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Synthesis of Blue Copper Sulfate Nanoparticles by Cold Plasma and Application to Bacteria

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Abstract: This research aims to prepare copper sulfate nanoparticles (CuSO4 NPs) experimentally by cold plasma method at a fixed concentration. Some tests and optical techniques have been performed for, ultraviolet-visible spectroscopy (UV-VIS), field emission scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy). The effect of the prepared nanoparticles on two types of bacteria: Escherichia coli and Staphylococcus aureus was studied. The results showed that nanoparticles prepared at a fixed concentration (2) mM at different exposure times (3 and 4) min had a polycrystalline cubic structure for all samples. In addition, the average crystal size changes directly with the change in exposure time to plasma at a fixed concentration (2). mM. On the other hand, the study showed that Gram-negative bacteria (Escherichia coli) were more resistant to the prepared copper sulfate nanoparticles. In contrast, Gram-positive bacteria (Staphylococcus aureus) were more affected by the prepared nanomaterial.

Introduction

Metal nanoparticles attract intense attention due to their outstanding properties and potential applications in the fields of catalysis, magnetism, electronics, biological activity, etc [1]. Most metal nanoparticles show modified chemical and physical properties, which depend on the nanoparticle size [2]. In recent years, these nanoparticles have received great attention in the field of medicine due to their unique properties and potential applications, from wound healing to cancer treatment, where copper nanoparticles have shown tremendous promise in revolutionizing medical treatments [3].

Copper nanoparticles possess unique properties that make them highly desirable for medical applications. Their small size gives them a large surface area to volume ratio, which enhances their reactivity and enables better interactions with biological systems [4]. Furthermore, copper nanoparticles exhibit excellent antimicrobial properties, making them effective against a wide range of pathogens, including bacteria, viruses and fungi. This property is particularly crucial in wound healing applications, where infection prevention is critical [5].

Escherichia coli (E. coli) is a bacterium commonly found in the intestines of humans and warm-blooded animals. Most strains of E. coli are harmless [6]. However, some strains, such as Shiga toxin-producing Escherichia coli, can cause severe foodborne illness[7]. This bacterium is transmitted to humans primarily through the consumption of contaminated food, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts[8].

Staphylococcus aureus is a type of Gram-positive bacteria that usually lives on human skin, in the nasal cavity, or in the respiratory tract. This type of bacteria is characterized by several characteristics: coagulase positivity, DNA degradation, and consumption of mannitol-type sugar. Although they do not always cause disease, one of the diseases caused by this type of germ is toxic shock syndrome, which leads to severe illness accompanied by fever, a widespread red rash, and other organs in the body being affected. Recently, new types of *Staphylococcus aureus* that are resistant to antibiotics have appeared, the most important of which is methicillin-resistant *Staphylococcus aureus[7]*.

Biomaterial

Is a nonviable material used in a medical device, intended to interact with biological systems. Biomaterial is Any material of natural or synthetic origin that intented to interface with tissue, blood or biological fluids, and intended for use in prosthetic, diagnostic, therapeutic or storage application without adversely affecting the living organism and its components [9,10]. There are many type of biomaterial, Polymeric biomaterials, Bioceramics, Metallic biomaterials, Biocomposite and Biologically based (derived) biomaterials.[11].

Exposure to body fluids usually implies that the biomaterial is placed within the interior of the body, and this places several strict restrictions on materials that can be used as a biomaterial, it should be nontoxic and non-carcinogenic.

There is an increasing demand for coatings with tailored and enhanced properties such as high hardness, wear and corrosion resistance, low friction, and specific optical or electrical properties [12]. Plasma Sputter deposition is a widely used thin film deposition technique, especially to obtain stoichiometric thin films (i.e. without changing the composition of the original material) from target material. Target material may be some alloy, ceramic or compound. Sputtering is also effective in producing nonporous compact films [13].

Metals and metallic alloy are susceptible to degradation by corrosion. The corrosion of metallic implant gives adverse effects to the surrounding tissues and to the implant itself. It produces chemical substances that are harmful to human organs and deteriorate the mechanical properties of the implant. Therefore corrosion resistance of a metallic implant is an important aspect of its biocompatibility [14].

Physical types of metallic biomaterials

The possession of metals and their alloys, strength, flexibility, and relatively easy re-formation into a high modulus, complex shape, production point, and vital materials made them suitable for carrying a large load without leading to permanent change in dimensions and large deformations [15]. Metals and their alloys are widely used as biomaterials because they are strong material, ductile, relatively easily formed into complex shape high modulus and yield point, make them suitable for bearing large load without leading to a large deformations and permanent dimensional change [16].

Bio-mineral materials continue to be widely used to manufacture surgical implants primarily for the same reason that they were initially chosen for these devices several decades ago. Possession of this class of fracture-resistant and high- strength material, assuming proper handling, provides in the long term reliable implant performance in major bearing situations. In addition to the ease of fabricating both complex and simple shapes using widely available and well- established manufacturing techniques (eg machining, casting, and forging), this has encouraged the use of metals in the fields of orthopedics and dentistry primarily, the two areas in which hardware is highly The most common loading although similar reasons have led to its use for cardiovascular devices (eg, artificial heart valves, blood conduits and other components of cardiac assist devices, and vascular stents), and neurovascular implants (aneurysm clips). In addition, it is preferable to use them in neuromuscular stimulation devices, and cardiac pacemakers are the most prominent examples of this. These preferred properties (formability, good resistance to fracture, electrical conductivity) are related to the interatomic metallic bonding that characterizes this class of materials [17, 18]..

Literature review

In (2015) Giovanni, M.,et al [19]. Detection of individual nanoparticles is of high importance to basic science as well as to applied one, i.e., for remediation efforts. Electrochemistry was shown previously to play an important role in detection, counting and measuring individual nanoparticles. Here we show that individual molybdenum nanoparticles can be detected and counted by chronoamperometry upon their impact on the carbon electrode surface. The size determination of individual nanoparticles by electrochemical measurement is consistent with the size determination by scanning electron microscopy.

In (2022) Hajimohammadi, S., et al [20]. Copper sulfate is not just known as a toxic substance it has a long history of use in the debridement of wounds because of its antiseptic properties and as an emetic agent in intoxication. It is still contained in intrauterine devices. Also, in many domestic and industrial products, copper is used. Poisoning with this substance is rare but can be severe and fatal, with up to 23% mortality rates. However, the rates are on the decline, and it is essential that physicians are aware of its lethal complications and management strategies. The most abundant route of poisoning is oral, but intravenous, cutaneous via wounds, and even intra-uterine, for abortion, routes have been reported. In our country, no cases of copper sulfate have been reported so far, and our patient’s symptoms were limited to gastrointestinal problem.

In (2021) Alengebawy, A., et al [21]. Acute oral toxicity (LD50) of copper sulfate is about 300 mg/kg in rats. The main complications of copper sulfate ingestion include intravascular hemolysis, methemoglobinemia, acute kidney injury, and rhabdomyolysis which is explained in detail below. Severe gastrointestinal effects may occur with acute overdosage In extreme or long-term overdosage, symptoms may be similar to those of Wilson’s disease, a disease in which the liver does not filter copper adequately and copper accumulates in the liver, brain, eyes, and other organs.

Dalefield, R. (2017). *Veterinary Toxicology for Australia and New Zealand*. Elsevier.

In (2017) Dalefield, R.[22] Gradually, high copper levels may cause life- threatening organ damage. Ingestion of more than 15 mg of copper has been reported to be toxic to humans . In a survey of human clinical case studies, 5.3 mg/day was the lowest oral dose at which local gastrointestinal irritation was seen. Ingestion of gram quantities of copper sulfate resulted in death by suicide, whereas less severe effects were reported from estimated copper doses of 40 to 50 mg from ingestion of carbonated beverages in contact with copper containers. Limited data are available on the chronic toxicity of copper. The hazard from dietary intakes of up to 5 mg/day appears to be low .

Aims of the work:

1. The current study aims to know the physical and chemical properties of copper sulphate nanoparticles.
2. Knowing the effect of copper sulphate nanoparticles on microscopic organisms, specifically *Escherichia coli* and *Staphylococcus aureus*

Concept Plasma

Plasma is a (partially) ionized gas that contain ingredients ions electrons and photons as well as radicals and molecules that are stimulated. A glow discharge is a kind of plasma that is defined as a partly ionized gas with almost equal positive and negative charge concentrations [23,24]. Low-pressure plasma technology has a wide range of applications in the microelectronics sector and the processing of materials surfaces. The field of glow discharge plasmas has seen a surge in attention in recent years.

Plasma, on the other hand, is the non-cellular fluid component of the blood, as defined in medicine and biology. The characteristics of ionic liquids in medicine and biology according to Irving Langmuir are similar to plasma in physical sciences. These charged particles have no plasma properties. Plasma particles in general cooperate and their algebraic total is zero. A few years ago plasma was employed in the medical industry for sterilizing, dental therapy, coagulation, and even plastic surgery [25].

Etching and semiconductor wafer processing, polymerization in optical fiber fabrication, treatment of fibrous materials, surface modification by deposition of diamond films and corrosion resistance coatings, plasma immersed ion implantation for plasma intruding flat plate displays high pressure arcs and jets for ceramic coating and plasma paralysis applications, and so on are some of these applications [26].

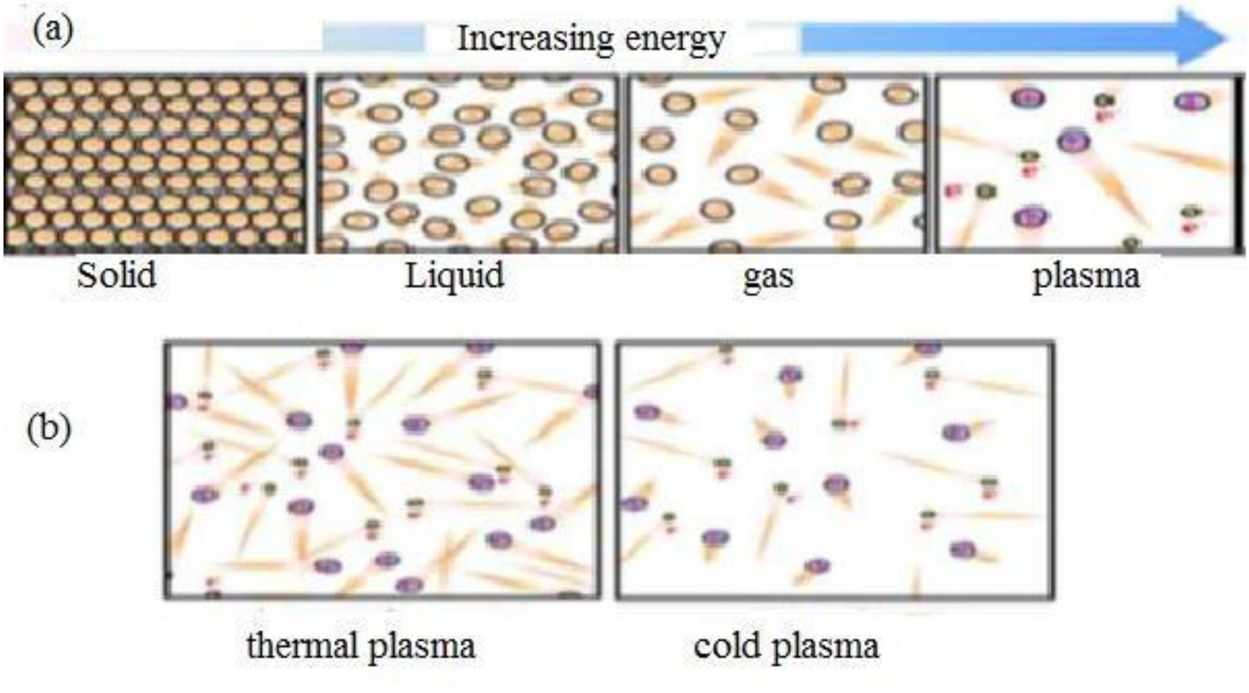


Figure (2-1): The physical portrayal of plasmas Thermal plasma Non-thermal plasma

a) Schematic illustration of the four fundamental states of matter. The triangular tails represent the thermal motion strength of particles.

b) Schematic illustration of the thermal plasma and the non-thermal plasma. Brown balls, violet balls, and iridescent balls represent the neutral atoms, the positively charged ions and electrons respectively [27].

Plasma can be completely ionized, as in the sun and the ozone layer or partially ionized, as in fluorescent lights that contain countless particles. Plasma temperature is determined by the thermal movements of electrons, atoms and ions. When molecule density is high due to massive collisions between electrons and substantial particles all particles are in a warm equilibrium because of the basic warm plasma [28]. This plasma has a high temperature, reaching thousands of degrees. Under barometrical weight conditions, this plasma is normally used [29]. However, if the plasma release is rapid another class of plasma emerges in which the electrons and overwhelming particles are unequal or unbalanced for this situation, known as Cold Atmospheric Plasma (CAP). In this type of plasma the temperature of small particles is much lower than that of electrons. This unpredictably unpredictable science leads to interactions between the CAP and natural frameworks such as living tissues and cells [30].

Collective effects

The molecule moves without incident until it collides with another molecule; this stage occurs when external forces are applied specifically to a molecule. The collisions of molecules with each other control and constrain the movement of molecules. Furthermore, currents generated by the movement of charges produce magnetic fields, which affect the movement of other charged particles located at a great distance while interacting with several close charged particles, resulting in a collective interaction [31]. Generally, in the rest frame of plasma, the electrical field is not strong, and the shielding between external and internal electrical fields are showing the plasma conductivity which is high from where the flux of plasma current that is enough to make the internal electrical field shorter, wherefore can consider the shielding as a dielectric phenomenon, and Debye length represents the scale of length for this shielding [32].

Debye length is a plasma description parameter that shows the measurement of distance for the electric field effecting a charged particle (at a nonzero potential) and which the surrounding particles are in felt with [48]. It's appropriate to identify the term of "Debye sphere" that means a sphere in inner of plasma which have a radius equal to λD through the charged particles, any originated electrostatic fields which are out-side a Debye sphere they effectively screened and it does significantly not contribute towards the existing of the electric fields which be at its center. Therefore every charge related to the plasma it will interact with the charges which are located in the inner Debye sphere collectively. The effects of Debye shielding regards the main significance to all kinds of plasma, even though it never happens in each medium that have charged particles.

Occurrences of plasma

Plasma can form in two ways: artificially or naturally. Artificial plasma is used in plasma televisions, lighting, semi-conductors, and synthetic fabric treatment, among other things. This form is primarily used for (i) the production of excited molecules such as excited hydrogen or singlet oxygen (ii) the production of electrons or charged ions, and (iii) the production of chemically active radicals or species such as nitric oxide ozone, molecular oxygen and etc [33].

Plasma generation

The plasma generation process by ionization in turn raises the ionization degree much greater than the value of thermal equilibrium, there are several methods for producing plasma, each of which is distinct from the others and is based on the photoionization process. While ionization occurs through the absorption of incident photons with energies greater than or equal to the energy of ionization absorption atom potential. The extra photon energy is converted into kinetic energy for the formed pair of ion and electron, and the ability of plasma generation improves with the advancement of laser techniques [34]. When for example a laser pulse with a short pulse (nanoseconds) is focused on a surface, plasma is produced by evaporating the material on the surface [35]. while in the case of gas discharge, through the ionized gas is applied an electric field, which in turn helps to produce high energies resulting from the process of accelerating free electrons that are sufficient to ionize another atom by the process of collisions. When the source of the ionization be switched off, then the ionization begins gradually decrease due to the process of recombination until it arrives at the value of equilibrium which compatible with the medium's temperature [36].

Properties of nanomaterials

The properties of mechanical electrical magnetic nanomaterials and the melting point are radically different from their counterparts in natural size, and this is due to the quantitative effect of nanomaterials and an increase in the surface-to- volume ratio, so the previous properties are greatly improved when the material is in the nanoscale, which has led to an increase in demand for Nanotechnology materials in industrial applications that rely on mechanical properties. The size and shape of nanoparticles have a large impact on nanomaterials. The size and shape of the nanoparticles also depend on the extent of the interaction with the stabilizers and the surrounding medium, as well as the style and method of preparation, it is very important to control, not only the size of the particles but also their shape and nature of their surface, the purity of the solvent substance and its cleanliness besides to the temperature of the solution, the concentration of the mineral salt, a factor Reduction and reaction time, all of these affect the size of the particles, and controlling the size and shape of nanoparticles remains an existing challenge [37]. At the nanoscale level, materials acquire new features and properties that have significant benefits in terms of applications, such as color change, electrical and magnetic properties. Thermal conductivity, insulation, melting point, light reflections, degree of hardness, strength, transparency… etc [38].

Biological properties

These materials can penetrate the hindrance and biological barriers that obstruct the arrival of medications and therapeutic drugs to the affected part (such as the membranes and the blood-brain barrier). Nanotechnology embraced Biotechnology which coincided with it, to form bionanotechnology which made exciting mutations in the innovation of advanced types of characterization devices employed to understand and analyze the structure of DNA for humans and viruses alike [39].

This naturally led to the knowledge of the behavior of diseases and viruses and the mechanics of their movement and transfers within the body and knowledge of the methods and tricks that they take to attack the components and molecules of living cells in organs of the body. The range of biological processes in cells of organ organisms at the level of parts of a single cell, the nanomaterials that are created are selective substances and suitable for interaction with those small biological molecules that make up the cell such as nucleic acids and proteins [39].

Among the most important characteristics of biological nanoparticles are improving biological compatibility, increasing the permeability and penetration ability of barriers that obstruct the arrival of medications and therapeutic drugs to the affected part such as membranes and the blood-brain barrier [40].

Structural Properties

The study of the crystal structure of elements is very important in order to know the nature of the regularity of atoms or crystals and calculating the size of the smallest cell in the crystal and knowing the locations of the atoms the crystals inside them, and this is done by studying the patterns of X-ray diffraction falling on the atoms of the elements [37].

Copper

Copper is a chemical element with the symbol Cu and atomic number 29. It belongs to the d-level elements and Is located at the top of the eleventh group elements in the periodic table[41]. Chemically, it is classified as a transition metal. Copper has a distinctive reddish-brown color. It is a soft, ductile and malleable metal. It is a good conductor of electricity and a good transmitter of heat as well. Copper is one of the few metals that can be found In nature in its free form, but It is also included in the composition of a number of minerals In the Earth's crust[42].

Natural abundance for Copper

Copper is classified in the giant stars. As a result, copper is up to 50 ppm, and comes at the age of fifth of its natural abundance[43]. Copper can sometimes find a free normal version of non-associated with other chemical elements. There is natural copper often in the Basalt lava, either in the form of cutting or positive cases, namely, named as crystalline. Some sources indicate that the weight of the largest mass of natural copper has reached 420 tons and was found in 1857 in Michigan[44].

However, copper is most likely found in nature associated with other elements in the form of minerals, especially within sulfide minerals such as chalcopyrite, bornite, digenite, covellite, and chalcosite; As well as within sulfo salt minerals such as tetrahydrite, tennantite, and energite; Among the arsenate minerals such as cornwallite and clinoclase; Among the carbonate minerals such as azurite and malachite; In addition to oxide minerals such as cuprite and tenorite; And halide minerals such as atacamite [45].

Physical properties of copper

Copper exists under standard conditions of pressure and temperature in the form of a metal with a blooming orange color. Upon prolonged exposure to air, it loses its luster and takes on a reddish-brown color, while its scratches have a blooming red color. Copper in general has a special and distinctive color, as the copper color is attributed to it; It is thus one of the few metallic elements with a color other than the gray or silvery color typical of most metals [44].

Copper is distinguished by its excellent conductivity and conductivity, as it ranks second after silver and before gold in terms of thermal and electrical conductivity among pure metals at room temperature [46]. This is because the resistance of electrons to transfer in metals at room temperature is mainly due to the scattering of electrons due to thermal vibrations of the crystal lattice, which is usually weak in soft metals. Copper is a good conductor of electricity, with an electrical conductivity value of 59.6 x 106 Siemens/meter.

Chemical Properties of Copper

Copper does not react with water, but it reacts slowly with oxygen in the air in an oxidation reaction, forming a brown to black layer on the surface of copper oxide, which, unlike rust in iron, protects the rest of the mass of the copper body from corrosion. Thus, this layer plays a velvet role[47]. A green layer of patina is formed on copper surfaces exposed to air for a long time, which is a substance that is chemically composed of basic copper carbonate, and is called copper rust. It is usually observed on the roofs of old buildings clad with copper, or statues made of copper and its alloys, as is the case with the Statue of Liberty. Copper loses its luster when it is exposed to some sulfur compounds, which react with them on the surface to form a number of different copper sulfide compounds [48]. Copper can exist in several oxidation states (namely 0, +1, +2, +3, and +4) in its chemical compounds; The most common are the oxidation states +1 and +2; The most stable in aqueous solutions is the +2 oxidation state. On the other hand, the +4 oxidation state is very rare[49].

Copper sulphate

Copper sulphate is a chemical that is easily accessible in many countries and is even sold without a prescription. It is commonly used in agriculture as a pesticide in the leather industry and to make homemade glue. Burning copper sulphate in homes and shops (as a good luck charm and for some religious activities) is a common practice among Buddhists and Hindus [50]. The light blue color of the aqueous form of copper sulfate crystals is tempting to children and is a frequent cause of unintentional poisoning. Wilson's disease is an autosomal recessive disorder characterized by excessive copper accumulation and caused by a variant in the gene that encodes the enzyme copper-ATPase[51]. Copper is found in the blood in two forms: bound to ceruloplasmin (85% to 95%), and the remainder is ―free,‖ loosely bound to albumin and other small molecules [52]. It can be used as an antifungal fungicide, as well as for the prevention and control of animal foot rot and the removal of pond algae [53]

The bacterial cell wall

There are two general categories of bacterial cell walls, first distinguished by Hans Christian Gram depending on their different retention of crystal-violet dye: Gram-negative or Gram-positive . Figure (2-2) shows the difference between the walls of the two bacteria strain type The structural distinction lies in arrangement of peptidoglycan that is the key part of membrane structure. Gram-negative bacteria wall shows a thin layer of peptidoglycan (about 2–3 nm) between the cytoplasmic membrane and the outer cell wall. E-coli is an example of gram negative bacteria. The outer membrane and peptidoglycan are connected to each other with lipoproteins. The wall of Gram-positive bacteria has a thick layer (about 20–50 nm) of peptidoglycan that is tied to teichoic acids that are unique to the Gram-positive bacteria cell wall. Staphylococcus aureus is an example of gram positive bacteria [54].

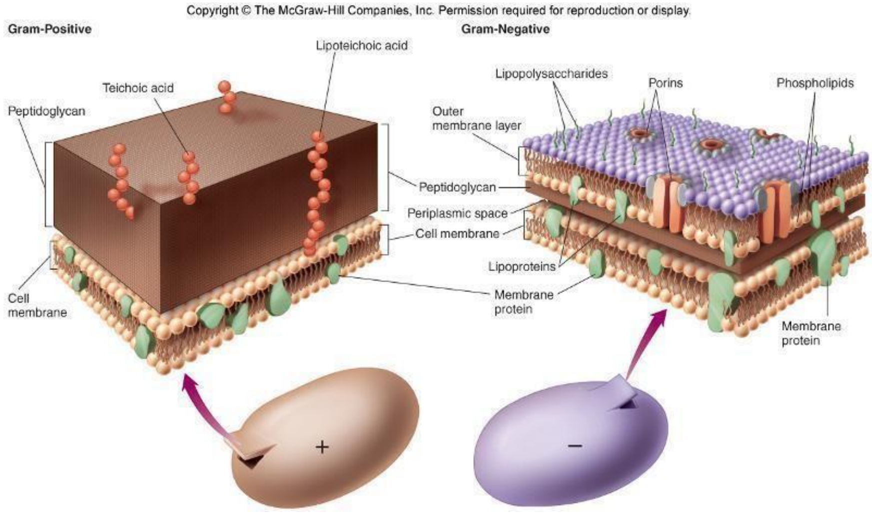


Figure (2-2): A Gram-positive and Gram-negative bacteria cell wall structure [120].

Introduction

This chapter includes the method and material used for synthesizing Copper sulphate nanoparticles (CuSO4) by atmospheric pressure plasma jet. Also it includes the instruments used for measurement and characterization of the properties of the resultant nanoparticles.

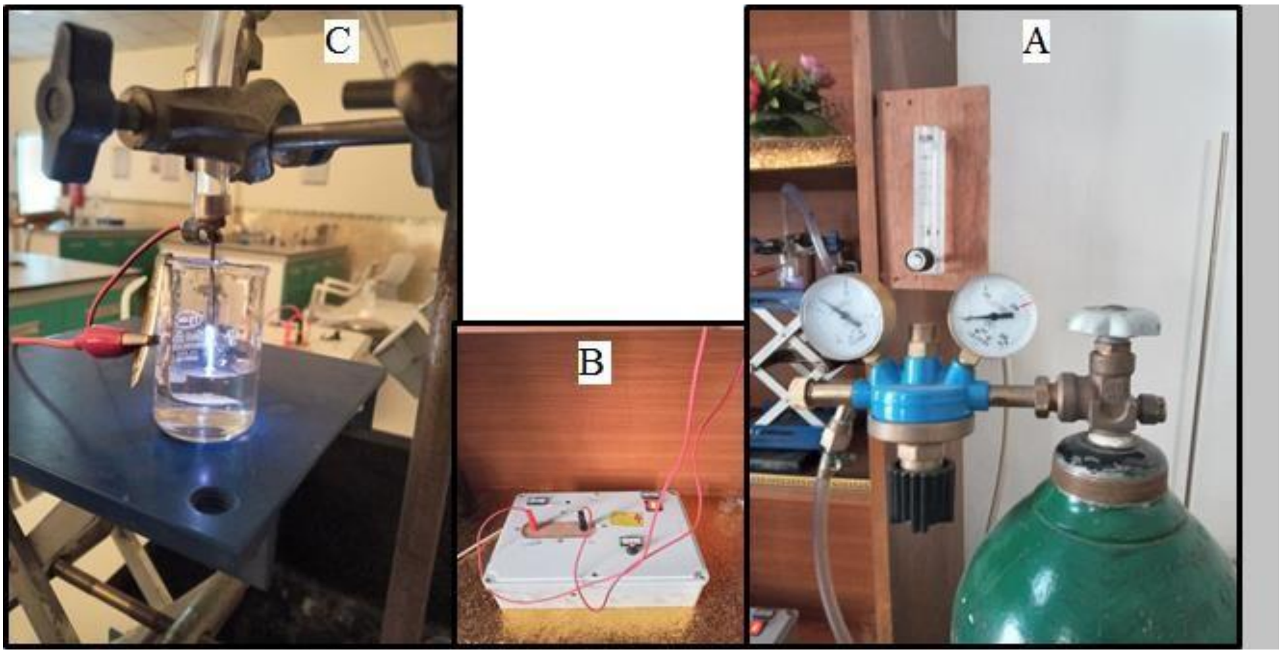


Figure (3-1) (present work): (a) argon gas. (b) Power supply. (c) Demonstrates the generation of plasma to produce nanomaterials

The system includes the following parts:

1. Capillary (with diameter 1mm) in which the gas flowed through it and connected to the negative terminal of the power supply, and placed at 1 cm above the liquid.
2. Stainless steel conductive length7 cm and width 5 mm strip ends with a 1 x 1 flat end that connects to the anode inside the aqueous solution.
3. DC Power supply with high voltage about 6 KV.
4. Argon gas bottle which is affordably available in the market, and contains a gas outlet containing a regulator to control the speed of exit gas.
5. Flow meter with a calibrator of (1-5) minutes/liter to control the Argon intake which connects to the hollow metal tube, we use a calibrator of 2.5 minutes/liter.
6. A glass vessel is used in this work as a cell. The stainless steel foil is placed in the glass vessel filled with 10 ml of solution. The depth of the stainless steel foil under the solution is about 1 cm.

Material Methods

3-2-1 Preparation of nanoparticles

To prepare the Copper sulphate nanoparticles ($CuSO_4$), we follow these steps: The argon gas tube is opened, the 1 mm diameter metal tube is fixed vertically by the catcher, after the process of the ($CuSO_4$) salts solution preparing with the demanded volume and concentration, the prepared form is placed on the stand under the metal tube as mentioned in detail as in figure (3-1).

The preparing is located on the holder beneath the metal tube. The distance between the tube nozzle and the liquid surface becomes 1cm when the beaker getting close to the metal tube. The gas quantity which inter inside of the metal tube is organized by flow meter which can be controlled from the control of speedometer and the gas tube. The voltage is produced by the system gradually increases till the case of the plasma is generated between the surface of the fluid and the tube [37].

Measurement Techniques

The morphology, structure, and optical properties of the synthesized nanoparticles for molybdenum nanoparticles are characterized by measurement instruments that are summarized as follows:

3-3-1 Ultraviolet-visible spectroscopy

After visual observation to note the change of color of the solution. The formation of nano-sized molybdenum particles in solution was investigated using a Chinese-made spectrometer (INOVIALAB, UV- visual-visual 1911 DB) as shown in figure (3-2). The samples containing molybdenum nanoparticles prepared by different methods were placed in a quartz cell with dimensions of 10 * 10 mm. The visible and ultraviolet spectra were recorded in the range of 200-700 nm. UV-Vis test was conducted at Anbar University - College of Science - Department of Physics.

Figure (3-2): The spectrorum (uv-vis) at the University of Anbar - College of Science - Department of Physics.

Introduction

This chapter includes the results and analysis of experimental measurements and technology used in producing Copper sulphate ($CuSO_4$) using cold plasma, at different concentrations (2 mM), with different times for plasma exposure to the solution (3 , 4) min. The structural and optical properties of the prepared nanoparticles were characterized. This chapter also includes the antibacterial effect of ($CuSO_4$) NPs on four type of bacteria (*staphylococcus and Escherichia coli*). Finally, includes the most important conclusions that were reached in the research, and future studies. Figure (4-1) shows the image of the ($CuSO_4$) NPs suspension in water prepared from Copper sulphate salts at different concentrations and different time. Equation used to calculate molarity

$$W = M \times m.w \times V_L \quad (4 - 1)$$

$$W = \frac{0.2}{1000} \times 249.68 \times \frac{100}{1000|} \qquad 0.00499 \text{ g}$$

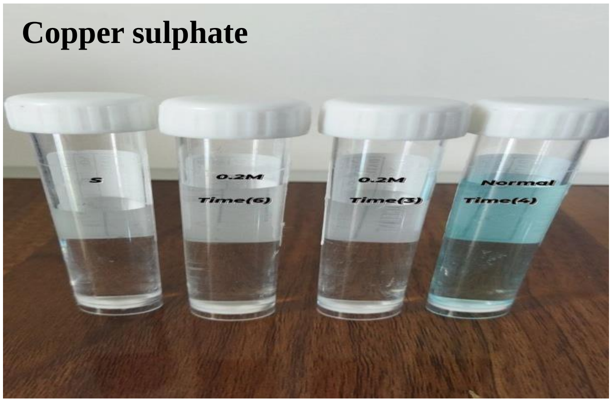


Figure (4-1): CuSO4NPs samples prepared from copper sulphate salts at fixed concentrations and different times

Characterization techniques

UV spectrometry of $CuSO_4$

The prepared Copper sulphate nanoparticles, an argon gas jet system, were diagnosed using UV-visible absorption spectroscopy. Figure (4-2) displays the UV-visible spectra of Copper sulphate dispersion in water prepared at different times (3 , 4) minutes at a concentration of 0.2 mM Copper sulphate salts. The optical absorption peak in Copper sulphate appeared at 220 nm in wavelength, and the intensity of the spectrum depends on the number, shape and dispersion of the particles. Increasing the time to 16 minutes led to the disappearance of the absorption peak due to the increase in the size of the particles and their leaving from the nanoscale.

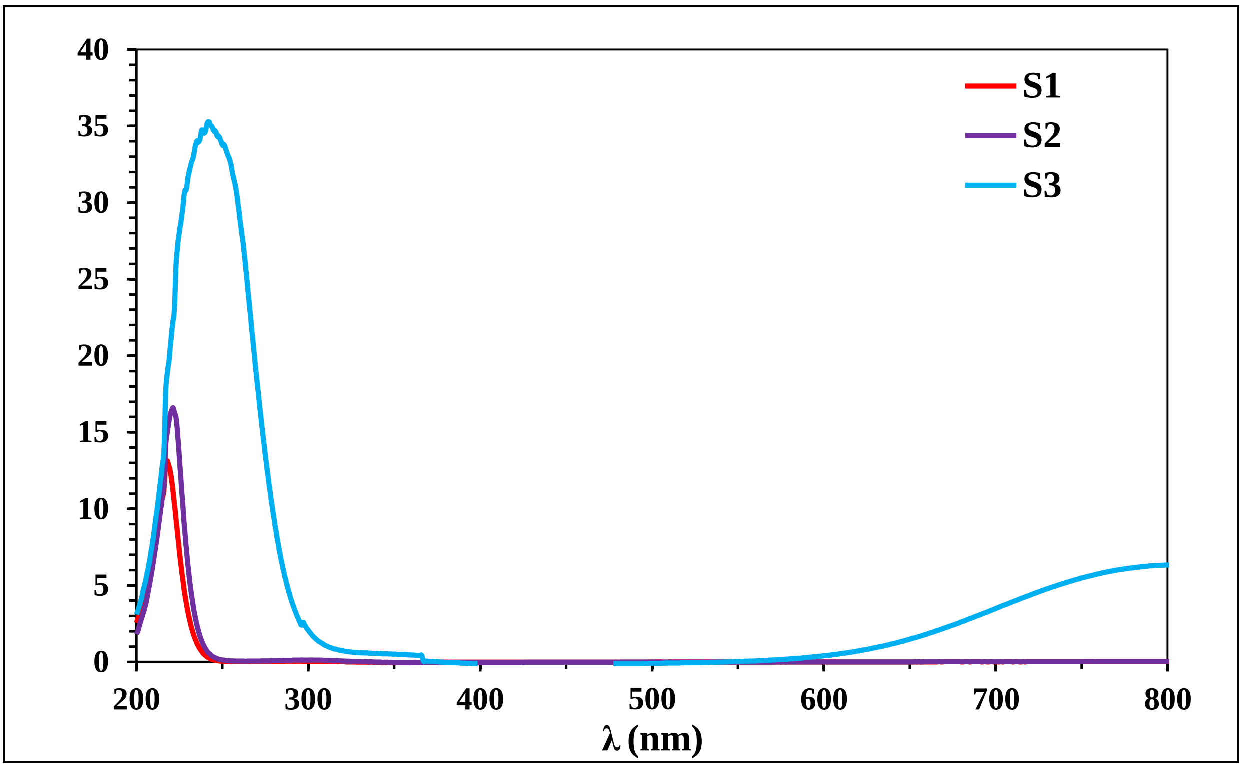


Figure (4-2): : UV-visible for CuSO4NPs prepared at same concentration of 2 mM and at different times.

SEM microscopy measurement

Figures (4-3) and (4-4) shows SEM images, with magnification, of cuso4 NPs prepared at the same concentration of .2 mM and at different reaction times. With constant concentration. Increasing the reaction time increases the particle size, as the seed crystals that have begun to grow act as a catalyst for the growth of more atoms and increase the size of the NPs.

Table (1-4) The difference between $cuso_4$ NPs at the same concentration of 2 mM and at different times.

Concentration (mM)	Time (min)	NPs Minimum (nm)	NPs Maximum)nm)
2	3	51.27	66.85
	4	50.27	73.75

Device sem

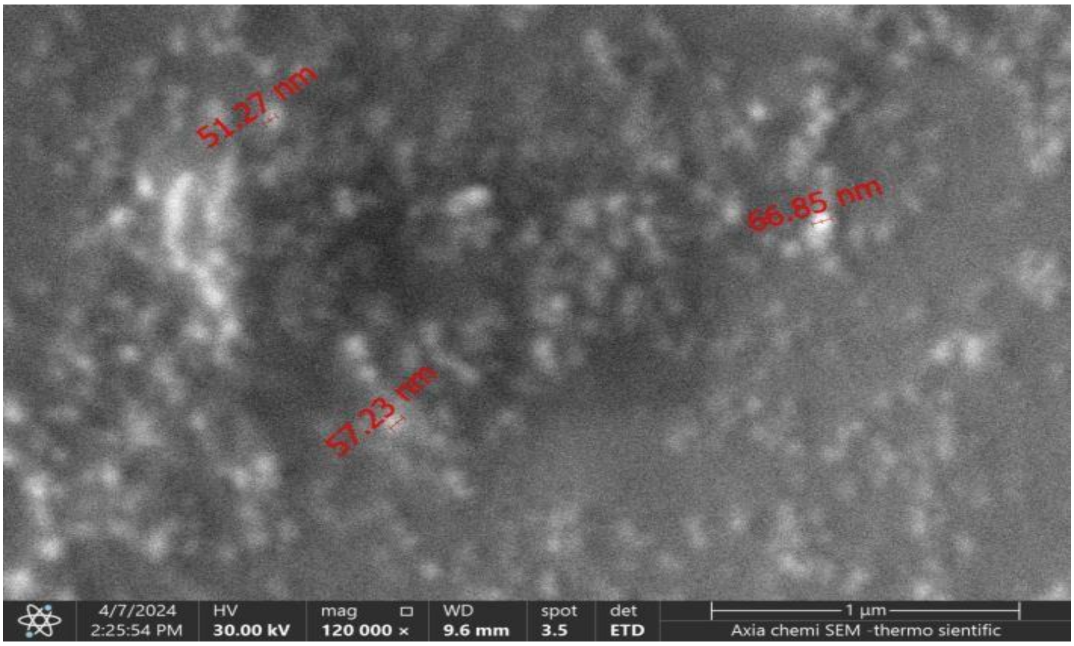


Figure (4-3): SEM analysis with magnification of cuso$_4$NPs prepared at the same concentration of 2 mM and at a time of 3 min.

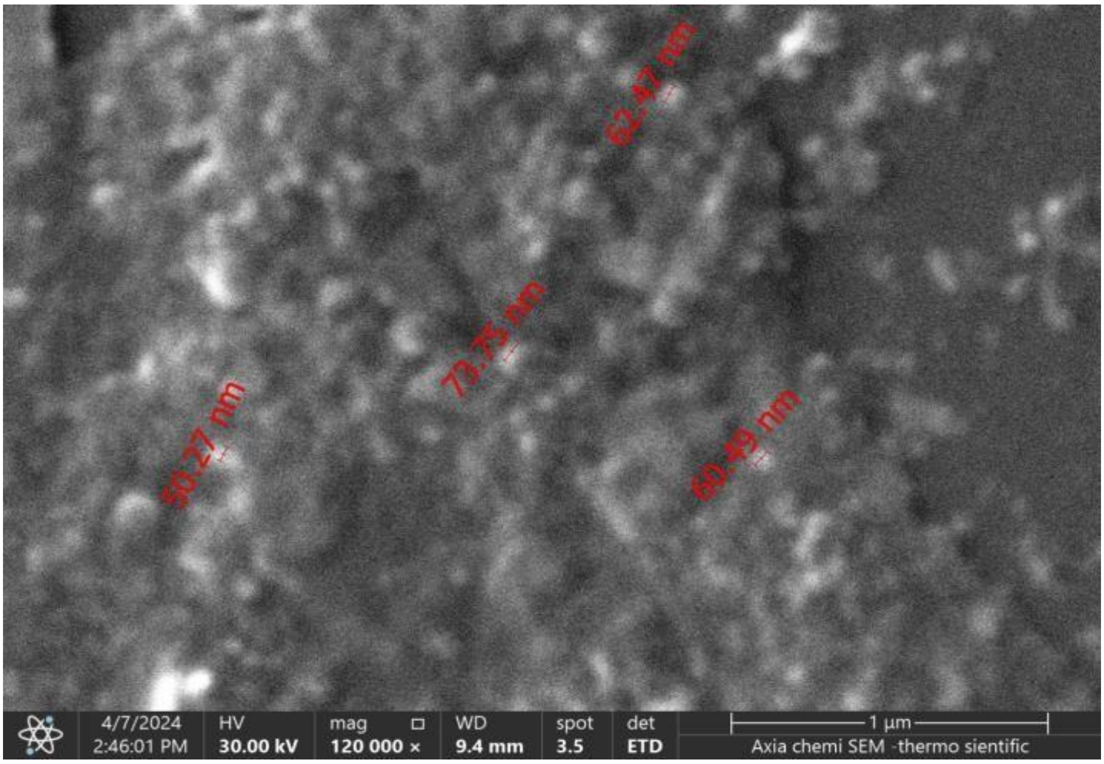
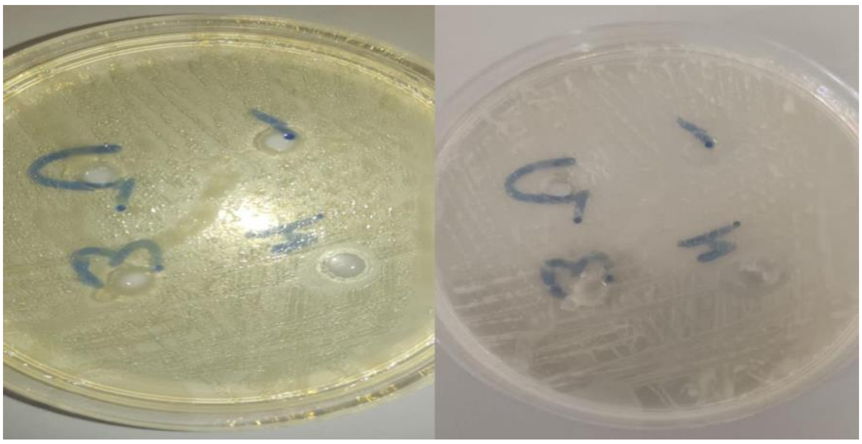


Figure (4-4): SEM analysis with magnification of cuso4NPs prepared at the same concentration of 2 mM and at a time of 4 min.

Biological activity of ($CuSO_4$)

After choosing the optimal time and concentration for copper sulphate nanoparticles, which are considered the most inhibiting bacteria, the concentrations used for copper sulphate salts (CuSO4 5H2O) were (2) mM. Copper sulfate nanoparticles showed greater antibacterial activity (Staphylococcus aureus) than their effect on bacteria (Escherichia coli), as the effect of this substance on these types of bacteria was small compared to its effect on the type of bacteria (Staphylococcus aureus), especially time (4 minutes) when exposed to cold plasma. Figure (4-5) shows the effectiveness of the nanomaterial in inhibiting the types of bacteria mentioned above. The effect of the nanomaterial on Gram- positive bacteria (Staphylococcus aureus) was more pronounced compared to Gram-negative bacteria, and this may be due to the difference in the composition of the cell wall. The cell wall of Gram-positive bacteria consists of a dense peptidoglycan layer (20–80 nm), consisting of linear polysaccharide chains linked by small peptides, thus building an additional rigid structure that retards the penetration of nanoparticles [55].



1- staphlyococcus **2- E-c0li**

Figure (4-5): Effect of cuSO₄ nanoparticles against: *1- Staphylococcus aureus* 2- *E-Coli1*.

Conclusions

1. Copper sulfate salts (at concentrations of 2 mM and at different times) were prepared using a plasma jet system, and the manufactured nanomaterials are easy to use, less time consuming, and non-toxic. Copper sulfate nanoparticles were prepared in an economical, clean and simple way.
2. When the exposure time to cold plasma increases, we notice an increase in crystal size.
3. The low cost of cold plasma technology makes it comparable to other technologies.
4. The prepared material was applied to two types of bacteria (Escherichia coli and Staphylococcus aureus).

Suggestions for Future Work

1. Studying the effect of the prepared Copper sulphate nanoparticles on fungi and rate.
2. Measuring the toxicity of Copper sulphate nanoparticles on mice and studying them as an antibacterial.

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