Anti-Bacterial Performance of Integrated CuO Nanoparticles Tested at Different Temperatures

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***Abstract*-CuO nanoparticles are synthesized by the Sol-gel method and are characterized by UV-visible spectroscopy; XRD, SEM, FTIR and anti-bacterial disk diffusion method are analyses. Here is an attempt to reduce and ensure a simple way to integrate CuO NPs at different temperatures using the precursor solution $CuCl_2$ dihydrate. XRD diffraction analysis revealed that synthesized CuO nanoparticles are in monoclinic structure with the particle size decreased with increases of temperatures. The SEM analyses conformed the particles are tablet tube like spherical shaped was observed. Blue shift absorption peak of about 376nm was observed in UV spectra analysis. The present of functional groups were observed in FTIR spectra. Anti-bacterial activity of CuO nanoparticles has been tested against various bacterias. Antibacterial efficacy of CuO particles is shown in gram positive and gram negative organisms, such as Staphylococcus saprophyticus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli.**

***Index terms*- CuO; Particle size, X-ray diffraction, SEM. FTIR, Antibacterial activity;**

I. INTRODUCTION

Nanotechnology research is quite attractive one to the researchers at various levels due to various physical, chemical and biological properties and is widely used in various field applications due to its unique environment, engineering, science, and technologies [1]. Nanotechnology involves imaging, measuring, modeling and manipulating matter at this length scale. Reduce the nanoparticle size and easily change nanoparticle shape, as compared to the macro and micro scale, so nanoparticle performed as the best catalyst with intense the strength and good efficiency [2], [3]. CuO nanoparticle is one of the important metal oxide semiconductors. But it can be used in many fields such as many infiltration, catalysts, tropical products, sensitivity materials, glasses, ceramics, porcelain resistors, magnetic storage media, and gas sensors, near infrared tiles, photographic spectroscopy and photosynthetic applications [4]. The most noticeable feature of the CuO NPs can be controlled during packages because they allow their applications to be designed. The synthesis method is an important parameter for control of particle size, morphology, crystallinity and in order to achieve this goal. Various concentrations of CuO nanoparticles are explored including sonochemical, hydrothermal, chemical pathway, and spin coating, and sol-gel techniques. in the midst of these methods, the Sol-gel method was used for a synthesis of CuO nanoparticles In this work [5], [6].

A sol-gel process can be described as "the formation of an oxide network through the poly condensation reactions of a molecular precursor in a fluid." A solution is a constant scattering of colloidal particles or polymers in a solvent when Particles can be transformed or crystallized [7]. An aerosol is a liquid of a powder, once in a gas grid. A gel has a three-dimensional continuous network, which connects to a liquid phase. A colloidal gel is made from a set of colloidal particles compared to the ceramic-ceramic system, with a reduced defect in the Sol-gel method at a low temperature of [8]. The sol-gel method is the simple and relatively fast method. This method is often used by the level of nanoparticles to ensure strict control. This method allows for enormous control of the shape and size of Nanoparticle. The idea behind sol-gel synthesis is to dissolve the compound in a liquid in order to bring it back as a solid in a controlled manner. Multi component compounds may be prepared with a controlled stoichiometry by mixing sols of different compounds. The sol-gel system prevents problems with the co-precipitation, which may be normal, which is a reaction and can be mixed at the atomic level. The results of easily stimulating small particles [9], [10].

Medical field of nanotechnology is another revolution of nanotechnology. Through this technique, we can safeguard human health in the best way. Nanotechnology is the study of a body's contents and the amount of root components. When a solid object passes to the nano-meter scale (smallest), the material receives a lot of energy [11]. Accordingly, all the devices that have been developed are of great potential. Biological zones can also be synthesized by nanotechnology. in future, all the devices will be created and produced by nanotechnology in the healthy, economic, and spacious atmosphere. This technology is used in medicine, chemistry and ecosystem, energy, communication, heavy industries and food industry in all sectors. Based on this, research and new courses are coming up [12].

Medical Nanotechnology detects various types of future technologies such as microscope, robotics, sensors, cameras and other devices that can be used in medical procedures, such as body piercing, protecting the patient's body inside and protecting individual cells. Medicare Nano

technology can tell you many achievements. For example, in the patient's body, nanoparticles can be cured by a particular area. Monitoring of molecules in biological zones, new formation, adjustment and control can be done by clinical nanotechnology [13], [14].

In this present work investigate the effect of the sol-gel method to prepare the CuO nanoparticles by using biological method. This nanoparticles have potential of antibacterial activities depends on the crystal size. it had considerable anti-bacterial activities. These results proved that the projected size-based bioactive component sol-gel method was sensitive, efficient, and convenient that the method may prove to be an effective solution to analyzing bioactive factor of bio synthesis method.

II. EXPERIMENTAL AND PREPARATION METHOD

CuO Nano powders were prepared by Sol-Gel method. Copper Chloride dehydrates $CuCl_2.2H_2O$, Sodium hydroxide NaOH and glacial acetic acid (CH_3COOH) is in analytical grades purchased from Merck. We have taken Copper (II) Chloride dehydrate $CuCl_2$ $2H_2O$ (0.2M) prepared with 100ml deionized water in 250ml beaker. The prepared solution heated at 80^OC with constant stirring and 1ml of glacial acetic acid (CH_3COOH) is added to above aqueous solution. 2M of sodium hydroxide NaOH pellet is heated for 5min and taken in burette to added drop wise in the above the mixture solution. The colour of mixture solution turned from blue to black immediately. The black precipitate is formed immediately. Obtained black precipitate repeatedly washed 4 times with deionized water and filtered using whatman No.1 paper. Subsequently, the washed precipitate dried at 100^oC for 24Hrs. finally the product were calcined at 400^oC, 450^oC and 500^oC, for 4Hrs. Finally, after calcinations process, the brown colour CuO nanoparticle was obtained.

Figure 1 Preparation of CuO Nanoparticles.

A Characterization Techniques

The crystalline phase and particle size of pure and precursor assisted CuO nanoparticles were analyzed by X-ray diffraction (XRD) measurement, which was carried out at room temperature by using SHIMADZU-XRD 6000 diffractometer system equipped with a Cu tube for generating Cu Kα radiation (k= 1.5406 Å). The incident beam in the 2-theta mode over the range of $10–80^O$, operated at 40 kV and 30 mA. The chemical structure was investigated by SHIMADZU-UV 1800 Fourier transform infrared spectrometer (FTIR) in which the IR spectrum was recorded by diluting the milled powder in KBr and the wavelength between 4000 and 400 cm^{-1} was used to assess the presence of functional groups in CuO. The morphology of the samples was characterized by SEM (Hitachi S-4500 SEM Machine) with EDAX. Antibacterial activity of CuO nanoparticles were analyzed by Mueller-Hinton Agar etc by Disc Diffusion Method.

III. RESULTS AND DISCUSION

A. Structural Analysis

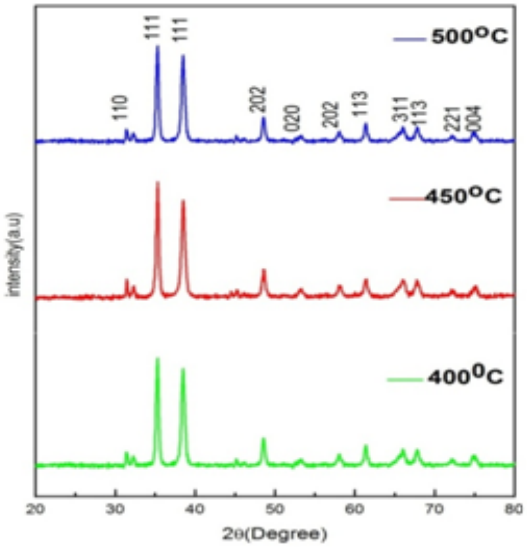

Figure.2. XRD pattern of CuO nanoparticles calcined at different temperatures (400oC, 450oC and 500oC)

The XRD analysis was employed to determine the structure and phase of the CuO nanopowder. Figure 3shows XRD spectra of the CuO nanoparticles grown at different calcination temperature 400^oC, 450^oC and 500^oC respectively. The XRD analysis was employed to determine the structure and phase of the CuO nanoparticle. All CuO diffraction peaks can be indexed to the monoclinic structure. No other peaks are detected within the detection limit of the XRD instrument. The intense and sharp peaks demonstrate that the as-obtained products are well-crystallized, which is confirmed by JCPDS card number 89-2530. The characteristic crystalline in nature, which represents the monoclinic structure of strong peaks,

35.16 º, 38.39º, and 48.42 º and the weakest peaks are 32.08 º, 53.85 º, 61.40 º, 65.14 º, 65.94 º, 67.71 º, 72.08 º and their hkl values are (111), (111), (202), (020), (113) (022) (311) (113) (221) and (004) [15]. Crystal size was measured from the XRD Data using the Debye–Scherrer formula

$$D = k\lambda / \beta \cos\theta \qquad (1)$$

k = 0.9 is a form factor; λ is the X-ray wavelength of Cu Kα radiation (1.54 Å), θ Bragg diffraction angle, and β is the FWHM of the respective at peak of top notch. The XRD peaks confirm that CuO formation is provided in monoclonic structure from each temperature [16], [17].

XRD pattern of CuO nanoparticles is demonstrated the enhancement of different crystal size with different temperatures. The variations of crystalline size and structural parameters are calculated with the different temperatures of the results are shown in tables.1. The size of crystalline size decrease in temperatures (400^{O}C and 450^{O}C) may be due to the nuclear spread. It has thermal treatment to break the bonds with the neighbors' atoms, and then some lattice distortion occurs during displacement. As the 500^{O}C temperature increases, the cells will receive more energy to give the more efficiency, so the size is increased [18], [19].

The values of lattice constants are higher than the bulk CuO, which is a strong indication of stress in the powder [20]. The structural parameters are calculated from the following equations

The microstrain (ε) is calculated using the relation,

$$\varepsilon = \frac{\beta cos\theta}{4} \qquad (2)$$

$$\text{Dislocation Density, } \delta = \frac{1}{D^2} \qquad (3)$$

$$\text{Stacking Fault, } SF = \left[\frac{2\pi^2}{45(3tan\theta)^{\frac{1}{2}}}\right]\beta \qquad (4)$$

The size of crystallite decrease with increase of different temperatures and stacking fault, dislocation density and micro strains are increase with decreases [21].The variation of the size of the crystal and SF, δ and ε is calculated and can be seen from Table.1.

In addition, The XRD pattern of CuO NPs common and similar to a single phase monoclinic structure of Lattice constant is calculated. Lattice constant results also decrease with increase when decrease with increase the particle size. The results are showed in Table 2. For $CuCl_2$ products, the basic crystal of integrated piles, such as CuO, is easily sensible, but the CuO peaks are strong after the synthesis process. The XRD concludes that CuO formation can be obtained by ensuring the simplest way to integrate CuO NPs from $CuCl_2$ dihydride in the Sol-gel system.

TABLE I

STRUCTURAL PARAMETER OF CUO NANOPARTICLE FORMED AT DIFFERENT TEMPERATURES

Temperature °C	FWHM	2θ	Crystal size(nm)	Stacking Fault	Dislocation Density $\delta \times 10^{14}$	Micro Strain (ε)
400	0.3871	35.1597	24.14	0.002867	17.6058	0.001519
450	0.3662	35.2973	22.47	0.003035	19.7957	0.001611
500	0.3776	35.2649	23.04	0.002956	18.8250	0.001571

TABLE II

VARIATION OF CRYSTALLITE SIZE AND LATTICE PARAMETERS WITH DIFFERENT TEMPERATURES

Temperature (°C)	A (Å)	b (Å)	c (Å)	Crystallite size (nm)
400	4.711	3.430	5.135	24.14
450	4.683	3.425	5.122	22.47
500	4.688	3.429	5.133	23.04

B. Surface Morphological Studies

Scanning electron microscope was used to oversee the monochromatic analyzes of CuO nanoparticles. SEM image of CuO nanoparticles micrographs in different temperatures of 400^{O}C, 450^{O}C, and 500^{O}C as shown in Figure 4. SEM observation shows that the average particle size is almost matched with XRD results. The presence of CuO NPs is agglomerated nano tablet tube shape for all the samples [22]. It can be shows that high affinities of agglomerations in low temperatures are 400^{O}C and 450 ^{O}C due to particles are uniformly distributed in CuO NanoParticles. At higher temperature (500^{O}C) was particles are highly agglomerated because higher temperatures to create oxygen relation defects to change the surface morphologies of the CuO NPS [23]. The SEM image clearly shows CuO nanoparticles at 450 degrees

temperature, similar to the homogenous and standardized size with clear crystal growth in the tablet tube form, such as a spherical shape.

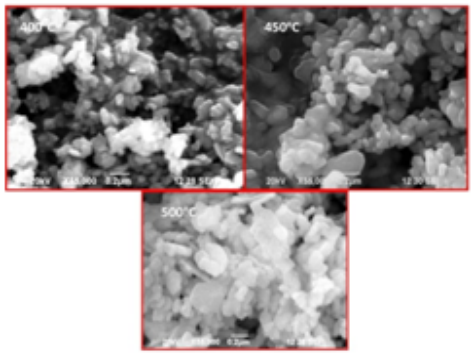

Figure 3 SEM Analysis of CuO Nanoparticles at different Temperatures (400°C, 450°C and 500°C).

Figure 4 CuO nanoparticles SEM image is represented by the temperature in 400 ° C, 450 ° C and 500 ° C of ×55,000 magnifications has been collected. From the SEM image of the Figure 4 (450 ° C) CuO-NPs, the particles are well-dispersed spherical, supplementary almost well distinct with the same uniform crystal system. There were also shows high cultural agglomerations. It explains the expansion of tightly constructed tower arrangement was clearly visible [24].

C. Elemental Analysis

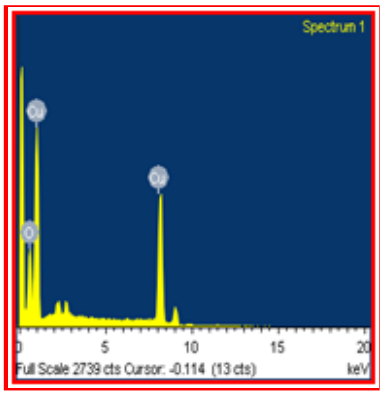

Figure 4 Represents Elemental Analysis

The chemical compositions of the CuO nanoparticles were analysed using energy dispersive X-ray analysis. EDAX spectra of the synthesized CuO nanoparticles reveal that there are strong peaks for Copper (Cu) and oxygen (O), which indicates the formation of pure CuO nanoparticles.

Atomic and weight percentages of Cu and O are 38.86at.%, 71.63wt% and 61.14at.%, 28.37 wt%, respectively. And these results agree with EDAX which do not show any other element peaks as shown in Figure 5. It was observed that the nanoparticles are composed only of Copper and oxygen.

D. UV Visible Absorbance Spectra

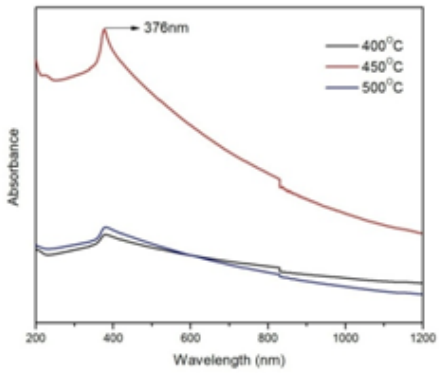

Figure 5 UV–Vis absorption spectra of CuO nanoparticles at different Temperatures (400°C, 450°C and 500°C).

The absorption of Figure 6 shows the CuO nanoparticles showing the peak absorbing around spectrum 376 nm. CuO nano particles exhibit exciton absorption (376 nm) due to excessive tropical bonding at room temperature [25]. Wavelength 376 nm absorption spectrum CuO Nano particles (378 nm) is slightly blue shift depending on the bulk value. The shifted weak blue emissions for (400^{O}C and 500^{O}C) might be due to surface defects in the CuO nanoparicles. This blue shift in the absorption edge causes the event of the quantum resistance of individual nano particles. The strong blue shift emission is observed in temperature of 450^{O}C, it may be less surface defect of the CuO Nanoparticles [26], [27].

E. FTIR Analysis

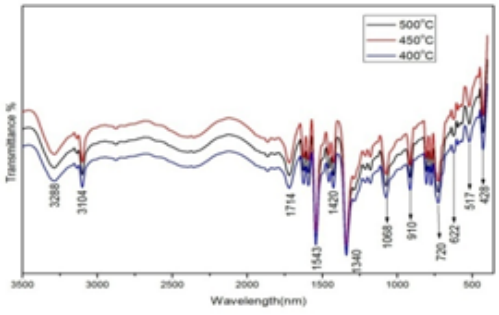

Figure 6 FTIR spectra of CuO nanoparticles calcined at different Temperatures (400°C, 450°C and 500°C).

FTIR spectra were recorded in solid phase using the CuO pellets technique in the range of 3500–400 cm^{-1}. FTIR spectra of CuO nanoparticles treated at 400°C, 450°C, and 500°C are shown in Figure 7. The following peak spectrum is 3,288 cm $^{-1}$ and 3104 CM^{-1}, attributed to intermolecular hydrogen bonding between the hydroxyl group of acids or alcohol compounds in the benzene ring. This is due to OH stretching on the surface of the nanostructured CuO crystals

[28]. The small sharp peak observed at 1714 $cm^{-1}$was assigned to C = O stretching of curcumin and the important sharp peak exhibited at 1543 $cm^{-1}$was assigned to curcuminaniline azomethine compound which was interacted with copper oxide nanoparticles and reached higher wave number [29]. The band obtained at 1420 cm^{-1} specifies C-N stretching and 1340 cm^{-1} are assigned to C–H deformation mode in alkanes [30]. The band located at 1068 cm^{-1} can be assigned to sulphate absorptions (v3) and (v1) respectively. While the bands located at 910 cm^{-1} due to sulphate compounds [31]. The peaks vibrations occurring at around 428 cm^{-1}, 517 cm^{-1}, 622 cm^{-1} and 720 c cm^{-1} for all the samples which can be attributed to the vibrations of Cu-O, which confirms the formation of highly pure CuO nanoparticles [32].

F. Anti Bacterial Activity

TABLE III

WELL DIFFUSION ASSAY OF CUO NPS AGAINST VARIOUS BACTERIAL STRAINS

Micro organism	Standa-rd Antibi-otic Disk*	Zone of inhibition (mm)		
		400^{O}C	450^{O}C	500^{O}C
Bacillus subtilis	21	12	20	10
Escherichia coli	23	14	21	15
Pseudomonas aeruginosa	20	10	18	13
Streptococcus pyogenes	20	11	17	11

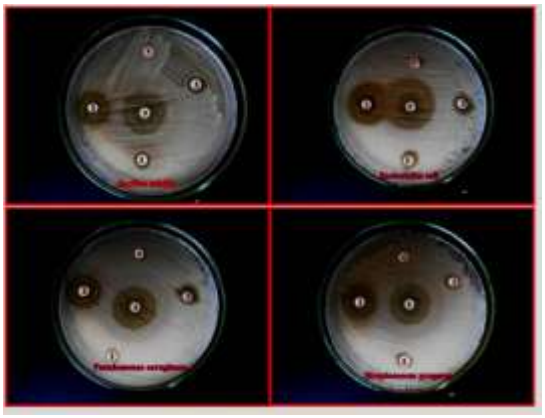

Figure 7 Antibacterial studies of CuO nanoparticles at different Temperatures (400^{o}C, 450^{o}C and 500^{o}C).

All the synthesized CuO were tested for their anti-bacterial activity against bacterial and fungal strains by disc diffusion method [33] with 50 µg/mL of the solutions prepared in Dimethyl sulfoxide (DMSO). The reason of choosing DMSO for antibacterial studies was that it has no effect on the above mentioned bacterial strains [34]. Using paper disc (8mm) in nutrient agar culture medium, 50µg/mL of the newly synthesized CuO were loaded through bacteria free micro pipettes.

Synthesized CuO nanoparticle is calcined at different temperatures and then investigated in antibacterial activity against four bacterial strains such as Staphylococcus saprophyticus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli through disk diffusion method as shown in Figure 8. The size of CuO nanoparticles in 400^{O}C, 450^{O}C and 500^{O}C calcined temperatures was significantly different. Figure 8 clearly shows that the calcined temperature 450^{O}C of the CuO nanoparticle showed the highest antibacterial activity is against like *Staphylococcus saprophyticus*, *Bacillus subtilis*, *Pseudomonas aeruginosa and Escherichia coli* better than the 400^{O}C and 500^{O}C. The 500^{O}C was better antibacterial properties against *Pseudomonas aeruginosa and Escherichia coli* whereas the 400^{O}C was better antibacterial properties against *E. coli.*

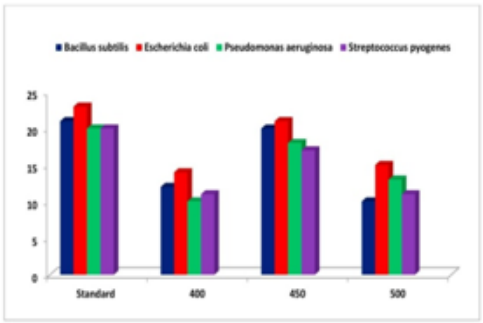

Figure 8 Variation of four Bacterial strains at different temperatures

While comparing the effect of nanoparticles synthesized at varying temperature ranges, the greatest inhibitory effect was recorded for 450°C of CuO nano particles, because the crystal size (22nm) is very small. We know that the bacterial cell is micrometer range but nanometer is a very smaller than the micrometer [35]. so that it has easily to cross the bacterial cell wall [36], [37]. In this study confirm that, the highest effect of antibacterial activity is obtained from the small size (22nm) of CuO nano particle i.e. 450^{O}C temperature. This concluded results are shown in Table 3 and Figure 9.

IV. CONCLUSION

Copper oxide nanoparticles were synthesized using modified sol-gel method. The crystallite size, morphology and Antibacterial activities were studied with various characterization techniques like X-ray Diffraction method, SEM Analysis, Elemental Analysis, UV Spectra, Fourier Transform Infrared(FTIR) and Disk Diffusion Analysis . The XRD spectra established the formation of single phase monoclinic CuO structure and also crystallite size was found to decrease up to 450°C calcined temperature. Minimum crystallite size of 22nm was observed in the case of CuO nanoparticles annealed at 450°C. The morphology studies supports the formation of the spherical shape which are believed to consist of particles aligned to tablet tube model and CuO nanoparticles possess grain like morphology and have a high regularity with high agglomeration as shows in SEM Analysis. The elemental measurements of percentage and stoichiometry ratio of CuO-NPs were confirmed by EDX analysis. The FTIR spectra confirmed the presence of metal-oxide bond. The Collections of all the characterization results are definite the regularity of synthesized CuO-NPs. In this study confirm that, the highest effect of antibacterial activity is obtained from the small size (22nm) of CuO nanoparticle ie 450^{O}C temperature. Finally, anti-bacterial Activity for CuO nanoparticles have been studied against several drug resistant bacteria, both gram positive and gram-negative organisms like *Staphylococcus saprophyticus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli*.

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