



Chemical synthesis of copper oxide nanoparticles and evaluation of antibacterial activity

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Abstract

Nanotechnology is presently one of the most dynamic disciplines of research in contemporary material science. Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles. Physical and chemical methods have been using high radiation and highly concentrated reductants and stabilizing agents that are harmful to environmental and to human health. Hence, biological synthesis of nanoparticles is a single step bio reduction method and less energy is used to synthesize eco-friendly NPs. The present study included the chemical reduction of copper acetate through glucose and testing for their antimicrobial activity. The aqueous copper acetate mixed with the reducing agent as glucose results in the synthesis of copper oxide nanoparticles proved by colour changes. Absorption spectrum of the incubated solution at different wavelengths ranging from 300 to 800 nm revealed a peak at 540 nm. High resolution scanning electron microscopic (SEM) image of Copper oxide nanoparticles (CuONPs). Spherical shaped CuNPs size at 42.28 nm. *Bacillus subtilis* bacteria strain was qualitatively assessed by the presence of inhibition zones (30µl: 5.50±0.38, 60µl: 7.50±0.52 and Standard 8.50±0.59). It is confirmed that copper oxide nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against bacterial diseases. Applications of CuO nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

Keywords: copper oxide nanoparticles, chemical synthesis, *Bacillus subtilis*, antibacterial

Introduction

Nanotechnology is a multidisciplinary field, as it combines the knowledge from different disciplines: chemistry, physics and biology amongst others (Schmid, 2006; Schmid, 2010) [1, 2]. The term "Nanoparticles" is used to describe a particle with size in the range of 1nm - 100nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles change in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc (Shakeel Ahmed *et al.*, 2016) [3]. The most successfully studied nanoparticles today are those made from noble metals, in particular Cu (copper), Ag (Silver), Ti (Titanium), Pt (Platinum), Au (Gold) and Pd (Palladium). Nanoparticles of noble metals, such as copper, gold, silver, and platinum, are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications (Ankanna *et al.*, 2010) [4]. Copper oxide is a compound from two elements copper and oxygen, which are block d and block p elements in periodic table respectively. In a crystal copper ion is coordinated by four oxygen ions. Copper (Cu) and copper oxide (Cu₂O) nanoparticles have attracted considerable attention because copper is one of the most important in modern technologies and is readily available (Xi *et al.*, 2010) [5]. Copper oxide is widely used in the field

of catalysis, superconductors, and ceramics as a kind of important inorganic materials. The particle size of nanometre copper oxide is between 1-100 nm (Motogoshi *et al.*, 2010) [6]. Compared with the ordinary copper oxide, nano CuO has peculiar physical and chemical properties such as: surface effect, superiority of the quantum size effect, volume effect and macroscopic quantum tunneling effect in magnetic, optical absorption, chemical activity and thermal resistance, catalysis, and the melting point. Nano copper oxide attracts more and more people's attention, and become one of the most extensively used inorganic materials (Suleiman *et al.*, 2013) [7]. In the present study aimed to synthesis and characterization of copper oxide nanoparticles using glucose.

Materials and methods

Chemicals

Copper oxide and glucose were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Synthesis of copper oxide nanoparticles

Copper oxide nanoparticles synthesized by the method of Hema *et al.*, (2013) [8]. A quantity of 0.01M copper acetate was dissolved in 20 mL of deionized water and subjected to stirring at a temperature of 45°C. Then 0.001M glucose was dissolved in 2 mL of deionized water which was then added drop wise into the reaction media with a feed rate of 0.42 mL/min to maintain the ratio of copper acetate and glucose as 1:4. The stirring was continued for 1 h. The temperature

of 45°C was maintained till the end of the reaction. After stirring maintained the pH 9.5. To adjust the pH, 1 M solution of NaOH in deionized water was added and kept for 30 minutes. Drying was carried out using a conventional oven at 110°C for 24 h to obtain copper oxide nanoparticles.

UV Visible spectroscopic analysis

The reduction of pure metal ions was examined under UV visible analysis. For UV spectrophotometer analysis, the sample is diluted to 1:10 with the deionized water. The reduction of pure metal ions were scanned in the wavelength ranging from 340-800 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. The peak values of the UV were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

Scanning Electron microscopy (SEM) Analysis

The particle size and morphology of nanoparticles were analysed by VEGA3-TESCAN SEM machine. The dried form of copper oxide nanoparticles were sonicated with distilled water, small droplet of copper oxide nanoparticles were placed on glass slide and permitted to dry. The ZEEISS-SEM machine was worked at a vacuum of the order of 10⁻⁵ torr. The accelerating voltage is 10 kV.

Determination of antibacterial activity

The antibacterial activity was performed by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) ^[9, 10].

Results and discussion

Nanophysics is commonly referred to as an intuition of Richard Feynman; in a famous conference of December 1959 by California Institute of Technology, he made forecasts around the possibility to control the matter and to realise devices at atomic scale, anticipating a great fields variety of scientific research and technical applications, that currently appear to be well developed (Di Sia, 2015) ^[11].

Synthesis of Copper oxide nanoparticles

The aqueous metal ion precursors from metal salts are reduced and as a result a colour change occurs in the reaction mixture. This is the first qualitative indication that nanoparticles are being formed. The intensity of color is directly proportional tonanoparticles production. The aqueous Copper acetate when exposed to glucose was reduced in solution, there by leading to the formation of Copper oxide hydrosol. During the visual observation, copper acetate and glucose stirred magnetically showed the green mixture after 1hr. The appearance of bluish green color is clear indication for the development of water soluble monodispersed copper oxide nanoparticles.

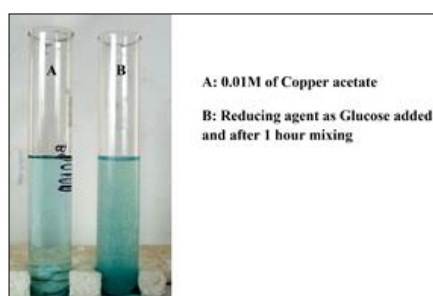


Fig 1: Processing of Copper oxide nanoparticle (CuONPs)

The time duration of change in colour varies from chemical to chemical. It is well known that Copper nanoparticles in aqueous solution due to excitation of surface plasmon vibrations in Copper nanoparticles (Mamun *et al.*, 2012) ^[12].

UV Visible spectroscopic analysis of Copper oxide nanoparticles

Ultraviolet/Visible/Infrared (UV/Vis/IR) spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample (a quantity known as the extinction, which is defined as the sum of absorbed and scattered light). In its simplest form, a sample is placed between a light source and a photo detector, and the intensity of a beam of light is measured before and after passing through the sample. These measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a "blank" – a cuvette filled with only the dispersing medium – to guarantee that spectral features from the solvent are not included in the sample extinction spectrum. Nanoparticles have optical properties that are sensitive to size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV/Vis/IR spectroscopy a valuable tool for identifying, characterizing, and studying these materials. Nanoparticles made from certain metals strongly interact with specific wavelengths of light and the unique optical properties of these materials is the foundation for the field of plasmonics. At nanocomposite we have developed numerical modeling algorithms that can be used to predict the optical properties of various nanoparticles allowing for comparison between theoretical and measured properties. Metal nanoparticles have free electrons, which yield a surface plasmon resonance (SPR) absorption band, due to the mutual vibration of electrons of metal nanoparticles in resonance with light wave. The appearances of the peaks show the characteristics of surface plasmon resonance of copper oxide nanoparticles. The band gap energy was determined based on the numerical derivative of the optical absorption coefficient. The fundamental absorption method refers to band to band transitions by using energy relation.

$$E = h \nu$$

Where h is the planks constant, $\nu = c/\lambda$, where c is the speed of light in vacuum and λ is the wave length of the spectrum. The relative percentage of scatter or absorption from the measured extinction spectrum depends on the size, shape, composition and aggregation state of nanoparticles. Metal nanoparticles may absorb light, scatter light, or both. As a general rule, smaller particles will have a higher percentage of their extinction due to absorption. For example, light scattering by small (2 nm diameter) particles is negligible; the optical spectrum of the nanoparticles, shown below in Figure 2, is almost entirely due to photon absorption by the metal.

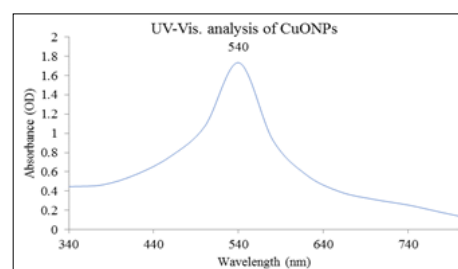


Fig 2: UV Visible spectrum of copper oxide nanoparticles

Absorption spectrum of the incubated solution at different wavelengths ranging from 300 to 800 nm revealed a peak at 540 nm. The UV-visible spectroscopy covers the UV range between 190 and 380 nm and the visible range between 380 and 800 nm. Both types of radiation interact with matter and promote electronic transitions from the ground state to higher energy states. Wavelengths between 300 and 800 nm are generally used for characterizing metallic nanoparticles ranging in size from 2 nm up to around 100 nm (Poinern, 2014) [13].

Scanning Electron Microscopic (SEM) analysis of copper oxide nanoparticles

Scanning electron microscopy (SEM) is one of the most popular and widely used techniques for the characterization of nanomaterials and nanostructures. SEM can be effectively used to characterize specimens down to a resolution of a few nanometres, with image magnifications achievable in the range of ~10 to over 300,000. In addition to information on surface topography, SEM can also provide useful information on chemistry, crystal orientation and internal stress distribution. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity. SEM can produce very high-resolution images of a sample surface, revealing details about less than 1 to 5 nm in size. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample. Under vacuum, electrons generated by a source are accelerated in a field gradient. The beam passes through Electromagnetic Lenses, focusing onto the specimen. As result of this bombardment different types of electrons are emitted from the specimen. A detector catches the secondary electrons and an image of the sample surface is constructed by comparing the intensity of these secondary electrons to the scanning primary electron beam. Finally the image is displayed on a monitor 42.28 nm.

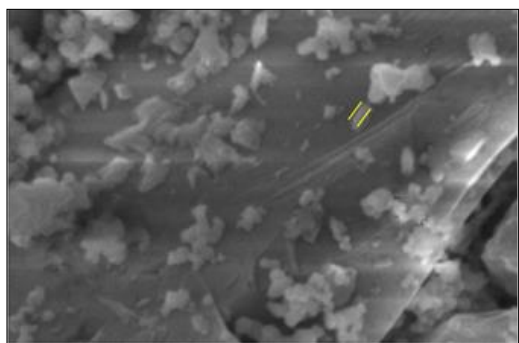


Fig 3: High resolution scanning electron microscopic (SEM) image of Copper oxide nanoparticles (CuONPs). Spherical shaped CuONPs size at 42.28 nm

Anti-bacterial Activity of Copper oxide nanoparticles

Microbes are truly the most underappreciated living organisms on Planet Earth. Billions of them can fit on a fingernail, and they make up more than half of the living biomass on the planet. The world we live in is one full of microbes. Microbes, whether they are good, bad, or benign, are certainly everywhere. This includes on our body, in our homes, far below the earth's surface and up to the atmosphere, in cold, cool, warm and hot and very hot places, and even in places without oxygen. Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. Microorganisms always

live in water (directly in aquatic environments, in water inside animals or plants, or in water around soil particles). They can eat all sorts of things, including oil, rocks, dead and living plants and animals (Needham, 2000) [14]. There are 4 major types of Microbes: bacteria, fungi, protists and viruses (Lynch and Hobbie, 1988) [15].

The *in vitro* antibacterial activity of the copper nanoparticles against these *Bacillus subtilis* bacteria strain was qualitatively assessed by the presence of inhibition zones (30µl: 5.50±0.38, 60µl: 7.50±0.52 and Standard 8.50±0.59) represented in the photographic fig 4. The inhibitory activities in culture media of the copper nanoparticles reported in Table 1 were comparable with standard antimicrobial *viz.* Chloramphenicol.

Table 1: Antibacterial Activity of Copper oxide nanoparticles

Microbial strain	CuONPs		Std. (30µl)
	30µl	60µl	
<i>Bacillus subtilis</i> (mm)	5.50±0.38	7.50±0.52	8.50±0.59

Values are expressed Mean ± SD for triplicates; Std. as Chloramphenicol

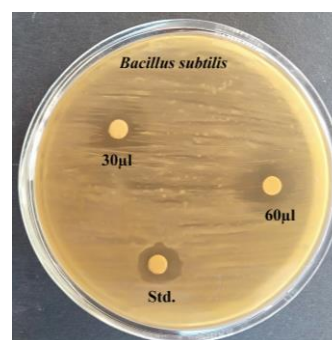


Fig 4: Antibacterial Activity of Copper oxide nanoparticles

Conclusion

It is confirmed that copper oxide nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against bacterial diseases.

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