Preparation, Characterization and Determination of Antimicrobial Activities of Cupric Oxide (CuO) Nanoparticles Using Citric Acid

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Abstract - The present research deals with a study on the synthesis and characterization of cupric oxide nanoparticles. The nanoparticles were synthesized by sol-gel method using citric acid. Magnesium nitrate and copper nitrate were used to obtain homogeneous solution. The solution was heated and stirred to obtain gel powder. The powder was dried in an oven at temperature of 105˚C to obtain constant weight. The resulting sample was thermally heated in muffle furnace at temperature of 500˚C for four hours to get required nanoparticles. The prepared nanoparticles were characterized by Energy Dispersive X-ray Fluorescence (EDXRF) and X-ray powder diffraction (XRD). Then determination of antimicrobial activities of the nanoparticle was carried out by Agar Well Diffusion Method. According to the XRD analyzed data, the average particle size of CuO nanoparticle was 23.21nm.

***Key words* : Cupric oxide nanoparticle, sol-gel method, XRD and EDXRF**

I. INTRODUCTION

In current years, researchers from the developed countries have focused to research various types of nanoparticles. Many questions may be asked about the nanoparticles. Among them, two questions should be asked and could be solved about nanoparticles. Firstly, what is nanoparticle? Secondly, why are researchers from many countries focusing on nanoparticle? Nanoparticle is a small particle that ranges between 1 to 100 nanometers in size. Owing to their very small size, nanoparticles have a very large surface area to volume ratio when compared to bulk material, such as powders, plate and sheet. The change in size can also affect the melting characteristics; gold nanoparticles melt at much lower temperatures (300^0C for 2.5nm size) than bulk gold(1064^0C). Moreover, absorption of solar radiation is much higher in materials composed of nanoparticles than in thin films of continuous sheets of material.

The use of nanomaterials spans across a wide variety of industries, from healthcare and cosmetics to environmental preservation and air purification. The healthcare field, for example, utilizes nanomaterials in a variety of ways, with one major use being drug delivery. One example of this process is whereby nanoparticles are being developed to assist the transportation of chemotherapy drugs directly to cancerous growths as well as to deliver drugs to areas of arteries that are damaged in order to fight cardiovascular disease. In aerospace, carbon nanotubes can be used in the morphing of aircraft wings. The nanotubes are used in a composite form to bend in response to the application of an electric voltage. Elsewhere, environmental preservation processes make use of nanomaterials too, such as nanowires. Applications of nanowires are being developed in flexible solar cells as well as to play a role in the treatment of polluted water. Absorption of solar radiation is much higher in materials composed of nanoparticles than in thin film of continuous sheets of material. In the cosmetic industry, mineral nanoparticles, such as titanium oxide are used in sunscreen for UV protection. The sports industry has been producing baseball bats that have been made with carbon nanotubes, making the bats lighter and therefore improving their performance. Furthermore, nanomaterials can be used in antimicrobial nanotechnology. Towels and mats used by sport peoples are made of nanomaterial in order to prevent illnesses caused by bacteria.

Bacterial strains that are resistant to current antibiotics have become serious public health problems that increase the need to develop new bactericidal materials. Therefore, nanoparticles have gained importance in the field of chemotherapy. This research focuses on the unique properties of nanoparticles and their mechanism of action as antimicrobial agents[1].

II. MATERIALS AND METHODS

A. Preparation of cupric oxide nanoparticles using citric acid

5.12 g of magnesium nitrate $Mg(NO_3)_2.6H_2O$ was dissolved in 50 mL of distilled water to obtain solution (A).

Solution (B) was prepared by dissolving 3.8 g of copper nitrate $Cu(NO_3)_2$ in 50 mL of distilled water. The solution (A) and (B) were mixed to obtain solution(I). 3.8gm of citric acid was dissolved in 100 mL of distilled water to obtain solution (II). Solution (I) and (II) were mixed together with 10mLof ethylene glycol to obtain a homogeneous mixture solution. The mixture solution was stirred and heated on a magnetic stirrer hot plate at 70-80˚C for 2 hours. The gel-powder was obtained. The gel-powder was dried in an oven at 105˚C until constant weight was obtained. The dried as prepared powder was calcined in a muffle furnace at 500˚C for 4 hours to obtain cupric oxide (CuO) nanoparticles.

B. Characterization of Cupric Oxide Nanoparticles

Crystal structure and phase analysis were performed by X-ray diffraction (XRD) using Rigaku, D-Max 2200, Japan in Department of Chemistry, Yangon University. The elemental compositions of prepared sample was confirmed by using EDXRF 700 spectrometer in Department of Chemistry, Monywa University.

C. Determination of Crystallite Size and Interatomic Spacing

The crystallite size of cupric oxide can be calculated by using Debye-Scherrer' formula,[2]

$$D = \frac{0.9\lambda}{\beta cos\theta}$$

The interatomic spacing can be calculated by Bragg's equation, $d = \frac{\lambda}{2sin\theta}$

Where, λ = the wave length of X-rays

(λ = 1.54056 ^{0}A for Cu/K-alpha 1)

θ = the diffraction angle

β = full width at half maximum in radian

D = average crystal size

d = interatomic spacing

D. Determination of Antimicrobial Activities of Cupric Oxide Nanoparticle

There are many types of microorganisms which cause various diseases to humans. Among of them, the antimicrobial activities of following six types of microorganisms were detected by CuO nanoparticles.

Table I Types of Microorganisms and Infection Diseases

No	Test Organism	Infection Diseases
1	*Bacillus pumilus* IFO 12102	Fever
2	*Baillus subtilus* IFO 90571	Fever
3	*Candida albicans* NITE 09542	Candidasis, Skin disease
4	*Eschericha coli* AUH 5436	Diarrhoea
5	*Staphylococcus aureus* AUH 8465	Food poisoning
6	*Salmonella typhi* AUH 7943	Typhoid fever

*1. Chemicalsandreagents:*Glucose, yeast extract peptone, Agar, Distilled water.

2. Apparatus and equipments: Autoclave, an incubator, hot plate, Petri-dishes, measuring cylinder, micropipette and clipper.

3. Screening of Antimicrobial Activities of Crude Extract by Using Agar Well Diffusion Method: Glucose 0.5g, yeast extract 0.3g, peptone 0.3g, agar 1.7g, 100mL of distilled water were added in a 250mL sterile conical flask and heated on hot plate until boil medium. Then, the mouth of the flask was plugged with a piece of cotton wool. This medium was sterilized in an autoclave at 121^{0}C for 45 minutes. After 45 minutes, a 0.1 mL test organisms were inoculated in to 20mL of medium agar at about 40^{0}C and were poured into the sterile petri- dishes at aseptic condition. After the agar became solid, cock borer was used to make the wells (8mm in diameter). Then extract sample (20μL) was introduced into the well and they were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. The clear zone surrounding the wells indicated the presence of antimicrobial active compound in the extracts which inhibit the growth of the test organisms[3].

III. RESULTS AND DISCUSSION

A. Characterization of Cupric Oxide Nanoparticle

1. EDXRF Analysis of Cupric Oxide Nanoparticle:

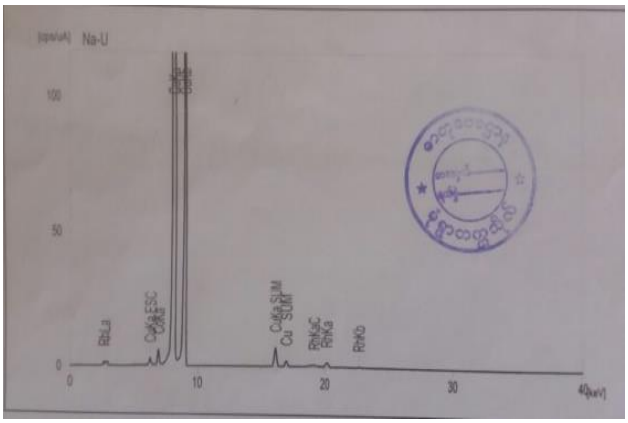


Fig .1 EDXRF spectrum of CuO nanoparticles

Table II Quantitative Results of CuO Nanoparticle

No	Analytes	Results (%)
1	Cu	99.779
2	Co	0.221

From the EDXRF data, the amount of copper and cobalt in prepared nanoparticles were found to be 99.779% and 0.221% respectively.

2. XRD Analysis of Cupric Oxide Nanoparticles:

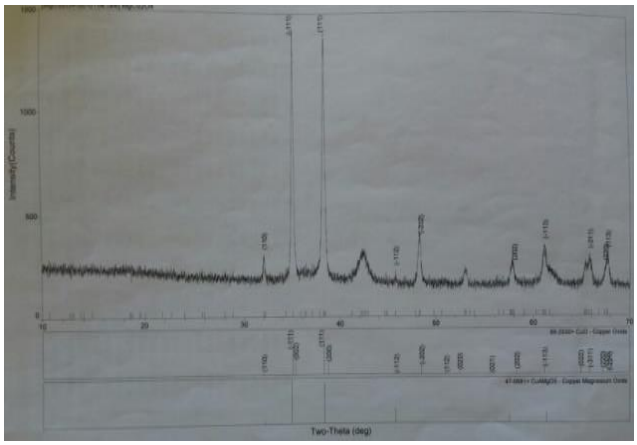


Fig . 2 XRD spectrum of CuO nanoparticle

Table III The Particles Size of CuO Nanoparticles

No	Bragg angle (2θ)	Miller indices (h k I)	(β) radiation	Inter planar spacing d (nm)	Particle size D (nm)
1	32.049	110	0.0043	0.279	33.817
2	35.359	002	0.0098	0.254	14.909
3	38.399	111	0.0059	0.234	24.759
4	38.658	200	0.0049	0.233	30.141
5	48.559	-202	0.0050	0.187	30.141
6	52.757	020	0.0037	0.173	42.015

Range of crystalline size = 14.909 – 42.015 nm

Average value = 23.21 nm

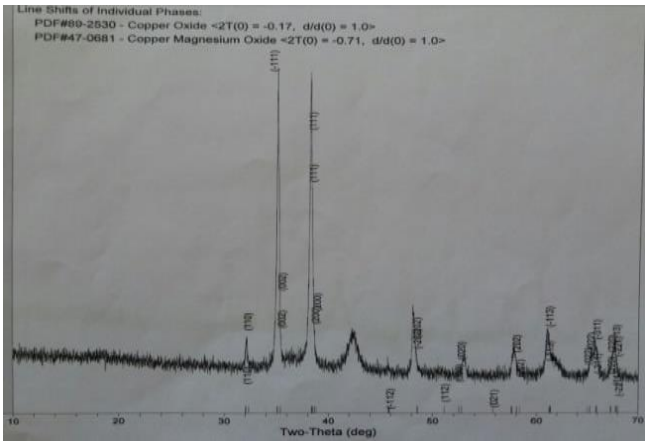


Fig .3 XRD spectrum of CuO nanoparticles

Table IV The phase ID Report of CuO nanoparticles

No	Bragg angle (2θ)	Miller indices (h k I)	Area (%)	Inter planar spacing d (nm)	Phase ID
1	32.049	110	10.7	0.279	CuO
2	35.359	002	21.8	0.254	CuO
3	38.399	111	78.2	0.234	CuO
4	48.559	-202	6.6	0.187	CuO
5	52.757	020	2.9	0.173	CuO

Prepared nanoparticles were to be CuO.

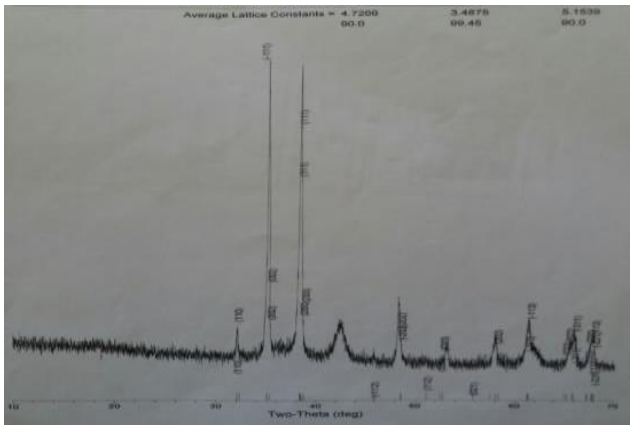


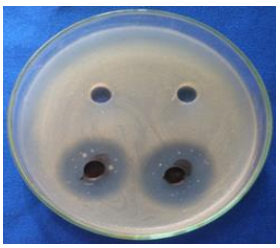
Fig. 4 XRD Spectrum of CuO nanoparticles

Table V Lattice Constants from Peak Locations and Miller Indices for CuO

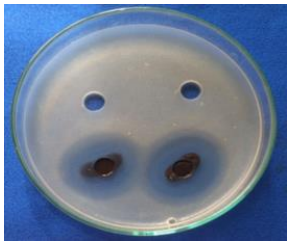
No	Bragg angle (2θ)	Miller Indices (h k I)	Inter planar spacing d (nm)	a-axis (nm)	b-axis (nm)	c-axis (nm)
1	32.04	110	0.279	0.472	0.348	0.515
2	38.39	111	0.234	0.472	0.348	0.515
4	48.55	-202	0.187	0.472	0.348	0.515
5	52.75	020	0.173	0.472	0.348	0.515
Average lattice constant				0.472	0.348	0.515

Average lattice constants calculated from XRD pattern for CuO nanoparticles were a = 0.472nm, b = 0.348 nm, c = 0.515 nm. The crystal structure is monoclinic.

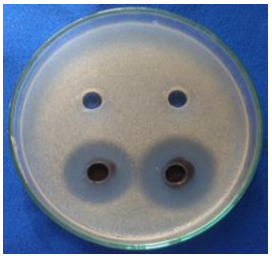
B. Determination of Antimicrobial Activities of Cupric Oxide Nanoparticles



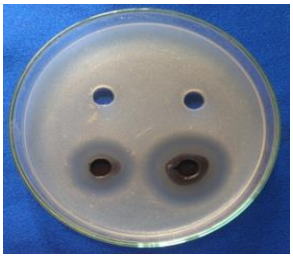
B.subtilis



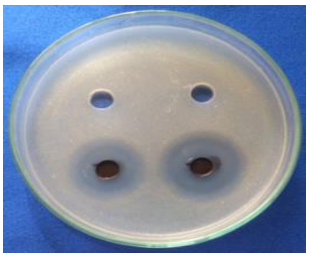
Candida.albicans



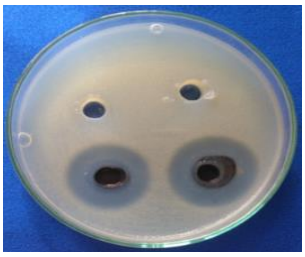
E.coli



Pseudomonas aeruginosa



Staphylococcus. Aureus



Salmonella typhi

Fig. 5 Investigation of antimicrobial activities of cupric oxide nanoparticle

In the figure, upper pair of holes of each dish shows the activity of blank solution (only distilled water). Right hole of lower pair in each dish indicates activity of CuO.

Table VI Results of Screening of Antimicrobial Activities of CuO Nanoparticles

No	Microorganisms	Inhibition zone diameter of samples (CuO) (mm)
1	*Bacillus subtilis* IFO 90571	26.40(+++)
2	*Candida albicans* NITE09542	28.91(+++)
3	*Escherichia coli* AUH 5436	29.61(+++)
4	*Pseudomonas aeruginosa*	27.80 (+++)
5	*Staphylococcus aureus* AUH 8465	24.75(+++)
6	*Salmonella typhi* AUH 7943	30.45(+++)

(-) No activity
(+) Low activity, 9-14 mm
(++) Medium activity, 15-20mm
(+++) High activity, 21mm - above

IV. CONCLUSION

In this research, cupric oxide nanoparticle was synthesized from magnesium nitrate and copper nitrate by sol gel method using citric acid. The prepared cupric oxide nanoparticle was characterized by modern sophisticated methods such as EDXRF, XRD. From the EDXRF data the 99.779% of copper was found in CuO nanoparticles. From the XRD results, the average crystalline size of prepared nanoparticles was 23.21nm. The crystal structure of CuO nanoparticle was monoclinic.

The cupric oxide nanoparticle was detected in antimicrobial activities by Agar Well Diffusion Method[3].

According to the screening of antimicrobial activities of crude extract by using Agar Well Diffusion Method, CuO showed high activity in all microorganisms such as *Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Staphylucoccus aureus* and *Salmonella typhi.*

ACKNOWLEDGMENT

I am extremely grateful to Dr Khin Maung Oo, Rector, University of Magway and Dr Thida Aung, Professor and Head, Department of Chemistry, University of Magway for their provision and suggestions of the research facilities. I also would like to express gratitude to my supervisor Dr Yi Yi Myint, Professor and Head, Department of Chemistry, University of Mandalay for her guidelines in my research works.

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