

Review Article

Copper: Synthesis Techniques in Nanoscale and Powerful Application as an Antimicrobial Agent

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Nanosized metal particles show specific physical and chemical properties that allow the creation of new composites materials, which are important for multiple applications in biology and medicine such as infections control. Metal nanoparticles, mainly copper, exhibit excellent inhibitory effect on Gram-positive and Gram-negative bacteria; therefore the exploration about the efficient, economical, and friendly environmental technics to synthesize inorganic nanoparticles is imperative. In this work a brief overview of the several methods is made including the comparison of the methods, mainly between sonochemical, microwave, and chemical routes. It allows determining the optimal parameters and technical conditions to synthesize copper nanoparticles with physical and chemical properties suitable for the oral bacterial inhibition.

1. Introduction

In recent years technology has been applied as a key partner in the emergence of the “nanoscience” more focused on the development of new methods to synthesize, study, analyze, and modify particles and nanosized structures, less than 100 nm. It has been shown that the physical properties of the metal nanoparticles are different from the bulk metal, made of the same atoms, which has taken great interest in their promising applications, such as incorporate antibacterial properties as well as their incorporation in pharmaceuticals and textiles and as photocatalysis [1], electrical conductors [2], biochemical sensors [3], and oxidative capacity [4, 5] so as to modify the surface properties of other materials [6] like cosmetic pigments [7].

Nanotechnology has opened a wide opportunity in the area of materials science and the incorporation of other

branches as photochemistry and electrochemistry to better understand its properties [8] has been necessary. The easy adjustment of the size of nanomaterials (<100 nm) [9] allows incorporating into a wide range of materials to improve their properties, such as size; therefore distribution and optical electric properties and potential biological applications are modified too [10]. The copper nanoparticles (Cu NPs) have been a strong focus on applications to health-related processes due to its antibacterial properties and antifungal activity in addition to their catalytic, optical, and electrical properties [11]. Cu NPs are often synthesized by dispersing polymers [12] and solvent evaporation [13]; in order to produce smaller nanometer sized particles, some methods have been suggested, for example, the use of ultrasound or organics separation and the use of solvents for extraction-evaporation or diffusion [14]. In recent years, the implementation of affordable friendly systems with the environment for

the synthesis of Cu NPs is a challenge due to the complex obtained metal nanoparticles instead of metal oxides [15, 16]. The development of Cu NPs is in constant growing and developing for future technologies [17]. The highlight of this review is related to the synthesis and characterization of Cu NPs techniques and their biological properties and applications in biomedical science.

2. Techniques for the Synthesis of Copper Nanoparticles

The development of materials at nanometric scale has been increasing in different fields. The properties of these nanomaterials are critical for the technological revolution worldwide, which mainly depend on the methods of synthesis for the potential applications such as the bactericidal and antifungal effect [18]. Some nanomaterials include those derived from metal oxides, metal salts, and metal hydroxides, such as copper oxide, zinc oxide, gold, silver, copper, magnetite and maghemite, titanium, and iron [7]. Metal-based nanoparticles represent an alternative for biomedical treatments, mainly in the fabrication of biomedical devices with antimicrobial coatings. A high antimicrobial activity of nanoparticles depends on the particle size that allows greater surface contact and a direct interaction with the membranes of pathogenic microorganisms. NPs are also used as drug delivery and ions agents as well as for diagnostic imaging [19]. Moreover, the increasing improper use of medications such as antibiotics has led the medical field to explore new alternatives of biocides to combat infectious diseases [20, 21].

The NPs are materials with different properties than those in the bulk form; these features made the nanoparticles have various applications in many fields such as electronics (nanowires and nanosensors), MRI (magnetic devices), pharmaceutical (drug-eluting), cosmetic (nanopigments), and catalytic and materials design (nanodevices) [19]. Silver and Cu NPs are the most usually reported with a high antimicrobial activity; it allows their incorporation in a variety of materials including titanium, polymers, and glasses [22, 23].

Generally, the methods for the synthesis of nanoparticles are usually classified into two categories: the physical and chemical techniques [24]. The physical method consists in the reduction of bulk solids to smaller portions in very fine grains through a grinding process, either by using acidic substances or by the application of energy sources. The grinding process is the most representative example of the physical methods, where highly efficient mills are used to separate the particles of nanometric sized. However it has not been a reliable system to obtain metallic nanoparticles because, generally, the obtained particles are larger than 100 nm, which could not be considered as nanometric size. Another disadvantage of this technique is that the applied energy for grinding must be continuous and it can infer energetic changes into solids producing a significant imbalance, which lead to a decrease of values in the energy activation [25]. In fact, probably it is one of the oldest methods but it is not currently used to obtain Cu NPs with regular size and defined morphology.

Physical techniques are unconventional methods (Table 1), such as those that require vacuum or plasma, sometimes

TABLE 1: Physical and chemical synthesis methods of Cu NPs.

| Physical methods | Chemical methods |
|--------------------------------------|----------------------------------|
| Ablation [85–87] | Colloidal microemulsion [48, 49] |
| Physical Vapor Deposition (PVD) [88] | Sonochemical [52, 89–91] |
| Wire discharge [83, 92] | Electrochemical [25, 40, 82, 93] |
| Grinding [94] | Microwave [43, 95–97] |
| Radiolysis [98] | Hydrothermal [46, 82, 99] |
| Aerosol [100] | |
| Mechanical attrition [101] | |

obtaining nanoparticles with low quality. Several physical techniques are incorporated during or after a chemical process, for example, the laser ablation requires a colloidal solution, which minimizes the chances of oxidation on the surface of the nanoparticles, and it must be placed in a vacuum chamber in order to remove or extract atoms from a bulk surface through emission of laser beam; this method is not feasible due to the complexity of the equipment and the use of high energy for the laser [26]. The number of pulses applied of the laser beam and the exposition time are important parameters to define the particle size. These parameters are in the range from 6000 to 10000 pulses in periods of 10 and 30 minutes [25]. The decomposition of volatile compounds inside a vacuum chamber or reactor is used for the deposition of atom by atom or molecule by molecule to form layers on a solid surface at subatmospheric pressure [27]. Unlike other physical techniques, in the Pulsed Wire Discharge (PWD), the ions are implanted on solid substrate by pulse electrical current [28, 29].

Chemical synthesis is currently the most used and functional method, which involves condensation of atoms or molecular entities from the gaseous phase or from a solution to obtain nanoparticles with specific size and morphology; these properties are adjusted with the synthesis parameters such as temperature, precursor concentration, stabilizer agent, or solvent [30]. This method for the Cu NPs synthesis shows the relation ratio between the precursor, the reducing agent, stabilizing agent, and the solvent (Table 2). Among the chemical methods, the chemical reduction of copper salts is easy and simple to obtain Cu NPs with controlled size and morphology [31–33].

The chemical reaction is sensitive to the aqueous media and air conditions, where the surface of the nanoparticles is highly oxidizing, so that sometimes it is necessary to use inert environmental conditions (nitrogen or argon atmosphere) or surface-active substances to protect the nanoparticles surface, like ligand agent, surfactants, soluble polymers, weak acids, and so forth (Figure 1) [30, 34].

Chemical reduction method and microemulsion route were used for the first time to synthesize gold metal, and they have been used to reduce other less noble metals such as copper by using mainly copper salts (sulfates, nitrates, and chlorides) and reducing agents (sodium borohydride,

TABLE 2: Chemical substances for the Cu NPs synthesis.

| Copper precursor | Reducing agent | Stabilizer | Medium | Temperature | Morphology and size (nm) and maximum absorption peak (nm) |
|---|--|---|--|-------------------------------|---|
| Copper nitrate [99] | Ascorbic acid | Chemical grade starch | Distilled and deionized water | Room temp. | Spherical, 5–12 nm |
| Copper nitrate (II) [42] | Cetyl trimethylammonium in isopropyl alcohol | Bromide, cetyl trimethyl ammonium (CTAB) | Aqueous | Room temp. | Spherical, 5–10 nm |
| Hydrated copper chloride (II) [102] | Cetyl trimethylammonium in isopropyl alcohol | Ascorbic acid | Acid inert atmosphere | Room temp. | semispherical, 5–12 nm |
| Copper nitrate (III) [18] | Sodium borohydride | Sodium hydroxide | Aqueous | Room temp. | Asymmetric, 9.25 ± 1.79 nm, |
| Copper pentasulfide (III) [42] | Cetyl trimethylammonium bromide | Ascorbic acid | Deionized water polyethylene glycol | Room temp. | 20 nm |
| Copper sulfate pentahydrate (III) [103] | Isonicotinic acid hydrazide | Ascorbic acid sodium hydroxide polyvinylpyrrolidone | Distilled water | 60–70°C | 6.95 nm |
| Copper chloride dihydrate [48] | Aluminum isopropoxide | Ascorbic acid | Ethanol | Room temp. | — |
| Copper (II) nitrate [74] | L-ascorbic acid | Chitosan | Distilled water | Microwave | 80–100 nm, 550 nm |
| Copper (II) sulfate [104] | Hydrazine monohydrate | Sodium dodecyl sulfate | Distilled water | Room temperature | 50–70 nm, 550 nm |
| Copper (II) nitrate [78, 99] | Ascorbic acid | Starch | Double-distilled water | Microwave | 5–12 nm, 579 nm |
| Copper (II) acetate [83] | Ethylene glycol | Tween 80 (polyoxyethylene-(80)-sorbitan monooleate) | (polyoxyethylene-(80)-sorbitan monooleate) | 190–200°C, 2–3 h | 45 ± 8 nm, 580 nm |
| Copper (II) nitrate [18] | Sodium borohydride | Tetra-n-octylammonium | Deionized water | Room temperature | 3–9 nm |
| Copper (II) chloride [105] | Sodium borohydride ascorbic acid | Polyvinyl alcohol | Deionized water | Room temperature | 3.5 nm, 516 nm |
| Copper (II) chloride [102] | Sodium borohydride | Ascorbic acid | Aqueous solution | Inert atmosphere | 5.3 ± 0.1 nm, 562 nm |
| Copper (II) acetate | Ethanol | L-ascorbic acid | Ethanol | Microwave irradiation (140°C) | 7–15 nm, 580–590 nm |
| Copper (II) nitrate [47] | | | | | |

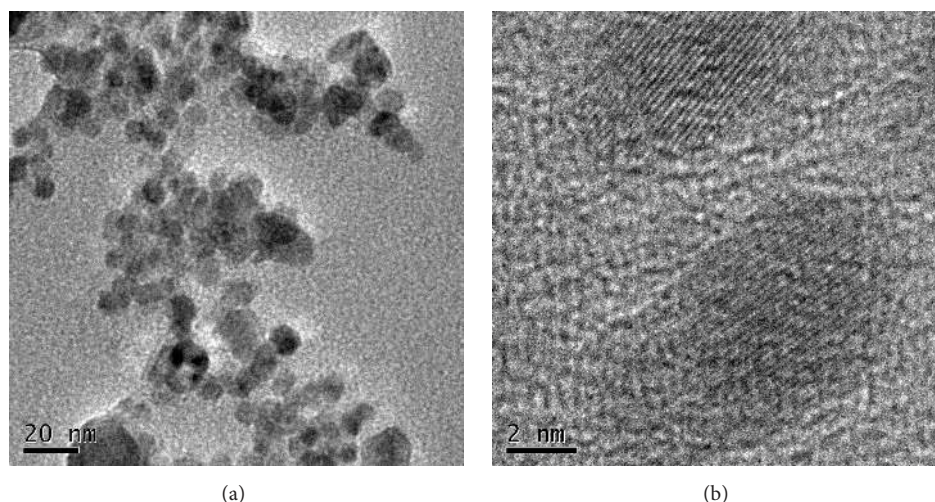


FIGURE 1: HRTEM of Cu nanoparticles. 1×10^{-2} M of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and 1×10^{-2} M of NaBH_4 , under nitrogen gas, were vigorously stirred for 2–3 h. Left image shows Cu NPs with sizes from 25 to 4 nm. Right image is a zoomed image of the characteristic size of Cu NPs.

isopropyl alcohol, ascorbic acid, and hydrazine) and, sometimes, by using stabilizing agents (polyvinyl pyrrolidone and polyethylene glycol) [35, 36]. Green chemical synthesis by using novel *Ginkgo biloba* Linn. leaves is a successful option to obtain stable spherical Cu NPs about 15–20 nm [37].

Microemulsion reduction method (Figure 2(a)) or colloidal synthesis involves the use of immiscible water-oil, oil-water, and water supercritical carbon dioxide forming surface-active microarrays [38]. Sonochemical reduction is based on ultrasonic waves (Figure 2(b)) with a frequency about 20 kHz to 10 MHz; the reaction is active by a physical phenomenon of acoustic cavitation [39]. The electrochemical synthesis (Figure 2(c)) is based on the application of an electrical current between two electrodes (cathode and anode) separated by an electrolytic solution; the reduction process occurs on the surface of the cathode electrode [40]. Microwaves and hydrothermal treatment are new alternatives to obtain regular particle size and morphology Cu NPs [41, 42]. The microwave technique consists in an electromagnetic energy with frequencies in the range of 300 MHz to 300 GHz [43–45], where the adequate amounts of energy influence directly the configuration of the Cu NPs. Finally, in the hydrothermal treatment, the chemical reaction requires a sealed autoclave, where the solvents are exposed at temperatures above their boiling points [46].

The synthesis techniques are also often classified into “bottom-up” and “top-down” by the direction of the nanoparticles formation. The “bottom-up” reaction begins from atomic level through forming molecules; however, in the opposite technique described as “top-down,” the scale of the resultant nanoparticles is larger, so that a mechanical process or the addition of acids is required to reduce the particle size. Usually, the “top-down” technique requires the use of complex and complicated instrumentation [47].

Regardless of the technique chosen for the synthesis of Cu PN, conditions should be controlled to adjust the particle

size (lower than 100 nm) and shape of the nanoparticles (nanofibers, nanotriangles, and nanospheres), because the wide distribution particle size modified their properties [48–50]. Shankar and Rhim found that nanofibers and triangle and spherical nanoparticles can be obtained by using basic or acidic solution, respectively, which presented different absorbing bands, the first in the range of 280–360 nm and the second from 240 to 280 nm [51]. They also found that both shapes of Cu presented inhibition against Gram-negative *E. coli* and Gram-positive *L. monocytogenes*, but nanofibers had stronger antibacterial activity than those triangle and spherical nanoparticles. The synthesis of metal NPs currently requires both physical and chemical systems to incorporate the best features and optimum properties to develop new materials. Certain characteristics can be obtained with notable differences between the physical and chemical methods; chemicals are more beneficial if what is sought is to obtain nanoparticles below 50 nm; both methods obtained effectively Cu NPs; however the properties conferred to nanoparticle size should be considered [52, 53]. Khodashenas and Ghorbani compared three methods (chemical, physical, and biological) concluding that the biological route is more ecofriendly, cheaper, and easier to obtain copper nanoparticles than physical and chemical ones [24].

3. Techniques for Characterization of Copper Nanoparticles

The observation of nanometric scale structures is carried out by different tests consisting of photons, electrons, neutrons, atoms, ions, or an atomically sharp tip, which has different frequencies, in the range from gamma to infrared rays or beyond. The resulting information can be processed to produce images or spectra that reveal topographic, geometric, structural, chemical, or physical details. Several techniques are available for the characterization of nanomaterials [32, 54].

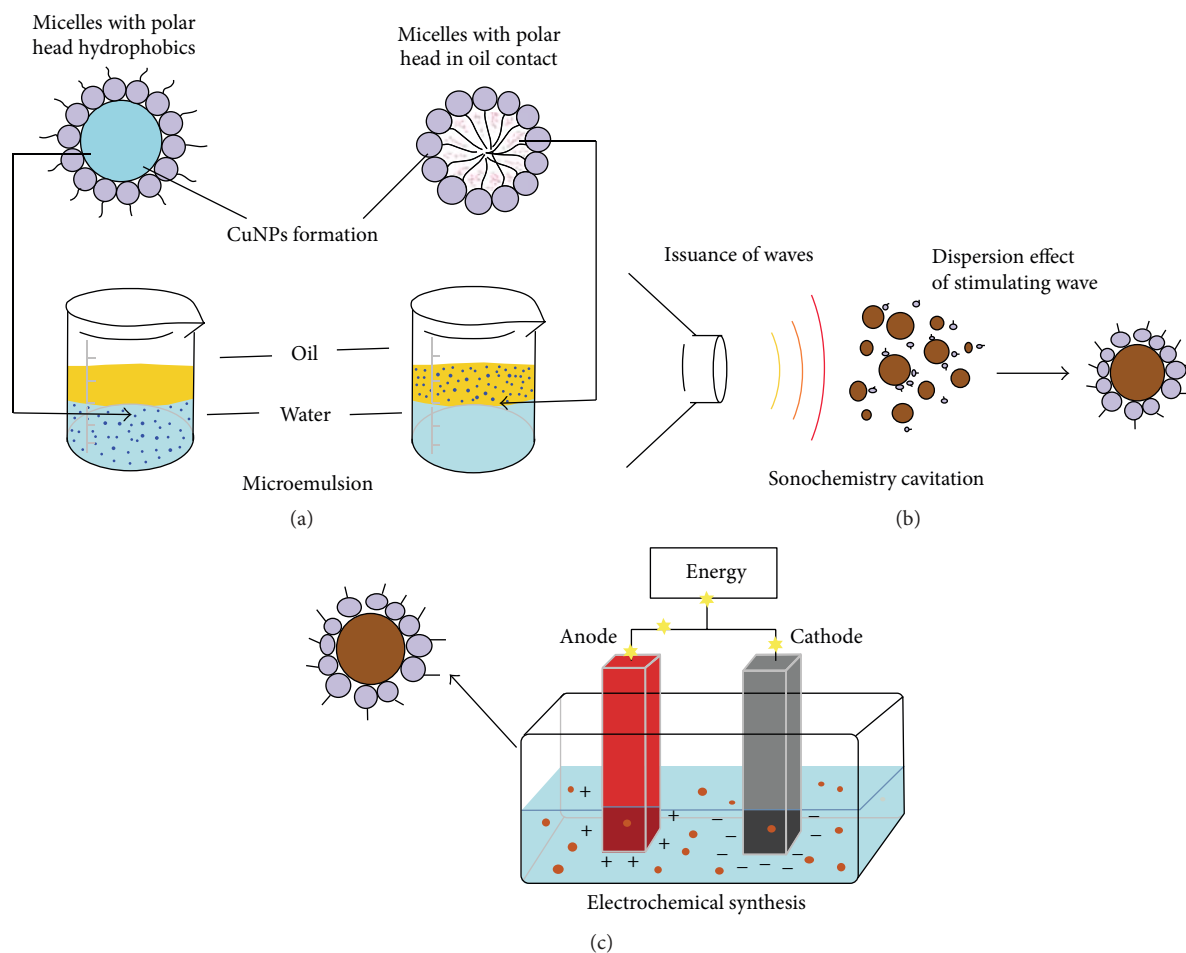


FIGURE 2: Synthesis of Cu NPs. (a) Microemulsion technique (oil-water/oil): the lipid micelles are surrounding the NPs giving the form of hydrophobic heads or hydrophilic tails, and the interaction of both resulted in spherical shape. (b) Sonochemical technique: applying energy as waves, the micelles found in the middle interacting with the surrounded copper resulting in spherical shape NPs. (c) Electrochemical technique: the interaction between opposite electric charges generated increased energy in the middle of the formation of NPs; these energy emissions are usually constants allowing the fact that between one issue and another the final structure is formed.

Cu NPs have been physically and chemically characterized in order to obtain the great amount of data to establish the physical and biological characteristics (Table 3). The diminutive sizes of Cu NPs are also their main disadvantage because it represents a challenge for the scientific community to achieve adequate physical and chemical characterization [49, 52].

The stability or ability to keep its size and shape as function of time is very important to keep the properties and potential applications as a “bioactive” material. Some relevant data such as the purity of phases, the distribution in the space, the chemical composition of its surface, and its crystallinity are analyzed by various methods, most of them from high energy with large resolutions [55, 56]. The importance of the Cu NPs analysis lies in the growing field of applications, making the knowledge of the nature of this new material imperative [57].

Metals such as copper colloids are generally absorbed in the ultraviolet-visible (UV-Vis) range due to excitation of surface plasmon resonance (SPR). Therefore, the UV-Vis

spectroscopy is a convenient method to characterize Cu NP [58]. Some of the colloidal metal materials are different under the macroscopic scale and show distinct absorption peaks in the visible region; copper, silver, and gold are metal with prominent absorption peaks. The Transmission Electron Microscopy (TEM) is the most common characterization technique to determine the shape and size of the Cu NPs. Although other methods such as Dynamic Light Scattering (DLS) and X-Ray Scattering at Small Angles (SAXS) are also used to measure particle size [59], only TEM analysis provides the real images of the morphology and shape of the nanostructures. The Scanning Electron Microscope (SEM) is an instrument that allows the observation of the inorganic materials providing morphological information. The main use of high resolution EDS/SEM ($\sim 100\text{\AA}$) is the ability to obtain three-dimensional images with large depth fields by a simple sample preparation [60]. The achievements in recent years allow the precise control over the generated structure in the synthesis of Cu NPs depending on the specific application.

TABLE 3: Characterization techniques and response for copper nanoparticles.

| Characterization technique | Copper NPs |
|--|---|
| UV-Visible Spectroscopy [52] | 566–580 nm |
| TEM, Transmission Electron Microscopy [6] | 10–20 nm/elongated aggregates, spheres 5–15 nm/rice, nanocrystals |
| HRTEM, High Resolution Transmission Electron Microscopy [30, 106, 107] | Atoms periodicity arrangement |
| AFM, Atomic Force Microscopy [6] | Topography |
| EDS/SEM, Electronic Data System/Scanning Electron Microscopy [106] | 8 KeV |
| XRD, X-Ray Diffraction [108–110] | Diameter estimated: 5–16 nm |
| Raman Spectroscopy [30, 107] | Raman shift: 430, 739, 1057, 1314, 1433, and 1459 nm (± 5 nm) |

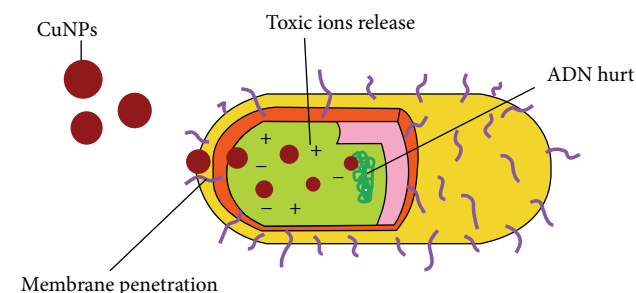


FIGURE 3: Possible Cu NPs action mechanism in a bacterium membrane.

TABLE 4: Minimum inhibitory concentrations (MIC) related to biological effect.

| Metal NPs | MIC | Size (nm) | Microorganism |
|---------------|----------------------|-----------|-------------------------|
| Cu [18] | 140 mg/mL | 6–16 | <i>S. aureus</i> |
| | 140–240 mg/mL | | <i>E. coli</i> |
| Cu [99] | 3.2 ± 0.41 mg/mL | 5–12 | <i>S. aureus</i> |
| | 1.6 ± 0.22 mg/mL | | <i>E. coli</i> |
| | 3.6 ± 0.43 | | <i>Salmonella typhi</i> |
| Cu [111] | 1.875–3.75 mg/mL | 50 | <i>S. aureus</i> |
| | 3.75 mg/mL | | <i>C. albicans</i> |
| Cu [112, 113] | 140 μ g/mL | | <i>S. aureus</i> |

4. Biological Behavior: Antimicrobial Effect and Toxicity

Since a decade, the metal and metal oxide nanoparticles such as silver, zinc, gold, or titanium dioxide have been used as antimicrobial agents. Currently, the behavior of nanometals at nanometric sizes against pathogenic organisms is still being studied [61]. The high antimicrobial activity of the Cu NPs has been shown in multiple studies focusing on the optimal size range of about 1 to 10 nm [62, 63].

The Cu NPs have been promising for medicine and dentistry fields due to their properties, specially their interaction with pathogens, their large active surface area, and their high chemical and biological reactivity [64].

Copper is an essential element for some metabolic processes but at low concentrations because large doses could be serious consequences in the metabolic performance. It can act as electron donor or electron acceptor in some enzymes due to its redox properties increasing its toxicity for bacterium, which is induced by Cu^{+1} and Cu^{+2} ions [65–67]. Some bacteria such as *Clostridium difficile* developed important mechanisms to protect themselves from the toxic effects of copper ions while they are in contact with the surface of the Cu NPs (Figure 3). A possible mechanism of bacterial resistance to copper is the formation of endospores, which allows its rapid diffusion. However, the antibacterial response of the copper is being studied, mainly in human pathogens [68].

The most analyzed bacteria species in contact with Cu NPs are *C. difficile*, *E. coli*, and *P. aeruginosa*, which were significantly inhibited with a particle size between 22 and

90 nm, finding a decrease in the viability cells of these microorganisms [18, 69, 70]. Gram-positive bacteria (e.g., *S. aureus*) are more sensitive to Cu NPs and Gram-negative bacteria (e.g., *E. coli*) are associated with ROS (Reactive Oxygen Species) expression by different sizes of Cu NPs. These features could change through free surface energy of the particles directly associated with size and morphology and the pH inner of cells too [71]. The antimicrobial effect is directly related to the nanoparticle size and minimum inhibitory concentration (MIC) (Table 4) and the oxidation degree of the surface [72].

The cell membranes of the microorganisms interact with the medium, so metal NPs especially Cu NPs will have some interactions to release metal ions that interfere with the processes of the DNA replication, cell membranes formation, cell division, and so forth, of certain microorganisms such as bacteria, which results in an antimicrobial effect [46, 73]. The action mechanism of the copper NPs occurs through the interaction of enzymes and -SH groups causing damage in the DNA and therefore oxidative stress generation [74–76].

5. Applications in Medicine

The bacteria resistance to antimicrobial drugs has been increased, and now it is considered one of the most important public health worldwide challenges [77]. Cu NPs offer a new strategy against drug antimicrobial resistance, reducing the cell adverse effects [37, 78]. In the medical field as well as a possible antibiotic penetrate common infections in the circulatory, respiratory, and digestive systems, it has also been

expanded to the oral cavity; the oral cavity provides habitat for a wide range of microorganisms including bacteria, yeast, and viruses associated with oral infections. Bacteria are the predominant components of the resident microflora and the diversity of species found in the oral cavity the wide ranges [70]. Currently, it has been reported that the incorporation of Cu NPs to orthodontics adhesives has showed significant bactericidal effects against *S. aureus*, *E. coli*, and *S. mutans*, without altering the shear bond strength, oppositely increased their adhesive properties by the addition as nanofiller [79].

Nanotechnology provides a cost-effective way to applying a surface treatment of metals oxidizing nanoparticles used as an antimicrobial substance. An innovative nanoparticle treatment may convert antimicrobial medical devices. Materials nanostructured have unique physicochemical properties promising for dental applications [80, 81]. Among the interesting properties are narrow size, high surface area, high reactivity, high biological interaction, and functional structures.

6. Future Trends

Cu NPs have excellent physicochemical properties, high electrical conduction and good biocompatibility and high surface activity, and therefore they are promising for magnetic nanodevices and multiple electronic and medical applications as well as their incorporation in materials and medicines [82]. Currently, researchers are looking for new routes to the synthesis of metallic nanoparticles such as copper in order to find new properties [83]. Microemulsion is the most common method for the copper NPs synthesis involving the use of tensioactive substances and organic solvents and a lot of energy and high costs [16, 17]. These are the main reasons that it seeks to develop new methods friendly with the environment [84] by incorporating low toxicity substances such as alginate used as stabilizing agent [38] or chitosan-silver-copper organometallic to form 33 bimetallic particles. The investigations are new trends to developing a system to obtain NPs that offer the least possible harm to the environment and which are inexpensive, but the road is still long and uncertain, although efforts are increasingly higher. As estimated for 2020, the production of nanoparticles focused on nanomaterials will be 20 times higher than that in the last 10 years [38].

Conflict of Interests

The authors report no conflict of interests for the present paper.

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