

A Chemical Reduction Synthesis And Characterization Of Copper Nanoparticles And Its Antibacterial Effect On Escherichia Coli Bacteria

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Abstract

In this paper Copper Nanoparticles have been synthesized by bottom up Chemical reduction route using Sodium Borohydride (NaBH_4) as a reducing agent and deionised water as solvent. X-ray diffraction, Raman spectroscopy, TEM, EDX measurements were taken to investigate the size, shape, lattice parameter, identification of compound present in sample, structure and elemental composition of synthesized copper nanoparticles. The initial formation of Cu NP's was confirmed by UV-visible spectrophotometer. The Ascorbic acid is used as antioxidants which prevent oxidation and agglomeration. The average sized of synthesized Cu nanoparticles by chemical reduction method is about 29 nm. The antibacterial activity of copper nanoparticles tested on E.coli bacteria.

Keywords: Chemical Reduction, TEM, EDX, XRD, UV-vis Spectrophotometer.

1. INTRODUCTION

Nanotechnology is the application of science and technology to control matter at the molecular level, which is also referred to as the ability for designing, production, characterization and application to structures, devices and systems by controlling shape and size at the nanometre scale.

Nanotechnology is the most promising technology that can be applied almost in all spheres of life, ranging from electronics, pharmaceutical, defense, transportations heat transfer [1] to sports and aesthetics. The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles usually referred to as particles with a size approximately extending from 1 nm up to 100 nm in length in at least one dimension exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The Cu NPs have special physical and chemical characteristics which include catalytic activity, optical properties, anti-microbial activity and electronic properties [2]. Copper nanoparticles can be fabricated by using different physical and chemical techniques such as chemical reduction [3, 4], laser ablation [5, 6],

electrochemical [4], thermal decomposition [7] and polyol method [8]. Copper nanoparticles due to their high surface to volume ratio are very reactive, can easily interact with other particles and increase their antimicrobial efficiency [9]. The shape, size and concentration of nanoparticles basically depend upon reaction kinetics, temperature, nature of capping agent, reducing agent concentration but also it strength and reaction media. In this paper we report a chemical reduction method to synthesized copper Nanoparticles. This method uses sodium borohydride and ascorbic acid as a reducing and capping agent. The obtained nanoparticle size in the range 29-30 nm. They were characterized by UV-vis spectroscopy, RAMAN, XRD and TEM.

2- EXPERIMENTAL

Materials

For present work we used analytical grade chemicals such as Copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), Ascorbic acid, sodium borohydride (NaBH_4), & Sodium hydroxide pellets (NaOH) were purchased from Merck India Pvt Ltd. All chemical were used as received without any purification.

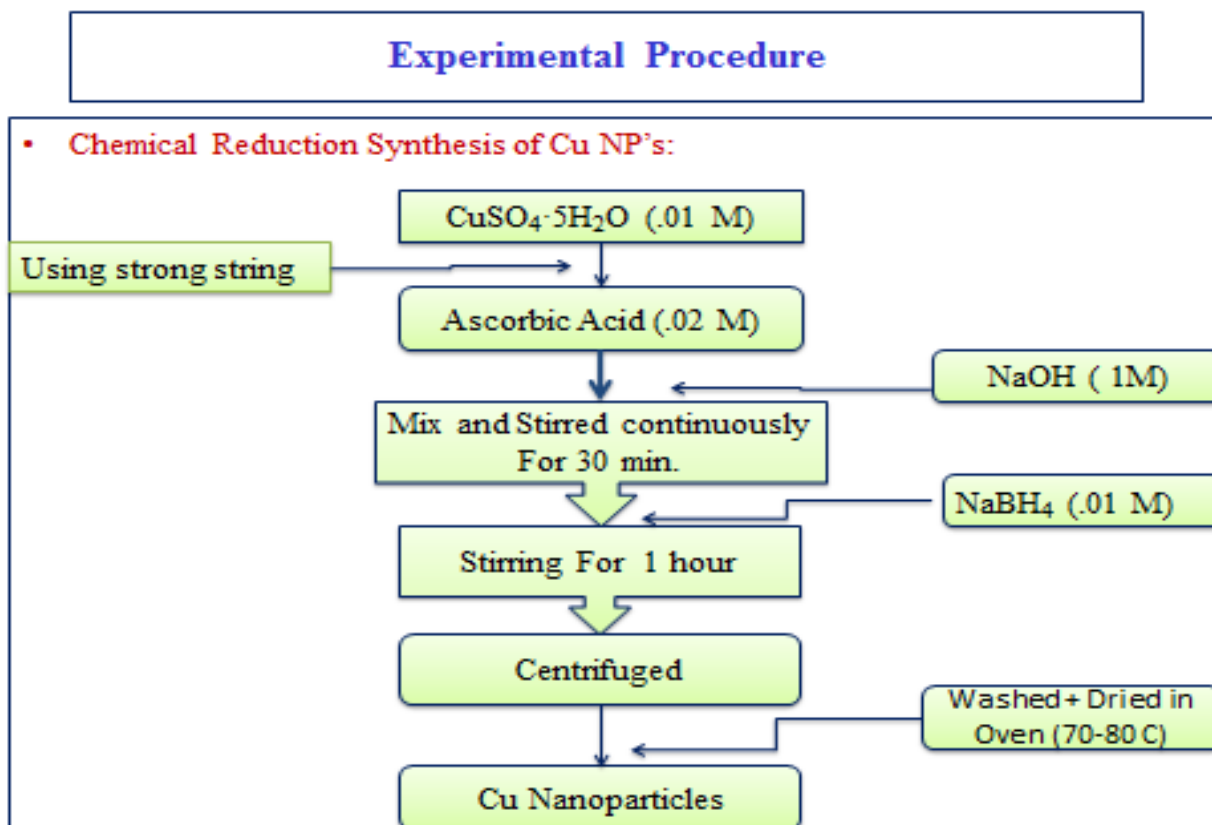
All solution was made with de-ionised water. Copper salts were used as a basic precursor,

NaBH₄ as a Reducing agent. NaOH was used to adjust PH between 9 to 12. Ascorbic acid used as antioxidant.

Synthesis of Cu Nanoparticles

In the synthesis procedure, copper nanoparticles were obtained by chemical reduction process using CuSO₄·5H₂O as precursor salt, ascorbic acid as a capping agent. The solution of all reactive material were prepared in de-ionised water. CuSO₄·5H₂O aqueous solution was prepared by dissolving (0.01M) in 100 ml deionised water also ascorbic acid solution (0.02M) was prepared in 100ml deionised water separately. The preparation starts with the addition of CuSO₄·5H₂O solution in ascorbic aqueous solution under continuous magnetic stirring. To adjust PH 1M solution of

NaOH in deionised water was added drop wise into the flask while stirring. After stirring for 30 min at 80°C 0.1M solution of NaBH₄ was added under continuous stirring for 1 hours. The color of the solution turned from initial blue to orange, orange, brown & finally black. After completion of reaction the solution was taken from stirrer and allowed to settle overnight. The resulting solution is centrifuged at 7000 Rpm for 5 min. The precipitates were separated from supernatant solution and washed with ethanol. Black color precipitates obtained and dried in oven at 70-80° C. After drying nanoparticles were stored in a plastic tube for further analysis. The flow chart of synthesis procedure is shown below:



Experimental procedure of synthesis of Cu NP's

Antibacterial Activity of copper nanoparticles on Escherichia coli bacteria

Preparation of media:

Mueller-Hinton Agar (MH) agar is a microbiological growth medium; it is generally in liquid state whereas MH broth is in solid state.

MH broth + Agar + distilled water = Media

Mueller-Hinton Agar (MH) agar preparation:

To prepare MH agar, 1.05 gm of MH broth and 1gm of agar were taken and dissolved in 50 ml of distilled water. Then this solution was kept on hot plate to dissolve agar in the solution. Afterwards the solution (media) was kept in petri plate to autoclave. In autoclave high pressures and temperature act on the solution, typical values of 15 atm. pressure and 137 degree temperature was set to achieve the final media.

Pouring of media:

After autoclave, process the whole work is carried in Laminar Air Flow (LAF) apparatus. In LAF the media is poured in the petri dish. Then the solution was left for some time to solidify for about 10 minutes. Then petri dish was sealed by Para film and left overnight.

On next day, E.coli (*Escherichia coli*) bacteria were spread in the petri plate with the help of loop. Then wells were made in petri dish, in which copper nanoparticle solution (0.01mg NPs dissolve in 5 ml deionized water) was introduced with the help of micropipette in amounts of 5 μ l, 10 μ l, 20 μ l. Then petri dish was kept in incubator at 37 $^{\circ}$ C for 24 hour.

3- RESULT AND DISCUSSION

(1) U-V visible analysis: UV-Vis absorbance spectroscopy has proved to be a very useful technique for studying metalnanoparticles because the peak positions and shapes are sensitive to particle size. The result obtained from UV-Visible spectroscopy analysis of the sample is presented in Fig 1. To study the stability of Cu colloidal solution in air, the absorption of Cu NPs was measured by UV-visible spectroscopy. It is the most important method of analysis to detect the Surface Plasmon Resonance property of CuNPs. The CuNPs formation is confirmed from the peak at 576 nm. The absorption band of copper nanoparticles has been reported in the range of 500-600nm in literature and this result similar with current result.

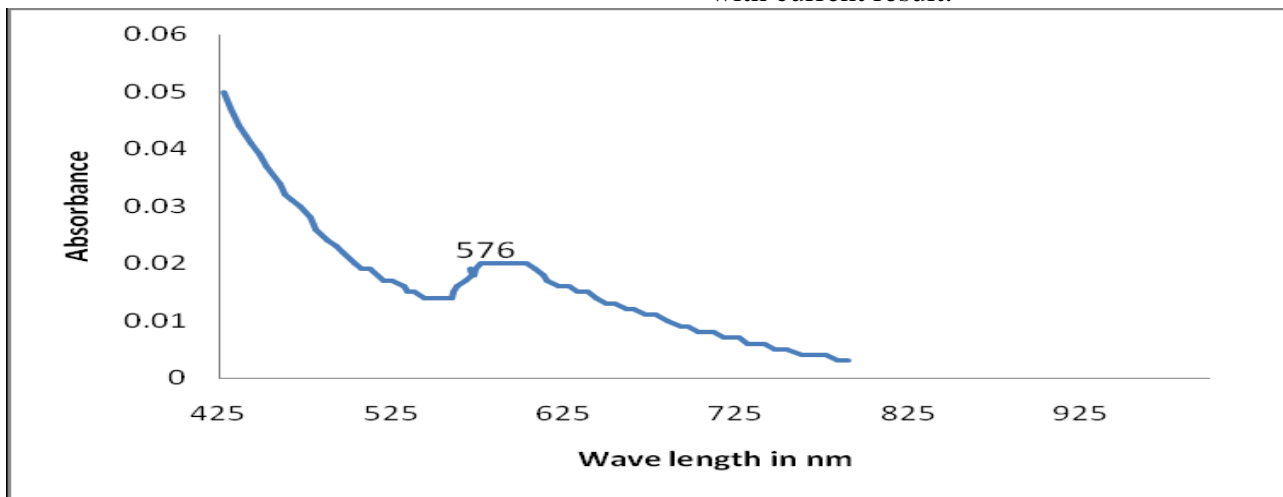


Fig 1 U-V vis spectra of Cu Nanoparticles

(2) Raman spectroscopy analysis: Raman spectroscopy is a powerful technique to identify the molecular species since most molecules have their own distinct spectrum, with different unique vibration frequencies. Raman Spectroscopy is a vibration spectroscopy technique used to collect a unique chemical fingerprint of molecules.

CuO nanoparticles belong to the C_{2h}^6 space group with two molecules per primitive cell. There are nine zone-centre optical phonon modes with symmetries $4A_u + 5B_u + A_g + 2B_g$

among which only three $A_g + 2B_g$ modes are Raman active. The Raman shift indicated in the graph given below at 282 cm^{-1} can be ascribed to be originating from the A_g mode while other peaks at 330 (shoulder to the main peak at 282 cm^{-1}) and 616 cm^{-1} represent B_g modes. Further, there is not feature available in the Raman spectra corresponding to the CuO nanoparticle, therefore we can say that copper is not in the oxide form. The Raman spectra of sample is shown below-

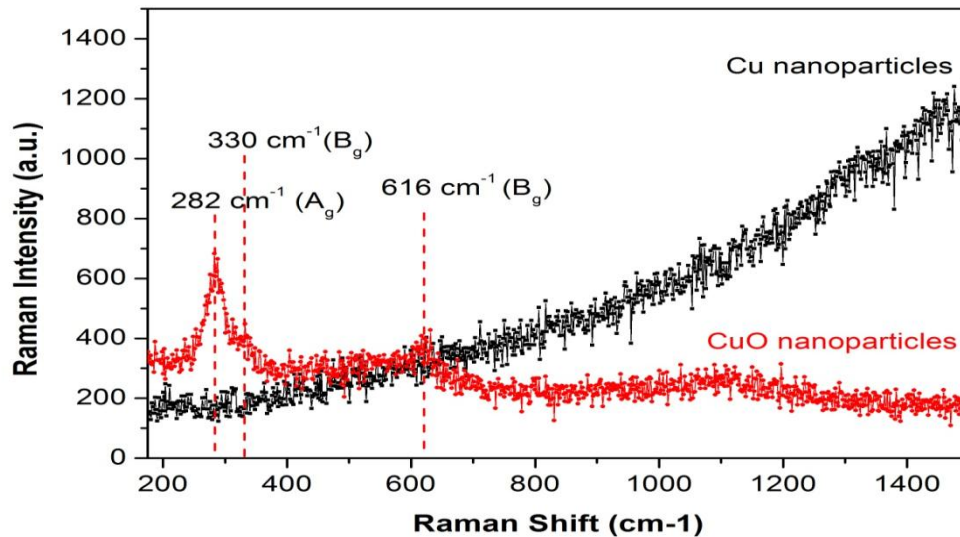


Fig 2: Raman spectra of Cu

(3) XRD Analysis: The crystal structure and phase composition of synthesized copper nanoparticles is analyzed by XRD, as shown in figure 3. As a primary characterization tool for obtaining critical features such as crystal structure, crystallite size, and strain, X-ray diffraction patterns have been widely used in nanoparticle research. The randomly oriented crystals in Nano-crystalline materials cause broadening of diffraction peaks. This has been attributed to the absence of total constructive and destructive interferences of x-rays in a finite sized lattice. Moreover, inhomogeneous lattice strain and structural faults lead to broadening of peaks in the diffraction patterns. X-ray diffraction spectra is carried using a Bruker D8- Advance diffractometer equipped with a source delivering a monochromatic Cu $K\alpha_1$ radiation ($\lambda = 1.54056 \text{ \AA}$). XRD analysis of prepared nanoparticles was performed on a PANalytical X'PertPro Software. The XRD pattern reveals that prepared nanoparticles are a mixture of metallic Cu and copper (I) oxide

(Cu_2O). The copper nanoparticles is synthesized via a chemical reduction method. The broadening of the X-ray diffraction lines, as seen in the figure, reflects the nano-particle nature of the sample. Figure shows the XRD pattern of the produced Cu nanoparticles which represents the formation of CuO nanoparticles with a monoclinic structure (JCPDS 036-0664 & 08-01268). The diffraction data exhibits that the copper nanoparticles have characteristic diffraction peaks (111), (200) and (220) at 2-theta value of 39.212° , 48.76° and 73.75° respectively. On the other hand, some diffraction peaks were indexed to cuprous oxide (Cu_2O) having corresponding peaks to (110), (220) and (310) at 2- θ value of 32.690° , 68.506° and 66.991° respectively. The presence of Cu_2O indicates the partial oxidation of copper nanoparticles with dissolved oxygen in the solution. The mean size of the crystalline Cu nanoparticles calculated from the major diffractions peaks using the Scherrer formula is about **29 nm**.

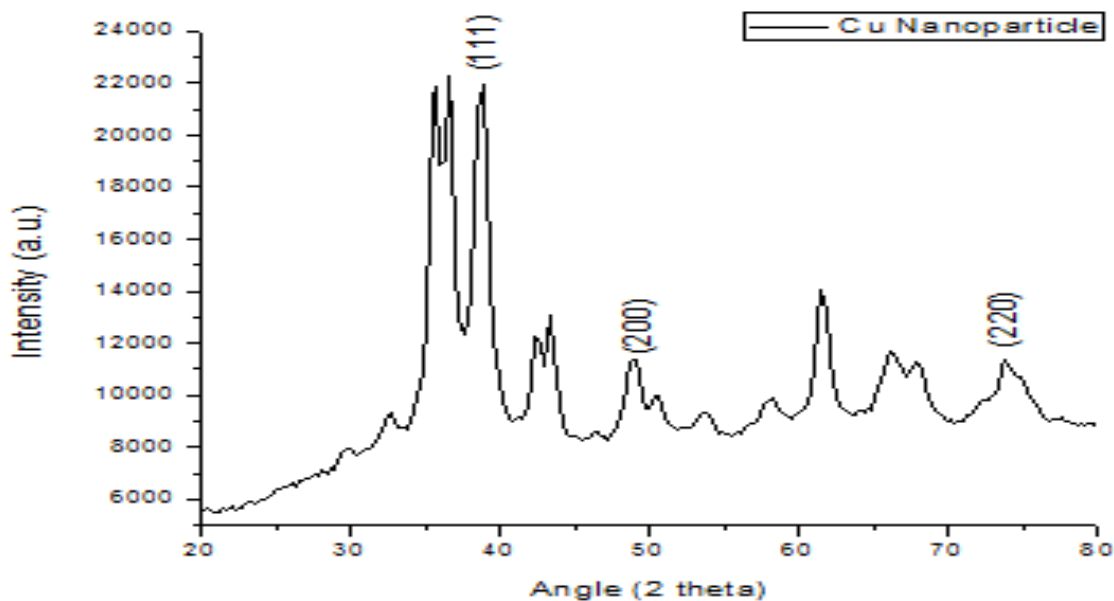
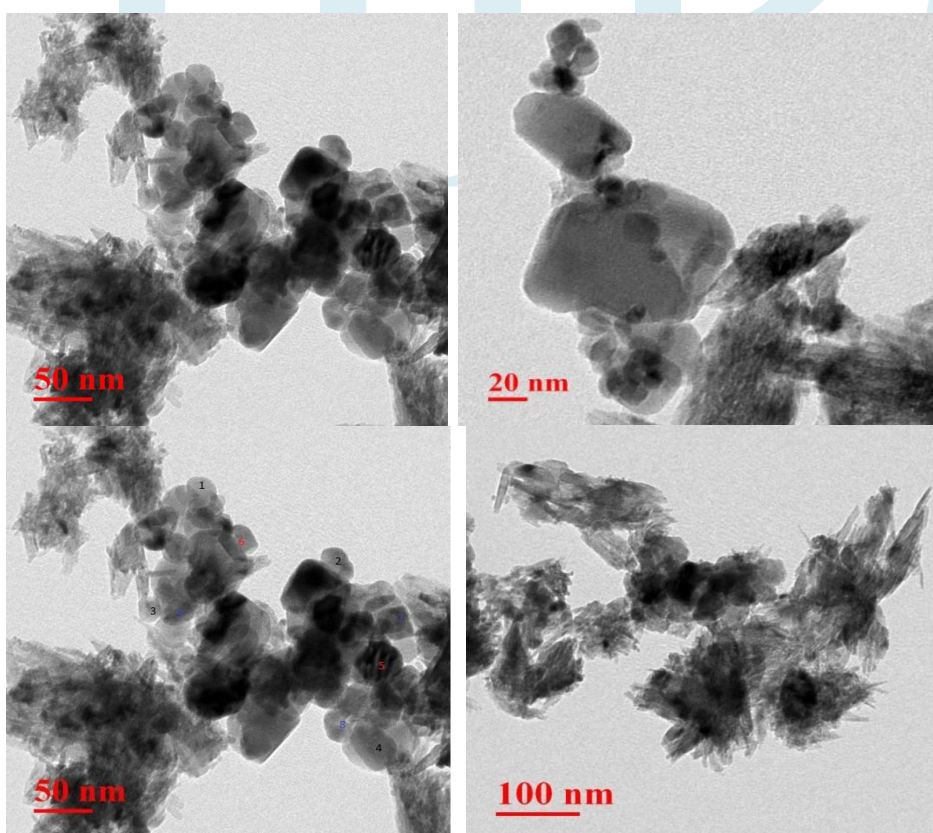


Fig 3: XRD Spectra of Cu/CuO nanoparticles

(4) TEM Analysis: Transmission electron microscopy (TEM) has been employed to characterize the size, shape and morphology of synthesized copper nanoparticles. Copper sulphate pentahydrate is found to be the best precursor that gives better result among other

salts used for the synthesis of copper nanoparticle. TEM images of the synthesized copper nanoparticles are shown in Fig 4. The average size of NP's are about **30nm**. The SAED pattern of sample has recorded.



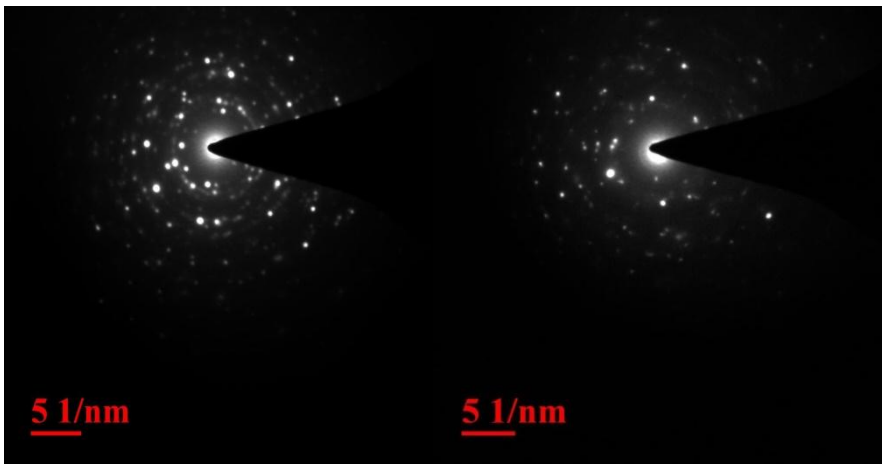


Fig 4(a): TEM micrograph of Cu sample

(5) Energy dispersive X-ray Analysis

Energy Dispersive X-Ray Analysis (EDX) is an X-ray technique used to identify the elemental composition of materials. Applications include materials and product research, troubleshooting, de formulation, and

more. EDX systems are attachments to Electron Microscopy instruments (Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM) instruments where the imaging capability of the microscope identifies the specimen of interest.

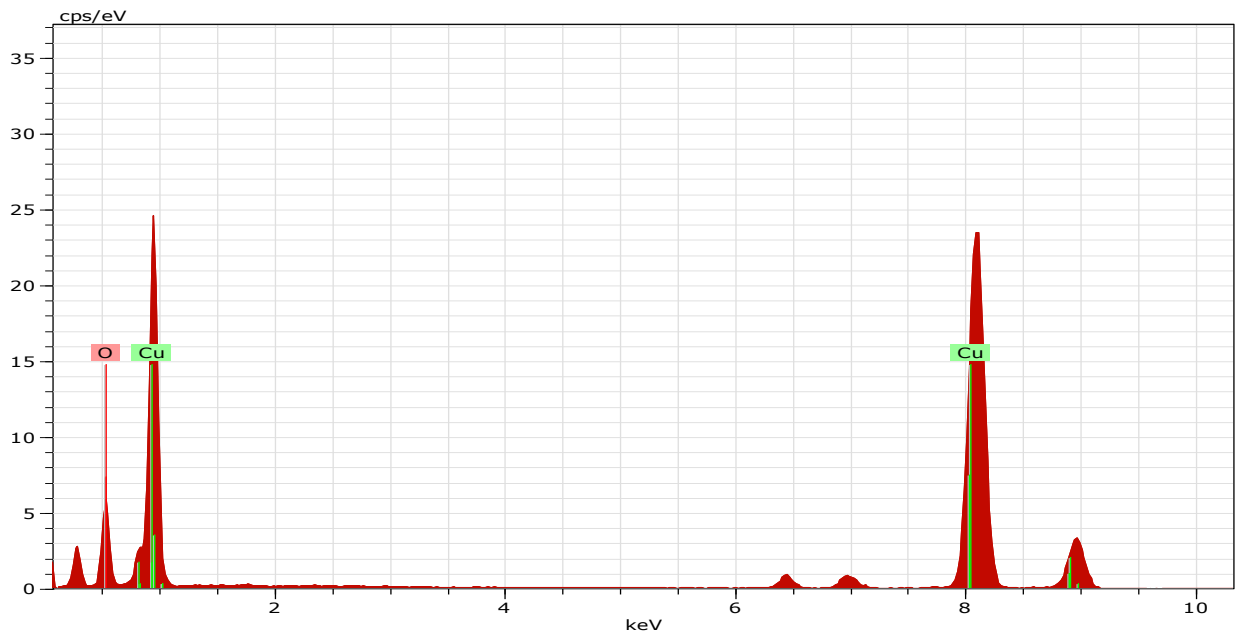


Fig 4(b): EDX Curve

Table1: Compositional analysis of EDX

El AN Series unnorm.C norm.C Atom. C Error (1 Sigma)
 [wt.%) [wt.%) [at.%) [wt.%)

Cu 29	K-series	89.19	89.19	67.52	2.72
O 8	K-series	10.81	10.81	32.48	0.36

Total: 100.00 100.00 100.00

From the above data of EDX show that the elemental composition of Copper is **67.52 %** while the Oxygen composition is **32.48%**, which predict the metallic structure of copper Nanoparticles but Oxide phase also present

there .The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analyzed.

4- APPLICATION

Antibacterial activity of copper nanoparticles on E.coli: The agar diffusion assay is a fast and simple to estimate the susceptibility of micro organisms toward an antimicrobial agent such as copper NP's. This test is based on the diffusion of the nanomaterial from high concentrations (well method) to the agar surface. It allows only a qualitative result about the susceptibility of the microbial strain. For fast grower microorganisms such as what we used in this study, the results interpreted after 24 h. incubation at 37°C, if the Cu NP's has activity,

clear zones (no growth of microorganism) will be observed around the well. The presence or absence of growth inhibition zone was interpreted as sensitive or resistant of microorganisms to the Cu NP's agent.

Clear Zone: The clear region around the well saturated with copper nanoparticles in the petri plate. Clear zone is a clear area around the well in which Cu NP's are filled. It can be easily measure by using a ruler or calliper.

Antibacterial activity is obtained only in 10µl well. The diameter of clear zone is **1.7cm** after 24 hour. The diameter of clear zone is **1.8cm** after 48 hour.

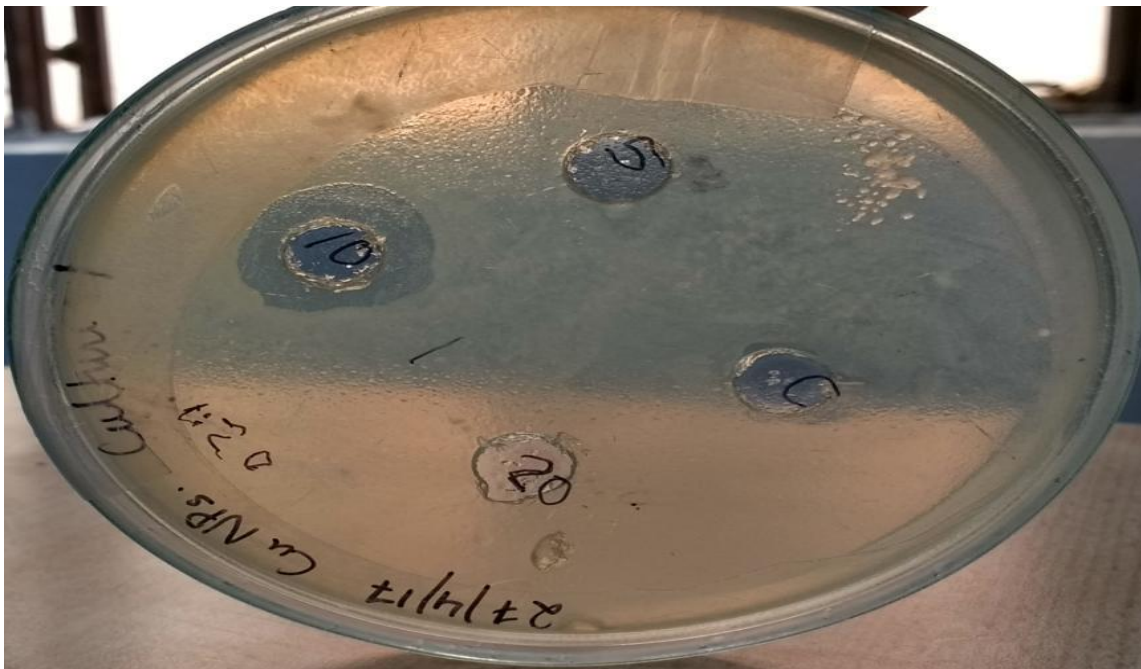


Fig 5: Antibacterial Activity of Cu NP's

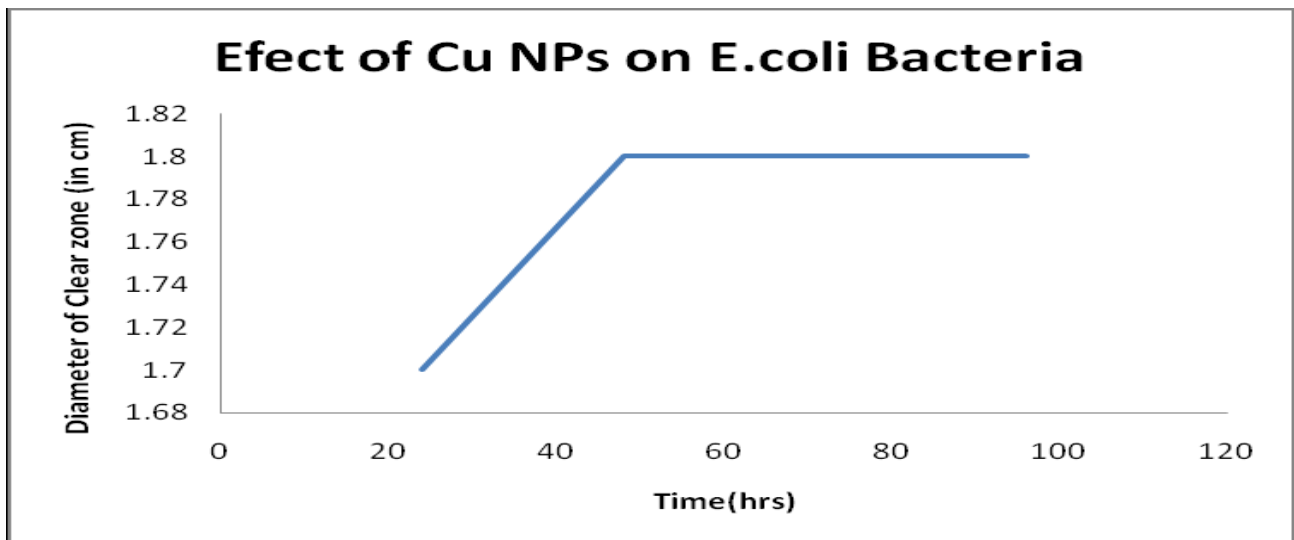


Fig 6: Graph between Time and Diameter of clear zone

5- CONCLUSION

In this work, synthesis of copper nanoparticles (Cu NPs) has been investigated by chemical reduction method (CRM). The average size of copper nanoparticles prepared by CRM is 28 nm to 29 nm. The absorption peak appeared at **576 nm** which confirms the formation of copper nanoparticles. The observed fcc XRD peaks for copper nanoparticles are ascribed the growth along different crystallographic planes. Another phase cuprous oxide (Cu₂O), also observed which shows the partial oxidation of copper nanoparticles with dissolved oxygen in the solution. This synthesis pathway is particularly suitable for large-scale synthesis of Cu and Cu₂O nanoparticles attributed to its simple process and low cost. This method has merits over other reported methods are easily available starting materials, inexpensive and procedure is easy to carry out any laboratory. It is probable that the development of improved synthetic methods for Cu and Cu₂O Nano crystals and more knowledge of their properties should lead to the great advancement in their applications such as catalysis, Biosensors and energy conversion. The morphology of Cu Nanoparticle shows the irregular needle shaped structure of particle also some regular spherical shaped particles is also present here. In antibacterial activity, the diameter of clear zone against **E.coli 1.7cm.** in 24 hour.

6- ACKNOWLEDGMENT

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