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SYNTHESIS OF COPPER NANOPARTICLES USING TRISODIUM CITRATE AND EVALUATION OF ANTIBACTERIAL ACTIVITY

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ABSTRACT: Nanotechnology is a multidisciplinary field, as it combines the knowledge from different disciplines: chemistry, physics, and biology amongst others. It is widely agreed that nanoparticles are clusters of atoms in the size range of 1–100 nm. Metal nanoparticles can be prepared by two routes, the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution are reduced in conditions favoring the subsequent formation of small metal clusters or aggregates. The present study included the chemical reduction of copper sulphate through trisodium citrate and testing for their antimicrobial activity. The aqueous copper sulphate exposed to the trisodium citrate results in the synthesis of copper nanoparticles, it was confirmed by the formation of brown colour. The pH and viscosity of the copper nanoparticles monitored and indicates the stability of the particles. Synthesized copper nanoparticles further confirmed in UV Visible spectrum and FTIR. The SEM analysis showed the particle size between 44.50 nm as well the spherical structure of the nanoparticles. Proven antibacterial activity of copper nanoparticles (CuNPs) against *E. coli* established.

KEYWORDS: Copper nanoparticles. Antibacterial activity, Copper sulphate, Trisodium citrate.

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1. INTRODUCTION

Nanotechnology is a multidisciplinary field, as it combines the knowledge from different disciplines: chemistry, physics, and biology amongst others [1,2]. Nanotechnology is the arts and science of manipulating matter at the atomic or molecular scale and holds the promise of providing significant improvements in technologies for protecting the environment [3,4]. Metal nanoparticles, due to their special properties and also small dimensions, find important applications in optical, magnetic, thermal, sensoric devices, catalysis, etc. Many metal nano particles are under active research because they posses interesting physical properties differing considerably from that of the bulk phase. The most common method employed for the synthesis of metal nanoparticles is the reduction of metal ions in solution [5,6]. The technology has excellent prospects for exploitation across the medical, pharmaceutical, biotechnology, engineering, manufacturing, telecommunications and information technology markets. Copper nanoparticles, due to their excellent physical and chemical properties and low cost of preparation, have been of great interest [7,8]. Copper nanoparticles have wide applications as heat transfer systems, antimicrobial materials, super strong materials, sensors and catalysts [9]. Copper nanoparticles are very reactive because of their high surface-to-volume ratio and can easily interact with other particles [10]. In the present study the synthesis of copper nanoparticles using Tri sodium citrate as reducing agent.

2. MATERIALS AND METHODS

Experimental

Copper sulphate ($CuSO_4$) and Tri sodium citrate ($Na_3C_6H_5O_7$) [Loba Chemi, India] have been used in the synthesis of Copper nanoparticles.

Preparation of copper nanostructures

To synthesize different-sized CuNPs, the spherical CuNPs were prepared according to the literature procedure by Fang *et al*. [11], by reducing aqueous $CuSO_4$ with sodium citrate at boiling temperature. In typical procedure, 40 ml of 0.001M $CuSO_4$ was heated to boiling. To this solution, 10 ml of 1% trisodium citrate was added drop by drop. The solution was heated at boiling point under continuous magnetic stirring for 30minutes. The solution was then cooled to room temperature. The reaction was allowed to take place for 24 hurs. The CuNPs in this solution were called citrate- CuNPs.

Characterization of copper sulphate nanoparticles

UV-Visible analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 340-960 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Fourier transform infrared Spectroscopy

To determine Fourier transform infra-red (FTIR) pattern of the $CuSO_4$ nanoparticles was freeze-dried and the dried powder was diluted with potassium bromide in the ratio of 1:100 and recorded the spectrum in Perkin Elmer FTIR Spectrum BX (Wellesley, MA, USA).

SEM analysis of copper nanoparticles

The scanning electron microscopy (SEM) analysis of freeze dried sample was performed by mounting nanoparticles on specimen stubs with double-sided adhesive tape and coated with platinum in a sputter coater and examined under VEGA3 SEM (Japan) at 10 kV.

Determination of antimicrobial activity

Antibiogram was done by disc diffusion method [12, 13] using samples. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from the culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing bacteria specie were spread on Nutrient agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude sample (50µl, 100µl, 150µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

3. RESULTS AND DISCUSSION

Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. Copper nanoparticles, due to their excellent physical and chemical properties and low cost of preparation, have been of great interest. Copper nanoparticles have wide applications as heat transfer systems, antimicrobial materials, super strong materials, sensors and catalysts. Copper nanoparticles are very reactive because of their high surface-to-volume ratio and can easily interact with other particles and increase their antimicrobial efficiency. In the present study to synthesize, characterization and antibacterial activity of copper nanoparticle using trisodium citrate.

Synthesis of Copper nanoparticles

The synthesis of Copper sulphate nanoparticles through trisodium citrate were carried out. Copper is used as reducing agent as Copper sulphate has distinctive properties such as good conductivity, catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). Copper sulphate and trisodium citrate were used as starting materials for the preparation of copper nanoparticles.

The copper colloid was prepared by using chemical reduction method [14]. The mechanism of reaction could be expressed as follows [15, 16]. The aqueous Copper when exposed to trisodium citrate was reduced in solution, there by leading to the formation of Copper sulphate hydrosol. The time duration of change in colour varies from chemical to chemical. It is well known that Copper nanoparticles exhibit in aqueous solution due to excitation of surface plasmon vibrations in nanoparticles [17] in Figure 1.

Figure 1: Processing of nanoparticle synthesis using magnetic stirrer

Ultraviolet/visible (UV/VIS) spectroscopy

The Copper nanoparticles shows a good transmittance in the visible region which enables it to be a good material for optoelectronic applications [18]. Spectroscopy is a tool for structure determination. It is essentially a technical procedure by which the energy differences between the allowed states of a system are measured by determining the frequencies of the corresponding light absorbed. The obtained spectra of the compound i.e. the response of a substance subjected to radiations of various wavelengths reveals the important properties of the compound [19]. UV-Vis-spectroscopy is used to measure the absorption or emission of radiation associated with the spatial distribution of electrons in atoms or molecules. For this purpose transmission is plotted against wavelength or frequency and is known as the transmission spectrum. The UV-Visible-transmission spectra were recorded using Systronic UV-Visible Spectrometer in the range 340 nm to 940 nm. Figures 2 show the transmission spectra Copper nanoparticles. From the spectra, it is observed that the show Copper nanoparticles good transmittance in the entire visible - regions.

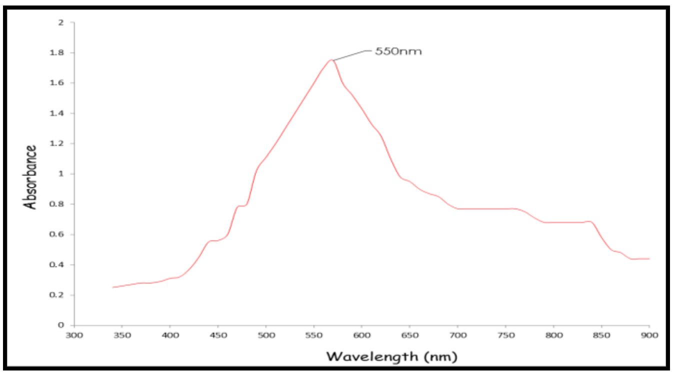

Figure 2: shows the UV Visible spectrum of CuNPs

The absorption spectra of CuNPs were compared with the absorption spectra of copper nanoparticles prepared using these extracts in order to reveal the formation of copper phyto-nanoparticles. The absorption spectra of copper nanoparticles were recorded after 24 hours after their preparation and exhibited absorbance peaks at 550 nm

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. Fourier Transform Infrared (FTIR) spectroscopy can effectively be used to measure the particle formation. It is found that the width and intensity of peaks in an IR spectrum have explicit dependence on the particle size. As particle size increases, the width of the peak decreases and intensity increases [20]. The FTIR spectra of reduced Copper sulphate show in the peaks of spectra in Figure 3. The band of carboxyl or carbonyl groups at 1660 to 1500 and 1390 to 1260 cm–1 region. This may be the reason for the reduction of the transmittance at this region in the case of spectrum of nanoparticles. The shift of the band from 1656 to 1586 indicates the formation of metal carbonyl groups. It is due to the stabilization of Cu nanoparticles by the –COO- group of trisodium citrate. This asymmetric shift can be comparable with the data presented by previous works [21]. According to them, when the citrate ligand bound to magnetite nanoparticles surfaces the antisymmetric stretching of -COO- at 1586 cm^{-1} almost remains the same but the symmetric -COO- stretching mode of citrate becomes redshifted and appears sharply at 1398 cm^{-1} [22].

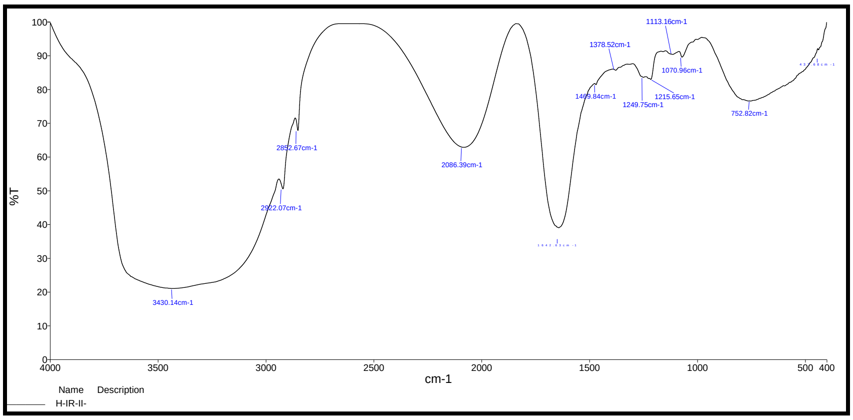

Figure 3: FTIR analysis of Copper nanoparticles

SEM analysis

SEM analysis was carried out to understand the topology and the size of the Cu-NPs, which showed the synthesis of higher density polydispersed spherical Cu -NPs of various sizes. The SEM image showing the high density copper nanoparticles synthesized by trisodium citrate further confirmed the development of copper nanostructures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM [23]. The SEM analysis showed the particle size 44.50 nm as well the spherical structure of the nanoparticles (Figure 4).

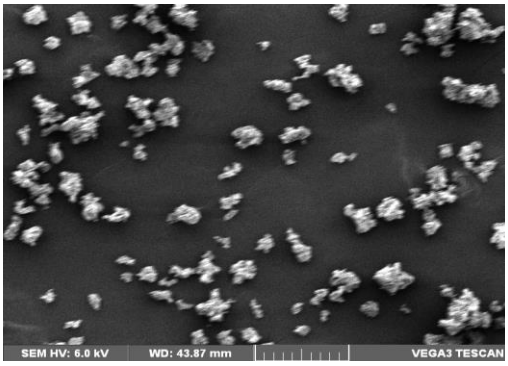

Figure 4: High resolution scanning electron microscopic (SEM) image of Copper nanoparticles (CuNPs). Spherical shaped CuNPs size at 44.50 nm

Antibacterial Activity of Copper 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 [24]. There are 4 major types of Microbes: bacteria, fungi, protists and viruses [25, 26]. The *in vitro* antimicrobial activity of the Copper nanoparticles against these bacteria was qualitatively assessed by the presence of inhibition zones represented in the photographic Figure 5. The inhibitory activities in culture media of the Copper nanoparticles reported in Table 1 were comparable with standard antimicrobiotic viz. Chloramphenicol.

Table 1: Antibacterial Activity of Copper nanoparticles

Microbe	50µl	100µl	150µl	Standard
Escherichia coli (mm)	5.23 ± 0.36	7.50 ±0.52	8.75 ± 0.61	9.25 ± 0.64

Values are expressed Mean ± SD for triplicates

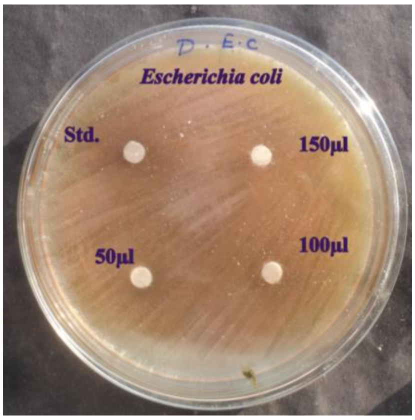

Figure 5: Antibacterial Activity of Copper nanoparticles

4. CONCLUSION

The present study exhibit a simple method of synthesis of copper nanoparticles from a novel primitive chemical source. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. Since the nanoparticles thus synthesized shows antimicrobial activity, they can be used in the field of pharmaceutical industry. Copper nanoparticles might be useful for the development of newer and more potent antimicrobial agents. All the above data's represented in our study contribute to a novel and unexplored area of nanomaterials as medicine.

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CONFLICT OF INTEREST

No conflict of interest

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