Evaluation of the Efficiency of Alumina Nanoparticles Prepared by Plasma Method against Some Pathogens of Skin Infections

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Abstract

This study included isolating and diagnosing the bacteria causing skin infections and using the plasma method to prepare alumina nanoparticles, testing their activity as an antimicrobial against gram-negative and gram-positive bacteria.

Twenty-five samples were collected from different skin infections, and these samples were diagnosed based on culture, microscopic characteristics, and biochemical tests. The positive growth result was 86% distributed among Staphylococcus spp., K. pneumoniae, Proteus mirabilis, and Acinetobacter baumannii. The properties of the prepared nanoparticles were studied using diagnostic devices represented by UV-visible spectroscopy, X-ray diffraction, and field emission scanning electron microscopy. The results of these examinations indicate the formation of pure alumina nanoparticles in a spherical shape with an average size ranging (20-55) nm. Where the peak of the absorption of alumina nanoparticles appeared at (250, 254). The activity of alumina nanoparticles was tested against gram-negative and gram-positive bacteria, it proved its high effectiveness as an anti-bacterial by measuring the diameters of the inhibition zones. Its effectiveness against gram-negative bacteria was greater than Gram-positive bacteria. Also, found alumina nanoparticles have antimicrobial activity greater than Gentamicin (GM).

Keywords: Alumina nanoparticles, skin, Plasma method, nanoparticles

1. Introduction

Skin is one of the largest and most various organs of the body representing about 15% of the total body weight of an adult. It is a complex barrier that performs many vital functions as it acts as a protective interface between the internal part of the human body and the external environment against chemical, physical, and pathogens, as well as preventing excessive water loss from the body and its role in thermoregulation and support of immune functions, and protection from ultraviolet rays by forming Melanin. Andersson et al. [1] Skin sites can be classified according to their physiological characteristics into oily, wet, or dry skin [2]. Anatomically, the skin consists of three layers: the keratinous layer, which consists of dead keratinized epithelial cells, the avascular epidermis, which consists mainly of keratinous (living) cells, and the dermis, rich in fibroblasts composed of collagen and rubbery fibers that provide strength and elasticity to the skin, and these layers differ greatly in their anatomy and function [3].

The indiscriminate use of antibiotics has led to the emergence of resistant microorganisms and the problem of bacterial resistance has become more serious, where antibiotics have begun to lose their effectiveness against microbes and thus constitute a threat to modern medicine [4]. Antibiotic resistance occurs when a microorganism is able to grow or survive in the presence of a concentration of an antibiotic that is usually sufficient to inhibit or kill organisms of the same species [5]. Bacteria use several mechanisms to make antimicrobials inactive such as enzymatic hydrolysis of antibiotics, functional group transfer, and oxidation and reduction processes [6].

The science that studies the possibility of changing materials and reducing their sizes to the nanoscale [7] to produce new materials that differ in their physical, chemical, and biological properties as a result of changing the size and shape of materials and the behavior of atoms and molecules. The synthesis conditions of nanoparticles directly affect their size, shape, diffusion, and distribution [8, 9]. Nanoparticles with a size of (1-100 nm) represent a new direction that is increasingly being developed and of interest for adoption in medical research [10, 11]. It has great importance in various fields of life due to its wide applications and distinctive properties that allow it to easily enter the plasma membrane through the withdrawal mechanisms that are used in the field of biomedicine. Nanomedicine aims to integrate modern nanotechnology with classical molecular tools and biotechnology to develop innovative therapy for disease treatment and tissue repair, rapid and highly sensitive diagnostic tools such as biosensors, surgical aids, and implantable biomaterials [12], and also used as antimicrobial agents. as well as, being useful for the separation and purification of cells and biological molecules, wound healing agents, and topical ointments for infection prevention [13]. Nanoparticle drug delivery is an important step for supervising on human biological systems using engineered nanostructures and nanodevices [14]. Over the past few decades, there has been great interest in developing biodegradable nanoparticles as effective drug delivery devices. Various polymers have been used in drug delivery research as they can effectively deliver drugs to the target site thus increasing therapeutic benefit with reducing side effects.

Different methods were used to prepare Nanomaterials which are chemical, physical, and biosynthesis methods. Lately, the technology of plasma is acquiring nanomaterials great attention as a prominent “eco-friendly” synthesis method that is considered a characteristic property when

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compared to gas, liquid, and solid phase synthesis approaches. On the other hand, in the applications of biomedical, the combination of plasma and nanomaterials are demonstrating many synergistic effects and efficiency in the treatment

2. Procedure

Isolation and Identification

Twenty-five samples were collected from patients with different skin infections of different ages and both sexes. The samples included swabs of acne and various skin infections. Samples were collected using sterile cotton swabs that were placed on a sterile transport swab for sampling and transportation to the laboratory. Then the diagnosis was made based on the culture, microscopic characteristics, and biochemical tests.

Preparation of alumina nanoparticles by aplasma method

-Preparation of the solution

Aluminum nitrate was used (a partial weight of 375.13 g/mol and a 99% purity) which was manufactured by the German company SKMA. A volume of 20 mL of 2 mM was prepared and the following equation (1) was used to calculate the required weight:

Concentration (mole) = (mass (g)/ (Molecular weight (g / mol) × volume (liter).

-Preparation of alumina nanoparticles

The argon gas tube is opened, and the 1 mm diameter metal tube is fixed vertically by the catcher, after the process of the aluminum nitrate solution preparation with the demanded volume and concentration, the prepared form is placed on the stand under the metal tube as mentioned in detail.

The form which is produced by the preparing process is located on the holder beneath the metal tube. The distance between the tube nozzle and liquid surface becomes 1mm when the beaker gets close to the metal tube.

The gas quantity inter inside the metal tube is organized by a flow meter which can be controlled from control of the speedometer and the gas tube. The voltage produced by the system gradually increases till the case of the plasma is generated between the surface of the fluid and the tube.

Antibacterial activity of nanoparticles test

The inhibitory activity of alumina nanoparticles prepared by the plasma method was tested using the diffusion method on Muller-Hinton agar medium based on Kirby-Bauer. Where 100 µl of bacterial suspension was taken and spread on agar Muller-Hinton and made holes in each dish and put 20 µl from a solution of alumina nanoparticles prepared at a concentration (2mM) in each hole and incubated for 24 hours at 37°C. After that, the diameters of the inhibition zones were measured around the holes.

Antibiotic test

The diffusion method was used to test the sensitivity and resistance of bacteria to antibiotics. Where the bacterial suspension was spread on aMuller-Hinton medium and incubated for 24 hours at 37°C, then the inhibition diameters were compared (CLIS, 2020).

3. Results and Discussion

Isolation and diagnosis

The results of the bacterial culture of samples isolated from different skin infections showed that 76% gave positive growth of bacterial culture, while 24% of them did not show any growth. The result of isolating bacterial strains was (68.4%) gram-negative and (31.6%) gram-positive, which is agree with [15]. The percentages of bacterial isolates were 31.6%, 26.3%, 21%, 15.8 and 5.3%) belong to Staphylococcus spp., K. *pneumoniae*, *Pseudomonas aeruginosa, Proteus mirabilis*, *Acinetobacter baumannii*, respectively and these results are in agreement with [16].

Aljanaby et al. [17] found that S. aureus was the most common isolate of bacteria, and these commensal bacteria may have a role as opportunistic pathogens when using immunosuppressive agents that reduce epidermal tissue defenses. Moreover, it is a common contaminant of the skin in addition to its ability to resist the antibiotics commonly used medically [18].

Diagnosis of Nanoparticles

UV-Vis Analysis of Alumina nanoparticles (Al2O3 NPs)

The results of this study showed the production of alumina nanoparticles by measuring the absorption spectrum of ultraviolet-visible rays within the range (200-800) nm. The absorption peak appeared at the wavelength (250 nm) at a concentration of 2mM, and this is one of the characteristic peaks of alumina nanoparticles, as shown in Figure (1) and this is consistent with [19].

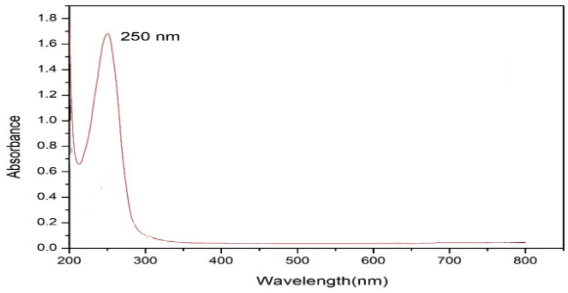
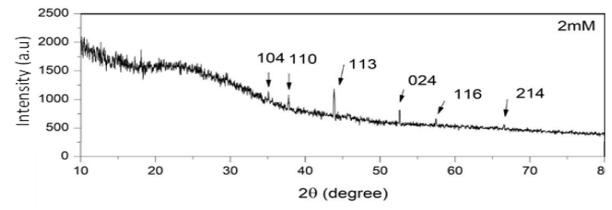


Figure (1): UV-visible absorption spectrum of alumina nanoparticles

X-ray diffraction analysis of the alumina nanoparticles (Al2O3 NPS)

Figure 2 shows the X-ray diffraction of alumina nanoparticles prepared using the plasma method. from the figure, we notice that the diffraction peaks were (104), (110), (113), (024), (116), and (214) at angles 35.3, 37.2, 43.8, 53.01, 57, 7, and 67.25, respectively, and these angles were identical to alumina nanoparticles when compared with JCPDS cards for both concentrations. The nature of the crystal structure of alumina nanoparticles is face-centered cubic (FCC) [20].



Concentration	D·(nm)	FWHM·(β)	2Theta
2·mM	38	0.2332	43.80

Figure 2: X-ray diffraction spectrum of alumina nanoparticles

Field emission scanning electron microscopy analysis

The morphology of the prepared alumina nanoparticles by plasma method was detected using field emission scanning electron microscopy. Figure (3) shows that alumina nanoparticles appear spherical with a diameter ranging between (15-50) nm, and we note the presence of clear masses which can be attributed to the precipitation method.

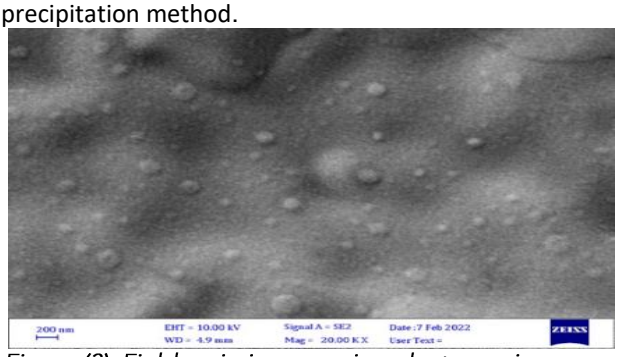


Figure (3): Field emission scanning electron microscope image of alumina nanoparticles.

Anti-bacterial Activity of Alumina Nanoparticles

Alumina nanoparticles showed high antimicrobial activity against Gram-negative and Gram-positive bacteria, and the inhibition diameters varied according to the type of bacteria, as shown in Table (2), and this is consistent with Sharma et al. [21], Ansari et al. [22]. Adsorption or diffusion of nanoparticles on the cell surface is the main penetration mechanism and adsorption can be achieved through the association of nanoparticles with negatively charged functional groups of proteins, which leads to protein destruction and cell death [23].

It was also observed that the antimicrobial activity of alumina nanoparticles against gram-negative bacteria is greater than that of gram-positive bacteria, which may be due to the difference in the structure of the cell wall as the gram-positive bacteria contain a peptidoglycan layer, which provides mechanical stability and is thicker and stiffer than gram-negative which usually contain a thin layer of peptidoglycan [24, 25].

Alumina nanoparticles showed high antimicrobial activity against all isolated bacteria compared with gentamicin antibiotic, where it found a high effect, especially on *Acinetobacter baumannii*, which showed high resistance to this antibiotic.

Gentamicin belongs to the group of Aminoglycosides. Bacteria resist this antibiotic through various mechanisms. The first mechanism includes inhibition of the antibiotic through an enzyme that changes the structure of the antibiotic by transferring a group functional to the antibiotic such as acyl, ribosyl, phosphoryl or Thiol, by changing the structure of the antibiotic by the enzyme nucleotide transferase. The second is reduce the permeability of the bacteria cell wall to the antibiotic and thus prevent it from entering the bacterial cell [26].

Table (1): Inhibition zone of alumina nanoparticles and Gentamicin (mm).

Bacterial Isolates	Plasma method	Gentamicin (GM)	
Klebsiella pneumoniae	24	11	**R**
Pseudomonas aeruginosa	21	17	**S**
Proteus mirabilis	19	8	**R**
Acinetobacter baumannii	20	0	**R**
Staphylococcus epidermidis	18	11	**R**
Staphylococcus schleiferi	24	20	**S**
Staphylococcus aureus	22	10	**R**

4. Conclusions

Alumina nanoparticles were successfully synthesized using the plasma method. Alumina nanoparticles showed high inhibitory activity against bacteria isolated from skin infections, and their activity against gram-negative bacteria was greater than gram-positive bacteria. Also, it was found that alumina nanoparticles have antimicrobial activity greater than the antibiotic Gentamicin (GM).

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