all trials.

Chapter Four

Results and Discussion

Chapter four
Results and discussion

4.1 Nanoparticles characterization

4.1.1 X-ray diffraction (XRD)

X-ray characterization was done for all samples to measure the particle size at λ= 1.54051 Angstrom (0.154051 nm) using the X-ray diffractometer, from this analysis, the following parameters can be determined: full width at half maximum-FWHM, peak intensity and peak position. By applying Scherrer equation: $d = K \lambda / ß \cos\theta_ß$

d: crystalline size (in nm), K: shape factor that has a typical value of about 0.9, λ: X-ray wavelength (1.5405 A^o = 0.154051 nm), β: full width at half maximum-FWHM (in radians), θ: Bragg angle [33]. The results for each sample are as the following:

4.1.1.1 X-ray characterization of zinc oxide nanoparticles

Zinc oxide nanoparticles size was determined from XRD diffraction pattern, which shows a hexagonal wurtzite phase of ZnO in Figure (4.1) that agrees with literature XRD analysis of wurtzite zinc oxide Figure (4.2) [33]. From four diffraction peaks located at: 35.95 °, 47.29 °, 56.30 ° and 62.65 ° then applying Scherrer equation.

The average particle size of zinc oxide nanoparticle (d) equals 15.64 nm.

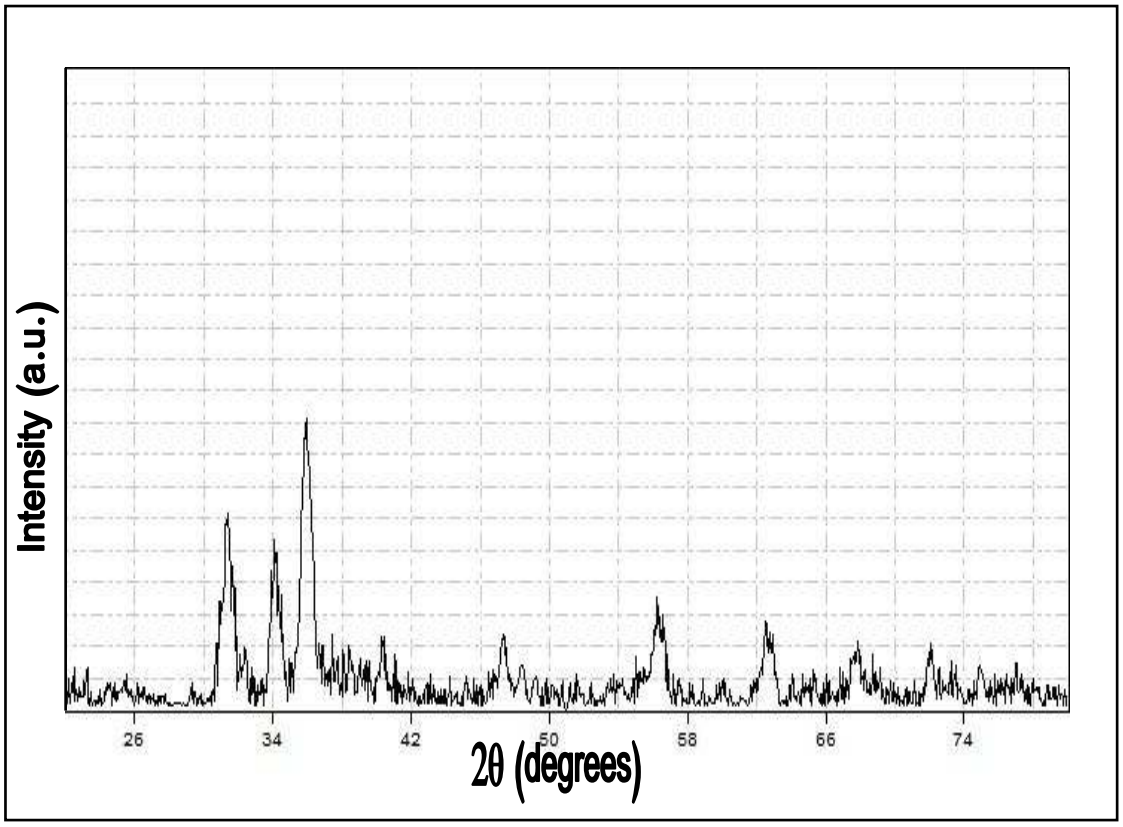

Figure (4.1): X-ray diffraction of zinc oxide nanoparticles.

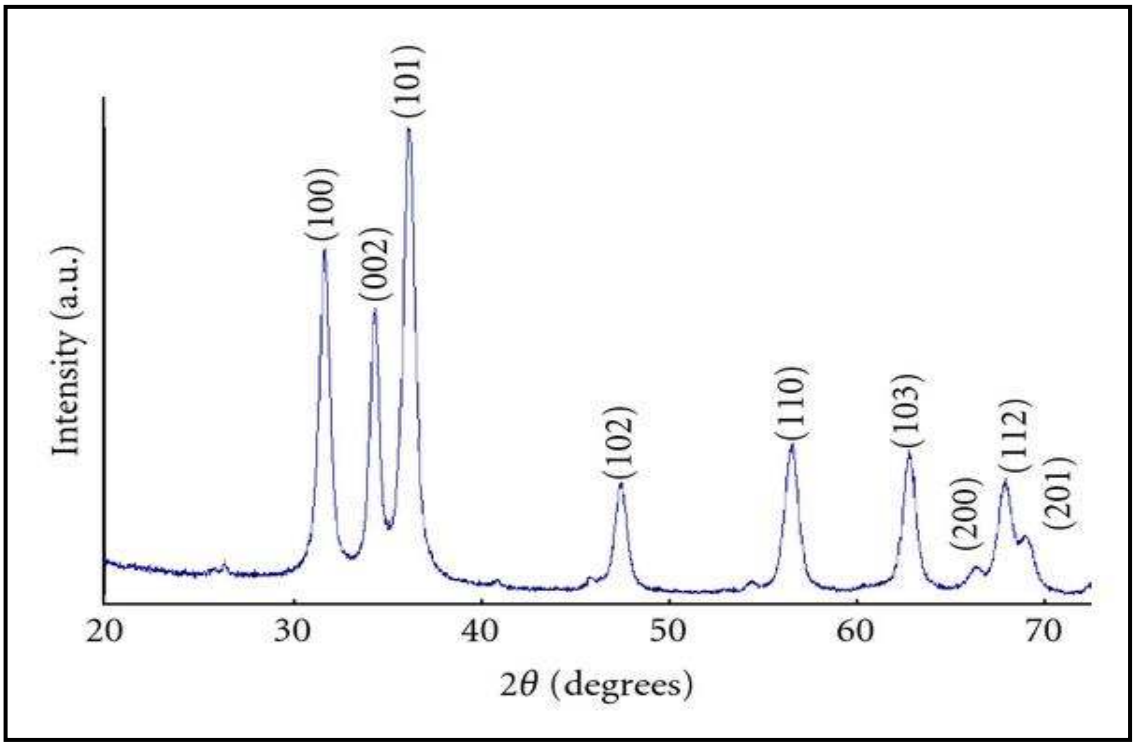

Figure (4.2): Literature X-ray diffraction of zinc oxide nanoparticles [31].

4.1.1.2 X-ray characterization of zinc oxide nanoparticles stabilized in TOAB

Zinc oxide nanoparticles stabilized in TOAB size was determined from XRD diffraction pattern, which appeared in Figure (4.3). From four diffraction peaks located at: 36.15 °, 47.51 °, 56.54 ° and 62.87 ° then applying Scherrer equation.

The average particle size of nano zinc oxide stabilized in TOAB (d) equals 13.76 nm.

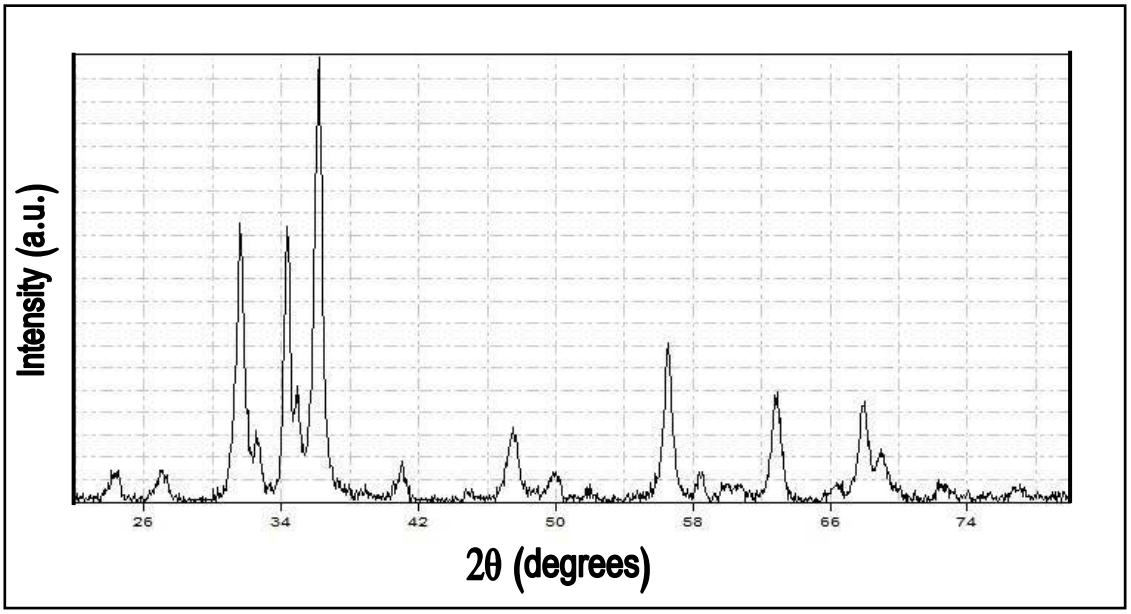

Figure (4.3): X-ray diffraction of zinc oxide nanoparticles stabilized in TOAB.

4.1.1.3 X-ray characterization of cobalt oxide nanoparticles

Figure (4.4) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles. The crystal structure of cobalt nanoparticles, which was determined to be predominantly face-centered cubic structure [40], that agrees with literature XRD analysis of wurtzite cobalt oxide [3].

From four diffraction peaks located at: 32.53 °, 38.01 °, 51.35 ° and 58.04 ° then applying Scherrer equation.

The average particle size of nano cobalt oxide (d) equals 15.22 nm.

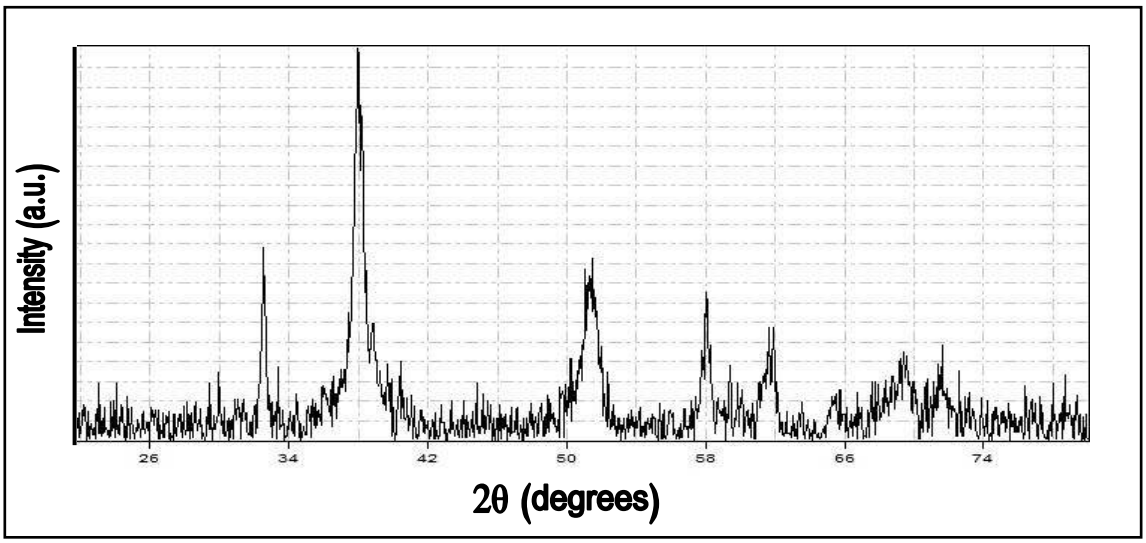

Figure (4.4): X-ray diffraction of cobalt oxide nanoparticles

4.1.1.4 X-ray characterization of cobalt oxide nanoparticles stabilized in TOAB

Figure (4.5) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles stabilized in TOAB. From four diffraction peaks located at: 32.27 °, 37.71 °, 51.14 ° and 57.70 ° then applying Scherrer equation.

The average particle size of nano cobalt oxide (d) equals 14.28 nm.

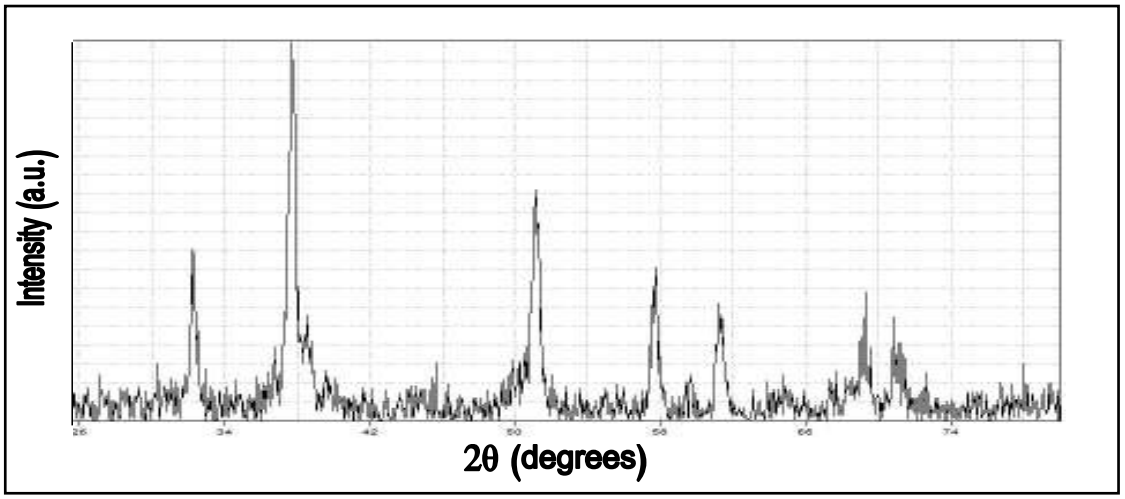

Figure (4.5): X-ray diffraction of cobalt oxide nanoparticles stabilized in TOAB.

4.1.2 Scanning Electron Microscopy characterization (SEM)

Scanning Electron Microscopy (SEM) characterization shows that the surface morphology of prepared nanoparticles at different magnifications, the SEM measurement was achieved for the following samples:

4.1.2.1 SEM characterization of zinc oxide nanoparticles

SEM pictures show the surface morphology of prepared ZnO nanoparticles at 5, 20 and 100 μm magnifications scale, these pictures in Figure (4.6) substantiate the approximate spherical to flakes-like particles shape of zinc oxide nanoparticles.

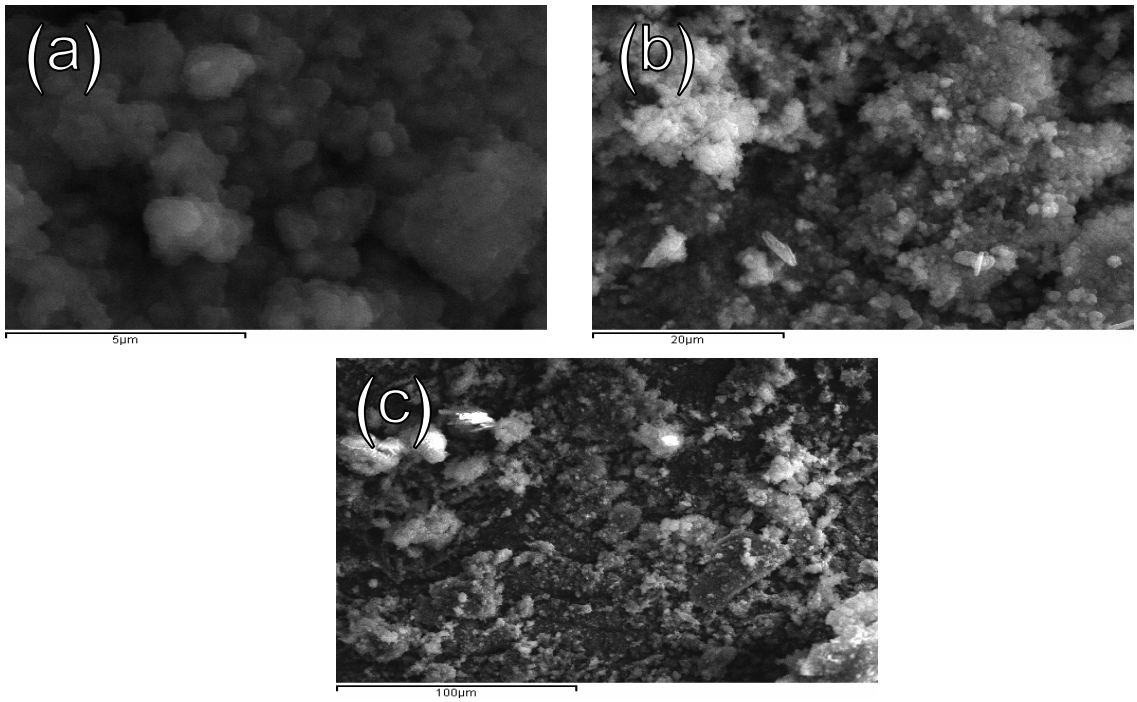

Figure (4.6): SEM pictures of ZnO nanoparticles at different magnifications (a, b & c)

4.1.2.2 SEM characterization of zinc oxide nanoparticles stabilized in TOAB

SEM pictures show the surface morphology of prepared ZnO nanoparticles stabilized in TOAB at 5, 20 and 100 µm magnifications scale, these pictures in Figure (4.7) substantiate the approximate spherical to flakes-like particles shape of zinc oxide nanoparticles stabilized in TOAB.

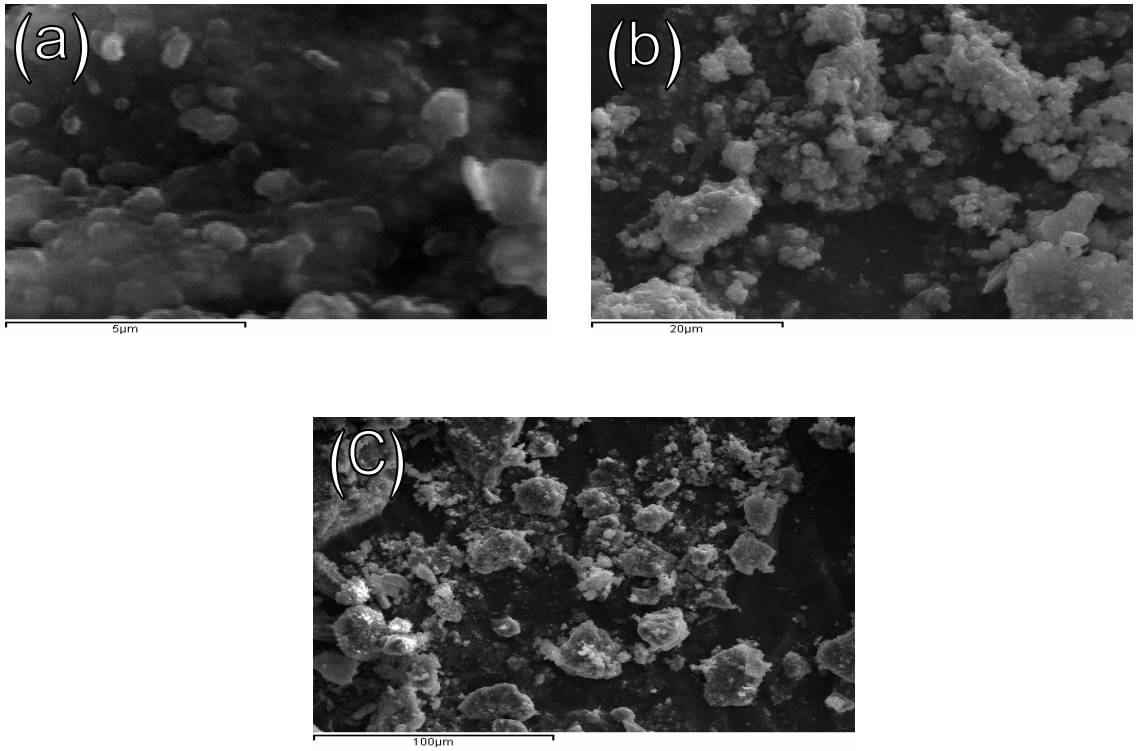

Figure (4.7): SEM pictures of ZnO nanoparticles stabilized in TOAB at different magnifications (a, b & c)

4.1.2.3 SEM characterization of cobalt oxide nanoparticles

The formation of cobalt oxide nanoparticles is confirmed by the SEM pictures for this metal oxide nanoparticles. These two magnifications scale (10 and 100 µm) of cobalt oxide nanoparticles in Figure (4.8) indicate the spherical to flakes-like particles shape of CoO nanoparticles.

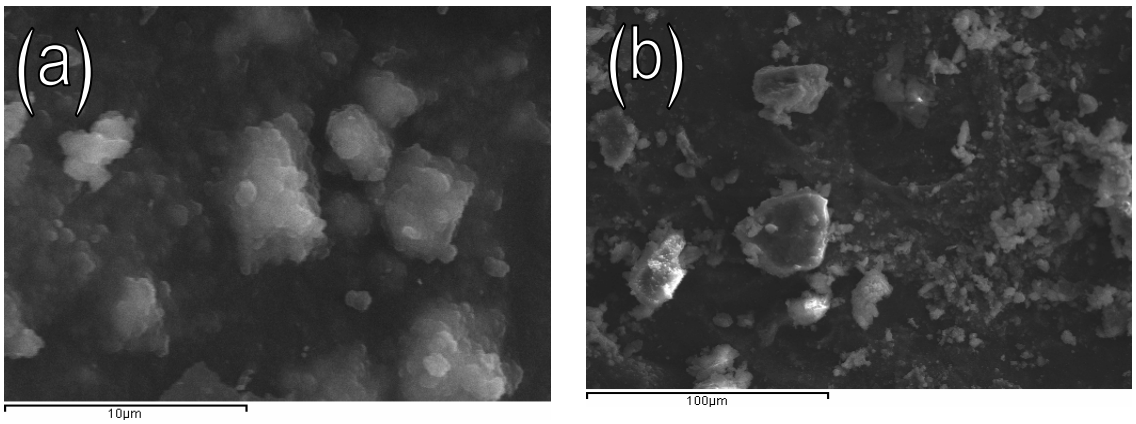

Figure (4.8): SEM pictures of CoO nanoparticles at different magnifications (a & b)

4.1.2.4 SEM characterization of cobalt oxide nanoparticles stabilized in TOAB

The formation of cobalt oxide nanoparticles stabilized in TOAB is confirmed by the SEM pictures for these stabilized metal oxide nanoparticles. These two magnifications scale (5 and 30 μm) of cobalt oxide nanoparticles stabilized in TOAB in Figure (4.9) indicate the spherical to flakes-like particles shape of CoO nanoparticles stabilized in TOAB.

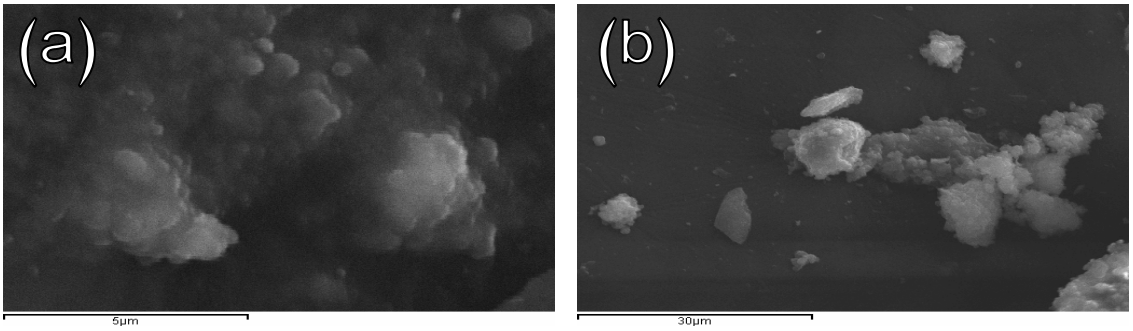

Figure (4.9): SEM pictures of CoO nanoparticles stabilized in TOAB at different magnifications (a & b)

4.1.3 Energy dispersive X-ray microscopy characterization (EDX)

Energy dispersive X-ray microscopy (EDX) is an analytical technique used for the elemental analysis of a sample. The following figures and tables show the results of this characterization:

4.1.3.1 EDX characterization of zinc oxide

EDX characterization of ZnO-NPs sample shows that each element has a specific atomic percentage, oxygen = 48.66, zinc = 46.74, and the remaining for silicon and copper. These are due to the substrate over which it was held to do SEM characterization, (Table. 4.1, Figure. 4.10):

Table (4.1): EDX analysis of ZnO-NPs

Element	Weight %	Atomic %
O K	19.59	48.66
Si K	3.03	4.29
Cu K	0.50	0.31
Zn K	76.88	46.74
Totals	**100.00**	

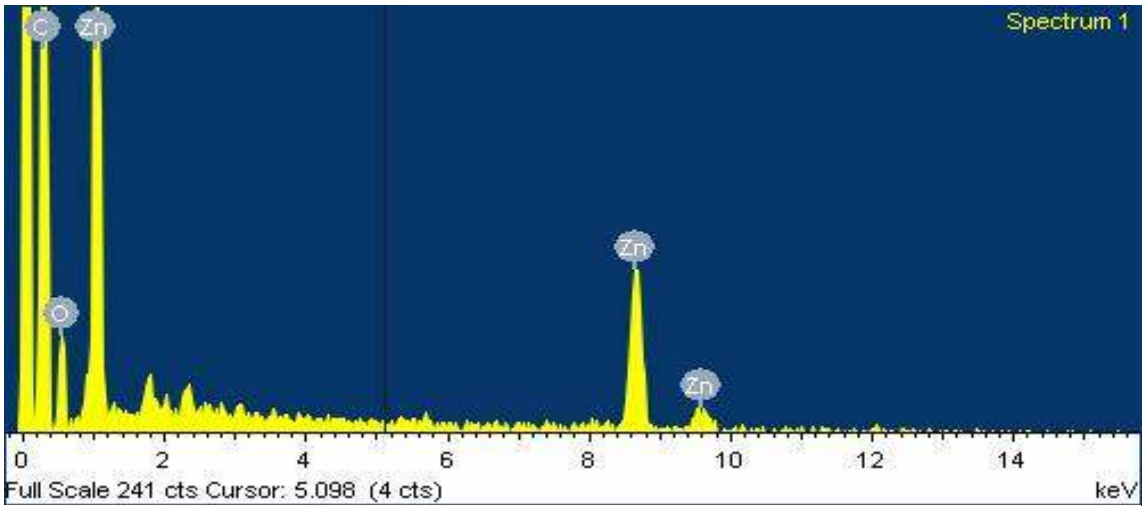

Figure (4.10): EDX image for ZnO nanoparticles.

4.1.3.2 EDX Characterization of zinc oxide stabilized in TOAB

EDX characterization of ZnO-NPs stabilized in TOAB sample shows that each element has a specific atomic percentage, oxygen = 2.64, zinc = 93.30, and the remaining for silicon and copper. These are due to the substrate over which it was held to do SEM characterization, (Table. 4.2, Figure. 4.11):

Table (4.2): EDX analysis of ZnO-NPs Stabilized in TOAB:

Element	Weight %	Atomic %
O K	0.67	2.64
Si K	0.70	1.59
Cu K	2.47	2.47
Zn K	96.16	93.30
Totals	**100.00**	

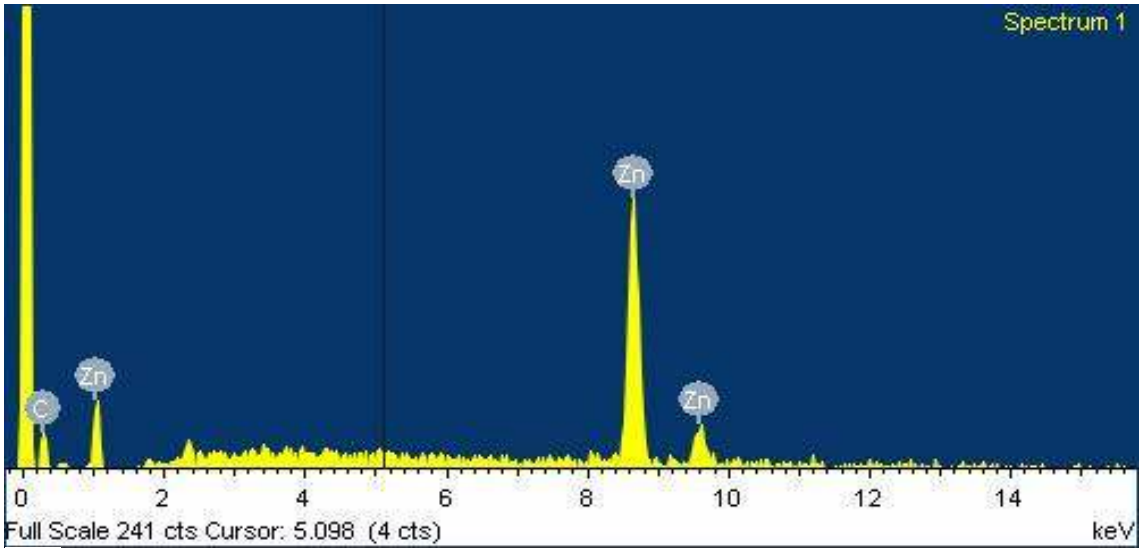

Figure (4.11): EDX image for ZnO nanoparticles stabilized in TOAB.

4.1.3.3 EDX Characterization of cobalt oxide nanoparticles

In table (4.3), EDX characterization of CoO-NPs sample shows that each element has a specific atomic percentage, oxygen = 50.09, cobalt = 48.50, and the remaining for silicon. This is due to the substrate over which it was held to do SEM characterization, (Table. 4.3, Figure. 4.12):

Table (4.3): EDX analysis of CoO-NPs

Element	Weight %	Atomic %
O K	21.66	50.09
Si K	1.07	1.41
Co K	77.27	48.50
Totals	**100.00**	

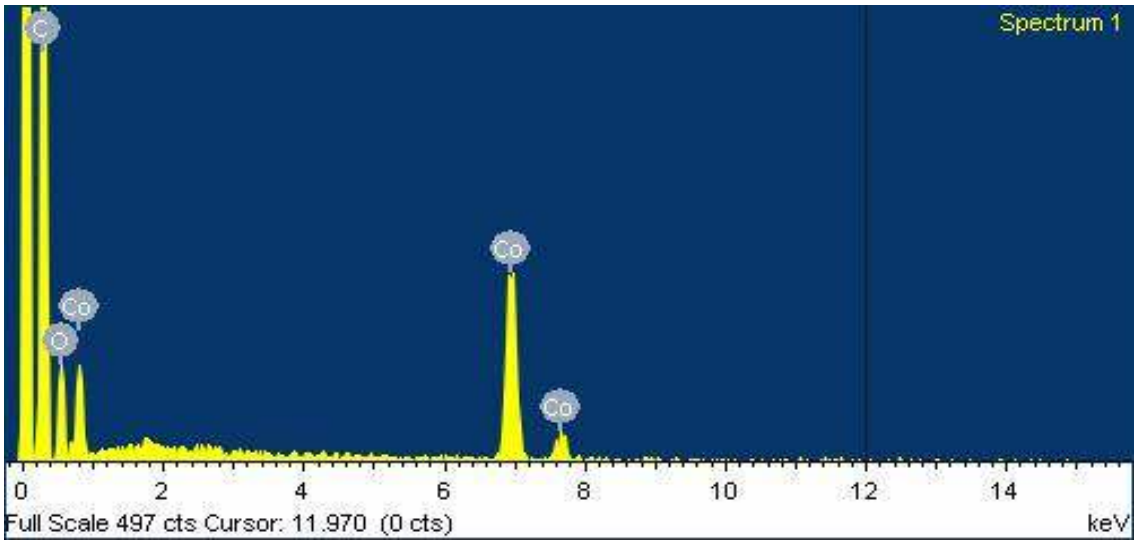

Figure (4.12): EDX image for CoO nanoparticles.

4.1.3.4 Characterization of cobalt oxide stabilized in TOAB

In Table (4.4), EDX characterization of CoO-NPs stabilized in TOAB sample shows that each element has a specific atomic percentage, oxygen = 20.41, cobalt = 73.44, and the remaining for silicon. This is due to the substrate over which it was held to do SEM characterization, (Table. 4.4, Figure. 4.13):

Table (4.4): EDX analysis of CoO-NPs stabilized in TOAB:

Element	Weight %	Atomic %
O K	6.76	20.41
Si K	3.58	6.15
Co K	89.66	73.44
Totals	**100.00**	

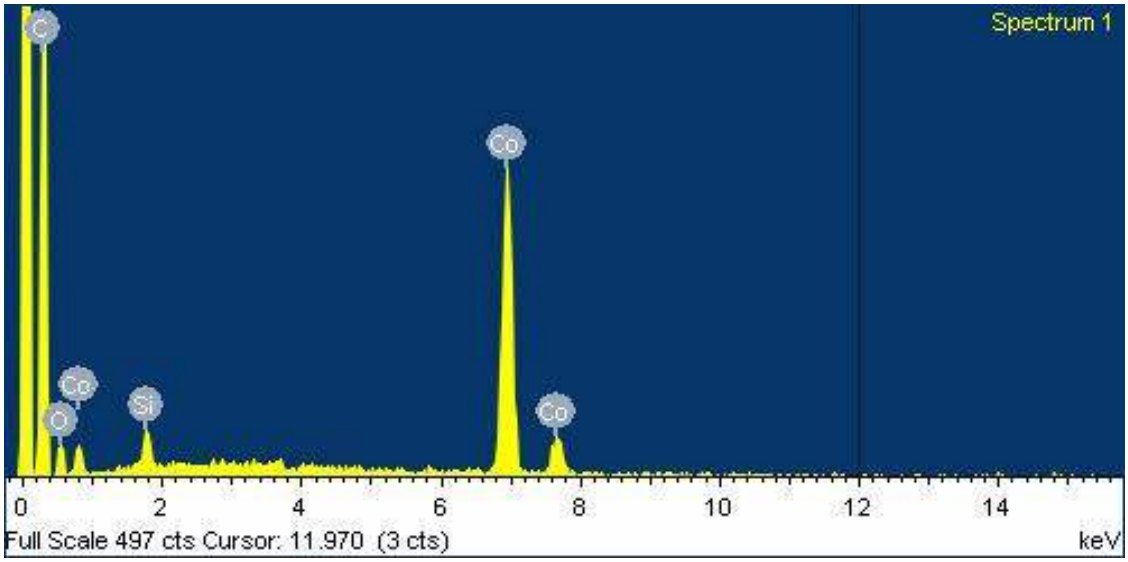

Figure (4.13): EDX image for CoO nanoparticles stabilized in TOAB

4.2 Antibacterial activity

Antibacterial activity to determine the MIC of each preparation was examined for each material used in this study by measuring the absorbance value for each test tube using the spectrophotometer at the λ of 625 nm. λ of 625 nm. It was determined as the appropriate λ of all studied bacterial strains using the spectrum option of the UV-spectrophotometer. All the preparations of the nanoparticles, the nanoparticles stabilized in TOAB and the TOAB stabilized nanoparticles mixed with the antibiotics were prepared in the same manner by dissolving in the NB.

The effect of the nanoparticles on the pH of the NB was determined to exclude the pH antibacterial activity. The pH of the NB before any addition was 6.4, whereas the addition of the nanoparticles to the NB increased the pH of the NB to a close pH of 7.4 and 7.1 for the ZnO and CoO, respectively. However, the addition of the nanoparticles stabilized in TOAB slightly increased the pH of the NB to a comparable pH to the NPs

of 6.73 and 6.53 for the ZnO stabilized in TOAB and CoO stabilized in TOAB, respectively.

4.2.1 The antibacterial effect of tetra-octyl ammonium bromide (TOAB)

The activity of TOAB (which is a member of Quaternary ammonium salts (QAS) disinfectant and used as the basic shell stabilizer for the postulated nanoparticles in this research) was measured by serial dilution method and used as comparative reference for the different related prepared nanoparticles, from (Table. 4.5, Figure. 4.14), the MIC for all bacterial isolates were 250 µg/mL (> 125 µg/mL).

Table (4.5): The antibacterial activity represented by absorbance for TOAB concentrations against *E. coli, S. aureus* and *B. subtillis*

	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	control
E. coli	0.000	0.000	0.700	0.720	0.790
S. aureus	0.000	0.000	0.718	1.247	1.140
B. subtilis	0.000	0.000	0.603	1.045	0.827

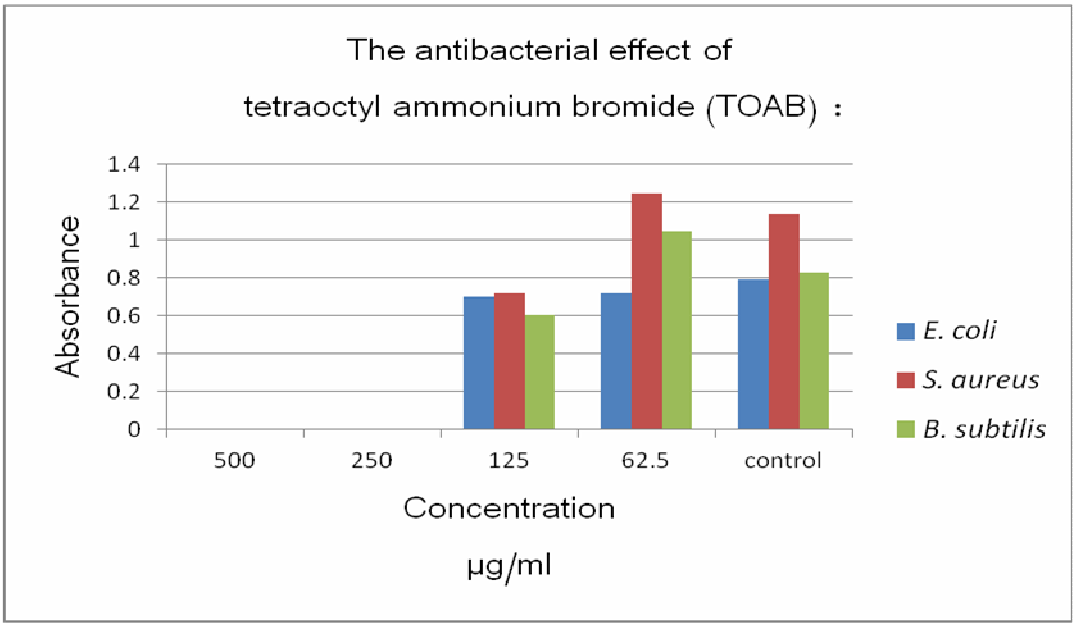

Figure (4.14): The antibacterial activity of TOAB against *E. coli, S. aureus* and *B. subtillis.*

The results shown in Figure (4.14) are in accordance to the action of QAS. Li Z *et al*., postulated that QAS penetrate and interrupt the bacterial membrane by their fatty alkyl chains and they increased the osmotic pressure between the high-ionic strength surrounding bacteria and the bacterial cytoplasm [23], and so the TOAB has antibacterial activity against gram positive and negative bacteria, also the MIC is the lowest against gram positive and spore forming bacteria, *S. aureus,* which has loose cell wall than gram negative bacteria which has complicated outer membrane structure in addition to cell wall to protect the gram negative against foreign molecules [9].

4.2.2 The antibacterial effect of dimethyl sulfoxide (DMSO)

DMSO was used as solvent for the nanoparticles, so its antibacterial activity was measured by serial methods, and the results detected that 7.5% (> 3.75%) was the percentage at which DMSO doesn't have any antibacterial effect for all bacterial isolates, the results were as in (Table. 4.6, Figure. 4.15):

Table (4.6): The antibacterial activity represented by absorbance for DMSO concentrations against *E. coli, S. aureus* and *B. subtillis*

	30.0%	15.0%	7.50%	3.75%	Control
E. coli	0.000	0.000	0.375	0.953	1.257
S. aureus	0.000	0.000	0.156	0.732	0.845
B. subtilis	0.000	0.000	0.450	1.009	0.972

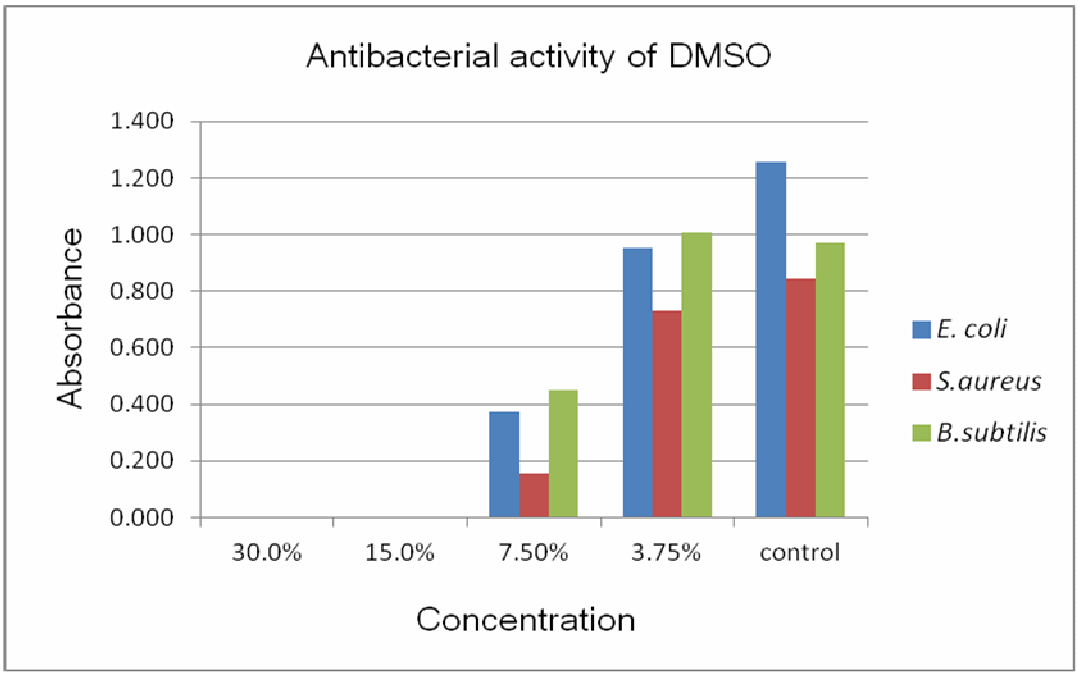

Figure (4.15): The antibacterial activity of DMSO against *E. coli, S. aureus* and *B. subtillis.*

DMSO was chosen to dissolve the nanoparticles that have low solubility in water, and it was found that DMSO has a marked antibacterial activity against a wide range of bacteria [6]. DMSO was prepared in 15% stock solution, that was used to prepare the serial dilutions of NPs at a concentration < 7.5% to exclude the antibacterial activity of the DMSO.

4.2.3 Antibacterial activity of nanoparticles

The main goal of this research is to compare the antibacterial activity of: zinc oxide and cobalt oxide nanoparticles, the antibacterial activity of these metal oxides with stabilizers (TOAB), then the antibacterial activity of the stabilized metal oxides mixed with three antibiotics types separately (Amoxicillin, Cephalexin and Streptomycin).

4.2.3.1 Zinc oxide nanoparticles

i. Antibacterial activity of zinc oxide nanoparticles:

The antibacterial activity of ZnO was higher against *S. aureus* and *E. coli* which have MIC = 165 µg/mL (> 82.0 µg/mL) than *B. subtilis* with MIC = 330 µg/mL (> 165 µg/mL) without any selective effectiveness against gram negative or positive bacteria, as shown in (Table. 4.7, Figure. 4.16):

Table (4.7): Antibacterial activity represented by absorbance for zinc oxide nanoparticles concentrations against *E. coli, S. aureus* and *B. subtilis.*

	660 µg/mL	330 µg/mL	165 µg/mL	82.0 µg/mL	41.0 µg/mL	control
E. coli	0.000	0.000	0.000	0.458	0.701	0.668
S. aureus	0.000	0.000	0.000	0.517	0.682	0.665
B. subtilis	0.000	0.000	0.185	0.355	0.383	0.461

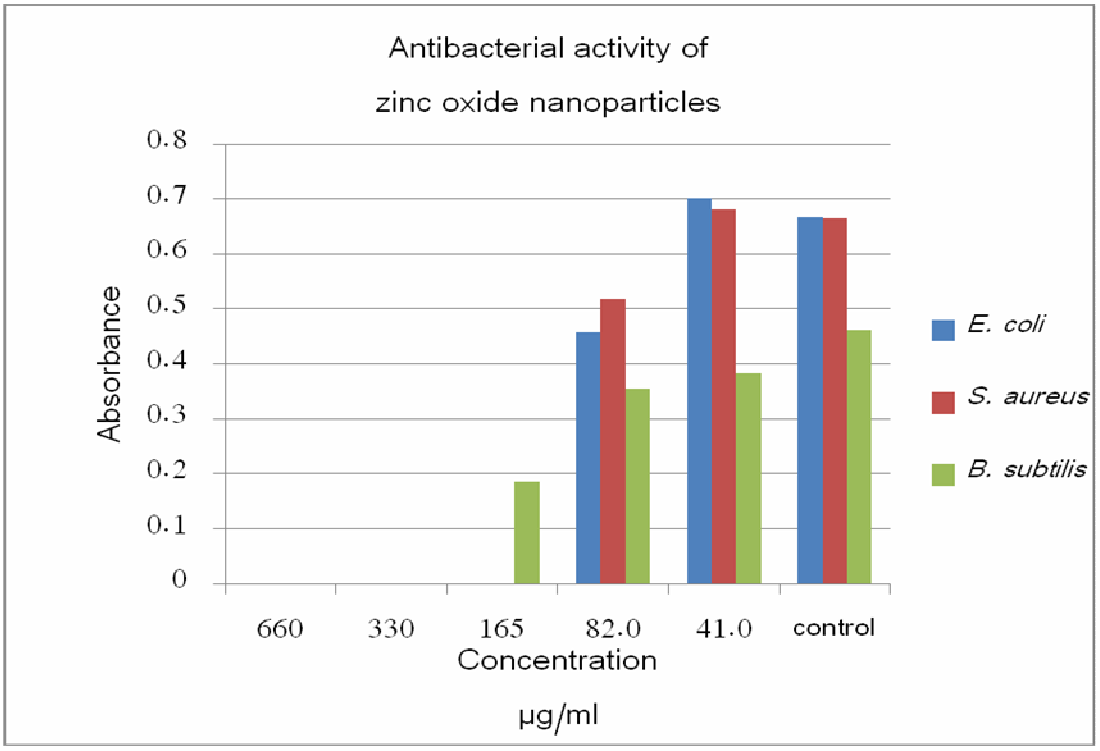

Figure (4.16): The antibacterial activity against ZnO-NPs for *E. coli, S. aureus* and *B. subtilis.*

Zinc oxide is one of dermatological ingredients in creams, lotion, but in nanotechnology zinc oxide plays a critical role slowing down the bacterial activity of gram positive and gram negative. The results in Table (4.7) show that zinc oxide nanoparticles have antibacterial activity against gram positive and gram negative at the same time, as a result of membrane disorganization which increases the membrane permeability and so the accumulation of nanoparticles in the bacterial membrane and cytoplasm regions of the cells [17] that could explain the relative higher MIC of *S. aureus* and *B. subtilis* as in Figure (4.16), from the Table (4.7) zinc oxide nanoparticles antibacterial activity increased as its concentration increased and this moves in accordance to the result of Wang *et al*. [38].

ii. Antibacterial activity of zinc oxide nanoparticles stabilized in TOAB:

Table (4.8) showed that the MIC = 100 µg/mL (> 50 µg/mL) for gram negative bacteria *E. coli* and gram positive bacteria *S. aureus*, and the MIC for *B. subtilis*, gram positive & spore forming = 200 µg/mL (> 100 µg/mL) which is higher than the previous bacteria: *S. aureus* and *E. coli*, results shown in (Table. 4.8, Figure. 4.17):

Table (4.8): The antibacterial activity represented by absorbance for ZnO-NPs stabilized in TOAB concentrations against *E. coli, S. aureus* and *B. subtilis*.

	200 µg/mL	100 µg/mL	50.0 µg/mL	25.0 µg/mL	12.5 µg/mL	control
E. coli	0.000	0.000	0.410	0.551	0.508	0.670
S. aureus	0.000	0.000	0.274	0.425	0.555	0.594
B. subtilis	0.000	0.124	0.350	0.581	0.749	0.972

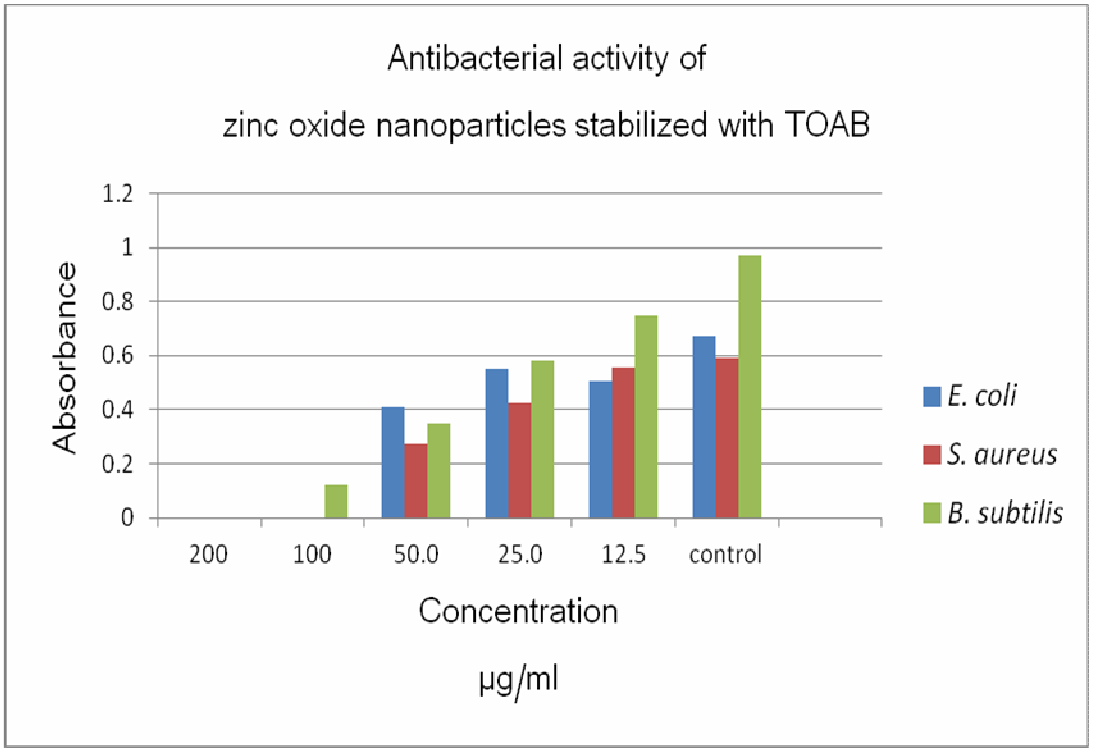

Figure (4.17): The antibacterial activity of ZnO-NPs stabilized in TOAB against *E. coli, S. aureus and B. subtilis.*

In this part, the monolayer stabilizer shell of TOAB which is a cationic surfactant, played a synergestic role that leads to the increment of nanoparticles effectiveness because they have a bioactive coating that slows down the antibacterial activity of gram positive and negative bacteria [10], for both gram positive and gram negative, the MIC for *S. aureus* and *E. coli* is 100 µg/mL (> 50 µg/mL) in the presence of TOAB with the zinc oxide nanoparticles whereas it was 165 µg/mL in the absence of TOAB (in the zinc oxide nanoparticles alone). *B. subtilis* MIC is also lower in the presence of TOAB (which equals 200µg/mL) than in the absence of TOAB (which equals 330 µg/mL).

4.2.3.2 Cobalt oxide nanoparticles:

i. Antibacterial activity of cobalt oxide nanoparticles:

Antibacterial activity of CoO are higher against *E. coli* with 82 µg/mL (> 41 µg/mL) MIC than *S. aureus* and *B. subtilis* 165 µg/mL (> 82 µg/mL) that observed in (Table. 4.9, Figure 4.18).

Table (4.9): The antibacterial activity represented by absorbance for CoO-NPs concentrations against *E. coli, S. aureus and B. subtilis*.

	660 µg/mL	330 µg/mL	165 µg/mL	82.0 µg/mL	41.0 µg/mL	control
E. coli	0.000	0.000	0.000	0.000	0.227	0.733
S. aureus	0.000	0.000	0.000	0.437	0.523	0.733
B. subtilis	0.000	0.000	0.000	0.353	0.493	0.573

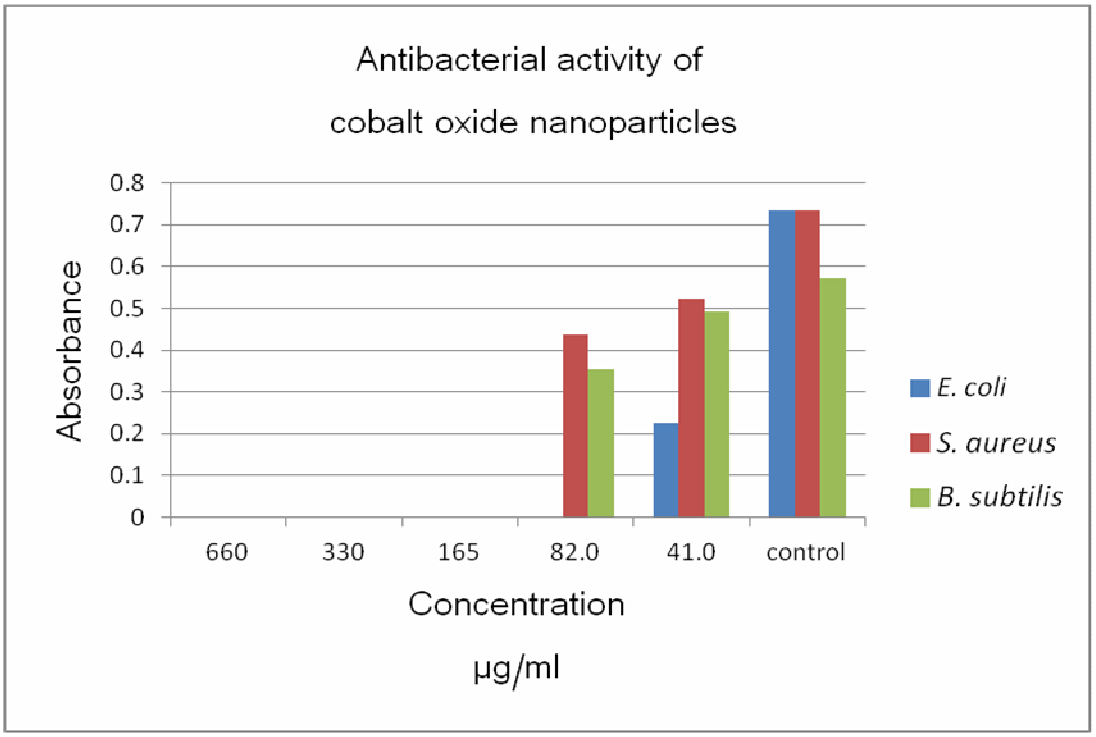

Figure (4.18): The antibacterial activity of CoO-NPs against *E. coli, S. aureus and B. subtilis*

The above results give a great improvement that cobalt oxide has antibacterial activity against gram positive and gram negative bacteria and

is clear against gram negative bacteria as in *E. coli*, that its MIC is 82.0 µg/mL while the MIC of *S. aureus* and *B. subtalis,* the gram positive bacteria, is 165 µg/mL.

Cobalt oxide nanoparticles are treated as a magnetic nanoparticles more than antibacterial agents, but as any nanoparticles, cobalt oxide nanoparticles exhibit a unique high surface area (surface/volume ratio).

ii. Antbacterial activity of cobalt oxide nanoparticles stabilized in TOAB:

From this Table (4.10) which shows the highest activity against gram negative bacteria *E. coli* which has MIC = 100 µg/mL (> 50 µg/mL), than the other positive bacterial types, *S. aureus* and *B. subtilis* which have MIC = 200 µg/mL (>100 µg/mL) as shown in (Table. 4.10 & Figure 4.19):

Table (4.10): The antibacterial activity represented by absorbance for CoO-NPs stabilized in TOAB concentrations against *E. coli, S. aureus and B. subtilis.*

	200 µg/mL	100 µg/mL	50.0 µg/mL	25.0 µg/mL	12.5 µg/mL	control
E. coli	0.000	0.000	0.393	0.530	0.750	0.708
S. aureus	0.000	0.120	0.390	0.396	0.477	0.596
B. subtilis	0.000	0.169	0.508	0.670	0.784	0.905

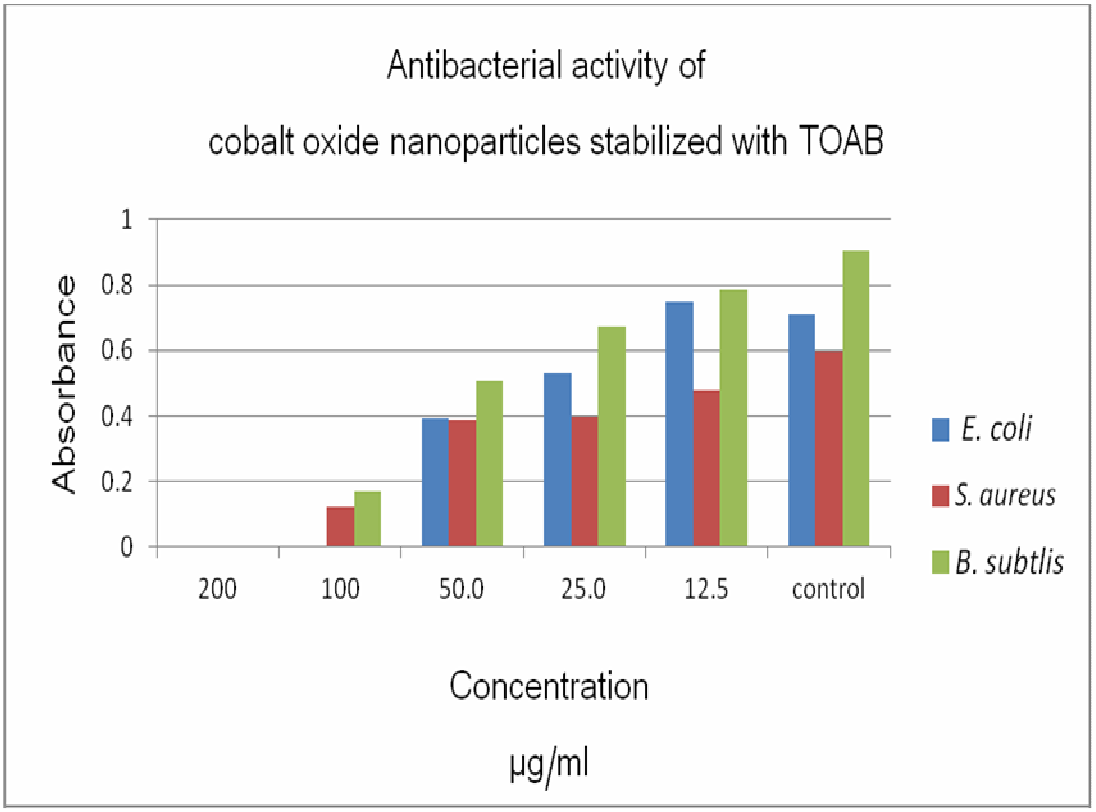

figure (4.19): The antibacterial activity of CoO-NPs stabilized in TOAB against *E. coli, S. aureus and B. subtilis*

As in the results of cobalt oxide alone, cobalt oxide stabilized in TOAB gives a clear antibacterial activity against gram positive and gram negative and its more obviously against gram negative *E. coli,* that its MIC = 100 µg/mL and for the other bacteria (*S. aureus* and *B. subtilis)* = 200 µg/mL, which means also that these bacteria have approximately the same MIC for cobalt oxide and cobalt oxide stabilized in TOAB.

4.2.4 Antibacterial activity of antibiotics:

Three kinds of antibiotics from three different classes were of our interest in this research, these antibiotics are: Amoxicillin, Cephalexin and Streptomycin.

Antibacterial activity for each three antibiotics types was measured by serial dilution against three bacterial types: *E. coli*, *S. aureus* and *B. subtilis* for each antibiotic as the following tables:

a. Amoxicillin:

The MIC of amoxicillin powder was examined using serial dilution technique before mixing it with the metal oxides (Table. 4.11, Figure. 4.20).

Table (4.11): The antibacterial activity represented by absorbance for amoxicillin concentrations against *E. coli, S. aureus and B. subtilis*

	81.4 µg/mL	27.2 µg/mL	8.80 µg/mL	2.90 µg/mL	0.98 µg/mL	0.29 µg/mL	control
E. coli	0.000	0.000	0.000	0.210	0.245	0.428	0.478
S. aureus	0.000	0.000	0.000	0.000	0.000	0.000	0.315
B. subtilis	0.305	0.331	0.330	0.350	0.995	0.968	0.940

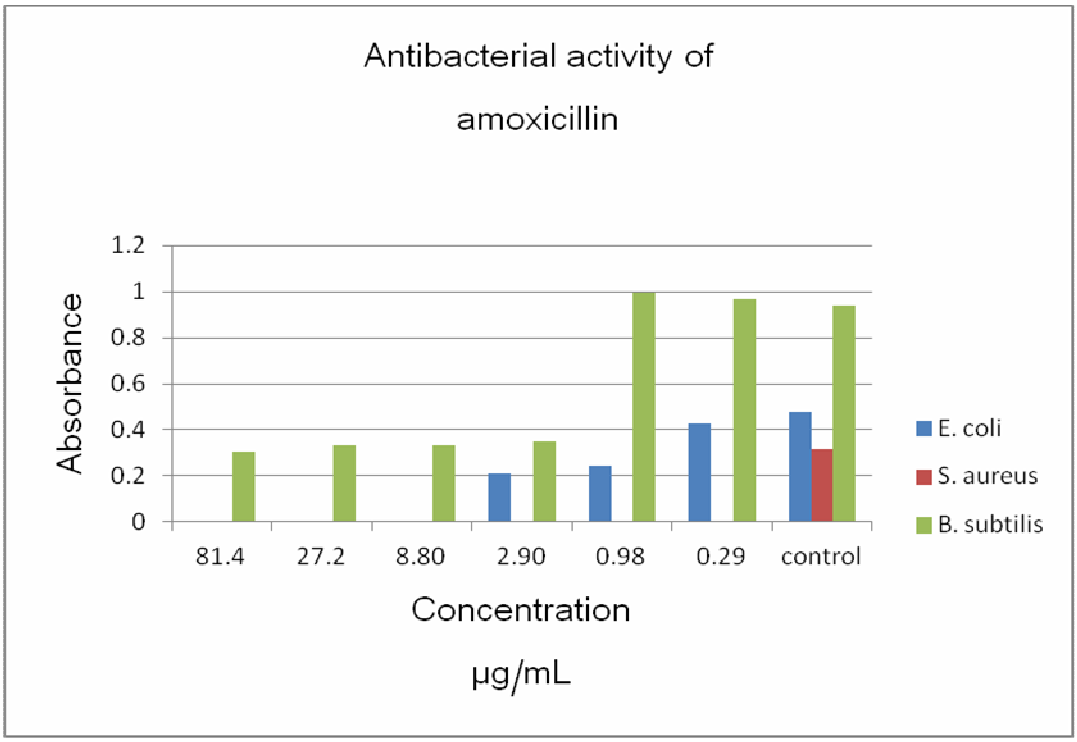

Figure (4.20): The antibacterial activity of amoxicillin against *E. coli, S. aureus and B. subtilis.*

In Table (4.11), it is clearly observed that amoxicillin has the highest antibacterial activity against *S. aureus* which has MIC < 0.29 µg/mL than *E. coli* which has MIC = 8.80 µg/mL (> 2.90 µg/mL), with approximately no effectiveness against *B. subtilis*, which means that *B. subtilis* has MIC > 81.4 µg/mL, so amoxicillin has the highest antibacterial activity against *S. aureus* which is gram positive bacteria then against *E. coli* which is gram negative bacteria and these results agree with literature results in [1] which said: amoxicillin has a broad spectrum of bactericidal activity against many gram-positive bacteria and a limited range of gram-negative bacteria.

b. Cephalexin:

The MIC of cephalexin powder was examined using serial dilution technique before mixing it with the metal oxides (Table. 4.12, Figure. 4.21)

Table (4.12): The antibacterial activity represented by absorbance for cephalexin concentrations against *E. coli, S. aureus* and *B. subtilis.*

	81.4 µg/mL	27.2 µg/mL	8.80 µg/mL	2.90 µg/mL	0.98 µg/mL	0.29 µg/mL	control
E. coli	0.000	0.000	0.175	0.190	0.333	0.371	0.478
S. aureus	0.000	0.000	0.000	0.360	0.507	0.600	0.315
B. subtilis	0.000	0.000	0.524	0.618	0.750	0.762	0.940

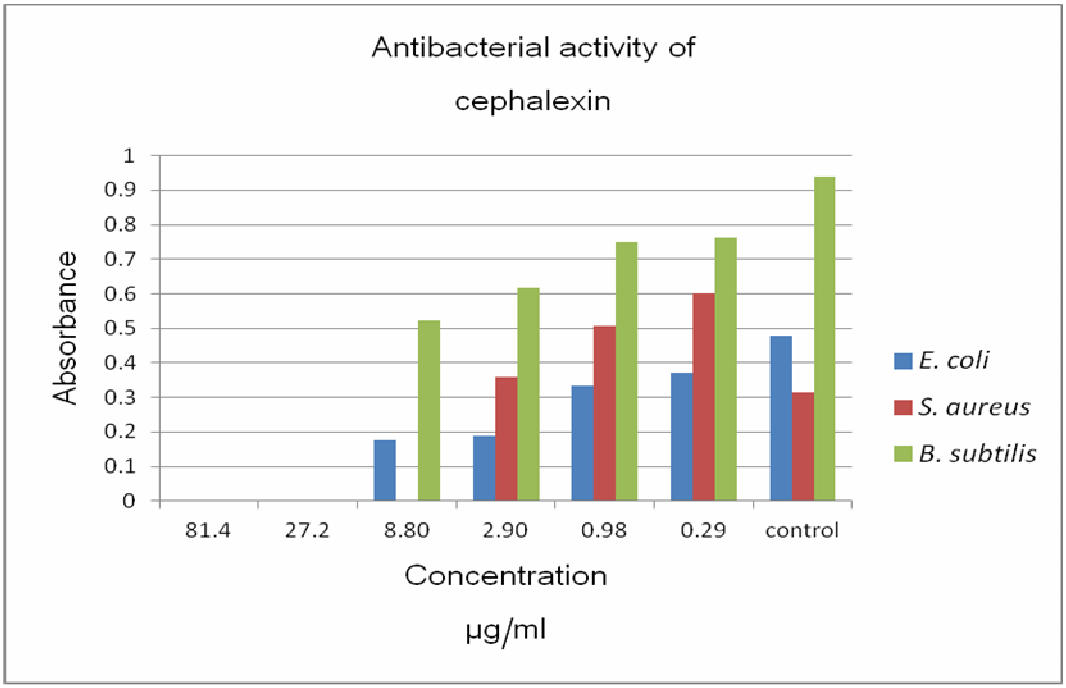

Figure (4.21): The antibacterial activity of cephalexin against *E. coli, S. aureus and B. subtilis*

From the above Figure (4.21) *S. areus* has the lowest MIC = 8.80 µg/mL (> 2.90 µg/mL) than *E.coli* and *B. subtilis* which has MIC = 27.2 µg/mL (> 8.80 µg/mL) more than the *S. areus*, these results coincide with Trevor A.J *et al*., who said: this medication is active against gram–positive cocci, including: staphylococci and streptococci [37].

c. Streptomycin:

The MIC of streptomycin powder was examined using serial dilution technique before mixing it with the metal oxides (Table. 4.13, Figure. 4.22).

Table (4.13): The antibacterial activity represented by absorbance for streptomycin concentrations against *E. coli, S. aureus* and *B. subtilis.*

	81.4 µg/mL	27.2 µg/mL	8.80 µg/mL	2.90 µg/mL	0.98 µg/mL	0.29 µg/mL	control
E. coli	0.000	0.000	0.000	0.000	0.000	0.215	0.478
S. aureus	0.000	0.000	0.000	0.000	0.000	0.000	0.315
B. subtilis	0.000	0.000	0.000	0.000	0.000	0.247	0.940

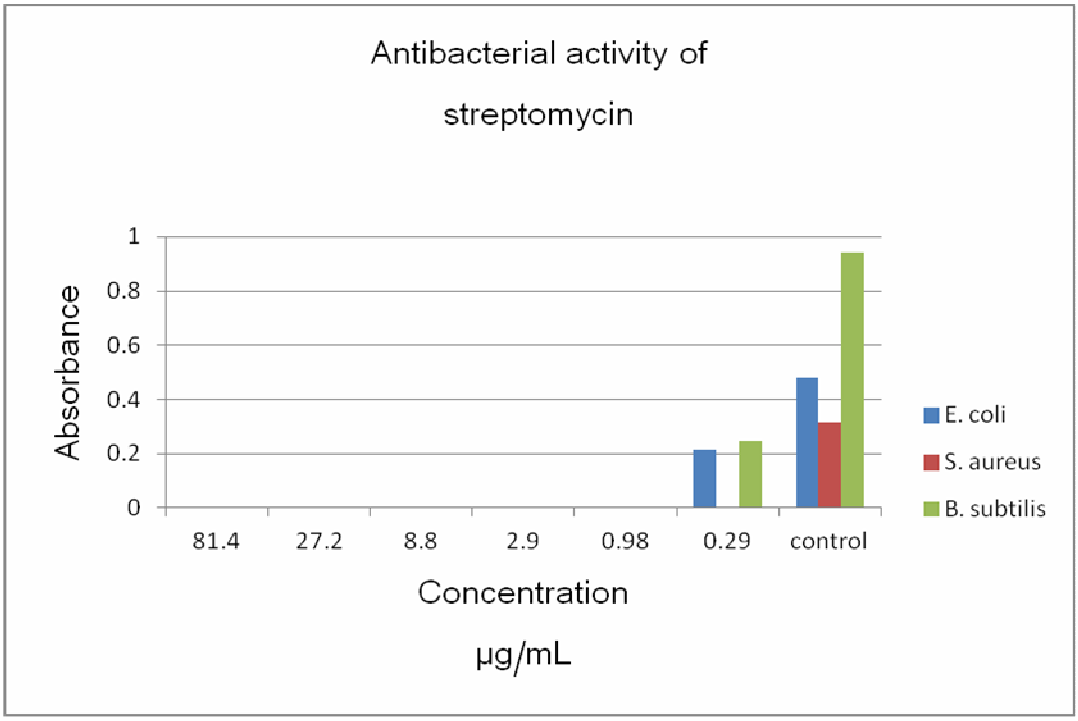

Figure (4.22): The antibacterial activity of streptomycin against *E. coli, S. aureus and B. subtilis.*

From the above Figure (4.22) *S. areus* has the lowest MIC < 0.29 µg/mL than *E.coli* and *B. subtilis* which has MIC = 0.98 µg/mL (> 0.29 µg/mL). So these results indicate that streptomycin can inhibit *S. aureus* at very low concentration.

4.2.5 The antibacterial activity of nanoparticles oxide stabilized in TOAB and mixed with antibiotics

Nanoparticles stabilized in TOAB are mixed with antibiotics to achieve synergestic effect against bacteria. Then by serial dilution, the MIC

of different prepared mixture can be detected by spectrophotometer at fixed λ equals 625 nm, the results are as the following:

4.2.5.1 The antibacterial activity of zinc oxide nanoparticles stabilized in TOAB and mixed with antibiotics

I. Antibacterial activity of zinc oxide stabilized in TOAB and mixed with amoxicillin:

After preparing zinc oxide that is stabilized in TOAB, it is mixed with specific amount of amoxicillin antibiotic, and by serial dilution for the three bacterial types the absorbance of each tubes are measured at λ = 625 nm. Then the MIC is determined for each bacterial type (Table. 4.14, Figure. 4.23).

Table (4.14): The antibacterial activity represented by absorbance for ZnO-NPs stabilized in TOAB and mixed with amoxicillin concentrations against *E. coli, S. aureus* and *B. subtillis.*

	400.00 µg/mL	80.000 µg/mL	16.000 µg/mL	3.2000 µg/mL	0.6400 µg/mL	0.1280 µg/mL	0.0256 µg/mL	control
E. coli	0.000	0.000	0.000	0.304	0.406	0.462	0.507	0.854
S. aureus	0.000	0.000	0.000	0.000	0.194	0.247	0.247	0.520
B. subtilis	0.000	0.000	0.220	0.312	0.306	0.375	0.411	0.610

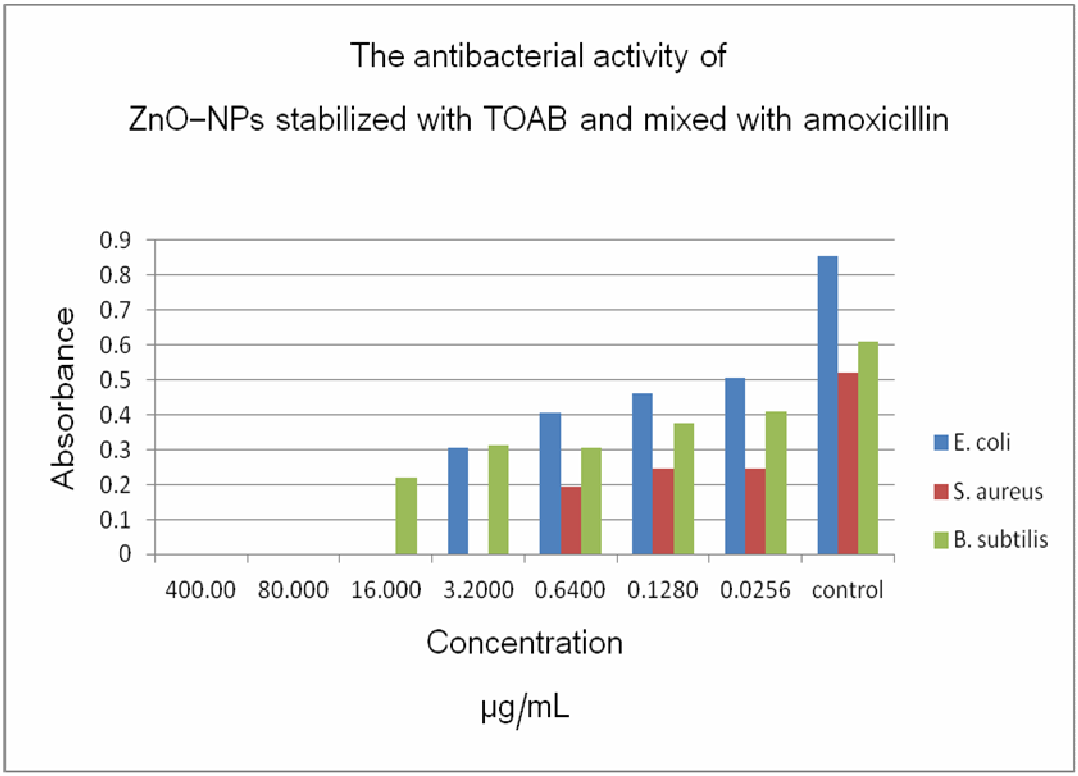

Figure (4.23): The antibacterial activity of ZnO-NPs stabilized in TOAB and mixed with amoxicillin against *E. coli, S. aureus* and *B. subtilis*

From Table (4.14) *S. aureus* has the lowest MIC which equals 3.20 µg/mL (> 0.64 µg/mL) then for *E. coli* which has MIC = 16.0 µg/mL (> 3.20 µg/mL) and the highest MIC is for *B. subtilis* which equals 80.0 µg/mL (> 16.0 µg/mL), when these results are compared with (Table. 4.8), so the synergestic effect of the combination between stabilized ZnO-NPs and amoxicillin are clear, that the MICs decreased for all bacterial isolates, especially for gram positive bacteria (*S. aureus* & *B. subtilis)*, may the reason that amoxicillin has broad spectrum against gram positive and limited action against the negative one, therefore the action of amoxicillin antibiotic is increased in the presence of ZnO-NPs.

II. Antibacterial activity of zinc oxide stabilized in TOAB and mixed with cephalexin:

From Table (4.15), it shows that *S. aureus* and *B. subtilis* which are gram positive bacteria has the lowest MIC for the cephalexin mixture with zinc oxide which equals 16.0 µg/mL (> 3.20 µg/mL) whereas *E. coli* the gram negative bacteria has MIC = 80.0 µg/mL (> 16.0 µg/mL), as the following in (Table. 4.15, Figure. 4.24).

Table (4.15): The antibacterial activity represented by absorbance for ZnO-NPs stabilized in TOAB and mixed with cephalexin concentrations against *E. coli, S. aureus and B. subtilis.*

	400.00 µg/mL	80.000 µg/mL	16.000 µg/mL	3.2000 µg/mL	0.6400 µg/mL	0.1280 µg/mL	0.0256 µg/mL	control
E. coli	0.000	0.000	0.186	0.236	0.328	0.442	0.436	0.570
S. aureus	0.000	0.000	0.000	0.191	0.212	0.232	0.233	0.437
B. subtilis	0.000	0.000	0.000	0.131	0.270	0.298	0.371	0.542

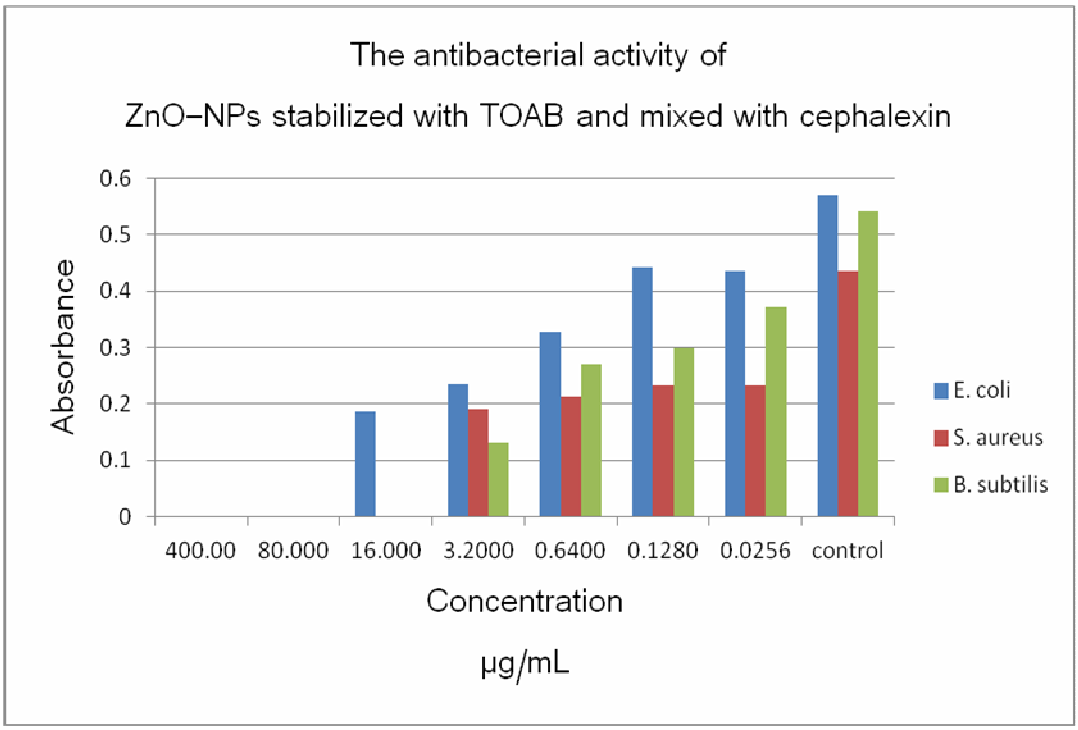

Figure (4.24): The antibacterial activity of ZnO-NPs stabilized in TOAB and mixed with cephalexin against *E. coli, S. aureus* and *B. subtilis*

In comparison the results of Table (4.15) with Table (4.8) for stabilized ZnO-NPs, the synergestic effect of the such combination (ZnO-NPs with cephalexin) is detected significantly for gram positive bacteria than the gram negative one.

III. Antibacterial activity of zinc oxide stabilized in TOAB and mixed with streptomycin:

Table (4.16) shows that *E. coli* and *B. subtilis* has the lowest MIC for the streptomycin mixture with stabilized zinc oxide which equals 17.2 µg/mL (> 3.40 µg/mL) whereas *S. aureus* has MIC = 86.0 µg/mL (> 17.2 µg/mL), as shown in (Table. 4.16, Figure. 4.25):

Table (4.16): The antibacterial activity represented by absorbance for ZnO-NPs stabilized in TOAB and mixed with streptomycin concentrations against *E. coli, S. aureus* and *B. subtilis.*

	430.0 µg/mL	86.00 µg/mL	17.20 µg/mL	3.400 µg/mL	0.680 µg/mL	0.130 µg/mL	0.027 µg/mL	control
E. coli	0.000	0.000	0.000	0.286	0.335	0.357	0.403	0.456
S. aureus	0.000	0.000	0.197	0.208	0.241	0.316	0.341	0.454
B. subtilis	0.000	0.000	0.000	0.198	0.209	0.264	0.330	0.395

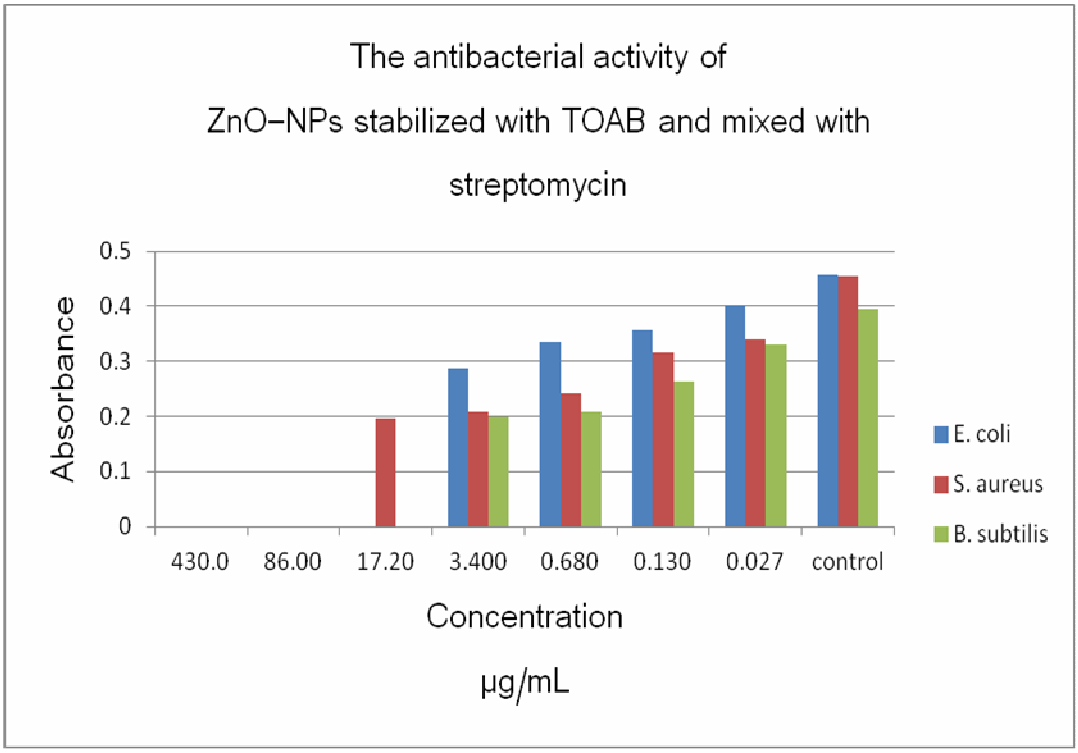

Figure (4.25): The antibacterial activity of ZnO-NPs stabilized in TOAB and mixed with streptomycin against *E. coli, S. aureus* and *B. subtilis*

These results can be discussed if this Table (4.16) is compared with Table (4.8), that the MIC of ZnO-NPs against *E. coli* and *B. subtilis* are decreased more than *S. aureus,* which indicate the synergestic effect of stabilized ZnO-NPs mixed with streptomycin. The previous results can be explained that streptomycin has antibacterial activity against gram negative bacteria as in *E. coli,* and *B. subtilis* which its growth inhibition is studied by Mandal A. and Majumdar S.K. 1968 to approve that, streptomycin slightly inhibited lactic and malic dehydrogenases of *B. subtilis*, and inhibited isocitric dehydrogenase to about 60% and inhibited 48% of its synthesis, but the streptomycin activity against *S. aureus* is limited to some strains as mentioned previously by [24].

4.2.5.2 The antibacterial activity of cobalt oxide nanoparticles stabilized in TOAB and mixed with antibiotics

I. Antibacterial activity of cobalt oxide stabilized in TOAB and mixed with amoxicillin:

Table (4.17) shows that *S. aureus* has MIC < 0.0256 µg/mL, whereas *E. coli* and *B. subtilis* have MIC = 16.0 µg/mL (> 3.20 µg/mL), (Table. 4.17, Figure. 4.26).

Table (4.17): The antibacterial activity represented by absorbance for CoO-NPs stabilized in TOAB and mixed with amoxicillin concentrations against *E. coli, S. aureus* and *B. subtilis.*

	400.00 µg/mL	80.000 µg/mL	16.000 µg/mL	3.2000 µg/mL	0.6400 µg/mL	0.1280 µg/mL	0.0256 µg/mL	control
E. coli	0.000	0.000	0.000	0.158	0.368	0.421	0.494	0.655
S. aureus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.380
B. subtilis	0.000	0.000	0.000	0.180	0.238	0.243	0.252	0.500

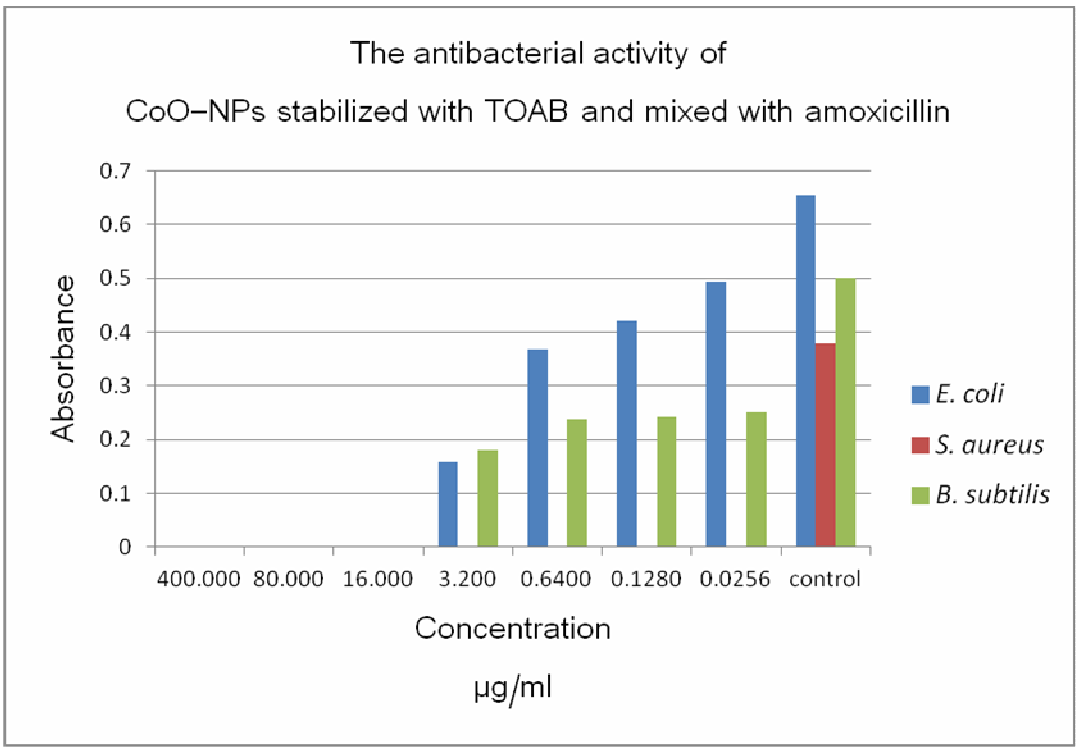

Figure (4.26): The antibacterial activity of CoO-NPs stabilized in TOAB and mixed with amoxicillin against *E. coli, S. aureus* and *B. subtilis*

From the comparison between this Table (4.17) and Table (4.10) the following can be seen: MIC of *S. aureus* decreased significantly then *B. subtilis* which are gram positive bacteria then *E. coli* which is gram negative bacteria, this means, the synergestic effect of stabilized CoO-NPs and broad spectrum against gram positive and limited one against gram negative, amoxicillin.

II. Antibacterial activity of cobalt oxide stabilized in TOAB and mixed with cephalexin:

Table (4.18) shows that *B. subtilis* has the MIC equals to 16.0 µg/mL (> 3.20 µg/mL), and the other bacterial types (*E. coli* and *S. aureus*) have MIC equals to 80.0 µg/mL (> 16.0 µg/mL) which is high in comparative to the other MIC as in (Table. 4.18, Figure. 4.27):

Table (4.18): The antibacterial activity represented by absorbance for CoO-NPs stabilized in TOAB and mixed with cephalexin concentrations against *E. coli, S. aureus* and *B. subtilis.*

	400.0 µg/mL	80.00 µg/mL	16.00 µg/mL	3.200 µg/mL	0.640 µg/mL	0.128 µg/mL	0.0256 µg/mL	control
E. coli	0.000	0.000	0.324	0.352	0.390	0.392	0.394	0.547
S. aureus	0.000	0.000	0.229	0.249	0.270	0.271	0.303	0.470
B. subtilis	0.000	0.000	0.000	0.209	0.222	0.239	0.257	0.456

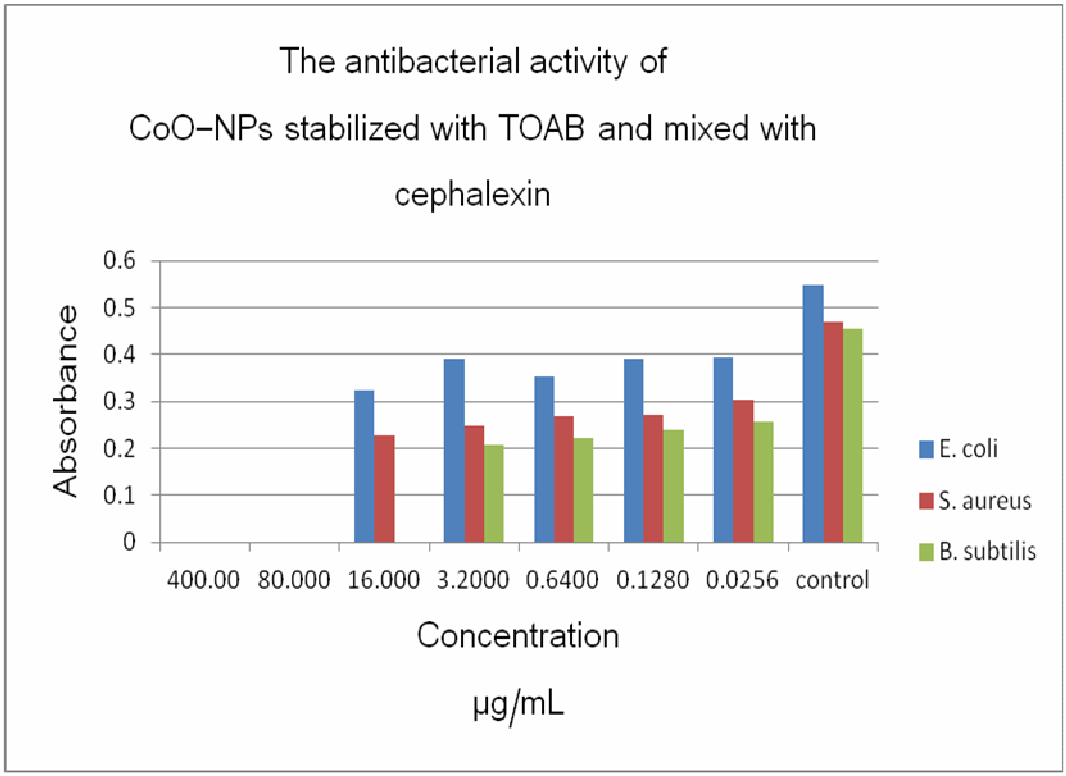

Figure (4.27): The antibacterial activity of CoO-NPs stabilized in TOAB and mixed with cephalexin against *E. coli, S. aureus* and *B. subtilis*

In Table (4.18), the MIC of *B. subtilis,* is the lowest one and if it is compared with Table (4.10), it is seen that the MIC has a significant jump from 200 µg/mL for the activity of stabilized cobalt oxide to 16.0 µg/mL for stabilized CoO-NPs mixed with cephalexin for *B. subtilis* , this can be explained that a gram positive antibiotic, cephalexin increase the activity of stabilized CoO-NPs for gram positive bacteria, first for *B. subtilis* then for *S. aureus*, which has a moderate jump of its MIC, from 200 µg/mL for the activity of stabilized cobalt oxide to 80.0 µg/mL for stabilized CoO-NPs mixed with cephalexin while the gram negative bacteria approximately has no effect from 100 µg/mL for the activity of stabilized cobalt oxide to 80.0 µg/mL for stabilized CoO-NPs mixed with cephalexin.

III. Antibacterial activity of cobalt oxide stabilized in TOAB and mixed with streptomycin:

Table (4.19) shows that *S. aureus* has the MIC equals to 0.680 µg/mL (> 0.130 µg/mL), and the other bacterial types (*E. coli* and *B. subtilis*) have MIC equals to 3.400 µg/mL (> 0.680 µg/mL) as in the following (Table. 4.19, Figure. 4.28):

Table (4.19): The antibacterial activity represented by absorbance for CoO-NPs stabilized in TOAB and mixed with streptomycin concentrations against *E. coli, S. aureus* and *B. subtilis.*

	430.0 µg/mL	86.00 µg/mL	17.20 µg/mL	3.400 µg/mL	0.680 µg/mL	0.130 µg/mL	0.027 µg/mL	control
E. coli	0.000	0.000	0.000	0.000	0.304	0.390	0.458	0.455
S. aureus	0.000	0.000	0.000	0.000	0.000	0.234	0.235	0.390
B. subtilis	0.000	0.000	0.000	0.000	0.361	0.519	0.537	0.552

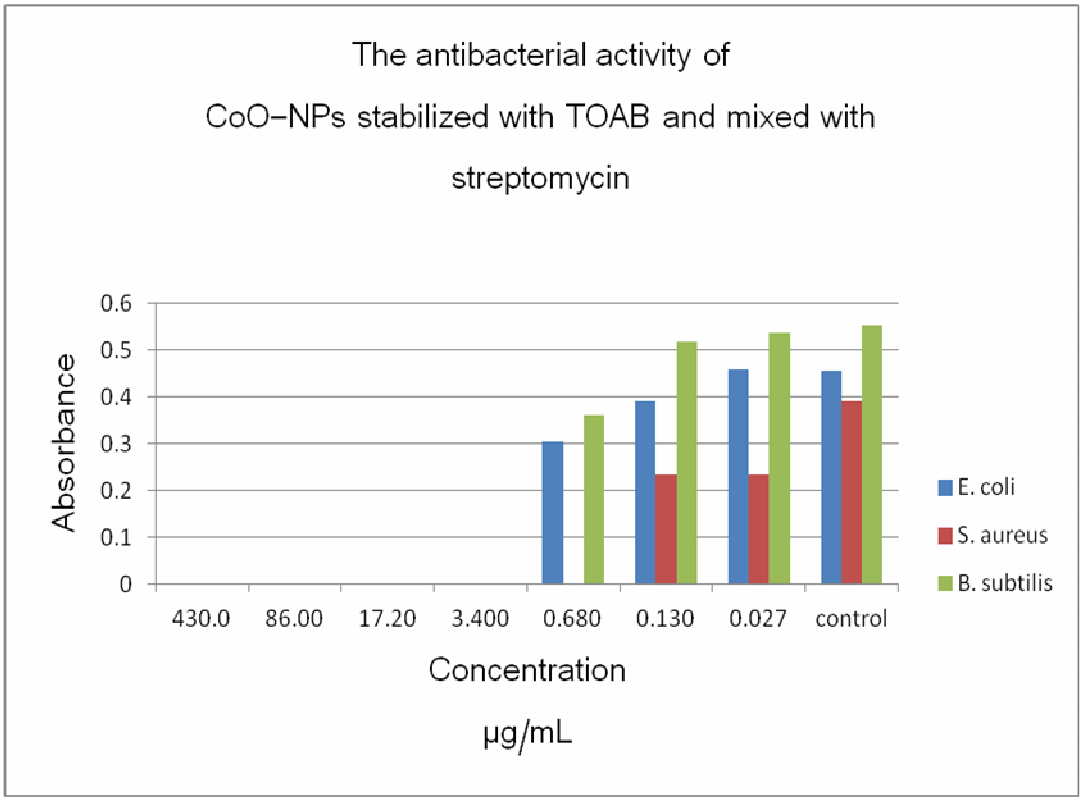

Figure (4.28): The antibacterial activity of CoO-NPs stabilized in TOAB and mixed with streptomycin against *E. coli, S. aureus* and *B. subtilis*

When this Table (4.19) is compared with Table (4.10), the synergestic effect of this novel combination is more significant in *S. aureus*, which has low MIC (0.680 µg/mL) in this mixture after the addition of streptomycin in comparison to high MIC (200 µg/mL) of this bacteria before antibiotic addition. Then for *B. subtilis,* the MIC decreased from 200 µg/mL to 3.40 µg/mL after the addition of streptomycin. Finally for *E. coli*, the MIC has moderate decreasing from 100 µg/mL to 3.40 µg/mL after streptomycin is mixed in this step.

Conclusion

In the following sections, antibacterial activity of zinc oxide nanoparticles (Table 4.20), antibacterial activity of cobalt oxide nanoparticles (Table 4.21) and comparison of zinc oxide & cobalt oxide NPs (Table 4.22) were compared to each other in accordance the usage of the controls.

Antibacterial activity of zinc oxide nanoparticles

Table (4.20) summarizes the antibacterial activity of ZnO nanoparticles preparations in comparison to TOAB and the used antibiotics that shows the following concluding points:

1) TOAB and ZnO-NPs have close antibacterial activity and the activity increased slightly when the ZnO-NPs stabilized in TOAB.

2) Mixing ZnO-TOAB with different antibiotics showed synergistic antibacterial activity that increased against both gram positive and gram negative bacteria, as the used ratios of the antibiotics to the NPs were ranged from 1:5 to 1:25.

Table (4.20): Comparison of the antibacterial activity of ZnO preparations against *E. coli, S. aureus* and *B. subtillis.*

	TOAB µg/mL	ZnO µg/mL	ZnO – TOAB µg/mL	Amoxi µg/mL	ZnO – TOAB – Amoxi µg/mL	Ceph µg/mL	ZnO – TOAB – Ceph µg/mL	Strep µg/mL	ZnO – TOAB – Strep µg/mL
E. coli	>125 (250)	>82.0 (165)	>50.0 (100)	>2.90 (8.80)	>3.20 (16.0)	>8.80 (27.2)	>16.0 (80.0)	>0.29 (0.98)	>3.40 (17.2)
S. aureus	>125 (250)	>82.0 (165)	>50.0 (100)	<<0.29	>0.64 (3.20)	>2.90 (8.80)	>3.20 (16.0)	<<0.29	>17.2 (86.0)
B. subtilis	>125 (250)	>165 (330)	>100 (200)	>>81.4	>16.0 (80.0)	>8.80 (27.2)	>3.20 (16.0)	>0.29 (0.98)	>3.40 (17.2)

The antibacterial activity of cobalt oxide nanoparticles

Antibacterial activity of CoO nanoparticles preparations in comparison to TOAB and the used antibiotics were summarized in Table (4.21), that have the following main points:

1) Stabilization of CoO-NPs with TOAB did not increase their antibacterial activity.

2) CoO NPs without stabilization were better against gram negative bacteria. However, mixing CoO-TOAB with different antibiotics showed a significant synergistic antibacterial activity against gram positive but not gram negative bacteria.

Table (4.21): Comparison of the antibacterial activity of CoO preparations against *E. coli, S. aureus* and *B. subtillis.*

	TOAB µg/mL	CoO µg/mL	CoO – TOAB µg/mL	Amoxi µg/mL	CoO – TOAB – Amoxi µg/mL	Ceph µg/mL	CoO – TOAB – Ceph µg/mL	Strep µg/mL	CoO – TOAB – Strep µg/mL
E. coli	>125 (250)	>41.0 (82.0)	>50.0 (100)	>2.90 (8.80)	>3.20 (16.0)	>8.80 (27.2)	>16.0 (80.0)	>0.29 (0.98)	>0.68 (3.40)
S. aureus	>125 (250)	>82.0 (165)	>100 (200)	<<0.29	0.0256	>2.90 (8.80)	>16.0 (80.0)	<<0.29	>0.13 (0.68)
B. subtilis	>125 (250)	>82.0 (165)	>100 (200)	>>81.4	>3.20 (16.0)	>8.80 (27.2)	>3.20 (16.0)	>0.29 (0.98)	>0.68 (3.40)

Comparison of zinc oxide & cobalt oxide NPs

The comparison of ZnO and CoO preperations were compared in the Table (4.22), that principally showed the CoO higher activity through the following:

1) Antibacterial activity of CoO (Table 4.21) were higher than ZnO (Table 4.20) without stabilization. However, stabilized ZnO with TOAB showed higher activity, which indicate that TOAB has better effect with ZnO and suppress the antibacterial activity of CoO NPs.

2) Mixing of the CoO and ZnO NPs with amoxicillin and cephalexin showed a close effect with a better effect for CoO-TOAB with amoxicillin and ZnO-TOAB with cephalexin against gram positive.

3) The most interesting point is the synergistic effect noticed for CoO-TOAB mixed with streptomycin in comparison to the ZnO NPs against both gram positive and gram negative bacteria.

Table (4.22): Comparison of the antibacterial activity of CoO in comparison to ZnO NPs preparations against *E. coli, S. aureus* and *B. subtillis*

	ZnO µg/mL	**CoO µg/mL**	**ZnO – TOAB µg/mL**	**CoO - TOAB µg/mL**	**ZnO – TOAB – Amoxi µg/mL**	**CoO – TOAB – Amoxi µg/mL**	**ZnO – TOAB – Ceph µg/mL**	**CoO – TOAB – Ceph µg/mL**	**ZnO – TOAB – Strep µg/mL**	**CoO – TOAB – Strep µg/mL**
E. coli	>82.0 (165)	>41.0 (82.0)	>50.0 (100)	>50.0 (100)	>3.20 (16.0)	>3.20 (16.0)	>16.0 (80.0)	>16.0 (80.0)	>3.40 (17.2)	>0.68 (3.40)
S. aureus	>82.0 (165)	>82.0 (165)	>50.0 (100)	>100 (200)	>0.64 (3.20)	0.0256	>3.20 (16.0)	>16.0 (80.0)	>17.2 (86.0)	>0.13 (0.68)
B. subtilis	>165 (330)	>82.0 (165)	>100 (200)	>100 (200)	>16.0 (80.0)	>3.20 (16.0)	>3.20 (16.0)	>3.20 (16.0)	>3.40 (17.2)	>0.68 (3.40)

This study showed a novel result concerning the lowest effective antibacterial concentration that can be reached from the combination of the

different nanoparticles stabilized in TOAB and antibiotics. This phenomena probably will have a tremendous effect in reaching low safe doses of both the nanoparticles and the antibiotics and escaping the current situation of emerging bacterial multidrug resistance.

Suggestions for future works

- Apply the results for an environmental and a biological model.
- Study different combinations of NPs, surfactants, antibiotics and bacterial strains.
- Other factors can be studied including temperature, incubation time, bacterial load, pH, light.
- Stabilize the nanoparticles with different biological surfactants other than chemical surfactants.

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Appendix

Antibiotics certificates of analysis

Medichem China Group Company
1905, 19/F., Ginza Plaza,
2A Sai Yeung Choi Street,
Mongkok, Kowloon, Hong Kong
Tel: 2780 5873 Fax: 2780 5974

MED CHEM CHINA GROUP

CERTIFICATE OF ANALYSIS

PRODUCT: AMOXYCILLIN TRIHYDRATE	
LOT: 3080022068	
MANUFACTURING DATE: Feb., 2010	EXPIRY DATE: Feb., 2013
PACKING: 25 KG DRUM	QUANTITY: 300 KG

TEST	SPECIFICATIONS	RESULTS
CHARACTERS:	A white or almost white crystaline powder.	White crystalline powder.
IDENTIFICATION:	Meets the requirements.	Meets the requirements.
APPEARANCE OF SOLUTION:	Meets the requirements.	Meets the requirements.
PH:	Between 3.5 to 5.5	4.4.
SPECIFIC OPTICAL ROTATION:	Between +290° to +315°.	+302°.
RELATED SUBSTANCE:	Individual impurity not more than 1.0%.	0.24%.
N, N-DIMETHYLANILINE:	Not more than 20 PPM.	Conforms.
WATER :	Between 11.5 to 14.5%.	13.0%.
SULPHATED ASH:	Not more than 1.0%.	0.21%.
ASSAY:	Between 95.0 to 100.5%.	100.4%.

REMARK: The above-mentioned product conforms to BP2005.

Date: Feb/2010

Dr. David Jiang
QA/QC Manager

For and on behalf of
美诺华中国集团公司
Medichem China Group Co
David Jiang

STARWAY PHARM CO., LTD.

CERTIFICATE OF ANALYSIS

DATE: JAN.20,2011

PRODUCT NAME	**CEFALEXIN POWDER**
BATCH NO.	**0410115068**
BATCH QUANTITY	**160KGS**
MFG DATE	**NOV.24,2010**
EXPIRY DATE	**OCT.2013**
PACKING	**20KG/DRUM**

TESTING ITEMS	STANDARDS	RESULTS
CHARACTERSTICS	A WHITE OR ALMOST WHITE POWDER	A WHITE CRYSTALLINE POWDER
IDENTIFICATION	MEETS THE REQUIREMENTS	PASS
CRYSTALLINITY	MEETS THE REQUIREMENTS	PASS
PH	3.0-5.5	4.8
WATER	4.0%-8.0%	5.40%
ASSAY (ON THE ANHYDROUS BASIS)	95.0%-103.0%	99.30%
SPECIFIC OPTICAL ROTATION	+149°-+158°	+156°
RELATED COMPOUNDS	MEETS THE REQUIREMENTS	PASS
DIMETHYLANILINE	MEETS THE REQUIREMENTS	PASS

CONCLUSION: **UP TO USP30 STANDARDS**

ISSUED BY : STARWAY PHARM CO., LTD.

For and on behalf of
STARWAY PHARM CO., LIMITED

Authorised Signatory

PIONEERS INTERNATIONAL TRADING LTD.

Nanjing Office: 1808, Jiangsu International Trade Building, 50 Zhonghua Rd., Nanjing,China

CERTIFICATE OF ANALYSIS

ORIGINAL

PRODUCT:	STREPTOMYCIN SULPHATE (ORAL GRADE)	INVOICE NO.:	TP1050211X
BATCH NO.:	HSO1003115	MFG.DATE:	MAR.21,2010
QUANTITY:	200BOU	EXP.DATE:	MAR.20,2014

SL.NO.	TEST	SPECIFICATION	RESULT
01	CHARACTERS	A WHITE OR ALMOST WHITE POWDER	A WHITE POWDER
02	IDENTIFICATION	A.TLC,COMPLIES	COMPLIES
		B.REACTION WITH FERRIC CHLORIDE SOLUTION,COMPLIES	COMPLIES
		C.REACTION WITH a-NAPHTHOL SOLUTION AND STRONG SODIUM HYPOCHLORITE SOLUTION,COMPLIES	COMPLIES
		D.REACTION WITH a-NAPHTHOL SOLUTION,COMPLIES	COMPLIES
		E.REACTION OF SULPHATES,COMPLIES	COMPLIES
03	APPEARANCE OF SOLUTION	CLARITY: ≤ 2# COLOR: NO MORE INTENSE THAN REFERENCE SOLUTION 3	<1# 5#
04	PH VALUE	BETWEEN 4.5 AND 7.0	5.6
05	STREPTOMYCIN B	NOT MORE THAN 3.0%	<3.0%
06	LOSS ON DRYING	NOT MORE THAN 7.0%	3.7%
07	SULPHATED ASH	NOT MORE THAN 1.0%	0.4%
08	SULPHATE	BETWEEN18.0% AND 21.5%	19.1%
09	COLOURIMETRIC TEST	NOT LESS THAN 90.0%	99.6%
10	ASSAY(DRIED SUBSTANCE)	NOT LESS THAN 720 UNITS/MG	753UNITS/MG
11	SODIUM METABISULPHITE	NOT MORE THAN 0.3%	0.011%
12	ABNORMAL TOXICITY	1MG/0.5ML,COMPLIES	COMPLIES

CONCLUSION:THIS PRODUCT COMPLIES WITH EP6.0

Rita

كلية الدراسات العليا
جامعة النجاح الوطنية

تحضير أكسيد الزنك وأكسيد الكوبالت بأحجام النانو و تغليفها بالمواد الفعالة سطحيا / المضادات الحيوية ودراسة تأثيرها كمضادات بكتيرية

إعداد
آلاء قاسم عبد الكريم الحاج قاسم

إشراف
الدكتور أمجد عز الدين حسين
الدكتور محمد عبد القادر سليمان

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في الأحياء بكليـــة الدراســـات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.
2013م

تحضير أكسيد الزنك وأكسيد الكوبالت بأحجام النانو و تغليفها بالمواد الفعالة سطحيا / المضادات الحيوية ودراسة تأثيرها كمضادات بكتيرية

إعداد

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إشراف

الدكتور أمجد عز الدين حسين

الدكتور محمد عبد القادر سليمان

الملخص

أدت زيادة المقاومة البكتيرية للمضادات الحيوية الى زيادة الطلب على ايجاد العوامل والعلاجات البديلة. من هذه العوامل البديلة استخدم العلماء المركبات والمواد النانوية والتي يتراوح أحجامها من1−100 نانومتر. أحد أكثر هذة المواد النانوية شهرة هو أكسيد الزنك النانوي والذي يعد من أشهر أكاسيد المعادن التي تمتلك العديد من الخصائص واهمها خصائص شبه الموصلة والنشاط المضاد للبكتيريا والذي حاز على اهتمام كبير كعلاج مضاد للجراثيم. من الناحية الأخرى يوجد العديد من المواد النانوية مثل أكسيد الكوبالت والذي لم يكن له نفس درجة الاهتمام كمضاد للبكتيريا كاكسيد الزنك ولكن له استخدامات واهتمامات بحثية اخرى مثل الموصلات المعدنية ولكن قليلا ما يوجد اهتمام به كمضاد للجراثيم.

في هذه الدراسة، تمت صناعة المركبين النانوين المذكورين اعلاه، مركبات أكسيد الزنك وأكسيد الكوبالت النانوية باشكال مختلفة لوحدها أو تثبيتها مع المعالجات السطحية أو خلطها مع مضادات الاموكسيلين والسيفلكسين والستربتوميسسن. دراسة التاثيير المضاد للبكتيريا لهذه المواد النانوية على عزلات بكتيريا الستافيلوكوكس والايشريشيا والباسيلس لمختلف هذه التحضيرات. أشارت هذه الدراسة الى زيادة التاثير على البكتيريا بعد تغليف المواد النانوية بالمركبات ذات التاثير السطحي ولكن التاثير كان أوضح بشكل كبير عند استخدام خليط من هذه المواد النانوية المثبته بالمواد السطحية مع المضادات الحيويه، الاموكسيلين والسيفلكسين والستربتوميسسن. يبدو ان ظاهرة الدعم من المواد النانوية المثبته بالمواد السطحية والمضادات الحيويه كانت السبب في الزيادة الكبيرة لهذه المواد كمضادات حيوية.

من المثير للاهتمام، أشارت نتائج هذه الدراسة إلى أن التاثير المضاد للبكتيريا لمركب أكسيد الكوبالت النانوي والمثبت بالمركبات الفعاله سطحيا والمخلوط بالمضادات الحيويه بان له تاثيرا أكبر بكثير مقارنة بالمركبات المحضره من أكسيد الزنك، ضد الثلاث أنواع البكتيريا المستخدمة في هذه الدراسة. لذلك، فتحت هذه الدراسة الباب الجديد للعثور على علاج سحري ضد سلالات البكتيريا المقاومة للأدوية المتعددة مع تأثير أقل سمية نتيجة استخدام جرعات عالية من المضادات الحيوية المختلفة والجسيمات النانوية.