

Review

Copper Nanoparticles: Synthesis and Characterization, Physiology, Toxicity and Antimicrobial Applications

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Abstract: Metallic nanoparticles are a new class of materials with applications in medicine, pharmaceutical and agriculture. Using biological, chemical and physical approaches, nanoparticles with amazing properties are obtained. Copper is one of the most-found elements and plays an important part in the normal functioning of organisms. Copper nanoparticles have superior antibacterial properties when comparing them to present day antibiotics. Moreover, apart from their antibacterial role, antifungal, antiviral and anticancer properties have been described. Although the mechanism of actions is not completely understood, copper nanoparticles can become a viable alternative in fighting multi-resistant bacteria strains. We hereby review the already existing data on copper nanoparticle synthesis, effects and mechanisms of action as well as toxicity.

Keywords: nanoparticles; copper; physiology; antibacterial activity; action mechanism; toxicity



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1. Introduction

While thinking about nanomaterials, two possible definitions come to mind. The more general one would consider nanomaterials as materials with the size of less than 100 nm. The second definition is much more specific and restrictive and mentions that nanomaterials develop characteristics that depend on their size [1].

Given their numerous and unique properties—including their role in energy conversion and storage, their catalytic properties and implications in surface treatments [2]—nanoparticles have started to be used as antimicrobial agents or alternatives to conventional antibiotics. There is extensive research on different materials, specifically metals, such as silver [3], gold [4], platinum [5], zinc [6], copper and their method of synthesis, as well as antibacterial properties [7]. Out of the metals, this paper focuses on copper and its properties.

2. Characterization Methods for Metal Nanoparticles

Nanoparticles can be characterized through various techniques. Each of them is useful to determine a property of the obtained nanoparticle. The chemical composition and concentration are not enough. Physical properties (size, shape and surface properties) need to be measured to be able to successfully reproduce experiments (Table 1). This paper presents several characterization methods and their use.

Various characterization methods are presented by Mourdikoudis et al. [8]. X-ray diffraction (XRD) is a characterization that started to be used recently for nanoparticles' characterization. It can provide information about the crystalline structure and grain size, nature of the phase and lattice parameters. While having good use, Chapman et al. have reported some limitations such as crystal growing difficulty, increased effort to obtain data on single conformation and low intensity [9].

Table 1. Nanoparticle characterization techniques.

Property	Technique
Size	X-ray Diffraction (XRD), Transmission electron microscopy (TEM), Dynamic light scattering (DLS), UV-Vis Spectroscopy (UV-Vis)
Shape	TEM, High-Resolution Transmission Electron Microscopy (HRTEM), Atomic force microscopy (AFM)
Surface charge	Electrophoretic mobility (EPM), Zeta potential
Size distribution	Dynamic light scattering (DLS), Atomic force microscopy (AFM), Differential centrifugal sedimentation (DCS)
Optical properties	UV-Vis, Photoluminescence (PL)
Magnetic properties	Vibrating sample magnetometry (VSM), Superconducting quantum interference device magnetometry (SQUID)

Transmission Electron Microscopy (TEM) transmits or scatters electrons from a thin sample using uniform current electron beams. TEM can provide accurate estimations of nanoparticle homogeneity, but it has difficulties when quantifying large numbers of particles. Another limitation is that it produces unreliable images due to orientation effects [8].

High Resolution Transmission Electron Microscopy became the most used technique for characterizing the internal structure of nanoparticles thanks to its high resolution [8].

A method used to characterize size, shape, concentration, agglomeration state and refractive index is UV-VIS spectroscopy. A reference material is used to measure the intensity of the reflected light and then compare it to the sample resolution [8].

Atomic Force Microscopy (AFM) is a technique used to create three dimensional images of surfaces. It is mainly used to determine the size of nanoparticles and it is cost effective, space saving and can be used to assess the interaction of nanoparticles in real-time [10].

FTIR spectroscopy has increased signal to noise ratio and reduced thermal deterioration when used to characterize nanoparticles. An improved version of this technique, attenuated total reflection (ATR-FTIR), is used for evaluation of chemical features on nanoparticles surface [11,12].

Particles with high charges tend to repel each other and form stable colloidal solutions. Zeta potential is used to analyze the stability of the formed colloidal solutions [8].

Electrophoretic mobility (EPM) is used to measure the surface charge of nanoparticles. Low values are associated with aggregations of iron oxide nanoparticles, while high values are associated with stable nanoparticles over long periods of time [8].

To measure nanoparticles' size in colloidal suspension, dynamic light scattering (DLS) is used. It is a powerful technique when used for real time observation of the aggregation process. When combined with differential centrifugal sedimentation (DCS), the measurements are accurate. A drawback is that it lacks the necessary resolution when working with small aggregates. It is, however, powerful because it is capable of measuring different sizes of nanoparticles at the same time [8].

Photoluminescence (PL) is light emission from matter after absorbing photons. PL is a good fit to study quantum dots and metal nanoclusters. It is used in optical labeling applications [8].

Methods used to measure magnetic properties are Superconducting quantum interference device magnetometry (SQUID) and vibrating sample magnetometry (VSM). SQUID is used to measure magnetic properties of nanoparticles such as magnetization saturation, remanence and blocking temperature while VSM is used as a function of magnetic field, temperature and time [8].

3. Copper and Copper Nanoparticles

Copper is one of the most abundant elements found on Earth. It has played an important part in history, given its many properties, like good electrical and thermal conductivity, high corrosion resistance and increased malleability. It has been used in ornaments, weapons and coins since the early 14th century [13].

Copper is an essential nutrient required for the normal functioning of the human body. The Food and Nutrition Board at the National Academies of Sciences, engineering and medicine have published the following recommended dietary allowance for copper, as represented in Table 2.

Table 2. Recommended daily copper allowance [14].

Age	Male	Female
0–6 months	200 µg	200 µg
7–12 months	200 µg	200 µg
1–3 years	340 µg	340 µg
4–8 years	440 µg	440 µg
9–13 years	700 µg	700 µg
14–18 years	890 µg	890 µg
19+ years	900 µg	900 µg

The human body can obtain copper from various sources, like shellfish, seeds and nuts, cereals, whole grain products and chocolate. Liquids like tap water can also contain copper; however, the copper concentration depends on the source of the water.

Although the human body can obtain the daily copper allowance from a multitude of sources, studies have shown that the average adult loses approximately 1.3 mg/day of copper.

Copper can be found in more than 30 types of protein, and it plays an important part in living organisms' metabolism. Numerous enzymes containing copper contribute to different body functions, such as oxygen transportation and iron homeostasis [13]. In addition, copper is also found in skin, bones and different body organs [15].

If it is ingested in higher quantities that exceed the human tolerance, copper becomes toxic and can cause hemolysis, jaundice, abdominal pain, nausea and, in extreme cases, death [16,17].

A common source of copper intoxication is tap water due to the piping used in water distribution either containing copper or being made of copper alloys.

On the other hand, copper deficiency is quite rare in humans. Different studies on both animals and humans show the results of copper deficiency include connective tissue disorders, osteoporosis and other bone problems, as well as increased risk of infection [18]. Another study highlighted that copper deficiency can lead to anemia and improper fetal development [15].

In addition to playing an important role for the human body, copper is also essential to the development of plants. Copper helps with protein regulation, photosynthetic electron transport, mitochondrial respiration and cell wall metabolism. When plants have a copper deficiency they present curled leaves, petioles bend downwards and permanent loss of turgor in young leaves. Contrarily, a high concentration of copper leads to toxicity, growth inhibition, photosynthesis interferences and oxidative stress [19]. This action will be further described in a later section (Mechanism of toxicity).

Copper nanoparticles (CuNPs) have obtained public interest due to their mechanical, electrical, magnetic and thermal properties and they have been used in water treatment, heat transfer systems and antimicrobial coating for surgical tools [20,21].

An advantage to using copper is that it is cheap and widely available, thus obtaining CuNPs is cost effective. One of the downsides of CuNPs is that when exposed to aqueous environments, they are susceptible to oxidation. Copper transforms into CuO and Cu₂O,

and converts to Cu^{2+} during preparation, imposing challenges in further synthesizing CuNPs in an ambient environment [22].

Yabuki et al. analyzed the oxidation of copper nanoparticles in relation to temperature. The threshold temperature was recognized between 190 and 200 °C. The analysis was then performed using nanoparticles treated at 170 and 240 °C for 200 min and after their chemical composition was studied. Below the threshold temperature, the resulted particles were mainly Cu_2O . Above the threshold temperature, Cu_2O was initially obtained, which then changed to CuO [23].

Pacioni et al. used copper sulfate to obtain 8 nm aqueous CuNPs. The authors have discovered that by using L-ascorbic acid (vitamin C, VC) in anaerobic conditions, VC is able to react with Cu^{2+} , reducing it to Cu^+ , thus stabilizing the CuNPs and delaying oxidation [24].

To avoid oxidation, Rajesh et al. [25] suggest using different protecting materials like polymers (poly vinyl alcohol, polyethylene glycol, poly vinyl pyrrolidone and organic ligands).

In a different study, the authors discuss the use of carbon-encapsulated copper nanoparticles. Carbon coating can prevent oxidation and have a few advantages over CuNPs, such as good compatibility with organics, highly pressure- and temperature-dependent electrical conductivity and excellent electromagnetic wave loss ability. The authors have concluded that the outside carbon shell can prevent oxidation of the copper inside at temperatures up to 900 °C [26].

4. Copper Nanoparticles Synthesis

Metal nanoparticles can be produced using green, chemical and physical methods. In order to obtain nanoparticles, especially copper nanoparticles, three elements are needed. First, a precursor that provides copper ions. Second, in order to obtain copper atoms a reducing agent is required to supply electrons. Given the correct temperature and pH conditions, the third component, the surfactant, aggregates the copper atoms resulted from the reducing agent into copper nanoparticles [20].

Depending on the source of electrons, we can categorize the synthesis methods into green, if the reducing agent is a biological organism (bacteria, molds, algae, plants), into chemical, if the reducing agent is a chemical compound and lastly physical, when the source is physical one, such as electricity [20].

4.1. Green Synthesis

Green synthesis of metallic nanoparticles is widely used because of its harmless obtaining method. It uses molecules in plants and microorganisms (bacteria, fungi) as a reducing agent. It has the advantage of using more eco-friendly materials, being cheaper than chemical synthesis, simpler, more rapid and sustainable. It is preferable to use plant extracts to obtain nanoparticles rather than using microorganisms because of increased difficulty in preserving cell cultures [27]. Moreover, it reduces the complex process of maintaining cell cultures and it is also suitable for creating large scale synthesis of nanoparticles [28].

Considering the use of nanoparticles in medicine, there is an increased need to use an eco-friendly method of obtaining as they are regarded as the next step in battling diseases. As Thiruvengadam et al. present, the nanoparticles obtained with the help of plants have excellent antimicrobial, anticancer, antidiabetic, anti-inflammatory and antioxidant activities [29].

Various types of plants can be used in the synthesis method of obtaining copper nanoparticles and the resulting nanoparticles have different antimicrobial properties. Table 3 shows the green synthesis of CuNP from different plants, their size and activity.

Table 3. Green synthesis methods of copper nanoparticles.

Used Substances	Size	Shape	UV-VIS	References
Copper(II) sulphate pentahydrate Citrus lemon fruits	28 nm	Spherical	579 nm	[30]
Extract of <i>O. sanctum</i> leaves Copper(II) sulphate pentahydrate	122.7 nm	Spherical	586 nm	[31]
Extract of <i>Rhuscoraria</i> L. fruits extract Copper(II) sulphate pentahydrate	70 nm	Spherical	568 nm	[32]
Extract of the flower <i>Milletta pinnata</i> Copper acetate solution	23 ± 1.1 nm	Spherical	378 nm	[29]
Leaf extract of <i>Ageratum houstonianum</i> Mill. Copper(II) chloride	80 nm	Cubic, hexagonal, rectangular	326 nm	[21]
Green tea extract Copper(II) chloride	15–30 nm	Spherical	580 nm	[33]
<i>Ginko biloba</i> L. leaf Copper(II) chloride	15–20 nm	Spherical	560–580 nm	[34]
<i>Magnolia kobus</i> leaf Copper sulphate pentahydrate	45–110 nm	Spherical	560 nm	[35]
<i>Syzygium aromaticum</i> bud Copper(I) acetate	12 nm	Spherical	580 nm	[36]
<i>Azadirachta indica</i> leaf Copper(II) chloride	48 nm	Cubic	560 nm	[37]
Ripened <i>Duranta erecta</i> fruit Copper(II) sulphate pentahydrate	76 nm	Spherical	588 nm	[38]
Tilia extract Copper(II) sulphate pentahydrate	27.6 nm	Spherical	563 nm	[39]
<i>Punica granatum</i> peel Copper(II) sulphate pentahydrate	15–20 nm	Spherical	585 nm	[40]
Copper(II) sulphate pentahydrate Cetyl trimethyl ammonium bromide Extract of seedless dates	78 nm	Spherical	576 nm	[20]

4.2. Chemical Methods

Chemical methods are the most widely used to obtain copper nanoparticles. One major drawback is the use of toxic materials during the synthesis phase. Considering that nanoparticles are being used more frequently and have increasing human contact, it is essential to develop environmentally friendly processes [41].

Various chemical methods are used to obtain nanoparticles, such as sonochemical reduction [42], hydrothermal synthesis [43], electrochemical [44] and chemical reduction [45]. The latter is the most commonly used one. It involves using hydrazine, ascorbic acid or sodium borohydride as a reducing agent. The chemical reduction method is often used to obtain CuNPs because it is simple, has high yield efficiency and requires limited equipment.

Abdulkin et al. [46] used reducing agents (NaH_2PO_2 , N_2H_4 and NaBH_4) and polymeric capping agents and obtained CuNPs with sizes between 3 and 9 nm. The authors discovered that the lack of water in nanoparticle synthesis had a small beneficial effect on the stability of obtained nanoparticles.

In another recent study, Alonso et al. [47] used anhydrous copper (II) chloride (135 mg, 1.0 mmol) in a suspension of lithium powder (14 mg, 2.0 mmol) under an argon atmosphere to obtain CuNPs.

By varying the concentration of copper salts, the reducing agent, the pH of the solution and synthesis temperature, we obtain nanoparticles with different sizes, shapes and activity [25].

Table 4 presents various substances used to obtain copper nanoparticles. The obtained copper nanoparticles are characterized using UV-VIS and their size and shape are recorded.

Table 4. Chemical synthesis methods of copper nanoparticles.

Used Substances	Size	Shape	UV-VIS	References
Na ₂ CO ₃ Copper(II) sulphate pentahydrate Methanol 2,2 diphenyl-1-picrylhydrazyl hydrate	15–30 nm	Nearly spherical	380 nm	[48]
Copper (II) sulphate pentahydrate Ascorbic Acid Chitosan Hydrazine NaOH	35–75 nm	Spherical	589 nm	[49]
Copper(II) sulphate pentahydrate Ascorbic Acid	45 nm	Spherical	266 nm—100 °C 269 nm—300 °C	[50]
Copper(II) chloride Sodium dodecyl sulphate Hydrazine hydrate Ammonia solution	15–100 nm	Spherical	566 nm	[51]
Copper(II) nitrate Isopropyl alcohol Cetyl trimethyl ammonium bromide	16 nm	Spherical	551 nm	[52]
	23 nm	Hexagonal	572 nm	[52]
	37 nm	Spherical	553 nm	[52]
Copper(II) sulphate pentahydrate Polyethylene glycol Sodium borohydride Ascorbic acid	4–10 nm	Spherical	562 nm	[53]
Copper(II) sulphate pentahydrate Sodium borohydride Ascorbic acid Sodium hydroxide	10 nm	Cubic	320 nm	[54]

4.3. Physical Methods

The most important physical synthesis methods are evaporation–condensation and laser ablation.

Nanoparticles obtained through physical synthesis present no solvent contamination and have uniform distribution which is an improvement over chemical synthesis. Very small nanoparticles can be obtained using evaporation–condensation (6.2–21.5 nm and 1.23–1.88 nm) but the process requires a lot of energy to increase the operating temperature and it is time-consuming [55].

The nanoparticles obtained with laser ablation have characteristics depended on the wavelength of the laser, the duration of its pulses, the laser fluence, the ablation time and the liquid medium. One study obtained nanospheres (20–50 nm) in water with femtosecond laser pulses at 800 nm [55].

Some disadvantages of physical obtaining methods are the requirement of expensive equipment and high use of energy, thus making them less popular than chemical or green methods.

5. Copper Nanoparticles and Their Antibacterial Role

Major health organizations such as The World Organization for Animal Health, the Food and Agriculture Organization and the World Health Organization agree on the growing threat posed by antimicrobial-resistant organisms. Taking into account that antimicrobial drugs are widely used, the rate of antimicrobial resistance is continuously growing [56].

Research has shown that resistance to antibiotics is rising and a number of known antimicrobial agents encounter resistance by some microorganisms. As a result, there is no antimicrobial agent that has no resistance to microorganisms. This rise has forced clinicians to use in vitro antimicrobial susceptibility tests for diagnosis purposes. Obtaining nanoparticles with antimicrobial characteristics is a must and they can be further used to fight antimicrobial resistant microorganisms endangering human and animal health (Figure 1) [57].

The US Environmental protection Agency has recognized copper as the first solid antimicrobial element due to its antimicrobial activity. It was first used to sterilize wounds and water, and it was later discovered it developed immunity to cholera for copper workers [58]. Considering the availability of copper and the fact that it has similar properties to silver and gold has made copper a better alternative. Additionally, it has been found that copper not only possesses antibacterial properties, but also has antifungal characteristics, which reduce the development of various microorganisms—such as *E. coli*, as later described in this article [59]. Research shows that copper and its composites have been used both due to their low-cost and effective methods for sterilizing liquids, surfaces, materials, textiles and human tissue for centuries [60]. However promising copper nanoparticles may be in the future, their stable synthesis is still challenging at the moment, as they undergo rapid oxidation in air or aqueous media [61].

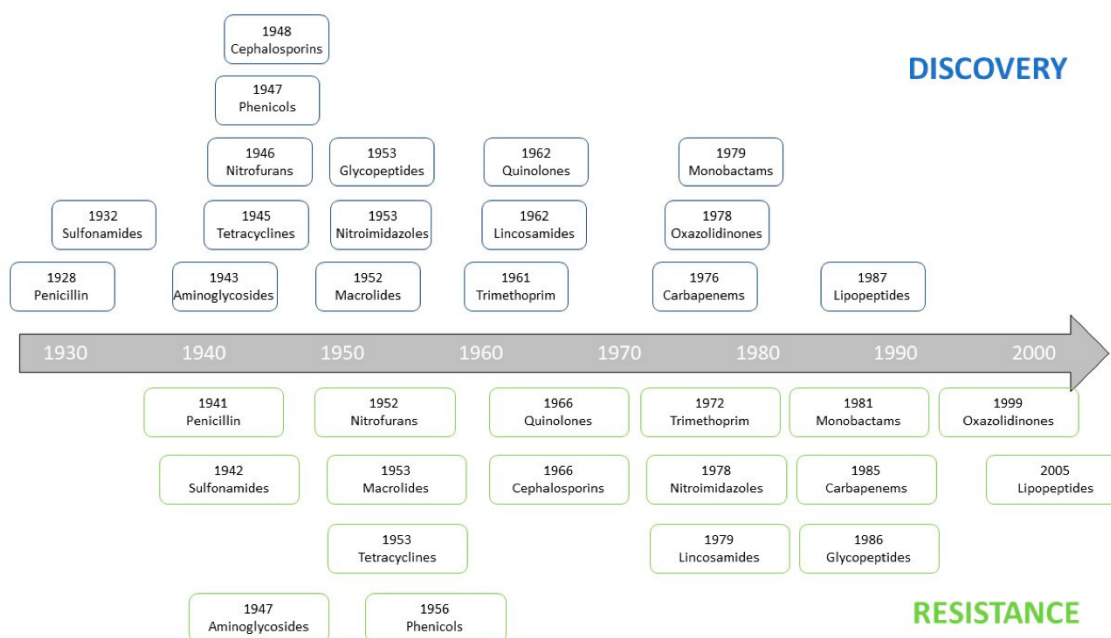


Figure 1. Antibiotics timeline [62].

5.1. Resistance to Antibiotics

At the beginning of 2017, the World Health Organization (WHO) published a list of a dozen bacteria which possess a very high resistance to antibiotics and who became threatening to human health. The bacteria were split into three categories, according to their urgency (Table 5).

Table 5. WHO criticality on pathogens and resistance to antibiotics.

Priority 1: Critical Urgency	Priority 2: High Urgency	Priority 3: Medium Urgency
	<i>Enterococcus</i>	
	<i>faecium</i> —vancomycin-resistant	
	<i>Staphylococcus</i>	
	<i>aureus</i> —methicillin-resistant, vancomycin intermediate and resistant	
<i>Acinetobacter</i>	<i>Helicobacter</i>	<i>Streptococcus</i>
<i>baumannii</i> —carbapenem resistant	<i>pylori</i> —clarithromycin-resistant	<i>pneumonia</i> —penicillin-non-susceptible
<i>Pseudomonas</i>	<i>Campylobacter</i>	<i>Haemophilus</i>
<i>aeruginosa</i> —carbapenem resistant	<i>spp.</i> —fluoroquinolone-resistant	<i>influenzae</i> —ampicillin-resistant
<i>Enterobacteriaceae</i> —carbapenem resistant, 3rd generation cephalosporin-resistant	<i>Salmonella</i>	<i>Shigella spp.</i> —fluoroquinolone-resistant
	<i>spp.</i> —fluoroquinolone-resistant	
	<i>Neisseria gonorrhoeae</i> —3rd generation cephalosporin-resistant, fluoroquinolone-resistant	

It is noticeable that the above list underlines the threat that Gram-negative bacteria pose, the *Enterobacteriaceae* in particular. From the total of 12 bacteria mentioned above, nine of them are Gram-negative: *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Helicobacter pylori*, *Campylobacter spp.*, *Salmonella spp.*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Shigella spp.*—and three of them are part of the *Enterobacteriaceae* family.

If we are to think about medication-resistant bacteria, Gram-negative are the bacteria which are most common in human infections. Researchers from the European Center for Disease Prevention and Control quantified Gram-negative bacteria being responsible for more than half a million infections out of a total number of over 650,000 and more than 24,000 deaths out of 33,000 during a time span of a year. On the other hand, the World Health Organization released communications mentioning that Gram-negative bacteria have developed and increased characteristics and abilities to find ways to increase resistance to treatments and transmit their genetic material to other bacteria, determining them to become resistant as well [62].

During the 1980s awareness emerged regarding the glycopeptide's (Vancomycin) resistance in Enterococci [63]. The main risk here was that there was a possibility for this resistance to transfer to Staphylococci as well—the *Staphylococcus aureus* infections already started to become a problem, at least in the United States. As a result, this was the first case of concern of antimicrobial resistance.

Moreover, since Gram-negative bacteria are more resistant and more numerous compared to Gram-positive bacteria, there are limitations in treating infections caused by GNBs.

This urgency has determined medical professionals to turn to old molecules to treat these drug-resistant bacteria infections, such as colistin. However, the situation improved during recent years, as other substances meeting the criteria of fighting against GNBs have emerged, among which are ceftolozane, ceftazidime and meropenem.

Since antimicrobial resistance is not a new subject and bacteria has always been able to adapt and develop, medicine was forced to react and research for new antibacterial molecules, such as copper- and silver nanoparticles. The role of copper nanoparticles against bacteria is discussed in the next section.

5.2. Mechanism of Action

Nanoparticles can reduce or stop the evolution of resistant bacteria because nanoparticles (NPs) target multiple biomolecules at once. Bacteria can be divided in two categories based on cell wall structure: Gram-positive and Gram-negative. Gram-negative bacteria have an additional outer membrane along a thin peptidoglycan layer in the cell wall, while the Gram-positive bacteria present only a thicker peptidoglycan layer in the cell wall.

Literature shows that Gram-positive bacteria have a higher resistance to the nanoparticle's mechanism of action. The assumption is that the difference in cell walls affects the bacteria's resistance. The Gram-positive bacteria present a thicker wall which acts as a protective layer which makes the interaction between nanoparticles and cell wall more negligible.

The additional outer layer of Gram-negative bacteria is coated in lipopolysaccharide, which has a negative charge. The ions released by nanoparticles have a positive charge, giving them a high affinity for the negative molecules present in the cell wall (Figure 2). This attraction leads to a buildup and intake of ions which lead to intracellular damage [64].

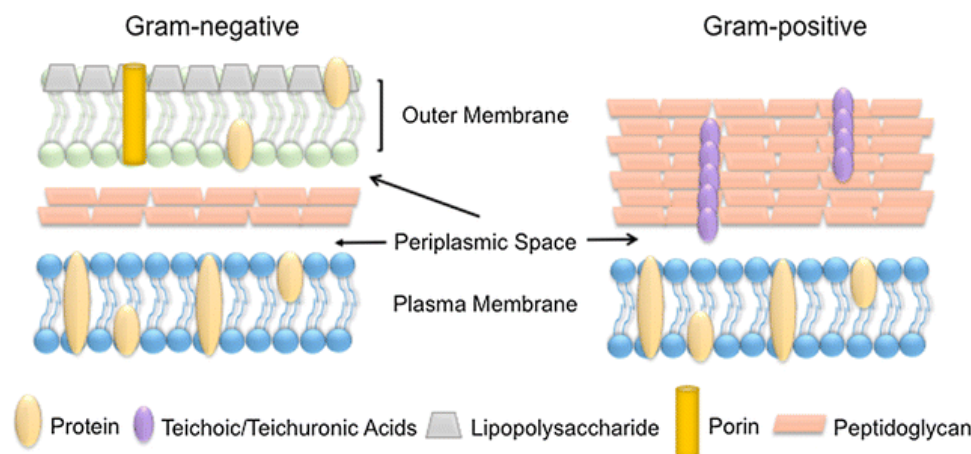


Figure 2. Gram-negative and positive bacteria [64].

Both types of bacteria have negatively-charged walls which influences the interaction between nanoparticles and the ions released. Studies with *Salmonella typhimurium*, a Gram-negative bacteria, show that the bacterium's cell wall is populated with a mosaic of anionic surfaces. This facilitates forming of areas with high concentration of nanoparticles binding to the cell wall which leads to an increased toxicity [64].

Besides the interaction of copper nanoparticles with bacteria's cells, copper ions can interact with DNA, intercalate with nucleic acid strands and can disrupt biochemical processes [65]. Ions of copper can also produce hydroxyl radicals which damage essential proteins [66].

It is worth remembering that bacteria accustomed to exposure to heavy metals show a higher resistance to metal nanoparticles such as TiO_2 , Al_2O_3 and carbon nanotubes. As an example, *E. coli* and *Cupriavidus metallidurans*, which are Gram-negative bacteria, *E. coli* was killed by all prior mentioned nanoparticles, while *Cupriavidus metallidurans* was resistant [64].

5.3. Comparison between the Antibacterial Activity of Metallic Nanoparticles

Research has focused on several metal nanoparticles that have antimicrobial activity, such as silver, copper and gold nanoparticles. Studies have shown these nanoparticles have antimicrobial activity against both Gram-positive and -negative bacteria such as *Staphylococcus aureus* and *Escherichia coli*, respectively [67,68].

Besides their antimicrobial activity on the aforementioned bacteria, these metals also influence *Bacillus subtilis* and are frequently used in medicine, dental materials, water treatment and even sunscreen lotions.

Most often, it is copper and silver nanoparticles that are compared because of their low cost, their stability from a physical and chemical standpoint and easiness of fusing with polymers [69].

While comparing silver and copper's effect from a bactericidal effect on various microbial strains, three types of bacteria stood out—*E. coli*, *B. subtilis* and *S. aureus*. Their antimicrobial effect was measured considering the inhibition zone by establishing the minimum growth inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) (Table 6).

Table 6. Antimicrobial effect of Ag and Cu nanoparticles on various microorganisms [68].

Culture	Strain No.	MIC (µg/mL)		MBC (µg/mL)	
		Ag	Cu	Ag	Cu
<i>Escherichia coli</i>	MTCC 443	40	140	60	160
<i>Escherichia coli</i>	MTCC 739	180	220	220	260
<i>Escherichia coli</i>	MTCC 1302	120	200	160	220
<i>Escherichia coli</i>	MTCC 1687	140	280	180	300
<i>Bacillus subtilis</i>	MTCC 441	40	20	60	40
<i>Staphylococcus aureus</i>	NCIM 2079	120	140	160	160
<i>Staphylococcus aureus</i>	NCIM 5021	120	140	160	160
<i>Staphylococcus aureus</i>	NCIM 5022	120	140	160	160

All the *S. aureus* strains presented the same level of sensitivity to silver and copper nanoparticles and no difference in strains was observed. However, while comparing *E. coli* with *S. aureus* it was observed the difference of sensitivity is less for silver as opposed to copper.

Similar results were found by Yoon et al. who reported their findings on the antibacterial effects of silver and copper nanoparticles against *E. coli* and *B. subtilis*, where in that case too, the copper materials proved to have superior antibacterial effect compared to the silver alloys [70].

Other studies have also shown the effects of copper and zinc nanoparticles as bactericidal agents. With this in mind, Sierra et al. discovered a high antibacterial effect against *S. mutans* of AgNP at a lower concentration than Au or Zn, for example, which would allow obtaining great results at a lower toxicity rate [71].

Nonetheless, copper is one of the least studied materials in terms of its antibacterial properties due to its increased instability in terms of oxidation and that it often forms complexes in relation with water molecules in aqueous environment [72].

5.4. Antifungal

For many years copper has been used as a material in the manufacturing process of pesticides, fungicides and fertilizers. Given the latest technology developments, it has been claimed that copper nanoparticles can be used as a good fungicide.

Studies reported that CuNPs can be used as fungicide against a broad range of plant fungi, such as *Fusarium* sp., *Phoma destructiva*, *Curcularia lunata*, *Alternaria alternate*, *Fusarium oxysporum*, *Penicillium italicum*, *Penicillium digitatum* and *Rhizoctonia solani* [73].

For example, CuNPs synthesized by a green method consisting of a chemical reduction of copper ions with ascorbic acid exhibit antifungal activity against *F. solani*, *Neofusicoccum* sp. and *F. Oxysporum*. The antifungal activity involved damages to the fungus' cell membranes and the intracellular production of ROS. Hence, this was a way of conducting an easy and cheap production method of CuNPs with high antifungal activity. Thus, copper can be used to control and damage threatening fungi to crop and forest species.

5.5. Antiviral

Several reports mention the antiviral activity of copper nanoparticles, meaning that the metal nanoparticles also have promising antiviral activities [17]. Fujimori et al. (2012) studied the antiviral potential of copper iodide particles with an average size of 160 nm against an influenza virus of swine origin (H1N1 pandemic in 2009) using plaque titration assay. They reported a dose-dependent activity of virus titer and found that the 50% effective concentration was approximately 17 g/mL within a 60-min exposure time. A later analysis shown the virus being inactive because of the virus proteins being degraded—hemagglutinin and neuraminidase—by copper iodide particles.

Thus, the authors concluded that these nanoparticles could be used to protect against viruses and may even be used to produce face masks, kitchen cloths or protection filters.

5.6. Anticancer

Aside from many different applications of copper nanoparticles, they have shown specific drug transport capabilities and high-efficiency photoluminescence capabilities, making them important materials for targeted delivery of imaging agents and anti-cancer drugs [17].

In their study of DNA potential degradation and anti-cancer activity of CuNP, the authors found that isolated DNA molecules' degradation is dose-dependent of copper nanoparticles by generating singlet oxygen [74]. Moreover, they observed that copper nanoparticles also have a cytotoxic effect on U937 and HeLa cells of histiocytic lymphoma in humans and cervical cancer origins by inducing apoptosis. They also mentioned that the named nanoparticles are able to degrade DNA even if other external agents are missing—such as hydrogen peroxide or ascorbate—in a single oxygen-mediated manner. Hence, copper nanoparticles are considered as good candidates for targeted therapy. Another advantage is the fact that the human body is able to handle the copper nanoparticles' metabolism since it is a micronutrient.

However, further developments are needed so as to advance the concept of nanoparticles technology into a practical application of the next generation of drug delivery system [75].

6. Copper Nanoparticles' Toxicity

Considering copper nanoparticles started being used as additives in lubricants, polymers, coatings, metal inks and others, we need to understand if these nanoparticles might have a negative impact on the environment, animals and humans.

A comprehensive understanding on how CuNPs induced adverse effects would be needed so that we can correctly assess the risks and then expand their safely use. More effort should be put into developing techniques to help understand the toxicity induced by CuO nanoparticles or dissolved Cu^{2+} . It is also important to reduce the toxicity by changing parameters such as particle size, surface characteristics and regulating the release of copper ions.

Copper's worldwide production was estimated to be 200 tons in 2010 and 570 in 2014 and it is estimated that by 2025 the production will reach 1600 tons. Once it reaches the environment, exposure and transfer between various organisms will increase.

6.1. Mechanism of Toxicity

The available literature discusses a few toxicity mechanisms such as: oxidative stress, DNA damage, lipid peroxidation, membrane damage, mitochondrial damage, possible leaching of meta ions and dissolution. The mechanism of action for CuO NPs is presented in Figure 3.

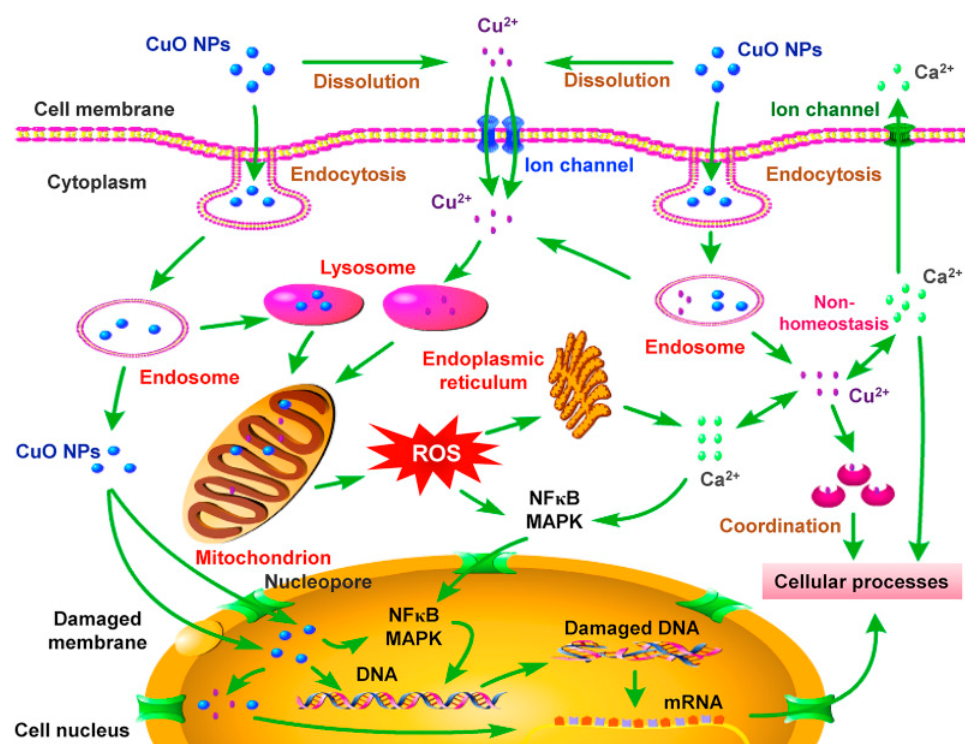


Figure 3. CuO NPs mechanism of action [76].

Reactive oxygen species (ROS) generation and oxidative stress are common toxicological mechanisms for cell damage induced by nanoparticles [77]. Small quantities of CuO nanoparticles are able to generate large quantities of ROS such as $O_2^{\cdot-}$, OH and H_2O_2 [78]. Once CuO NPs enter the mitochondria, they trigger membrane perturbing and ROS generation.

Particle dissolution is correlated with particle size, surface area, chemical composition, pH, temperature and organic matter. The CuO NPs that enter the nucleic acid can release more Cu^{2+} that lead to oxidative damage and DNA damage.

6.2. Toxicity in Microorganisms

Microorganisms are responsible of decomposing organic matter. They play an important role in food chains and it is important to understand the effects of CuNPs on microorganisms. Huo et al. [76] studied the toxicity of nano and bulk CuO to microorganisms. The authors concluded that the nano sized CuO nanoparticles are more toxic than the bulk format. The results are summarized in Table 7.

Table 7. Toxicity of nano and bulk CuO nanoparticles.

Nano CuO NP	Bulk CuO	Microorganism	Notes
21.6 mg/L	2031 mg/L	<i>Saccharomyces cerevisiae</i>	8 h exposure EC_{50}
20.7 mg/L	1297 mg/L	<i>Saccharomyces cerevisiae</i>	8 h exposure EC_{50}
13.4 mg/L	873 mg/L	<i>Saccharomyces cerevisiae</i>	24 h exposure EC_{50}
68 mg/L	3894 mg/L	<i>Vibrio fischeri</i>	30 min EC_{50}

Huo et al. [76] focused next on the impact of exposure method and medium on the toxicity of CuO nanoparticles. The authors found that the toxicity varies with the nanoparticle's aggregation, electrostatic repulsion or ion dissolution due to the chemistry of the exposure medium.

Huo et al. [76] reviewed a number of studies to understand algae toxicity. The authors conclude that copper nanoparticles are again more toxic than the bulk CuO form. It is also worth mentioning that stronger toxicity is related to CuO nanoparticles dissolution.

6.3. Toxicity in Animals

Most recently, Copper nanoparticles have also been studied in rapport with animals, namely how copper nanoparticles supported on titania might impact and influence the porcine ovarian cells [79]. The study concluded that the copper nanoparticles not only had a benefic impact on the granulosa ovarian cells, but also increased ovarian cell proliferation, turnover, viability and hormone release. Since the standalone copper nanoparticles are generally considered to possess increased toxicity, the potential application of CuNPs/TiO₂ could be a safe replacement of toxic CuNPs and a novel bio stimulating agent of reproductive processes in animals.

On the other hand, if we are to analyze the impact of copper nanoparticles effect on liver in rats, a high dose of nano-copper can cause serious liver damage, as levels of AST and ALP increased significantly. CuNPs can breach different biological zones, enter blood circulation and accumulate in liver—oxidative stress being the main cause of organ cytotoxicity caused by nano-copper [80].

6.4. Toxicity in Humans

The four main ways of human exposure to nanoparticles are inhalation, dermal penetration, ocular exposure and ingestion [81]. Depending on particle size, nanoparticles are able to remain in the lung tissue and can cause enhanced oxidative stress and inflammatory responses due to irritation. The authors have determined that copper nanoparticles (23.5 nm) were more toxic than microparticles (17 µm) because nanoparticles are able to penetrate the body more easily [17].

Individual copper nanoparticles are able to move between cells or permeate across cellular membranes and eventually enter the blood stream. The circulatory system then plays an important role in spreading the nanoparticles from the initial site of exposure to different parts of the body and eventually accumulating in organs.

Rhode et al. investigated the toxicity levels between CuNPs, CuO NPs and soluble CuCl₂ in leukemic cell line HL60 [82]. They show that CuNPs induced higher toxicity compared to the other two. CuNPs released more ionic copper than CuO NPs and conclude that the higher toxicity is attributed to a combination of both nanoparticles and copper ions. After 2 h of exposure, DNA oxidation, intracellular ROS and mitochondrial damage was observed. Eventually cell death was determined to be through necrosis.

Laha et al. show that CuO NPs induce cell time and dose dependent autophagy against human breast cancer cells with the formation of autophagolysosomes [83].

7. Applications and Prospects

Two fields where copper nanoparticles can be used to prevent infections and diseases are hospitals—as seen below in Figure 4—and transportation. Copper nanoparticles can be used in wall coatings, clothing, equipment and bedding to help fight against the spread of infections especially against microorganisms that have developed a resistance to standard antibiotics and disinfectant solutions [67].

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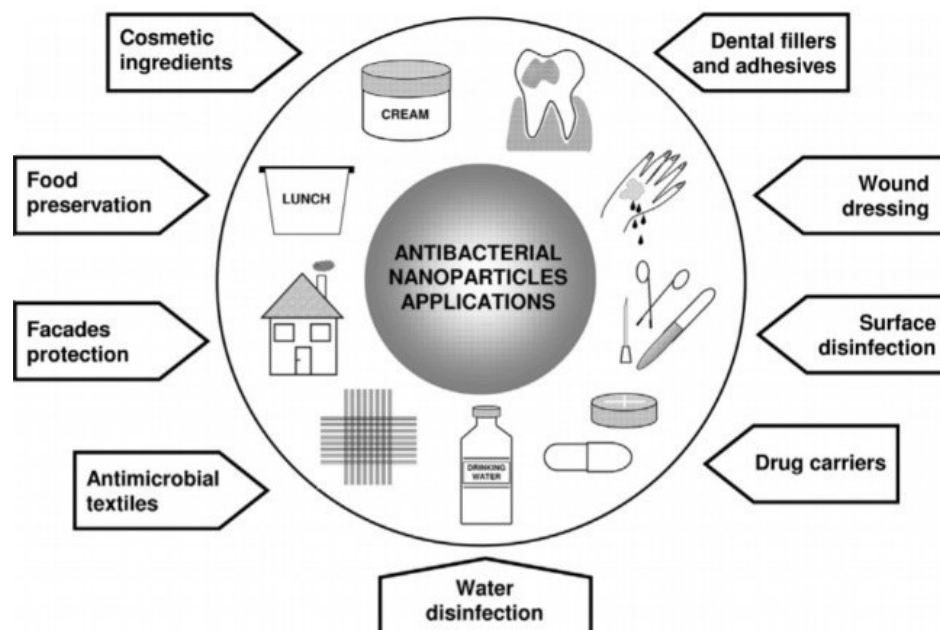


Figure 4. Antibacterial nanoparticles applications [84].

Agarose polymer films with CuNPs can be used with great success in antimicrobial filters and coatings. Agarose can form gels and films; therefore, the resulted materials can be successfully used as food packaging, sanitation and fabrics [85].

Another use for materials improved with CuNPs is to extend food shelf life and improve food quality by using biopolymer films such as chitosan. They are effective in reducing fluid concentration of two microorganisms that affect food quality [86].

Fibers such as cotton cellulose loaded with CuNPs can be used as wound dressing, personal care products, protective suits, different clothing and also in military and bio-defense [87].

Copper nanoparticles were demonstrated to be effective in fighting a range of bacterial pathogens and to stop infections. The number of potential applications is enormous, and it allows for further development of this field of study. For example, in combination with other nanoparticles, e.g., AgNp, copper nanoparticles have demonstrated an important effect in curing bovine mastitis [88]. Further studies need to be conducted in order to assess the safe use of copper nanoparticles, considering their potential toxicity.

Additionally, copper nanoparticles may also be considered for agricultural purposes—for the growth of wheat crops, in order to match the high demand given by the increase in population. Thus far, it has been demonstrated that the application of 30 parts per million on the soil may increase the growth of wheat crop. Nonetheless, extensive research is needed in order to determine what is the best concentration, application mode and time [89].

Given copper nanoparticles' high oxidation rates, recent attention has been given to copper nanoparticles carbon encapsulation—CECNp. They may be able to resist oxidation and temperatures of up to 900 °C and also show good compatibility with organic materials, making them feasible to use as conductive fillers, electromagnetic wave shielding materials and catalysts [26].

Using stabilized or polymer dispersed CuNPs has better control on ion releases. Another aspect that helps with better control is if the nanoparticles are interacting with the polymer or further stabilization using a core shell structure [90].

8. Conclusions

A selection of studies on copper nanoparticles, their antimicrobial activity and toxicity, and potential uses have been reviewed in the present paper. We have looked at how we can obtain copper nanoparticles using various methods and compared copper nanoparticles with other metallic nanoparticles. Given copper's unique properties—antibacterial, antifungal, anticancer—and high availability, additional studies on its toxicity and stable synthesis would need to be performed in order to better understand its potential applications and effects. As the current paper focuses on copper nanoparticles, we have looked at how copper nanoparticles can be obtained using green, chemical and physical methods. For each of the methods, based on the researched materials, we noted the used materials and the resulted nanoparticles with their corresponding properties.

As bacteria can be divided in two categories based on cell wall structure: Gram-positive and Gram-negative, Gram-positive bacteria have a higher resistance to the nanoparticle's mechanism of action. On the other hand, Gram-negative bacteria possess an increased risk, considering the majority of human infection and deaths are caused by it. Given their increasing drug resistance, further efforts are needed to develop ways to counter this resistance.

Copper nanoparticles interact with the bacteria's cell wall, DNA, nucleic acid. Nanoparticles are responsible for ROS generation and oxidative stress. This eventually leads to cell death.

We then discussed the impact of various metals on two bacteria: *Escherichia coli* and *Staphylococcus aureus*. For their antimicrobial activity, we have looked at MIC and MBC. Copper nanoparticles are prone to oxidation, therefore further studies should look into the antibacterial activity of CuNPs and not on CuO particles.

Copper nanoparticles can be successfully used to combat different fungi, to treat viral diseases and as a delivery system for cancers. The benefit is that the human body is already capable of digesting copper nanoparticles; however, future work is still needed. However, copper nanoparticles also have levels of toxicity which might limit their use. Further studies can look at how varying particles' characteristics affects the toxicity and delivery of copper ions.

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