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Faculty of Graduate Studies

Size- dependent Antibacterial Activity of Cobalt Oxide Nanoparticles

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Dedication

To the person who meant and continue to mean so much to me although you are no longer of this world, to your soul that is always with me, to you my brother Anas I dedicate this thesis

Acknowledgment

It would not have been possible to write this thesis and to finish this master's program without the conciliation from Allah Subhanah Watalla, who is to be thanked for everything in my life.

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الإقرار

أنا الموقع أدناه موقعة الرسالة التي تحمل العنوان:

Size- dependent Antibacterial Activity of Cobalt Oxide Nanoparticles

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت اليه الاشارة حيثما ورد، وان هذه الرسالة ككل أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب 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.

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Date: ٩/٦/٢٠١٥م **التاريخ:**

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List of Symbols

Symbol	Abbreviation
Aº	Angstrom
CoO	Cobalt oxide
$CoCl_2 \cdot 6H_2O$	Cobalt(II)Chloride Hexahydrate
E. coli	*Escherichia coli* bacteria
FWHM	Full width at half maximum
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
NA	Nutrient Agar
NB	Nutrient Broth
Nm	Nanometer
NP	Nanoparticles
PVP	Polyvinylpyrrolidone
S. aureus	*Staphylococcus aureus* bacteria
TOAB	Tetra octyl ammonium bromide
XRD	X-ray diffraction

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Abstract

One of the major health problems that has been showing up recently, was due to the emergence of new bacterial strains that posses resistant to common antibiotics, scientist find out that with the unique properties of nanoparticles, a nanoscale material ranging from 1-100 nm, it become possible to use them as alternative antibacterial agents, and the antibacterial effectiveness of several metal oxide nanoparticles has been proved, were cobalt oxide nanoparticles is among one of these metal nanoparticles that can be used as antibacterial agent.

As it well known that nanoparticles behavior is strongly governed by the size and composition of the particles, in this study cobalt oxide nanoparticles were prepared by a simplified salt reduction method where variations in the preparation conditions lead to the production of cobalt oxide nanoparticles in different forms and different sizes, the nanoparticles alone, were three samples were obtained with an average sizes ranging from (25.25nm, 21.61nm, 20.19nm), and three nanoparticles samples stabilized in Tetra octyl ammonium bromide (TOAB) with average sizes ranging from (23.08 nm, 19.47 nm, and 19.03 nm), and nanoparticles

samples stabilized in Polyvinylpyrrolidone (PVP) with average sizes ranging from (20.87 nm, 17.73 nm, and 17.09 nm).

The antibacterial inhibitory and bactericidal effects of all prepared cobalt oxide nanoparticles samples with different sizes were examined against Gram-positive *S. aureus* and Gram-negative *E. coli* both reference bacterial strains and clinical bacterial isolates.

The overall experimental results indicate that cobalt oxide nanoparticles appear to have a promising results as antibacterial inhibitory effects against tested microorganisms were its related inhibitory effects were varied depending on the variations in the type of tested microorganism were it was more inhibitory toward *S. aureus* more than toward *E. coli*. A significant different in size-dependence of antimicrobial activity was observed, in which the fine scale differences in size of the prepared cobalt oxide nanoparticles can alter their related antibacterial activity. Moreover, the usage of the reference and the clinical bacterial isolates showed the great need to be carful that the clinical isolates could have a more robust antibacterial resistance.

Key words: cobalt oxide nanoparticles, Tetra octyl ammonium bromide, Polyvinylpyrrolidone, *S. aureus*, *E. coli*.

Chapter One

Introduction

1.1 Motivation and Project Aims

In recent times and with the new advances in the field of nanotechnology, it has brought nanosized material to the fore with their increased application in industrial, medicine and therapeutic purposes[1][2]. Previous studies showed that because of the unique properties of nanoparticles at the nanoscale level and their enhanced bioactivity due to their large surface area in comparison to volume ratio[3][4], they can be used in various medical applications [4]. In addition nanotechnology make it possible for the implication of NPs in a wide variety of scientific areas such as energy conversion, catalysis ,medicine and water treatment [5].

One of the new aspects of nanotechnology is its ability to develop new antibacterial agents via the synthesis of NPs [3]. One of the reasons lies behind chosen NPs as alternative antibacterial agents is due to their high surface area in comparison to volume ratio which leads to characteristics that allow these NPs to interact with pathogenic bacteria and act as effective bactericidal agents specially against bacterial resistant strains that are found to be responsible for large number of bacterial health problems, deaths and hospitalization [6][7].

Metal oxide NPs are consider among one of the most newly developed materials that are found to be applicable and can be used in medical

applications[1], catalysis, optics, electronics, environmental and biotechnology[5]. Through studied conducted on these NPs it become relevant that these NPs with their unique biological, chemical, and physical properties they can be used to address a number of challenges in the field of nanotechnology[1]. More over With the Rapid growth in the field of nanotechnology[8]. The emergence of nanotechnology as a field of science, scientist find out that it become possible to explore the antibacterial activity of metal oxide NPs [9], where recent studies indicates that metallic NPs are being explored for their potential use as antimicrobials [1][10]. Recent studies have shown that metal oxide nanoparticles such as ZnO, MgO, TiO_2, SiO_2, CuO and CoO NPs have an apparent antibacterial activity and can be used as antimicrobial agents because of their effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistant. In addition, they provide mineral elements essential to human cells[11][6].

Nanosized Cobalt based NPs resides among one of the newly developed materials with their promising applications in information storage, magnetic fluids [12], and as they display a wide range of interesting size dependent, structural, electrical and magnetic properties, and such as other NPs with their high surface area to volume ratio, they showed high chemical reactivity and make them suitable for catalysis, with their further application in biomedicine [13].

Cobalt oxide NPs in particular is known to be widely used in different fields such as magnetic, gas sensors, lithium ion batteries, catalysis and electrochemical [14], and they have shown to possess antibacterial activity and several studies reported that they can be used as bactericides for water disinfection [15]. Currently, cobalt nanoparticles are commonly treated as magnetic nanoparticles more than antibacterial agents where only few works have focused on the investigation of the related Antibacterial activities of cobalt oxide NPs [16]. Horst et al., examined the antibacterial activity of cobalt oxide, nickel, zinc oxide, copper oxide, iron oxide, and titanium dioxide NPs against *E. coli* using two methods: culturing in liquid media containing one of these NPs 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 activity properties from titanium, iron and copper oxide [46].

Many methods can be used for the production of cobalt oxide nanoparticles such as thermal method, precipitation method, chemical pyrolysis process and sonochemical method [14]. The chemical reduction method with its simple equipment and short and easy process for industrial production can be used for the production of cobalt oxide nanoparticles [17]. In this study, preparation of uniformly sized, monodispered, and sized tunable cobalt oxide NPs, was accomplished by using a simplified cobalt salt reduction process, first without adding any protective agents, in the other part of the study PVP, and TOAB protective agents were added.

Synthesis of NPs with defined sizes and morphologies can be controlled by manipulation in the preparation conditions such as temperature, solvent, pressure, and the choice of stabilizing agent [18], which make it possible to manipulate the reaction conditions under which cobalt oxide NPs were prepared and make it possible to prepare cobalt oxide nanoparticles with various sizes[4]. Several discoveries revealed that as the size of the prepared nanoparticles is altered their related properties are altered as well, at the nanoscale diameter, the ultra small particle size lead to increase in the surface area per mass which allow for an immediate contact with ambiance[19].

Correlation between the nanoparticles sizes and there antibacterial properties were reported in several studies [20][16][21], In a study where ZnO NPs were prepared in different sizes and shapes, and variations in size, and shapes of the prepared ZnO NPs was due to variation in used zinc salts, in which results indicate a variation in its corresponding antibacterial activities as it interacts with bacteria, where good antibacterial activity found to be size dependent [20].

The study of NPs in the biological context, revealed that as the particle size decrease their will be an increased and enhanced biological activity per given mass compared to large particles [9], this was proved in Previous studies on different NPs which showed that as the size of these NPs is reduced, they appear to be more efficient in inhibition the growth of bacteria, where three metal oxide NPs of ZnO, CuO and Fe_2O_3 were

prepared and their antibacterial activity was compared, where results showed that ZnO NPs with smaller size, showed a higher antibacterial activity, and it was more efficient in inhibiting the growth of bacteria[22]. In another study where ZnO NPs were prepared and showed a significant antibacterial activity against *S. aureus*, the related antibacterial activity of the prepared ZnO NPs was found to be size dependent once it interact with bacteria, and results also revealed that as the size of the prepared ZnO NPs reduced, it showed higher antibacterial activity[6]. In another study where antimicrobial activity of ZnO NPs was investigated, results indicated that the antibacterial activity of ZnO NPs increased with decreasing particle size[11][10].

A study on silver NPs showed that the size and shape of prepared silver NPs affects its antibacterial activity, and the results indicated that the small size had more antibacterial activity, this is because as the size is reduced, it will easily penetrate the cell wall, and the large surface area to volume ratio results in a large number of atoms that will be in immediate contact with bacteria and react with the cell [23]. In another study in which silver NPs where prepared , and the antibacterial activity of the as-synthesized silver NPs was tested against *E. coli* and *S. aureus* bacterial strains, where it found to exhibit strong antibacterial activity against used bacterial strains, and the related antibacterial activity of silver NPs was found to be size dependent, where, and particles of different sizes showed different rate and extent of bacterial growth inhibition, and the study revealed that the smaller the size of the as-synthesized Ag NPs, the larger the surface area that come

in contact with bacteria resulting in higher percentage of interaction with bacteria and growth inhibition [16], in another study silver NPs interaction with bacteria found to be dependent on the size and shape of the prepared NPs [24].

The effects of small differences in size of prepared nanoparticles toward microorganisms have not been well investigated, and as previous studies revealed that there is a size dependent correlation between the prepared nanoparticles and there functional activities, it is important to investigate the antibacterial activities of the prepared cobalt oxide nanoparticles that exhibit different sizes. In particular, the size dependent antibacterial properties should be studied to evaluate their interaction with human pathogenic bacteria. *Escherichia coli* and *staphylococcus aureus* that considered among the most pathogens representing the gram negative and positive bacteria respectively that the antibacterial properties of nanoparticles is of great concern to investigate against these two kinds of bacteria as they cause a wide range of serious infections and diseases [20].

The main objectives of this study are:

1) Size selective synthesis of cobalt oxide nanoparticles with and without surfactants, using chemical reduction technique.

2) Stabilization of cobalt oxide NPs with Tetra octyl ammonium bromide (TOAB).

3) Stabilization of cobalt oxide NPs with Polyvinylpyrrolidone (PVP).

4) Studying the antibacterial activity of the prepared cobalt oxide NPs with and without TOAB and PVP surfactants, against *Escherichia coli* and *Staphylococcus aureus* reference bacterial strains and clinical bacterial isolates.

Chapter Tow

State of Art

2.1 Nanotechnology

Nanotechnology is the study and application of technology at the nanoscale level which is utilized across several science fields such as biology, physics, chemistry, materials science, and engineering. In recent years, nanotechnology has emerged as one of the most promising field of sciences; it is unique because of its capability to manipulate matter to the nanoscale diameter ranging from 1-100 nm [5][1][2][25][26].

Nanoparticles are zero-dimensional nanostructures [27], as the size of these particles is reduced to the nanoscale structure, these particles exhibit properties and behaviors significantly different to their corresponding bulk materials of the same chemical composition[26][5][1]. Frequently, nanometer-size metallic particles show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts, due to their high surface-to-volume ratio. Thus, these nanoparticles have been the subject of substantial research in recent years [27] [5][1][3][2][24][28][14][29][22][8][30], where these properties found to be manipulated suitably for its desired application [1].

Currently, several antimicrobial drugs are used to kill microbes or prevent the growth of microbes. However, with the worldwide use and abuse of the most commonly used antibacterial drugs , microorganisms, especially

bacteria, are becoming resistant to more and more antimicrobial agents[1][24][10][16],which represent a major health problem in hospital settings , as these new resistant pathogenic strains are the under laying cause of increased mortality and morbidity and this become one of the major health concerns [1][22][10], for example bacterial strains that known to cause tuberculosis (TB), are found to be resistant to previous effective antibacterial treatment [31][32][3],and E.coli is found to be intrinsically resistant to therapeutic levels of penicillin G, the first β-lactam introduced into clinical practice, because of its outer membrane barrier. *E. coli* is also resistant to several different classes of antibiotics with distinct mechanisms of action[31].

Scientist find that it is important to look for alternative agents that can be used to control bacterial infections within both gram positive and gram negative bacterial resistant strains [10], a great deal of interest was given to metal oxide nanoparticles that exhibits unique properties with enhanced bioactivity, where scientist find out that with the unique properties of NPs at the nanoscale level it's become possible to develop new generation of nanoparticles as alternative antibacterial agents in order to control bacterial resistant problem [22][16][7][32][33] with the possibility for the implantation of nanomaterials for the antimicrobial medical therapeutic and diagnostic purposes[6].

There are many advantages among using inorganic metal NPs as antimicrobial agents, due to their stability ,robustness and long self-life,

where the antimicrobial activity of different NPs have been studied among different pathogenic and some non pathogenic bacteria such as *E. coli* and *S. aureus*, and among a group of inorganic oxides that have been tested for their antimicrobial properties such as TiO_2, ZnO, MgO, CaO, Al_2O_3, Ag_2O and CeO_2 [20]. Furthermore where scientist became capable to prepare modified NPs with better efficiency and facilitate their application in different fields such as bioscience and medicine [24].

Little is known about the adverse effects of the prolonged exposure to NPs on human health, so before the large scale production and implication of NPs there must be more knowledge about their adverse effects, where metal based NPs are found to have effects on cells, where the small size of NPs allow them to easily penetrate skin, brain, lungs and cause adverse side effects, so more in vivo and in vitro studies must be conducted to study toxic effects of NPs [1].

Toxicity of NPs was studied, where ZnO NPs are found to be nontoxic and biosafe with its wide implication and used as drug carriers and cosmetics, where previous several reports have shown the harmful effects of nanomaterials on cells, but low concentration of ZnO NPs were found to be non toxic[20], and Ag NPs appear to have more toxicity against organism in the sequence Ag > Hg > Cu > Cd > Cr > Pb > Co > Au > Zn > Fe > Mn > Mo > Sn [3]. Toxicity of cobalt based NPs in which several studies showed the interaction of these NPs with cells and tissues and their corresponding adverse effects [8].

2.2 Stabilizers

During the course of preparation of NPs, aggregation of these NPs need to be prevented, which make it possible to prepare NPs in a size of few nanometers[29]. Stabilization which can be achieved by coating these particles with capping molecules, without these molecules, it will result in irreversible particle aggregation [34], where These surfactants appear to play an important role as a controller of the crystal growth, and controller of the crystal shape as well [35].

These additives as they play in important role, they could be a bilayers of surfactant ions that lead to electrostatic stabilization, or surface capping legends or polymers that give a protective layer which provides protective coverage to prevent agglomeration [4]. Several studies revealed the importance of using these capping molecules during preparation process of NPs, in order for agglomeration to be prevented, In a study performed on silver NPs, those NPs were prepared with well controlled size by chemical reduction method, in which silver ions are reduced by reductants and stabilizing or protecting agents were used to prevent these NPs from agglomeration [23]. And more over Several approaches have been used to prepare cobalt NPs by using a mixture of a reducing and capping agents offering a method to synthesize NPs where stable metal collides can be prepared [34].

An important component of metal salt reduction processes is the protective agent. Protective agents such as surfactants that form a layer of molecular

membrane around the nanoparticles and polymers that provide steric hindrance between nanoparticles are added during the reaction to inhibit particle agglomeration and to control the particle growth, where this method was used for Cobalt NPs preparation with controlled size and shape, in which atoms are produced in solution, collapse into NPs, where their shape and size are controlled by means of a surfactant, a molecule which will strongly absorb selectively or not to the nanocrystal surface [35].

PVP is consider among one of the surfactants that used in NPs stabilization, it is a polymer that can strongly bind to a metal surface, affects its formation and the prepared particles size [29], The role of PVP is very important in order to keep those particles isolated and stable for months even at room temperature [23].

NPs in colloidal solution can be stabilized by adsorbed PVP, in a study where PVP was used as a stabilizer and sodium borohydride as reducing agent during silver NPs preparation, where the role of PVP was to stabilize the NPs, in the solution, and it bounded to the surface of silver NPs during their preparation, PVP was expected to inhibit the further aggregation of silver NPs and to stabilize the dispersed silver NPs in the solution. As results showed that the amount of PVP in the solution , and its molecular weight both appear to influence the average size and PVP-adsorbed surface of the prepared silver NPs [23], and furthermore , in another study where Ag NPs were prepared and stabilized by PVP[16].

A study on the formation process of silver and palladium NPs, in order to study the effect of metal ion and reducing agent concentration and the presence of a polymer on nucleation and nucleus growth, where it was observed that, when PVP is used a smaller particles are obtained, where PVP molecule can strongly bind to a metal surface and inhibiting its growth by mean of collision, as results revealed that the Particles size decrease as there is an increase in the concentration of the stabilizing polymer and the reducing agent [29].

2.3 Cobalt oxide Nanoparticles

Cobalt based nanoparticles among one of the promising metal oxide nanoparticles, appear to exhibit unique size dependent structural, magnetic, electronic and catalytic properties [36]. Cobalt Nanoparticles as one of the magnetic NPs, which consider as an active area of research with their potential implication in various technological applications such as information storage devices, magnetic fields and catalysis, data storage and sensors [9], [34]. Currently, cobalt nanoparticles are commonly treated as magnetic nanoparticles more than antibacterial agents where only few works have focused on the investigation of the related antibacterial activities of cobalt oxide NPs [16]; however, similar to other nanoparticles they have high surface area to volume ratio, which enhances their chemical reactivity making it possible to use them in biomedicine as antibacterial agents [10].

Cobalt based NPs in general and Cobalt oxide NPs in particular are currently attracting enormous interest owing to their size and shape dependent outstanding properties, where these magnetic, optic, catalytic, and electronic properties of nanomaterials depend strongly on their size, structure, and shape and they are destined to find their place in medical biotechnology because of their magnetic properties [25][8][37].

2.4 Size -dependent antibacterial activity of nanoparticles

Recent advances in the area of nanotechnology make it possible to control the reaction conditions under which these nanoparticles are produced, further more it becomes possible to prepare NPs with specific size and shape, by manipulation that done at the nanometer scale. Several factors such as temperature, surfactant and precursor cooperate in order to manipulate nanoparticles with specific shape, size, and stability, in addition to that chemical and physical properties, of metal NPs are found to be strongly influenced by the preparation conditions , such as how the metal ions will interact with the reducing agent, and the adsorption of stabilizing agent with metal NPs [5][22].

Different factors were studied during the preparation of cobalt oxide NPs to see how these factors will affect the structure and morphology of the prepared NPs , these factors are : effect of PH , synthesis temperature , capping agents , annealing temperature , and different usage of cobalt salts [13]. The role of cobalt precursor, the surfactant , the working temperatures, all of which affect the resulting structural and magnetic properties of cobalt

NPs, where Reduction of cobalt salts and the thermal decomposition of a cobalt carbonyl are among the most commonly methods used to prepare NPs [12]. A study investigated the effects of surfactant, coating on the shapes and sizes of cobalt NPs, where different types of surfactants were used in order to produce different shapes and sizes of CoO NPs [38], where in this study we reported the synthesis of cobalt NPs by using various types of surfactants.

At the nanometer size, Most of the unique and unexpected properties of NPs is due to their small sizes with high surface area to volume ratio [28][7]. Previous studies showed that nanoparticles properties can be changed by altering their size, where the changes in size result in changes in surface area to volume ratio, as a result the surface area increase , an increase in the reaction sites on the particles, more than those with lower surface area the functional activities and the related unique physical, chemical and biological properties of these nanoparticles will be alternatively changed as the size of these particles is altered [22][9]. So Precise control of the prepared particle size in order to investigate their related physical and chemical properties is needed, and efforts are made in order to control the size of the prepared NPs by changing the preparative conditions [12].

The size of nanoparticles is significant in their function as antibacterial agents and having a large surface area of the particles in contact with bacterial effluent can result in varying level of activity (e.g., silver

nanoparticles), and give them the ability to interact more closely with bacterial membrane [10][9]. This has led to the production of large variety of nanoantibacterial agents with different properties depends on their particle size [20][21].

The antibacterial activity of NPs is mostly depends on the size of NPs and further more on their stability and concentration that is added to the growth medium, since this can provide greater retention time for bacterium – nanoparticles interaction Where the size of the prepared NPs seems to play an important role in affecting the antibacterial activity and its interaction with bacteria, Many publication showed that as the size of the prepared NPs is reduced, a good antimicrobial activity was reported, So we need to control the size of the prepared NPs and study its corresponding effects on its antimicrobial characteristic [28][22].

The acting mechanism of NPs against bacteria can differs due to differences in the respective bacterial strains, and the drug resistant ones and their respective defense mechanism, first of all the properties of bacteria can affect its susceptibility to NPs, for example the bacterial cell wall itself can play an important role, since its divided into two main categories gram positive and gram negative which differs in that as it known that the wall of gram positive bacteria has a thick layer of peptidoglycan attached to teichoic acids, in the other hand gram negative bacteria has a thin layer of peptidoglycan with an outer membrane that posses a selective permeability[32]. As gram-negative bacteria *E. coli* has

an outer membrane outside its peptidoglycan layer, that is not found in gram positive bacterial strains, which act as selective permeability barrier[24]. Results showed higher gram –negative bacterial strain resistance against such nanomaterials over gram positive bacterial strains [22].

Also the NPs type itself can affect its interaction with bacteria for example *E. coli* is more susceptible to CuO NPs than *S. aureus* and *Bacillus subtilis*, more over Ag NPs has a higher antibacterial activity against *E. coli*, more than Cu NPs against *E. coli* and *S. aureus* [32].

As it is little known about the acting mechanism of NPs on bacteria, the studies gave possible suggestions depending on the morphological changes and structural changes in the bacterial cell wall [24]. Where NPs toxicity against bacteria need to be further studied, most studies show that the toxicity of NPs comes from the fact that they can attach to the bacterial membrane through electrostatic interaction and disrupt the bacterial membrane integrity, further more toxicity of NPs depends on surface composition, Intrinsic properties and the bacterial species [32].

Silver NPs which show a good antibacterial activity against *E. coli*, for more understanding for how these NPs affects bacteria, the growth of bacteria, the morphological changes in the bacterial cell wall were analyzed as bacteria exposed to silver NPs. The study showed that when *E. coli* was exposed to different concentrations of silver NPs it affects the bacterial cell membrane structure, and further more it affects the activity of some

enzymes as its observed in bacterial cell wall by transmission electron microscopy and scanning electron microscopy [24].

Study of copper NPs effects on Micro-organism (it showed that copper NPs that was prepared by electrolysis method has an enhanced antibacterial activity on *E. coli* more than those copper NPs that are prepared by chemical reduction method), where slight change in surface area results in enhancement in the antibacterial activity of copper NPs, and as the result showed that also the antibacterial activity of copper NPs changed depending on the type of bacteria whether its gram negative or gram positive bacterial strains [9].

2.5 Bacterial strains

Staphylococci, among the pathogenic bacterial strains, are the common bacteria that cause a wide variety of infections and diseases especially skin and mucus membranes [10]. On the other hand, *E. coli*, enteric bacteria that is currently more implicated in many digestive tract and urogental infections with the high incidence of septicemia cases. The antibacterial activity of wide range of NPs has been mostly studied against pathogenic strains of *E. coli* and *S. aureus* [22].

2.5.1 Staphylococcus aureus

Staphylococci are Gram-positive bacteria, that are characterized by an individual cocci, that appear to be divided into more than one plane to form grape like clusters, that are non-motile, non-spore forming facultative

anaerobes, and grow by aerobic respiration, or by fermentation, staphylococcus genus include 36 species, nine of which are divided into subspecies, however *Staphylococcus aureus* and *Staphylococcus epidermidis* are the two most characterized and studied strains [39].

Most of the genus staphylococci are catalase positive and oxidase negative differs from those of genus streptococci that catalase negative, and with a different cell wall composition, they appear to be tolerant to high concentration of salts therefore they inhabits the skin. Pathogenic *S. aureus* is a member of the genus *Staphylococci*, which is named *aureus* due to the golden colour appearance as it grown on solid media, coagulase positive because of its ability to produce coagulase and clot blood, which distinguish it from other members of the genus *Staphylococci* that coagualse negative such as *S. epidermis*. Further more, it has a cell wall that appear as a tough protective coat, with an amorphous appearance and 20-40 nm thick [40].

S. aureus is considered one of the major human pathogens, and consider as one of the main leading cause to morbidity and mortality in both nosocomial and community acquired infections, it persistently colonize the anterior nares of about 20-25% of the healthy adult population, while as many as 60% are intermittently colonized [41], with its of great importance due to its high capacity to acquire resistance to antibiotics [39], and cause a wide variety of infections and diseases. To date the *S. aureus* genome databases have been completed for 7 strains, 8325, COL, MRSA, MSSA,

N315, Mu50, and MW2, and the average size of the *S. aureus* genome is 2.8Mb [10].

2.5.2 Escherichia coli

Enteric *Escherichia coli* (*E. coli*) is known to be both a natural flora of humans, and more over an important pathogens that considered a significant cause of morbidity and mortality worldwide [42] [31].

Upon its identification in 1885, it has become one of the mostly studied bacterial species. *E. coli* strains are comparatively easy to grow under both aerobic and anaerobic condition and manipulate in the laboratory, as it appear to be amenable to genetic manipulation, and naturally can acquire mobile genetic elements. Some of *E. coli* isolates are considered part of the beneficial normal flora of the intestine, but some strains appeared to acquire pathogencity mechanisms to cause broad spectrum of diseases in humans and animals. For example, *E. coli* strains that can cause diarrhogenic or extraintestinal (ExPEC) infections in humans. ExPEC infections are primarily urinary tract (caused by uropathogenic *E. coli*, UPEC) and sepsis/ meningitis (caused by neonatal meningitis *E. coli*, NMEC) [42] , where it is more commonly found as inhabitant of the gastrointestinal tract of humans and warm-blooded animals [43][31]. *E. coli* is used in a wide variety of applications both in the industrial and medical area [31].

Chapter Three

Experimental Part

3.1 Chemicals and Materials

Cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) was purchased from Sigma-Aldrich Company (catalogue# 544167), Tetraoctylammonium bromide (TOAB): ([CH3(CH2)7]4N Br) was purchased from Aldrich Company with purity 98% (catalogue # 294136), Polyvinylpyrrolidone (PVP) was purchased from Alzahra factory, Sodium hydroxide (NaOH) was purchased from Frutrarom Company, Nutrient broth (catalogue # M001_500G) was purchased from Hi media.

3.2 Samples preparation

Several approaches have been used to prepare Cobalt Oxide NPs. as thermal method, precipitation method, pyrolysis process and sonochemical methodare hard to control the function properties of NPs with low yield; NPs with more cost effective and environmental friendly methods [14], chemical approaches such as chemical reduction method, electrochemical techniques, and photochemical reduction methods are among the most commonly used[5]. Recently liquid phase reduction of metal salts is one of the preferred method [29].

Chemical approach is considered as one of the best preparation methods to prepare cobalt oxide NPs, with its advantages of increasing the functional efficiency for its use in technology, and help better understanding the

crystal growth with a required shape, size, and phase purity by controlling surface energies [14], where aggregation need to be prevented during the course of preparation of NPs, when aggregation is prevented it become possible to prepare NPs in a size of few nanometers [29].

Chemical reduction method is the most common method that is used to prepare metallic NPs in ionic liquid in general [4], where the reduction of metals, in chemical reduction method is the most commonly applied method for preparation of Ag NPs, where the shape, size and the size distribution is strongly depend on the strong and weak tendency of organic substrate to reduce silver salts, where reductants in Ag NPs preparation such as borohydride was used [44].

Mainly used reducing agents during chemical approaches such as hydrogen gas, hydrazine and sodiumborohydride ($NaBH_4$) are often used as reductants in the preparation method of NPs [29] [4].

In this study, preparation of uniformly sized, monodispered, and sized tunable cobalt oxide NPs, was accomplished by using a simplified cobalt salt reduction process, first without adding any protective agents, in the other part of the experiment PVP, and TOAB protective agents were added.

In achieving the objectives, during the preparation of cobalt oxide NPs, the size of the prepared NPs was tuned by varying the amount of the stabilizing agent, and the concentration of the reducing agent used during the course of preparation.

3.2.1 Cobalt oxide nanoparticles preparation

In this part, three experiments were performed to prepare three cobalt oxide NPs samples without adding any protective agents, where the size was controlled by variations that took place in the concentration of the reducing agent (Table 3.1) used during the course of preparation.

$CoCl_2$ solution were prepared by dissoloving about 1.0 gm of $CoCl_2.6H_2O$ in 50 ml D.W, mixed and stirred at constant temperature 75ºC, in shaking water bath, using a stirring rate of 160 rpm, and under inert gas atmosphere (N_2).

Different NaOH solution concentrations were prepared and added to $CoCl_2$ solution to prepare the different samples (Table 3.1). .

Table (3.1): Variations in the concentration of the reducing agent used during the preparation of cobalt oxide nanoparticles

Samples	Concentration of reducing agent
Sample 1	1 M
Sample 2	2 M
Sample 3	2 M

Furthermore, 0.2 gm of NaBH4 was added to complete the reaction. The preparation process lasted for two hours with the same temperature and stirring rate.

PH during the reaction must be >9 so it was monitored each time interval (10 min), the reaction solution was allowed to be completed and the pH during that was 13-14.

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

3.2.2 Cobalt oxide nanoparticles stabilized in TOAB preparation

In this part, three experiments were performed to prepare three cobalt oxide NPs samples were TOAB was used as protective agent, and the size was controlled by variations that took place in the amount of the protective agent that was used during their preparation (Table 3.2).

The first step were started by dissolviing about 0.8 gm of $CoCl_2.6H_2O$ in 50 ml D.W, mixed and stirred at constant temperature 75ºC, in shaking water bath, using a stirring rate of 160 rpm, and under inert gas atmosphere (N_2).

The three samples were prepared by variations that took place by the reducing agent and the amount of TOAB (Table 3.2). Sample 1 was prepared by the addition of 0.112 gm of TOAB and 2 M NaOH reducing agent. Sample 2 and 3 were prepared by the addition of 0.05 gm of TOAB and 2 M and 1 M NaOH reducing agent, respectively.

TOAB solution was prepared by dissolving 0.05 gm of TOAB in 50 ml D.W and added it to $CoCl_2$ solution. Here 1 M NaoH solution was prepared by dissolving 4 gm NaOH in 100 ml D.W where 80.0 ml of this solution was being added to $CoCl_2$ and TOAB solution, where Table (3.2), illustrates the variations in the preparation conditions for the three samples.

Table (3.2): Variations in the concentration of the reducing agent and the amount of TOAB (gm) that was used during the preparation of cobalt oxide nanoparticles stabilized in TOAB.

Samples	Amount of TOAB (gm)	Concentration of reducing agent
Sample 1_TOAB	0.112	2 M
Sample 2_TOAB	0.05	2 M
Sample 3_TOAB	0.05	1 M

More over 0.2 gm of $NaBH_4$ was added to complete the reaction during the preparations of the three samples, pH during the reaction must be >9 so it was monitored each time interval (10 min), the reaction solution was allowed to be completed and the pH during that was 13-14.

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

3.2.3 Cobalt oxide nanoparticles stabilized in PVP preparation

In this part, three experiments were performed to prepare three cobalt oxide NPs samples were PVP was used as protective agent, where as the amount of PVP in the solution and its molecular weight, both of which can influence the average size of the prepared cobalt oxide NPs.

CoCl2 solution were prepared by dissolving of about 0.85 gm of $CoCl_2.6H_2O$ in 50 ml D.W, mixed and stirred at constant temperature 75ºC, in shaking water bath, using a stirring rate of 160 rpm and under inert gas atmosphere (N_2). PVP solution was prepared by dissolving a specific amount of PVP in 50 ml D.W and added it to $CoCl_2$ solution.

Different amounts of PVP surfactant was used during the course of preparation of the three samples as seen in Table (3.3) , for sample 1 , 0.0552 gm of PVP was used , and for sample 2 about 0.1208 gm , and sample 3, 0.2111 gm of PVP was used.

Table (3.3): Variations in the amount of PVP (gm) that was used during the preparation of cobalt oxide nanoparticles stabilized in PVP

Samples	Amount of PVP (gm)
Sample 1_PVP	0.0552
Sample 2_PVP	0.1208
Sample 3_PVP	0.2111

Here 1 M NaoH solution was prepared by dissolving 4.037 gm NaOH in 100 ml D.W where 80.0 ml of this solution was being added to $CoCl_2$ and PVP solution, where 0.2 gm of NaBH4 was added to complete the reaction, pH during the reaction must be >9 so it was monitored each time interval (10 min), the reaction solution was allowed to be completed and the pH during that was 13-14.

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

3.3 Samples Characterization

The prepared cobalt oxide nanoparticles without surfactant, nanoparticles stabilized in TOAB, and the ones that are stabilized by PVP all were characterized by XRD, that was done in the lab of Jordan University of Science and Technology.

3.3.1 X-Ray diffraction (XRD)

X-ray powder diffraction, as an analytical technique was used for phase identification of the prepared cobalt oxide nanoparicles with and without surfactant by using Rigaku Ultima IV XRD diffractometer.

3.4 Antibacterial activity

3.4.1 Bacterial isolates and Bacterial reference strains

In this study the prepared cobalt oxide nanoparticles, surfactant combinations were investigated against Gram- negative *E. coli* (ATCC 8739) and Gram-positive *S. aureus* (ATCC 6538) which were purchased from American Type Culture Collection (ATCC), conserved in the lab and used as reference strains.

Furthermore, the antibacterial activity assessment was performed on *E. coli* and *S. aureus* bacterial isolates, which were isolated from clinical samples with further diagnosis in microbiological labs at An-Najah National University according to standard diagnostic method.

3.4.2 Bacterial culture preparation

3.4.2.1 McFarland preparation

The number of bacteria in the prepared bacterial suspensions used, was adjusted according to 0.5 McFarland standard, in which the number of bacteria will be within 1.5X 10^8 CFU/ml, the prepared bacterial suspension turbidity was adjusted to give an absorbance between 0.08 to 0.1, when it’s

too turbid it was diluted with more diluents, and in case it is not turbid enough more bacteria were added.

3.4.2.2 Nutrient broth preparation

NB was used in serial dilutions preparations of nanoparticles, and bacterial cultures preparation, it was prepared by dissolving 13.0 gm of nutrient broth powder in 1.0 L distilled water, followed by dissolving it by heating on a Bunsen burner, moreover sterilization of NB at 121ºc for 15 min was performed and confirmed using sterilization indicator tapes.

3.4.3 Serial dilution preparation and MIC determination

The MIC was determined using serial two-fold dilutions of cobalt oxide nanoparticles, surfactant combination, in which a specific amount of the prepared nanoparticles was dissolved in Nutrient broth, followed by transferring a specific amount from the nanoparticles / NB mixture from one tube to another and with discarding the last amount, which gave us a serial dilution ranging from 2500µg/ml to 1.22µg/ml, where an inoculated NP-free broth was used as negative control. 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 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.

The MIC then was defined as the lowest concentration of the NP that completely visually inhibits the growth of the bacteria; The MIC measurements were done in duplicate in order to confirm the value of MIC. In brief, MIC for each tested bacteria, a series of dilutions was prepared for test and reference samples and tubes were assessed visually for growth. No growth tube with the most dilute NP preparation was taken as the practical MIC value.

3.4.4 MBC determination

After determination of MIC for the prepared cobalt oxide NPs and the surfactant combination, a specific amount was transferred from tubes in which no visible growth was observed, and seeded in Muller Hington Agar, that are not supplemented with NPs, which were incubated for 24 h at 37ºC, the MBC end point then was defined as the lowest concentration of NP that kill 99.9% of the initial bacterial population.

Chapter Four
Results and Discussion

4.1 Nanoparticles Characterization

4.1.1 X-ray diffraction (XRD)

X-ray powder diffraction patterns were taken in reflection mode CuKα (λ=1.5406Aº) radiation in 2 range [14], it was done for all samples in order to measure the particle size at λ = 1.5406 Aº, when using the x-ray diffractometer, and after this analysis is done, the following parameters can be determined:

Full width of half maximum-FWHM, peak intensity and peak position, and by applying Scherrer equation: $d=K\,\lambda/\beta\cos\theta_{ß}$

d:crystalline size (in nm), K:shape factor that has a typical value of about 0.9 , λ:x-ray wavelength (1.5405Aº=0.154051 nm), β: Full width of half maximum-FWHM (in radions), θ : Bragg angle.

4.1.1.1 X-ray characterization of cobalt oxide nanoparticles

X-ray diffraction pattern of the as-synthesized cobalt oxide nanoparticles was analyzed to investigate the phase structure along with its crystallinity as illustrated in Figures (4.1), (4.2), (4.3), the peaks were indexed to pure phase with a face-centered cubic structure, which corresponds to literature XRD analysis of wurtzite cobalt oxide[14].

Sample 1

From four diffraction peaks located at: 32.42º, 37.94 º, 51.53º, 58.07º, as illustrated in Figures (4.1), then applying Scherrer equation

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 25.25 nm

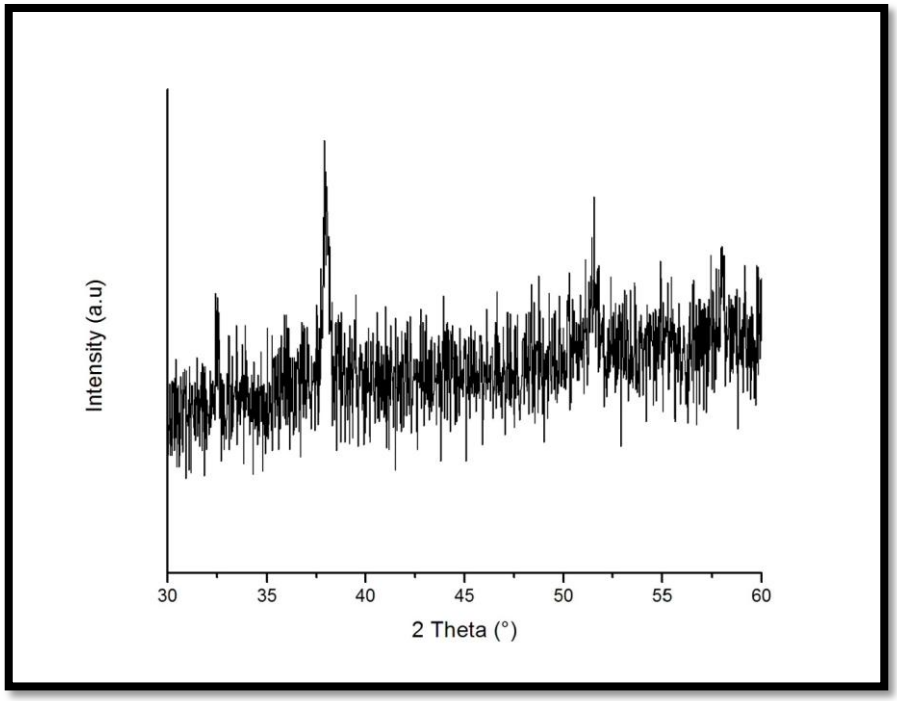

Figure 4.1 X-ray diffraction of cobalt oxide nanoparticles, sample 1 (25.25 nm).

Sample 2

From four diffraction peaks located at: 32.55º, 37.90 º, 51.51º, 57.99º, as illustrated in Figures (4.2) and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 21.61 nm.

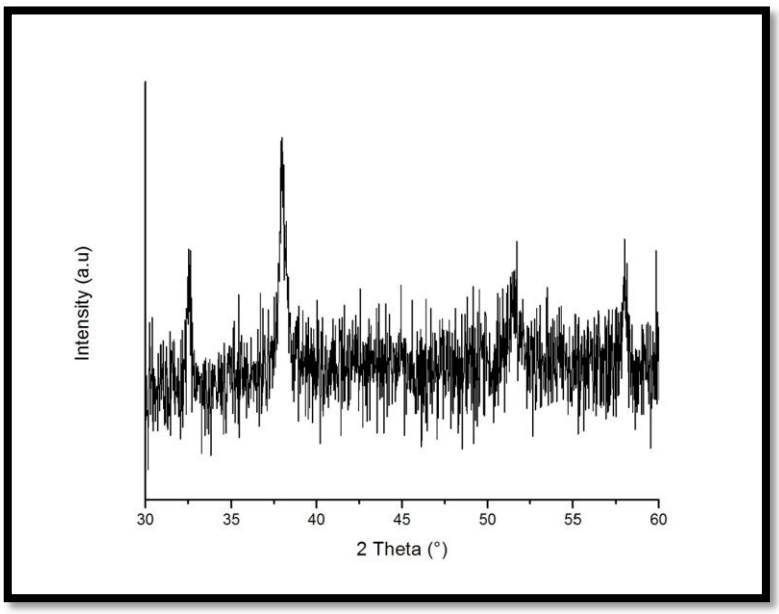

Figure 4.2 X-ray diffraction of cobalt oxide nanoparticles, sample 2 (21.61 nm).

Sample 3

From four diffraction peaks located at: 32.51º, 37.96 º, 51.37º, 58.27º, as illustrated in Figures (4.3) and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 20.19 nm.

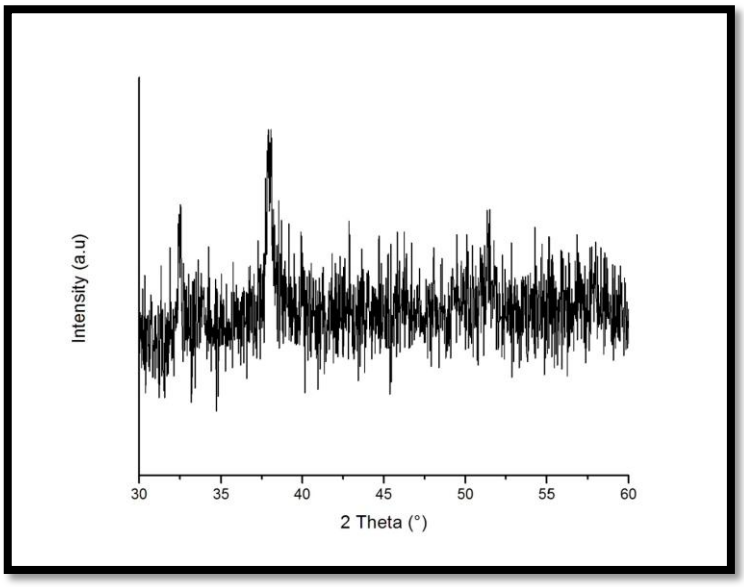

Figure 4.3 X-ray diffraction of cobalt oxide nanoparticles, sample 3 (20.19 nm).

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

Sample 1

Figure (4.4) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles stabilized in TOAB. From four diffraction peaks located at: 32.51º, 37.88º, 51.9º, 58.50 º, and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 23.08 nm

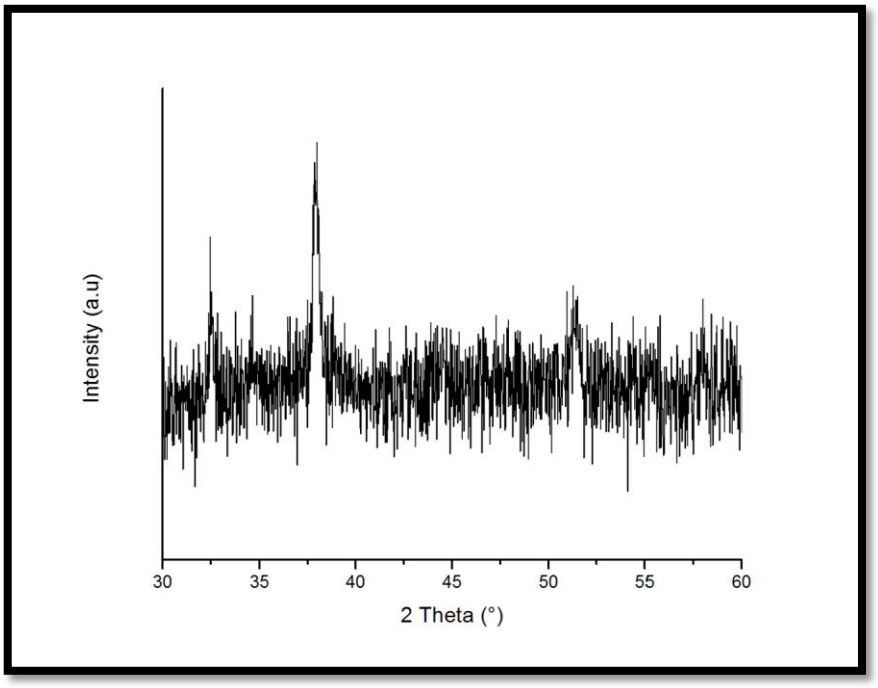

Figure 4.4 X-ray diffraction of cobalt oxide nanoparticles stabilized in TOAB, sample 1 (23.08 nm).

Sample 2

Figure (4.5) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles stabilized in TOAB. From four diffraction peaks located at: 32.48º, 37.97º, 51.47º, 58.02 º, and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 19.47 nm.

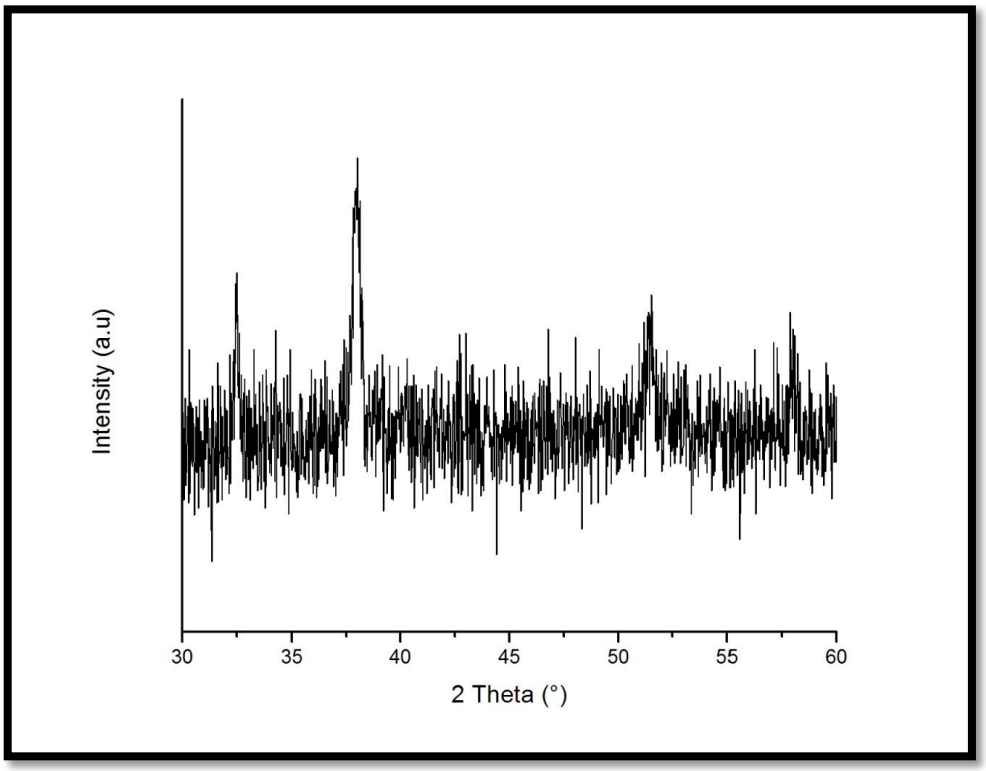

Figure 4.5 X-ray diffraction of cobalt oxide nanoparticles stabilized in TOAB, sample 2 (19.47 nm)

Sample 3

Figure (4.6) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles stabilized in TOAB. From four diffraction peaks located at: 32.48º, 37.87º, 51.46º, 58.02 º, and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 19.03 nm

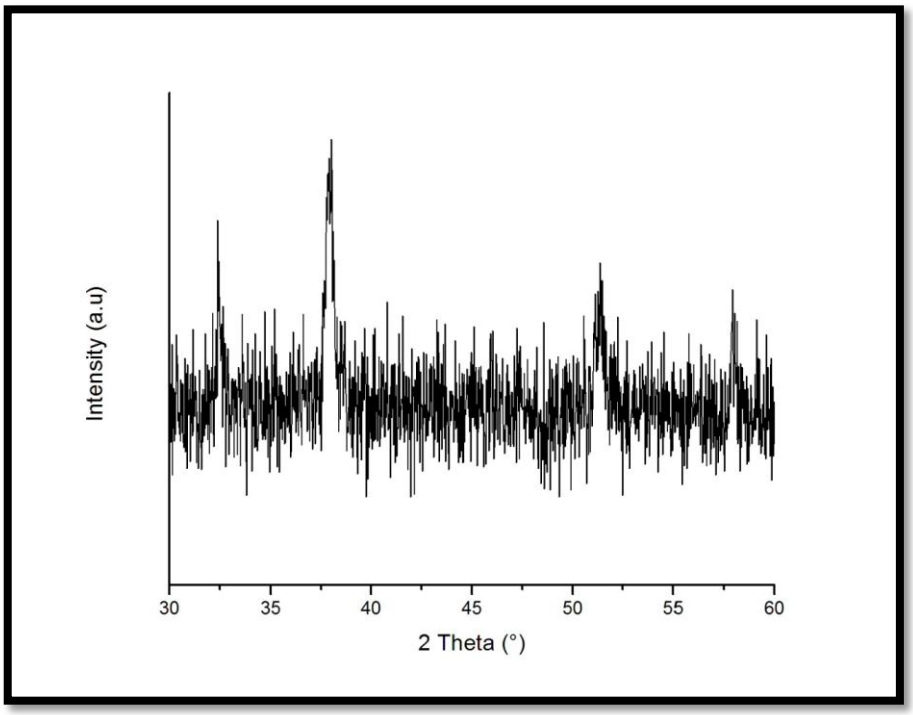

Figure 4.6 X-ray diffraction of cobalt oxide nanoparticles stabilized in TOAB, sample 3 (19.03 nm).

4.1.1.3 X-ray characterization of cobalt oxide nanoparticles stabilized in PVP.

Sample 1

Figure (4.7) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles stabilized in PVP. From four diffraction peaks located at: 32.45º, 37.84º, 51.25º, 57.7 º, and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 20.87 nm.

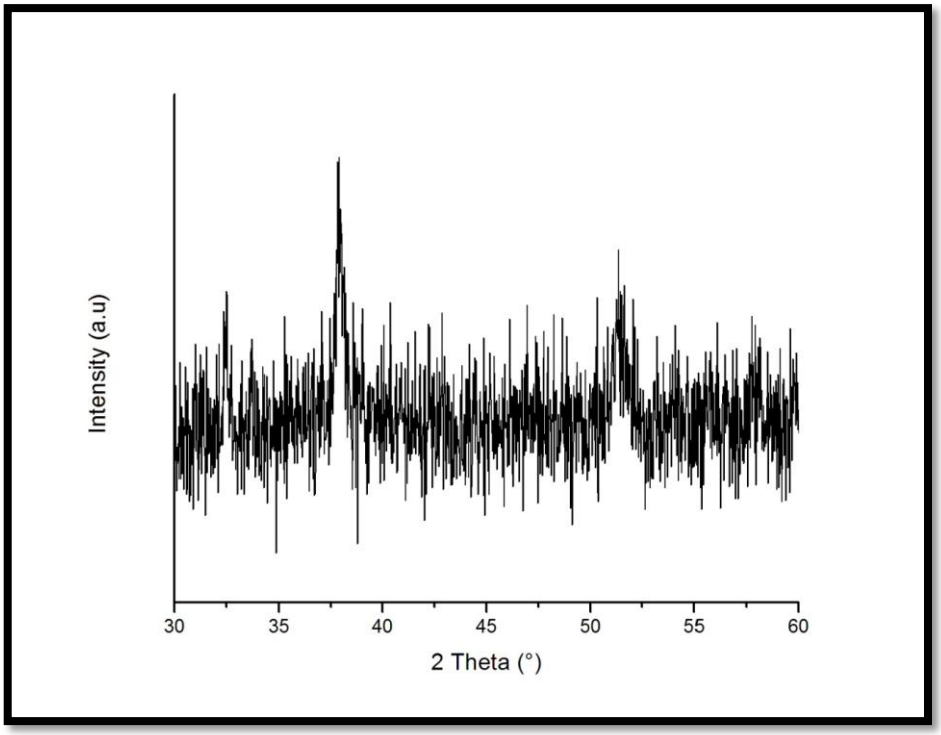

Figure 4.7 X-ray diffraction of cobalt oxide nanoparticles stabilized in PVP, sample 1 (20.87 nm).

Sample 2

Figure (4.8) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles stabilized in PVP. From four diffraction peaks located at: 32.44º, 37.92º, 51.38º, 57.92 º, and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 17.73 nm.

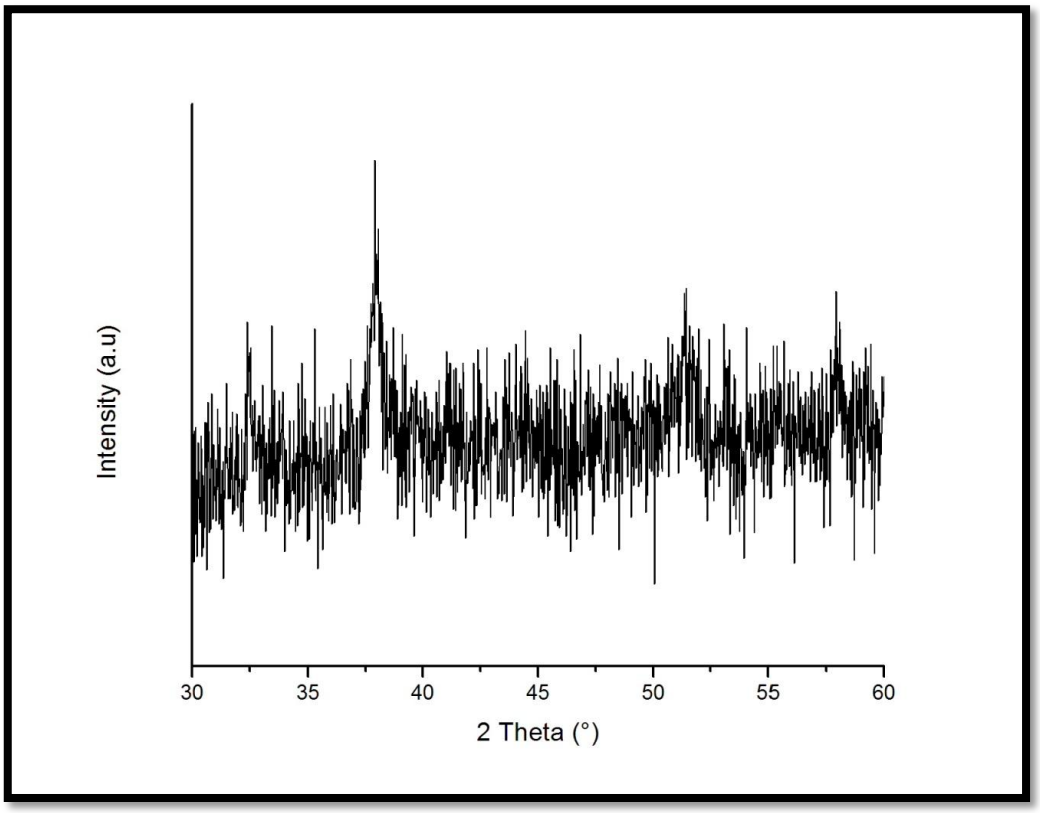

Figure 4.8 X-ray diffraction of cobalt oxide nanoparticles stabilized in PVP, sample 2 (17.73 nm).

Sample 3

Figure (4.9) shows the definite line broadening of XRD peaks for cobalt oxide nanoparticles stabilized in PVP. From four diffraction peaks located at: 32.48º, 37.85º, 51.7º, 57.99 º, and then applying Scherrer equation.

The average particle size of the as-synthesized Cobalt oxide NPs (d) equals 17.09 nm

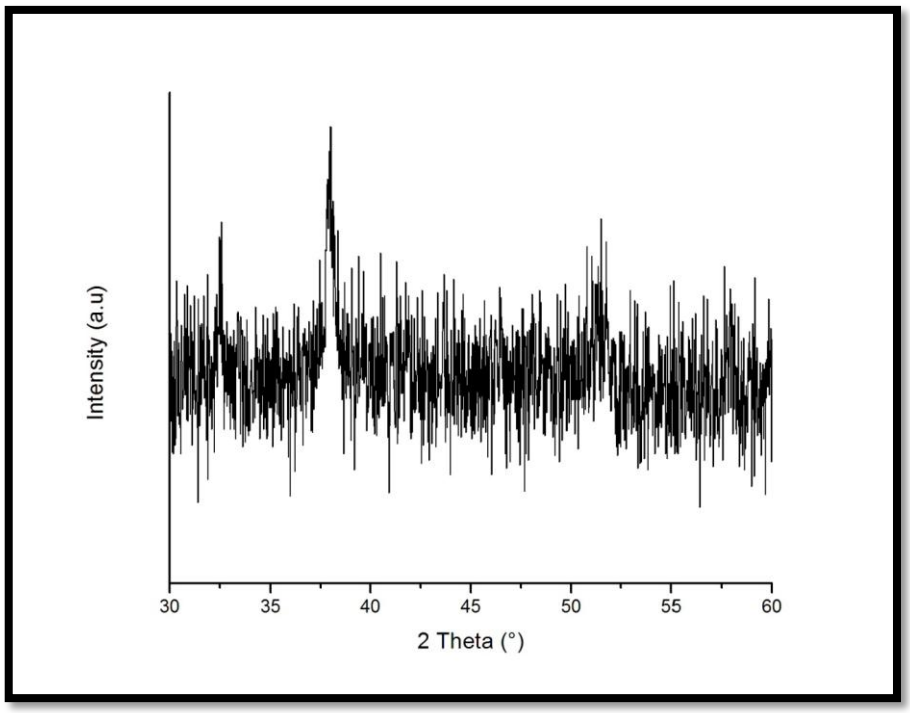

Figure 4.9 X-ray diffraction of cobalt oxide nanoparticles stabilized in PVP, sample 3 (17.09 nm)

4.2 Antibacterial activity of nanoparticles

4.2.1 Antibacterial Activity of cobalt oxide Nanoparticles

4.2.1.1 Minimum inhibitory concentration Determination

MIC of cobalt oxide NPs, used against S. aureus (ATCC 6538) and E. coli (ATCC 8739) bacterial reference strains

In this section, the antibacterial behavior of three samples of cobalt oxide nanoparticles with three different sizes (25.25 nm, 21.61 nm, 20.19 nm) was investigated against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739)

reference bacterial strains, where the MIC for each NPs sample with different size was measured.

Results in Table (4.1) shows the corresponding MIC of each sample of prepared cobalt oxide nanoparticles with different size, in which cobalt oxide NPs with an average size of 25.25 nm shows a MIC of 156 µg/ml against *S. aureus* ATCC 6538, in the other hand two cobalt oxide NPs samples with almost similar average size of about 21.61 nm, and 20.19 nm shows the same antibacterial activity for the two samples with a MIC of 78 µg/ml against *S. aureus* ATCC 6538. when the antibacterial behavior of the as-synthesized three cobalt oxide NPs samples was investigated against *E. coli* ATCC 8739, cobalt oxide NPs with an average size of 25.25 nm shows a MIC of 312.5 µg/ml, and samples with average similar size of 21.61 nm, 20.19 nm, showed a MIC of 156 µg/ml.

Table (4.1): Sizes of prepared cobalt oxide NPs (nm), and their corresponding Minimum inhibitory concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538)

	Sizes of Prepared Cobalt Oxide NPs (nm)		
Bacteria	25.25 nm	21.61 nm	20.19 nm
S. aureus (ATCC 6538)	156	78	78
E. coli (ATCC 8739)	312.5	156	156

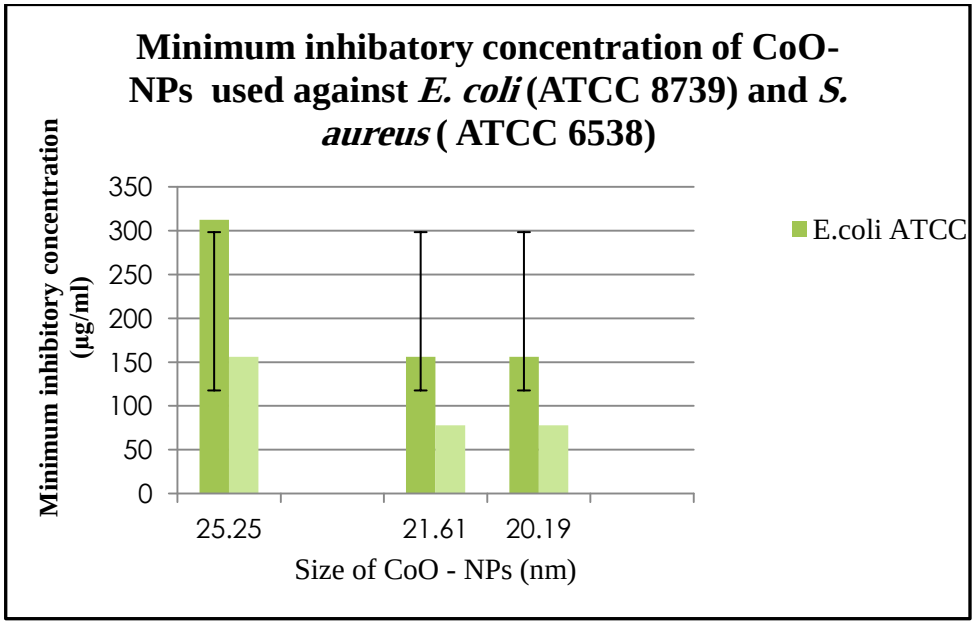

Figure (4.10): Antibacterial activity of cobalt oxide NPs of different sizes represented by Minimum inhibitory concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538)

Results in Figure (4.10) indicate the apparent antibacterial activity of cobalt oxide nanoparticles against both types of bacterial reference strains *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538), where the related inhibitory effects of these samples was found to be size dependent, in which NPs with larger size 25.25 nm showed a lower inhibitory effect against both *E. coli* and *S. aureus* reference bacterial strains, while cobalt oxide NPs samples with smaller sizes 21.61 nm and 20.19 nm appear to have a higher inhibitory effect as it interact with bacteria, and as these two samples have almost the same size, their inhibitory effect were the same against both types of bacterial reference strains these results agree with the fact that the antibacterial activity of NPs is known to be a function of the surface area in contact with microorganisms, where as there is a reduction in size of these particles lead to large surface area to volume ratio which enhance their interaction with microbes [1] .

In the other hand, results indicate that cobalt oxide nanoparticles samples were more inhibitory toward *S. aureus* than toward *E. coli*, so the inhibitory effect of NPs is also appear to be changed as it interacts with different bacterial strains, and this also agree with the fact that the properties of bacteria can affect its susceptibility to NPs [32]. As gram-negative bacteria *E. coli* has an outer membrane outside its peptidoglycan layer, that is not found in gram positive bacterial strains, which act as selective permeability barrier and affect its susceptibility to NPs [24].

MIC of cobalt oxide NPs, used against S. aureus and E. coli clinical bacterial isolates

In this section, the antibacterial behavior of three samples of cobalt oxide nanoparticles with three different sizes (25.26 nm, 21.61nm, 20.19nm) was investigated against *S. aureus* and *E. coli* clinical bacterial isolates. Where the MIC for each NPs sample with different size was measured.

Results in Table (4.2) shows the corresponding MIC of each sample of prepared cobalt oxide nanoparticles with different sizes where it's antibacterial behavior was investigated against *E. coli* and *S. aureus* clinical bacterial isolates, in which cobalt oxide NPs with an average size of 25.25 nm shows a MIC of 156 µg/ml against *S. aureus* clinical isolate, in the other hand two cobalt oxide NPs samples with almost similar average size of about (21.61 nm, and 20.19 nm) shows a MIC of 78 µg/ml.The antibacterial behavior of the as-synthesized three cobalt oxide NPs samples was investigated against *E. coli* clinical isolate, cobalt oxide NPs with an average size of 25.25 nm shows a MIC of 625 µg/ml, and samples with average similar size of (21.61 nm , 20.19nm), showed a MIC of about 156 µg/ml.

Table (4.2): Sizes of prepared cobalt oxide NPs (nm), and their corresponding Minimum inhibitory concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates

	Sizes of Prepared Cobalt Oxide NPs		
Bacteria	25.25 nm	21.61nm	20.19 nm
S. aureus clinical	156	78	78
E. coli clinical	625	156	156

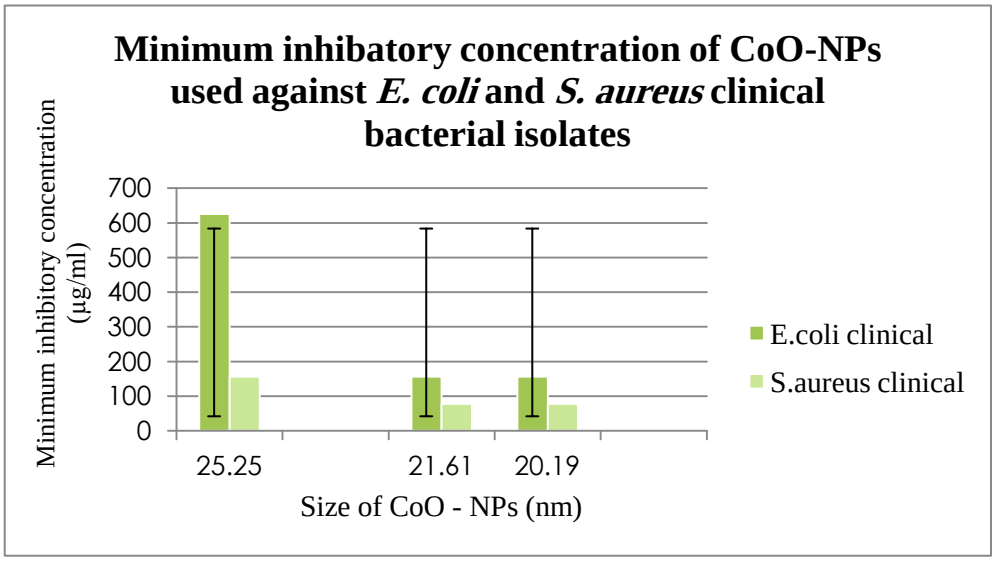

Figure (4.11): Antibacterial activity of cobalt oxide NPs of different sizes represented by Minimum inhibitory concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical Bacterial isolates

In a similar manner to the antibacterial activity of the cobalt oxide nanoparticles against both types of the ATCC bacterial isolates, Figure (4.11) indicate the apparent antibacterial activity of cobalt oxide nanoparticles against both types of clinical bacterial isolates, and the related inhibitory effects of these samples was also found to be size dependent, which agree with studies showed that the antibacterial activity of NPs increase as these particles reduced in size, as a result of increase in their surface area to volume ratio [22]. In which NPs with larger size 25.25

nm showed a lower inhibitory effect against both *E. coli* and *S. aureus* when compared with cobalt oxide NPs samples with smaller sizes. So as size of the prepared NPs was changed a different inhibition rates were observed in the same way as indicated earlier against ATCC bacterial isolates. However the MIC of the larger size of 25.25 was higher two times against the clinical isolate of *E. coli* bacterial isolate that could indicate the clinical isolates higher tendancy of being more resistant even the difference is not significant.

4.2.1.2 Minimum bactericidal concentration determination

MBC of cobalt oxide NPs, used against S. aureus (ATCC 6538) and E. coli (ATCC 8739) reference bacterial strains

In this section, the antibacterial behavior of three samples of cobalt oxide nanoparticles with three different sizes (25.25nm, 21.61nm, 20.19 nm) was investigated against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739) reference bacterial strains, where the MBC for each NPs sample with different size was measured.

Results in Table (4.3) shows the corresponding MBC results of each sample of the prepared cobalt oxide nanoparticles with different sizes, in which cobalt oxide NPs with an average size of 25.25 nm shows a MBC of 312.5 µg/ml against *S. aureus* ATCC 6538, in the other hand two cobalt oxide NPs samples with almost similar average size of about (21.61 nm, and 20.19 nm) shows the same antibacterial activity for the two samples

with a MBC of 156 µg/ml against *S. aureus* ATCC 6538. The antibacterial behavior of the as- synthesized three cobalt oxide NPs samples was investigated against *E. coli* ATCC 8739, cobalt oxide NPs with an average size of 25.25 nm shows a MBC of 625 µg/ml, and samples with average similar size of (21.61 nm, 20.19 nm), shows the same MBC of 312.5 µg/ml.

Table (4.3): Sizes of prepared cobalt oxide NPs (nm), and their corresponding Minimum bactericidal concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538).

	Sizes of Prepared Cobalt Oxide NPs		
Bacteria	25.25 nm	21.61 nm	20.19 nm
S. aureus (ATCC 6538)	312.5	156	156
E. coli (ATCC 8739)	625	312.5	312.5

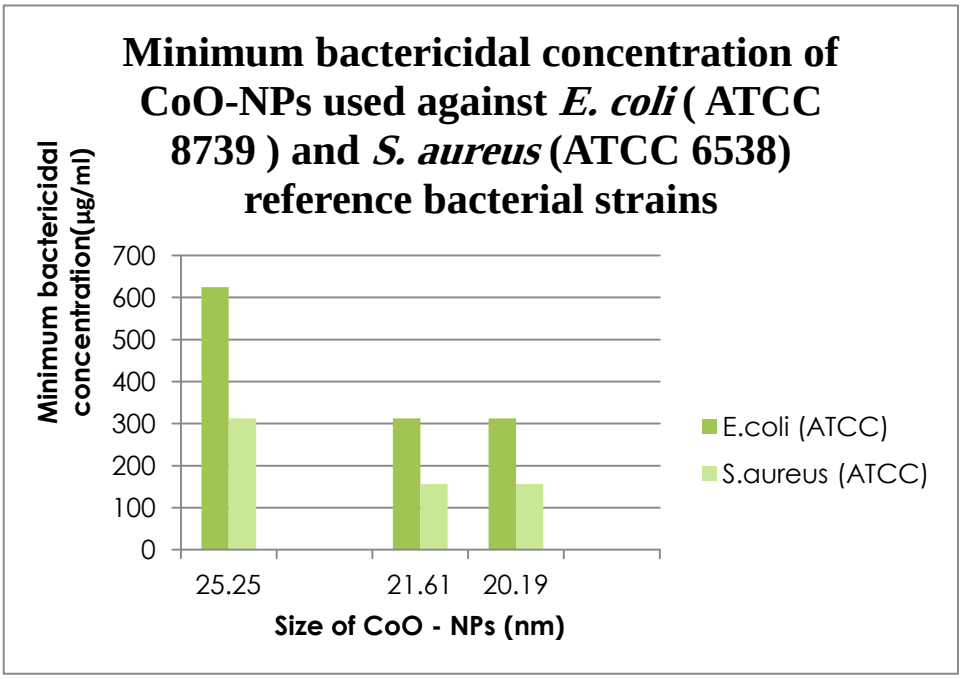

Figure (4.12): Antibacterial activity of cobalt oxide NPs of different sizes represented by Minimum bactericidal concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538)

Results in Figure (4.12) indicate the apparent bactericidal effect of cobalt oxide nanoparticles against both types of reference bacterial strains (*E. coli* ATCC 8739 and *S. aureus* ATCC 6538), where results indicate that the related bactericidal effects of these samples was also found to be size dependent, in which NPs with larger size 25.25 nm showed a lower bactericidal effect against both *E. coli* and *S. aureus* when compared with cobalt oxide NPs samples with smaller sizes that appear to posses higher bactericidal effect and the effect again is higher against gram positive *S. aureus* bacteria. MBC in comparison to MIC was doubled that reflect the bactericidal not the bacteistaic properties of the CoO NPs.

MBC of cobalt oxide NPs, used against S. aureus and E. coli clinical bacterial isolates

In a smillar manner to the antibacterial activity seen in the MBC of CoO NPs against ATCC bacterial isolates; table 4.4 and figure 4.13 shows the MBC of CoO NPs against the clinical bacterial isolates that the antibacterial activity were identical to MBC seen against the ATCC bacterial isolates. This result confirmed that the difference seen in the MIC between ATCC and clinical bacterial isolates, especially of the larger size against E. coli that was not significant.

Table (4.4): Sizes of prepared cobalt oxide NPs (nm), and their corresponding Minimum bactericidal concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates

	Sizes of Prepared Cobalt Oxide NPs		
Bacteria	25.25 nm	21.61nm	20.19 nm
S. aureus clinical	312.5	156	156
E. coli clinical	625	312.5	312.5

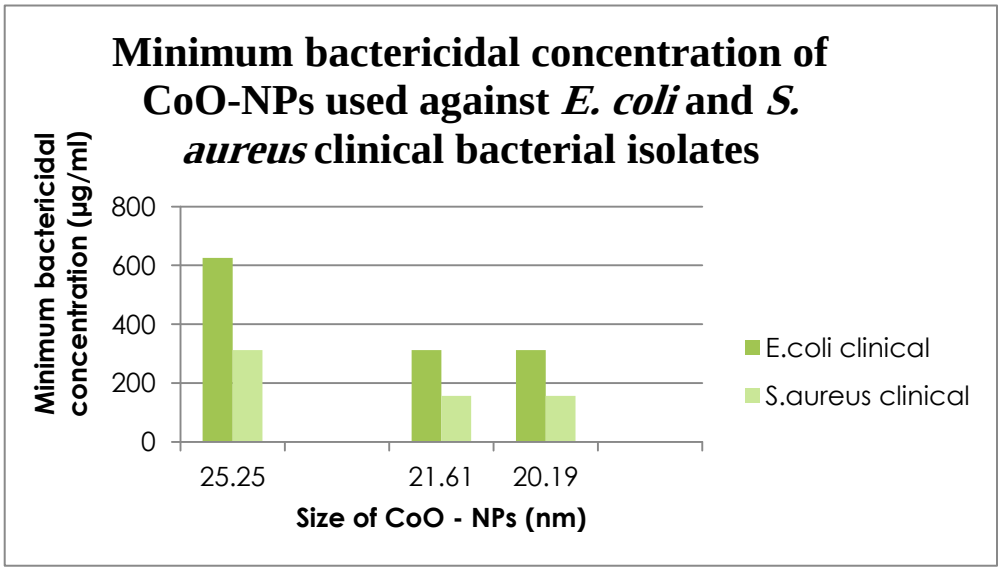

Figure (4.13): Antibacterial activity of cobalt oxide NPs of different sizes represented by Minimum bactericidal concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates.

4.2.2 Antibacterial Activity of Cobalt Oxide Nanoparticles stabilized in TOAB

4.2.2.1 Minimum inhibitory concentration determination

MIC of cobalt oxide NPs stabilized in TOAB and used against S. aureus (ATCC 6538) and E. coli (ATCC 8739) bacterial reference strains

In this section, the antibacterial behavior of three samples of cobalt oxide nanoparticles with three different sizes (23.08 nm, 19.47 nm, and 19.03 nm) and stabilized in TOAB was investigated against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739) reference bacterial strains, where the MIC for each NPs sample with different size was measured.

Most of the related properties of nanoparticles are dependent on their size and shape [27], in which results in Table (4.5) summarize the corresponding MIC for each sample, in which cobalt oxide NPs with an average size of 23.08 nm showed a MIC of 78 µg/ml when it interact with *S. aureus* ATCC 6538, where as the inhibitory effect of the other two samples of sizes equal to 19.47 nm and 19.03 nm, gave a MIC of 39 µg/ml, as the size of prepared NPs was slightly changed, its related inhibitory effect against *S. aureus* ATCC 6538 was altered as well. On the other hand as the antibacterial behavior of the three cobalt oxide NPs with different sizes was examined upon its interaction with *E. coli* ATCC 8739, cobalt oxide NPs with an average size equal to 23.08 give a MIC equal to 156 µg/ml, and the same MIC was observed when cobalt oxide NPs with sizes 19.47 nm and 19.03 nm were tested,which equals to 78 µg/ml.

Table (4.5): Sizes of prepared cobalt oxide NPs (nm) stabilized in TOAB, and their corresponding Minimum inhibitory concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538)

	Sizes of Prepared Cobalt Oxide NPs stabilized in TOAB		
Bacteria	23.08 nm	19.47 nm	19.03 nm
S. aureus (ATCC 6538)	78	39	39
E. coli (ATCC 8739)	156	78	78

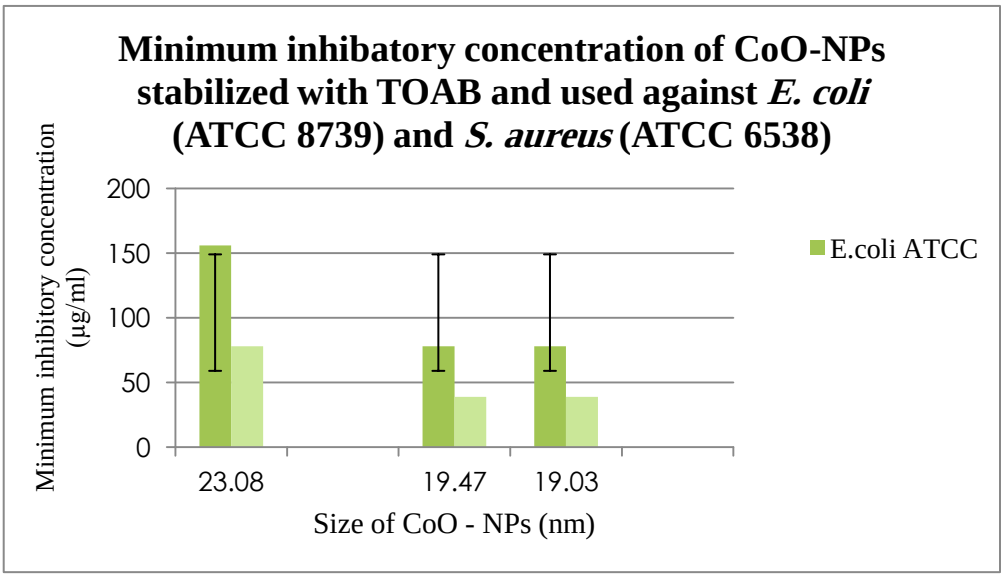

Figure (4.14): Antibacterial activity of cobalt oxide NPs stabilized with TOAB, of different sizes represented by Minimum inhibitory concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538)

Results in Table (4.5) and Figure (4.14) indicate the apparent antibacterial activity of cobalt oxide nanoparticles stabilized in TOAB against used bacterial strains that was in consistency with the nonstabilised CoO NPs in the matter of the particle size and the kind of bacterial strains. However, the effect of TOAB addition to the NPs was doubled against both strains which could be a good choice for stabilizing the NPs and getting better antibacterial activity.

MIC of cobalt oxide NPs stabilized in TOAB and used against S. aureus and E. coli clinical bacterial isolates

In this section, the antibacterial behavior of three samples of cobalt oxide nanoparticles with three different sizes (23.08 nm, 19.47 nm, and 19.03 nm) and stabilized in TOAB was investigated against *S. aureus* and *E. coli* clinical bacterial isolates, where the MIC for each NPs sample with different size was measured.

Results in Table (4.6) summarize the corresponding MIC of each sample of prepared cobalt oxide nanoparticles with different sizes and stabilized in TOAB where it's antibacterial behavior was investigated against *E. coli* and *S. aureus* clinical bacterial isolates, in which cobalt oxide NPs with an average size of 23.08 nm shows a MIC of 156 µg/ml against *S. aureus* clinical isolate, in the other hand two cobalt oxide NPs samples with an average size of about 19.47 nm , and 19.03 nm shows the same MIC which equal 78 µg/ml.The antibacterial behavior of the as-synthesized three cobalt oxide NPs samples was investigated against *E. coli* clinical isolate, cobalt oxide NPs with an average size of 23.08 nm shows a MIC of 312.5 µg/ml, and samples with average size of 19.47 nm, 19.03 nm , showed a MIC of about 156 µg/ml.

Table (4.6): Sizes of prepared cobalt oxide NPs (nm) stabilized in TOAB, and their corresponding Minimum inhibitory concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates

	Sizes of Prepared Cobalt Oxide NPs stabilized in TOAB		
Bacteria	23.08 nm	19.47 nm	19.03 nm
S. aureus clinical	156	78	78
E. coli clinical	312.5	156	156

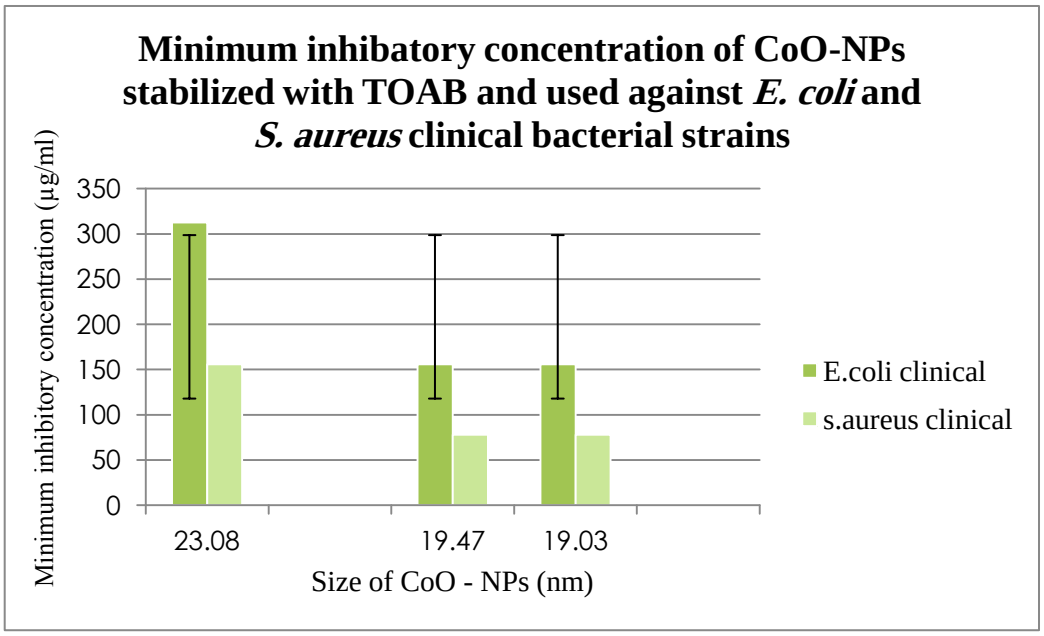

Figure (4.15): Antibacterial activity of cobalt oxide NPs stabilized with TOAB, of different sizes represented by Minimum inhibitory concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates.

Results in Table (4.6) and Figure (4.15) indicate the apparent antibacterial activity of cobalt oxide nanoparticles stabilized in TOAB against both types of bacterial clinical isolates (*E. coli* and *S. aureus*), where MIC improved in the TOAB against both ATCC bacterial isolates, there was almost same result with TOAB and without TOAB against clinical bacterial isolate even at the larger size of CoO_TOAB has lower effect

against *E. coli* that could be explained by the size itself which was smaller with TOAB, therefore will have a higher effect. This result could indicate an important issue that there will be a challenge to to solve such difference in the behavior of the same bacterial strains that could need more investigation to know the exact reasons behind this difference.

4.2.2.2 Minimum bactericidal concentration determination

MBC of cobalt oxide NPs stabilized in TOAB and used against S. aureus (ATCC 6538) and E. coli (ATCC 8739) bacterial reference strains

In this section, the antibacterial behavior of three samples of cobalt oxide nanoparticles with three different sizes (23.08 nm, 19.47 nm, and 19.03 nm) and stabilized in TOAB was investigated against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739) reference bacterial strains, where the MBC for each NPs sample with different size was measured.

Results in Table (4.7) summarize the corresponding MBC for each sample, in which cobalt oxide NPs with an average size of 23.08 showed a MBC of 312.5 µg/ml when it interact with *S. aureus* ATCC 6538, where as the bactericidal effect of the other two samples of sizes equal to 19.47 nm and 19.03 nm, gave a MBC of 156 µg/ml, as the size of prepared NPs was slightly changed, its related bactericidal effect against *S. aureus* ATCC 6538 was altered as well. In the other hand as the antibacterial behavior of the three cobalt oxide NPs with different sizes was examined, it shows that as the size of the prepared NPs changed, it also affect it is bactericidal

effect against *E. coli* ATCC 8739, where NPs with an average size equal to 23.08 nm, had a bactericidal effect equal to 625 µg/ml, and samples with an average size equal to 21.61 nm, and 20.19 nm had a bactericidal effect equal to 312.5 µg/ml.

Table (4.7): Sizes of prepared cobalt oxide NPs (nm) stabilized in TOAB, and their corresponding Minimum bactericidal concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538) .

	Sizes of Prepared Cobalt Oxide NPs stabilized in TOAB		
Bacteria	23.08 nm	19.47 nm	19.03 nm
S. aureus (ATCC 6538)	312.5	156	156
E. coli (ATCC 8739)	625	312.5	312.5

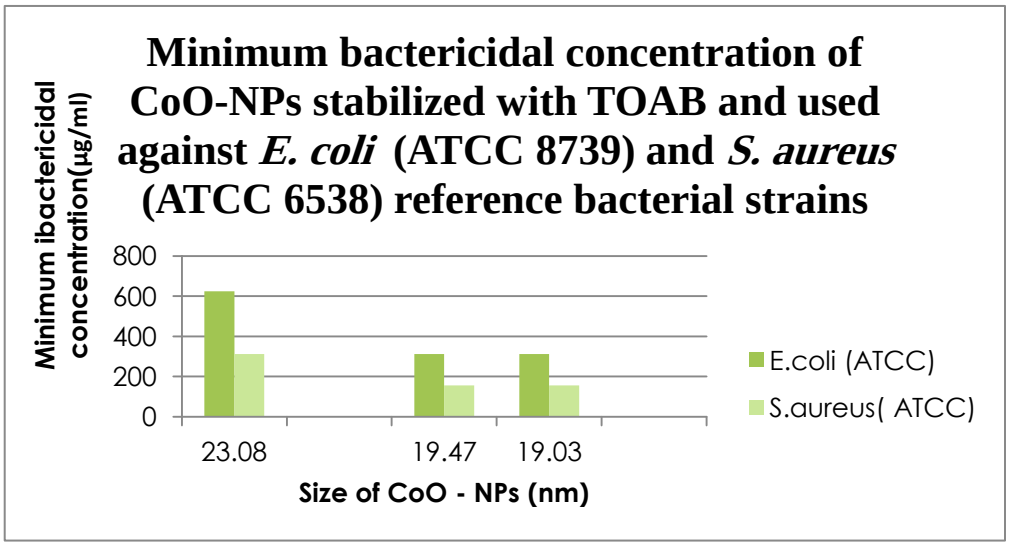

Figure (4.16): Antibacterial activity of cobalt oxide NPs stabilized with TOAB, of different sizes represented by Minimum bactericidal concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538).

Results in Figure (4.16) indicate the apparent bactericidal effect of cobalt oxide nanoparticles stabilized in TOAB against both types of bacterial reference strains *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538), cobalt oxide NPs samples with different sizes appear to have different bactericidal effect as it interact with bacteria ,and the related bactericidal effect of these samples was found to be altered as the size of the NPs is changed, further more results indicate that cobalt oxide nanoparticles stabilized in TOAB show a higher bactericidal effect against *S. aureus* (ATCC 6538) more than it against *E. coli* (ATCC 8739) for each NP sample size.

MBC of cobalt oxide NPs stabilized in TOAB and used against S. aureus and E. coli clinical bacterial isolates

As seen in the antibacterial activity of the MBC of CoO_TOAB NPs against ATCC bacterial isolates; table 4.8 and figure 4.17 shows the MBC of CoO NPs against the clinical bacterial isolates, the antibacterial activity represented by MBC were identical against the ATCC and the clinical bacterial isolates.

Table (4.8): Sizes of prepared cobalt oxide NPs (nm) stabilized in TOAB, and their corresponding Minimum bactericidal concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates.

	Sizes of Prepared Cobalt Oxide NPs stabilized in TOAB		
Bacteria	23.08 nm	19.47 nm	19.03 nm
S. aureus clinical	312.5	156	156
E. coli clinical	625	312.5	312.5

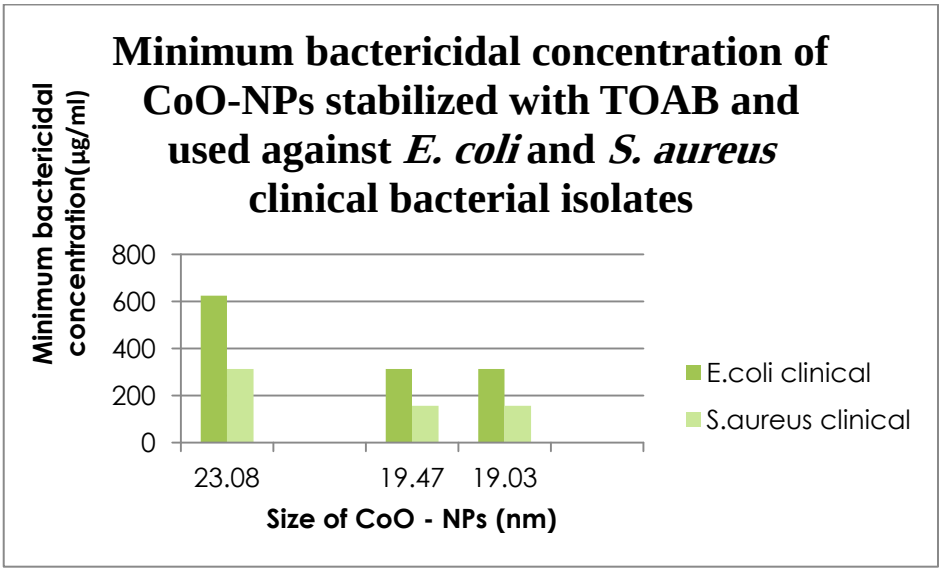

Figure (4.17): Antibacterial activity of cobalt oxide NPs stabilized with TOAB, of different sizes represented by Minimum bactericidal concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates.

4.2.3 Antibacterial Activity of Cobalt Oxide Nanopartclies stabilized in PVP

4.2.3.1 Minimum inhibitory concentration determination

MIC of cobalt oxide NPs stabilized in PVP and used against S. aureus (ATCC 6538) and E. coli (ATCC 8739) bacterial reference strains

The antibacterial behavior of three samples of cobalt oxide nanoparticles stabilized in PVP with three different sizes (20.87 nm, 17.73 nm, and 17.09 nm) was investigated against *S. aureus* (ATCC 6538) and *E. coli* (ATCC 8739) reference bacterial strains, where the MIC for each NPs sample with different size was measured.

Table (4.9) summarize the corresponding MIC for each sample, in which cobalt oxide NPs stabilized in PVP with an average size of 20.87 nm showed a MIC of 312.5 µg/ml, where 17.73 nm and 17.09 nm, gave a MIC of 19.5 µg/ml against *S. aureus* ATCC 6538. The inhibitory effect of this small size against *S. aureus* ATCC 6538 with 19.5 µg/ml is interesting in that if the size can be controlled to a smaller size will probably give amore prominent inhibitory effect. NPs with an average size equal to 20.87 nm gave a MIC equal to 625 µg/ml and other NPs samples with sizes equal to 17.73 nm,17.09 nm gave the same MIC equal to 312.5 µg/ml.

Table (4.9): Sizes of prepared cobalt oxide NPs (nm) stabilized in PVP, and their corresponding Minimum inhibitory concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538) .

	Sizes of Prepared Cobalt Oxide NPs stabilized in PVP		
Bacteria	20.87 nm	17.73 nm	17.09 nm
S. aureus (ATCC 6538)	312.5	19.5	19.5
E. coli (ATCC 8739)	625	312.5	312.5

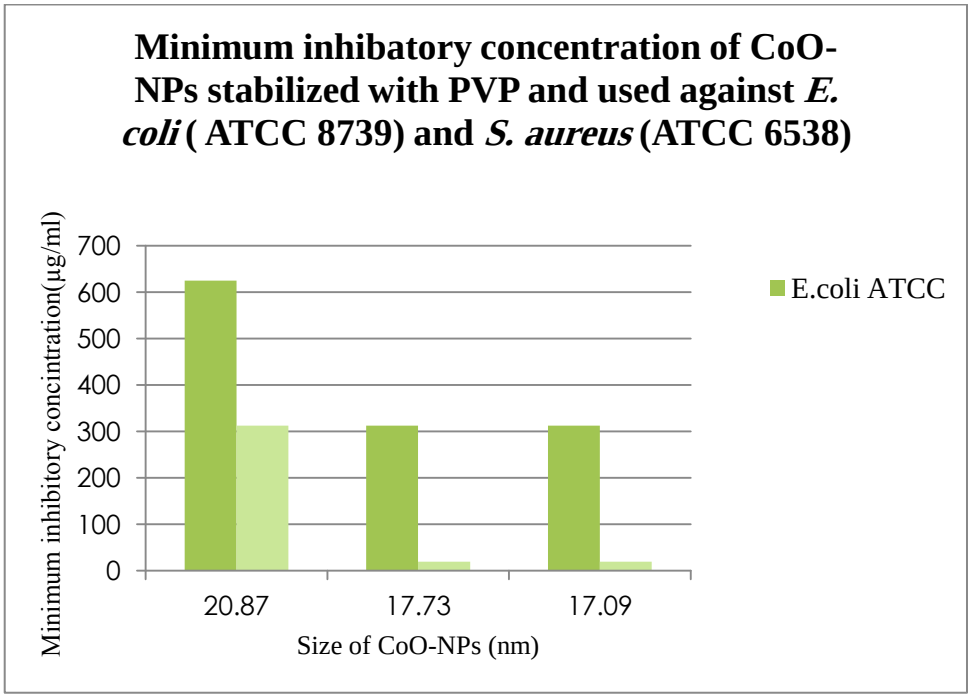

Figure (4.18): Antibacterial activity of cobalt oxide NPs stabilized with PVP, of different sizes represented by Minimum inhibitory concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538).

Figure (4.18) indicate the apparent antibacterial activity of cobalt oxide nanoparticles stabilized in PVP and used against both types of bacterial reference strains *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538). The most apparent effect was seen in the smaller sizes against *S. aureus* (ATCC 6538) that was more prominent than that seem with *E. coli* (ATCC 8739).

MIC of cobalt oxide NPs stabilized in PVP and used against S. aureus and E. coli clinical bacterial isolates

The antibacterial behavior of the three samples of cobalt oxide nanoparticles stabilized in PVP with three different sizes (20.87 nm, 17.73 nm, and 17.09 nm) was investigated against *S. aureus* and *E. coli* clinical bacterial isolates, where the MIC for each NPs sample with different size was measured.

The corresponding MIC of each sample of the prepared cobalt oxide nanoparticles with different sizes and stabilized in PVP where it's antibacterial behavior was investigated against *E. coli* and *S. aureus* clinical bacterial isolates are shown in Table (4.10). Cobalt oxide NPs with an average size of 20.87 nm shows a MIC of 625 µg/ml against *S. aureus* clinical isolate, the other two cobalt oxide NPs samples with an average similar size of 17.73 nm and 17.09 nm shows the same MIC of 312.5 µg/ml. Even, as it showed in the earlier sections, that the effect is always better against gram positive *S. aureus*, the effect against the gram negative *E. coli* in this trial is the same for both two kinds of the isolates of clinical origin to confirm our notion that it should be considered for the usage of both reference and clinical isolates to have a better idea about the mechanisms of antibacterial activity against different bacterial strains.

Table (4.10): Sizes of prepared cobalt oxide NPs (nm) stabilized in PVP, and their corresponding Minimum inhibitory concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates.

	Sizes of Prepared Cobalt Oxide NPs stabilized in PVP		
Bacteria	20.87 nm	17.73 nm	17.09 nm
S. aureus clinical	625	312.5	312.5
E. coli clinical	625	312.5	312.5

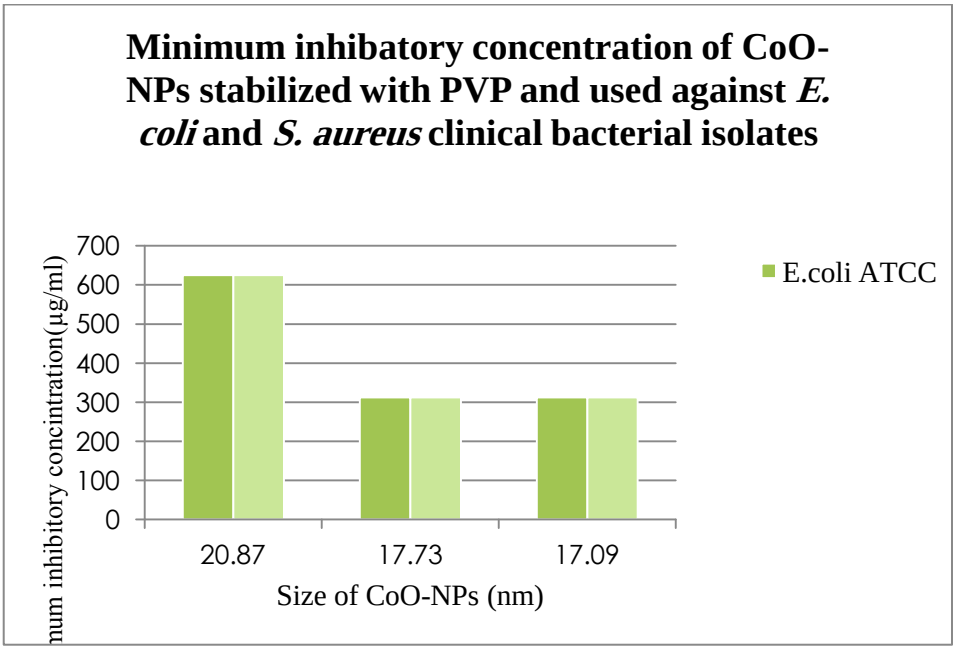

Figure (4.19): Antibacterial activity of cobalt oxide NPs stabilized with PVP, of different sizes represented by Minimum inhibitory concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates

As shown in Figure (4.19) the apparent antibacterial activity of cobalt oxide nanoparticles stabilized in PVP against both types of bacterial clinical iolates *E. coli* and *S. aureus* was the same and the difference is limited in comparison to what seen against the gram positive ATCC bacterial isolate shown in the previous section as shown in figure (4.18).

4.2.3.2 Minimum bactericidal concentration determination

MBC of cobalt oxide NPs stabilized in PVP and used against S. aureus (ATCC 6538) and E. coli (ATCC 8739) bacterial reference strains

The minimum bactericidal concentration of three samples of cobalt oxide nanoparticles with three different sizes 20.87 nm, 17.73 nm, and 17.09 nm and stabilized in PVP was investigated against *S. aureus* (ATCC) and *E. coli* (ATCC) reference bacterial strains.

MBC for each sample as in Table (4.11) was consistent with the corresponding MIC as seen in table (4.9) in that the smaller size gave a prominent effect against gram positive bacteria, with the average size of 20.87 nm showed a MBC of 625 µg/ml and the other two samples of sizes equal to 17.73 nm and 17.09 nm, gave a MBC of 39 µg/ml. MBC against *E. coli* ATCC 8739 with the NPs average size of 20.87 nm gave a MBC equal to 1250. The other two samples with different sizes 17.73 nm and 17.09 nm samples gave a MBC against *E. coli* ATCC 8739 equal to 625 µg/ml.

Table (4.11): Sizes of prepared cobalt oxide NPs (nm) stabilized in PVP, and their corresponding Minimum bactericidal concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538).

	Sizes of Prepared Cobalt Oxide NPs stabilized in PVP		
Bacteria	20.87 nm	17.73 nm	17.09 nm
S. aureus (ATCC 6538)	625	39	39
E. coli (ATCC 8739)	1250	625	625

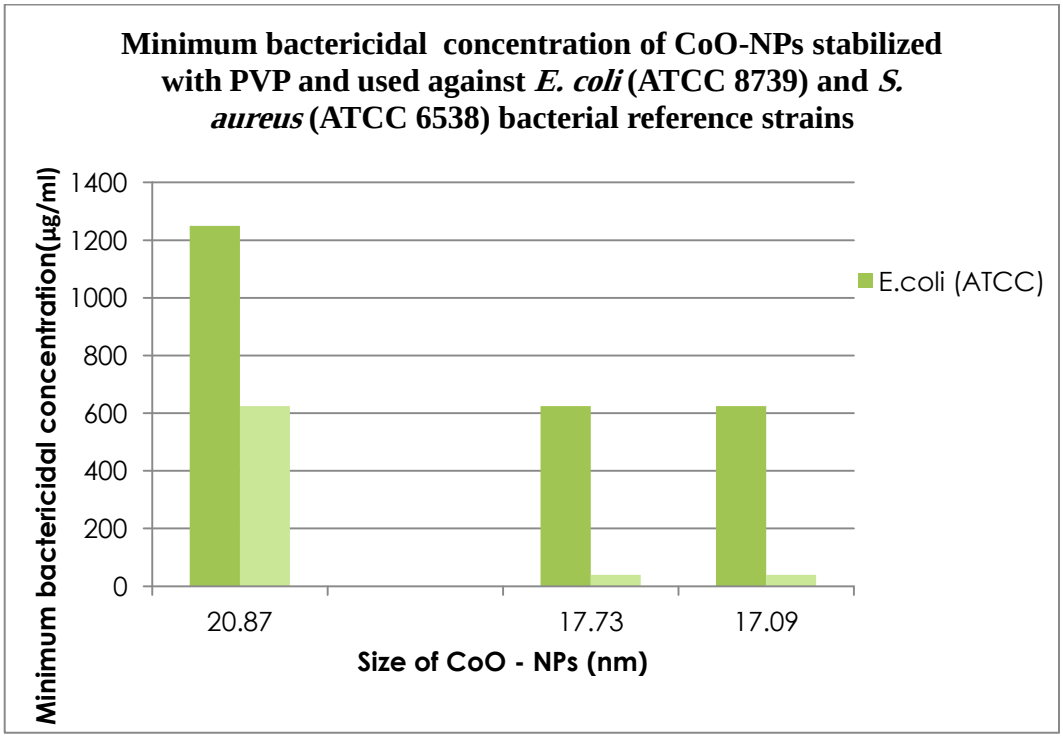

Figure (4.20): Antibacterial activity of cobalt oxide NPs stabilized with PVP, of different sizes represented by Minimum bactericidal concentrations (µg/ml), used against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538).

The apparent bactericidal effect, Figure (4.20) of cobalt oxide nanoparticles stabilized in PVP and used against both types of bacterial reference strains *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538) was two time higher than the MIC, which indicate the bactericidal not the bacteristatic property for the cobalt oxide nanoparticles stabilized in PVP. However, as seen in the MIC effect, Figure (4.18), the difference is very high between the larger size and the smaller size of the NPs.

MBC of Cobalt Oxide NPs stabilized in PVP and used against S. aureus and E. coli clinical bacterial isolates.

The antibacterial behavior of the three samples of Cobalt Oxide nanoparticles stabilized in PVP with three different sizes 20.87 nm, 17.73

nm, and 17.09 nm was investigated against *S. aureus* and *E. coli* clinical bacterial isolates.

MBC against both clinical bacterial isolates, *S. aureus* and *E. coli*, were the same, Table (4.12), in which cobalt oxide NPs with an average size of 20.87 nm shows MBC of 625 µg/ml, the other two cobalt oxide NPs samples with an average similar size of about 17.73 nm, and 17.09 nm shows MBC of 312.5 µg/ml.

Table (4.12): Sizes of prepared cobalt oxide NPs (nm) stabilized in PVP, and their corresponding Minimum bactericidal concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates.

	Sizes of Prepared Cobalt Oxide NPs stabilized in PVP		
Bacteria	20.87 nm	17.73 nm	17.09 nm
S. aureus clinical	625	312.5	312.5
E. coli clinical	625	312.5	312.5

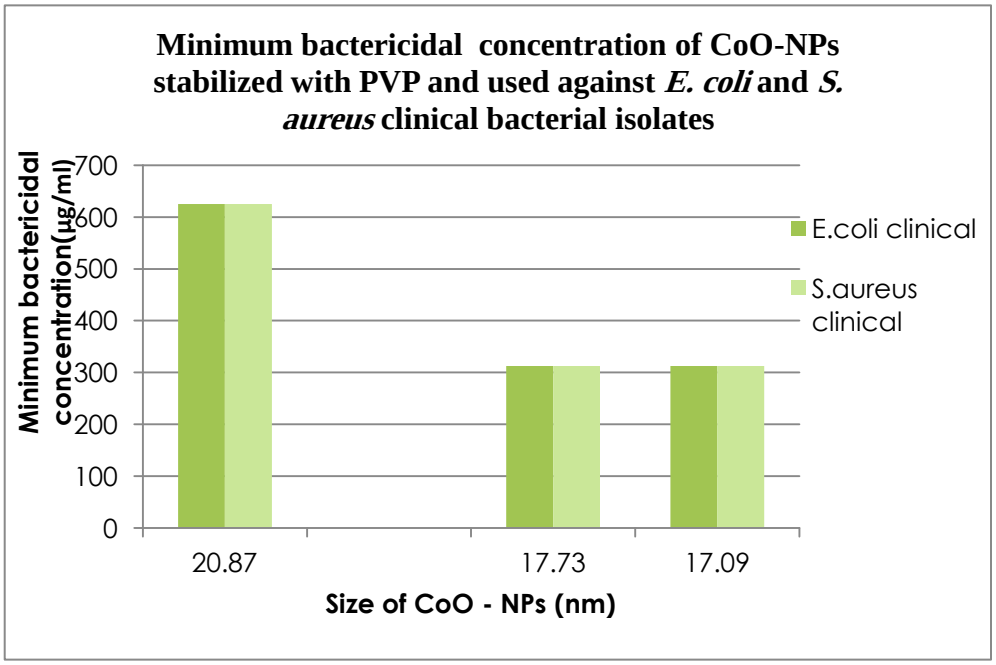

Figure (4.21): Antibacterial activity of cobalt oxide NPs stabilized with PVP, of different sizes represented by Minimum bactericidal concentrations (µg/ml), used against *E. coli* and *S. aureus* clinical bacterial isolates

Antibacterial activity of the CoO NPs stabilized in PVP, as shown in Table (4.12) and Figure (4.21) were identical against both bacterial isolates that was different from the rest of the used nanoparticles through out this study. Morover, perfect bactericidal was noted with the same MIC and MBC.

Chapter five

Conclusion

Investigating the interactions that take place between nanoparticles and different microorganism is of great importance in order to provide a comprehensive, understanding of nanoparticles toxicity and explore their potential for novel usage and applications. It is well known that nanoparticles behavior is mediated by the compositions and the size of the nanoparticles, though the effects of small differences in size against biological cells have not been of great concerns for most researchers in the field [45].

Most of the unexpected and unique properties of NPs is due to their small sizes with high surface area to volume ratio, previous studies showed that nanoparticles properties can be changed by changing their size, where the changes in size result in changes in surface area to volume ratio, as a result the surface area increase, an increase in the reaction sites on the particles, more than those with lower surface area and more over the functional activities and the related unique physical, chemical and biological properties of these nanoparticles will be alternatively changed as the size of these particles is changed [22][9].

In this study as cobalt oxide nanoparticles with and without surfactant were prepared by salt reduction method, and their antibacterial behavior was studied. These NPs were studied against both gram-negative *E. coli* and gram-positive *S. aureus* reference strains and clinical bacterial isolates, by

measuring the corresponding MIC (µg/ml), and MBC (µg/ml) for each cobalt oxide NPs sample with different size.

This study showed that these NPs can act as antibacterial agents against both gram-negative *E. coli* and gram-positive *S. aureus* clinical isolates and reference strains. A significant difference in size dependence was observed between clijnical and reference isolates that was most clear with the PVP stabilized CoO NPs. Antimicrobial activity against both types of bacteria was more prominent against gram positive, in which results indicate that changes in size of the tested NPs will result in changes in its corresponding inhibitory effect on bacteria, due to changes in surface area to volume ratio that lead to changes in their functional activities as antibacterial agents, which also appear to be differed based on the microorganism tested, the drug resistant ones and their respective defense mechanism [32]. As gram-negative bacteria *E. coli* has an outer membrane outside its peptidoglycan layer, that is not found in gram positive bacterial strains, which act as selective permeability barrier [24]. This study results was consitenet with most of the previous studies showed higher gram –negative bacterial strain resistance against such nanomaterials over gram positive bacterial strains [9], [22], [24], [32], [45].

Overall, the experimental results suggest that cobalt oxide nanoparticles could be developed as antibacterial agents against wide range of microorganisms to control and prevent the spreading and persistence of bacterial infections and the fine scale differences in size can alter their

related antimicrobial activity. Moreover, the clinical isolates were more resistant than the reference strains that gave direct clues for the great need for careful investigation and future trends for evaluating the applicability of the usage of the NPs for medical and clinical usage.

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Chapter Six

Outlook

As it well known that nanoparticles behavior is highly governed by the size and compositions of the particles, it is important to more precisely control the size of prepared nanoparticles of interest in order to prepare them in wide range of sizes, and investigate their interactions with various microorganisms that are commonly found in environmental settings, in the light of changed size. Further more, different combinations of nanoparticles, surfactants and bacterial strains can also be studied. In addition to the effect of nanoparticles size on its behavior as antibacterial agent, other factors can be studied such as the shape of nanoparticles, their stability and concentration that is added to the growth medium, and explore their potential for novel applications.

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كلية الدراسات العليا

دراسة تأثير الأحجام المختلفة من مركّبات أكسيد الكوبالت النانوية كمضادات بكتيرية

إعداد

هبة عوض عزّات مالول

إشراف

د. أمجد عزالدين حسين

د. محمد سليمان

قدمت هذه الأطروحة إستكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس – فلسطين

2015

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الملخّص

تعدّ مقاومة البكتيريا لكثير من المضادات الحيوية المستخدمة أحد أبرز المشاكل الصحية حديثة الظهور، أستخدم العلماء المواد النانوية، والتي تتراوح أحجامها ما بين 1-100 nm، لتكون بديلا للمضادات الحيوية في القضاء على البكتيريا. ولقد ثبتت فعالية عدة جزيئات منها أكسيدات المعادن كمضادات للبكتيريا مثل أكسيد الكوبالت النانوية والتي لم يتم العمل عليها بشكل مكثّف رغم إمكانية استخدامها كمضادات للبكتيريا.

في هذه الدراسة، تم تحضير أكسيد الكوبالت النانوية باستخدام طريقة مبسّطة لاختزال الملح؛ حيث أنّ التغيرات في ظروف التحضير أدّت إلى إنتاج أكسيد الكوبالت النانوية في أشكال وأحجام مختلفة. وكما هو معروف جيدا أن سلوك الجسيمات النانوية محكوم بحجم وطبيعة مكوّنات تلك المواد. تمّ تحضير ثلاث عينات من الجسيمات النانوية بمتوسط أحجام تتراوح ما بين (25.25 nm, 21.61 nm, 20.19 nm)، وتم تحضير ومعالجة العينات بمعالجات سطحية مثل بروميد الأمونيوم رباعي الأوكتيل (TOAB) والبوفيدون (PVP).

تم فحص الآثار التثبيطية لعينات أكسيد الكوبالت النانوية ذات أحجام مختلفة كمضادات البكتيريا وقاتلة لبكتيريا المكورات العنقودية الذهبية إيجابية الجرام والإشريكية القولونية سالبة الجرام في السلالات البكتيريا المرجعية والعزلات البكتيرية السريرية على حد سواء.

أظهرت النتائج التجريبية الشاملة أن جزيئات أكسيد الكوبالت النانوية لديها تأثيرات عالية كمضادات بكتيرية ضد الميكروبات المفحوصة، ومن الممكن تطويرها كمضادات للبكتيريا. كما

أنّ اثارها التثبيطية قد تباينت اعتمادا على الاختلافات في نوع من الكائنات الحية الدقيقة المختبرة؛ حيث أنّ آثارها التثبيطية كانت ضد بكتيريا المكورات العنقودية الذهبية أعلى من الإشريكية القولونية. وقد لوحظ وجود اختلاف جوهري في علاقة حجم الجزيئات مع نشاطها المضاد للبكتيريا؛ حيث أنّ وجود فروقات طفيفة في حجم جزيئات أكسيد الكوبالت النانوية بإمكانها تغيير نشاطها المضاد للبكتيريا المتعلق بها.