Antibacterial activity and physical features of some nano metals and their oxides

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Abstract
In this paper, nanomaterials and their oxides have been prepared using a simple chemical mixing method for use as antibacterial agents (Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae). The synthesized nanoparticles were characterized using different techniques like FESEM, optical properties and XRD. The results of the composition showed that the nanomaterials had diameters ranging from (50-70) nm.

Introduction
As the field of nanomedicine emerges, there is a deficiency of research surrounding the topic of nanoparticle (NP) toxicity, particularly concerned with mechanisms of action. NPs have increasingly been used in industry over the past few decades with usages varying from food additives [1] to drug administration [2]. The continuous emergence of bacterial resistance has challenged the research community to develop novel antibiotic agents. Among the most promising of these novel antibiotic agents are metal NPs, which have shown strong antibacterial activity in an overwhelming number of studies. Generally, antibiotic-resistant bacteria appear in a relatively short period of time even when new antibiotics are released into the market. However, it is hypothesized that NPs with antibacterial activities have the potential to reduce or eliminate the evolution of more resistant bacteria because NPs target multiple biomolecules at once avoiding, the development of resistant strains. Many studies have found that Gram-positive bacteria are more resistant to NP mechanisms of action [3-7]. It is hypothesized that the differing cell walls are the reason this phenomenon exists. In the case of Gram-negative bacteria, such as Escherichia coli, bacterial cells are covered by a layer of lipopolysaccharides (1–3 µm thick) and peptidoglycans (~ 8 nm thick). This arrangement may facilitate the entrance of released ions from NPs into the cell. On the other hand, Gram-positive bacteria such as Staphylococcus aureus possess a peptidoglycan layer much thicker than Gram-negative bacteria, spanning over 80 nm with covalently attached teichoic and teichuronic acids. The cell wall destruction that occurs from physical interaction between NPs and the cell wall is more detrimental for Gram-negative bacteria as they lack the thick peptidoglycan layer found in Gram-positive bacteria that could possibly act as a protective layer. Another potential reason for Gram-negative susceptibility to NPs is that
Gram-negative bacteria are coated with lipopolysaccharide molecules, which carry a negative charge. These negatively charged molecules have a higher affinity for the positive ions that most of the NPs release, leading to a buildup and increased uptake of ions, which then cause intracellular damage. Both Gram-positive and -negative bacteria have a negatively charged cell wall, a characteristic that is hypothesized to influence the interactions between the cell walls of the bacteria and NPs or ions released from them. Studies performed in Gram-negative bacteria such as Salmonella typhimurium showed that the cell wall is populated with a mosaic of anionic surfaces domains rather than a continuous layer [8]. Thus, a potential binding of a high number of NPs on these negative anionic domains may increment the focal toxicity because of the relatively high NP concentrations in these areas. Moreover, combined studies of electrophoretic mobility and mathematical calculations determined that E. coli is more negatively charged and rigid than S. aureus [9].

**Method Section**

Magnesium nitrate, Calcium nitrate, Silver nitrate and Iron nitrate was resaved from these minerals nanoparticles were prepared by simple chemical method using citric aside as solvent with distilled water is not ionic, the solution was prepared by added (2gm) from magnesium nitrate to (50ml) from citric aside and (50ml) from distilled water, Then the solution was kept under constant stirring using magnetic stirrer for (constant string 2hours) for complete dissolution of contents at room temperature.

**Cultivation method for Antibacterial activity**

Antibacterial activity was done by the disc diffusion method using the hung of bacteria spread on nutrient agar. Dip the swab into the broth culture of the organism. Softly squeeze the swab against the tube inside to remove spare fluid. Use the swab to streak agar plate or a nutrient agar plate for a lawn of growth. This is best complete by streaking the plate in one direction, then streaking at right angles to the first streaking, and lastly streaking diagonally. We end by using the swab to streak the outside diameter of the agar. The inoculated plates were incubated at suitable temperature for 24 hours. Antibiotic discs can be placed on the surface of the agar using a dispenser that dispenses multiple discs at the correct distance apart, or by obtaining single discs and placing them on the surface of the agar using flame disinfected forceps. The antibacterial activity was estimated by measuring the zone of inhibition against the test organisms. Zone of inhibition is the area in which the bacterial growth is stopped due to bacteriostatic effect of the compound and it measures the inhibitory effect of compound towards a particular microorganism. Lastly we measure (mm) diameters of zones of inhibition of the control strain and test with a ruler, thickness [9].

**Results and Discussions**

The particle size and morphology of synthesized nanoparticles were diagnosed using the optical microscope. (Fe, Mg, Ag and Ca) nanoparticles were characterized in fig. (1), observed particles seemed to be small spherical agglomeration.
Figure (2) showed X-ray diffraction for metal oxides nanoparticles were prepared using chemical method. This technique gives an indication about the grain size, strain and dislocation density of the prepared nanoparticles.

Fig (1): Optical microscope for metal oxide nanoparticle prepared using chemical method

Figure (2) showed X-ray diffraction for metal oxides nanoparticles were prepared using chemical method. This technique gives an indication about the grain size, strain and dislocation density of the prepared nanoparticles.
Fig (2): Shows the XRD pattern of the nanoparticle prepared by simple chemical method.
These studies have confirmed the stability of nanoparticles. Shows sharp peak at (3699.47 cm$^{-1}$) is attributed to the OH ant symmetric stretching vibration in Mg(OH)$_2$. The peaks at (478.35 cm$^{-1}$) are assigned to the Mg–O stretching vibration. The absorption peak at (1415.75 cm$^{-1}$) corresponds to the Mg–OH stretching vibration. The other absorption peak at (1651.75 cm$^{-1}$) Fig. S3B represents the FT-IR spectrum of CaO, and it displayed wide and intense bands at 500 cm$^{-1}$, 1482 cm$^{-1}$ and week band at 877 cm$^{-1}$ corresponding to Ca-O stretching, which is in consistence with the literatures11-13. The peak at 3657 cm$^{-1}$ is attributed to the absorption of -OH groups present on the surface of CaO$^{-1}$) and (3437.15 cm$^{-1}$) due to the bending and the stretching vibration of water, respectively. The peak at (435 cm$^{-1}$) is assigned to the Fe–O stretching vibration. The absorption peak at (1504.48 cm$^{-1}$) corresponds to the Fe–OH stretching vibration. The other absorption peaks at (1654.92 cm$^{-1}$) and (3483.44 cm$^{-1}$) due to the bending and the stretching vibration of water, respectively. To characterize the synthesized nanoparticle and also to compare the results with the commercial powders. The FTIR result of the raw eggshell showed broadband cantering at 1415.52 cm$^{-1}$ which is a characteristic of C–O bond showing a bond between the oxygen atom of carbonate and calcium atom [30]. In addition, there were two sharp bands at 711.62 and 875.54 cm$^{-1}$ showing C–O bond [30]. The peaks of raw eggshell were in correspondence with commercial CaCO$_3$ except for the broadband at 2360.48 cm$^{-1}$ which represents the N–H bond caused by the amines and amides present in the protein fibre of the eggshell membrane [31]. On the other hand, CaO nanoparticles showed peaks at 1444.42 cm$^{-1}$, 1064.51 cm$^{-1}$, and 863.95 cm$^{-1}$ which were ascribed to C–O bond indicating the carbonation of calcium oxide nanoparticles [18,24]. The absorption peak at 3639.02 cm$^{-1}$ has also resulted due to the O–H bond from water molecules on the surface of the nanoparticle [18,24]. The tiny peak at 2343.09 cm$^{-1}$ might be due to atmospheric CO$_2$ [32]. This peak has also been seen in commercial CaO and Ca(OH)$_2$. 

![Image A](image-url)
Fig (3): Shows the FTIR of the nanoparticle prepared by simple chemical method.
Antibacterial activity of metal oxide NPs against E.coli and S.aureus were also studied by measuring the diameter of inhibition zone (DIZ) using well disc diffusion method and the results are shown in (Fig. (4)) with the presence of metal oxide NPs, there was a clear formation of a inhibition zone, as no significant bacterial growth area around the well present. The diameter of inhibition zone for metal oxide NPs against E.coli and S.aureus were found to be 20± 4 mm, 16 ± 4 as we show in table (1), respectively. The antibacterial activity of metal oxide NPs against E.coli and S.aureus often the same, which is well compatible with previous searcher [13].
Bacteria type | Fe | Mg | Ag | Ca
---|---|---|---|---
Staphylococcus aureus | 14 | 16 | 25 | 20
Staphylococcus epidermidis | 22 | 23 | 26 | 22
Escherichia Coli. | 18 | 21 | 28 | 16
Klebsiella Sp. | 15 | 20 | 24 | 14

**Conclusion**

The present paper is comparative and comprehensive about the activity of NP materials. In order to explain the interaction of bacterial cells and nanoparticles, TEM, XRD, FTIR and the bacteria activity was performed, the results show death of bacterial cells after interaction with the nano-matrials (Fe, Mg, Ca, Ag).

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