Synthesis, Characterization and Antibacterial Studies of Co$_3$O$_4$ and Ag doped Co$_3$O$_4$ Nanoparticles Synthesized from E-waste

Dr. B.V. Mohan Kumar[1] and Dr.M.Giridhar[2]

[1]Department of PG Studies in Chemistry, I D S G Government College, Chikkamagaluru.

[2]Department of PG Studies in Chemistry, Government Science College, Hassan.

Abstract—**Nano sized metal particles have got much attention due to its unique electrical, optical and magnetic property which depends on the size and shape of the particles. In this connection synthesized Co3O4 and Ag doped Co3O4 nanoparticles, and were confirmed from XRD, SEM, EDS and FT-IR spectroscopy. Ag doped nanoparticles were evaluated for their antibacterial activity against *S.Aures* and *E.coli* bacteria. The results showed that synthesized Ag doped Co3O4 nanoparticles exhibit excellent antibacterial effect compared to undoped Co$_3$O$_4$ nanoparticles.**

Index Terms—**Nanoparticle, XRD, SEM, Antibacterial.**

I. INTRODUCTION

Functionalized nanoparticles are prepared by functionalization of small organic compound. Nano sized metal particles have got much attention due to its unique electrical, optical and magnetic property which depends on the size and shape of the particles. Nano particles sizes range between 1 to 100 nanometer in size have properties unique from their bulk equivalent. the field of nanotechnology is emerging as a rapid growing technology with its applications in various fields for manufacturing material at nano scale level. It also finds applications in the fields such as drug delivery, nano medicines, biosensor and bio nanotechnology, magnetic storage device, organic image etc [1].

Synthesis of nano sized drug particles is of almost important in the development of new pharmaceutical products in general, the small size of nanoparticles leads to a lower concentration of point defect compared to their bulk [2].

Due to their size decrease in dimensions to atomic level, their properties change. These possess unique Physiochemical, optical and biological properties, which can be manipulated for desired application[3-7].

Properties such has luminescence, conductivity and catalytic activity depend on the size of nanoscale particles. The easiest method that has been used for the preparation of metal nanoparticle is a chemical reduction of metal salts[8]. For nanoparticles synthesis, we make use of soluble metal salt, a reducing agent and Capping agent (which prevents further growth of the particles or aggregation).

High power rechargeable lithium-ion battery (LIB) is a device that meets certain demands of our today's technological world, acting as the source of device like mobile phones, laptop, computers, electrical vehicles (EVS), hybrid electric vehicles (HEVS), and grid level power systems.

Because of their high energy density, long life spam, light height and compatibility, the grab attention of people. The first commercial LIB was invented by Sony in 1991 which replaced by the other type of batteries.

The exciting methods for recycling of CO and Li from the spent LIB's, focusses on pyrometallurgy, hydrometallurgy, or biohydrometallurgy process[9-13].

In hydrometallurgical process, the dismantled electrodes are first dissolved in concentrated acids followed by recovery of metal Ions by selective precipitation. Therefore, recycling of constituent metal ion in LIB'S has become important towards the development of green technologies the dissolutions of active cathode

material is done using strong acids such as to 2M H_2SO_4 ,4M HCL, 1M HNO_3 has leaching agent.

The leaching efficiency of co is highest in HCl when compared to H_2SO_4 and HNO_3 at 80 °c. The efficiency of the leaching process was increased by Adding H_2O_2(5-,20vol%) as a reducing agent in acidic medium.

LIB's consist of lithium intercalating material has anode (usually graphite carbon) and lithium metal oxide as a cathode. The most widely used cathode material in LIB is lithium cobalt oxide ($LiCoO_2$). This is characterized by high specific energy density and its durability. They contained valuable metals, organic chemicals and plastics in ratio proportions 5-20% of Co ,5-10% of Ni and 5-7% Li and 7% of plastics, in which the contents of valuable metals higher than those in natural ores [14-20].

II. EXPERIMENTAL

Materials and Method: The antibacterial activity of test compounds was screened against the above-mentioned strains and the following materials were used for the testing.

1. Nutrient agar in conical flask.

2. Sterilized petri dishes

3. L shaped glass rod, micropipette with tips and corn borer

4. 70% alcohol

5. Sterile test tubes for the preparation of solutions of test compounds in desired concentrations.

6. Inoculum

7. Standard Ampicillin 5 mM

8.Glycine and ascorbic acid(leaching)

9.Sodium hydroxide

Preparation of media: Nutrit agar media was prepared by dissolving bacteriological peptone (1.0%), meat extract (0.5 %), Agar (2%) and sodium chloride (0.5 %) in distilled water. The pH of the solution was adjusted to 7.0 -7.4 by using sodium hydroxide (40 % approximately, 0.25 ml for 100 ml of nutrient broth) and then sterilized for 30 minutes at 15 lbs pressure in an autoclave.

Preparation of Ag doped Co Oxide Nanoparticles:

a) Dismantling and separation:

To prevent self-ignition and short circuiting the spent Lithium-ion batteries were discharged completely and dismantled to separate the cathode and anode material coated on aluminum and copper foil respectively, the cathode material was uncurled and cut into small pieces. The cathode material was separated from the aluminum foil filtered and heated at 700 °C for two hours to burn off the Organics such as carbon and poly vinylidene fluoride. The Powder $LiCoO_2$ was then ground for 30 minutes to obtain smaller particles with higher surface to increase leaching efficiency.

b) Dissolution of $LiCoO_2$ in mixture of glycine and ascorbic acid:

The cathode material of 2 gram was subjected for chemical dissolution using mild organic acids, an aqueous mixture of glycine and ascorbic acid which were taken in a round bottom flask with the volume of 100 ml and the flask was placed in a water bath to control the reaction temperature during leaching. The flask was fitted with the vapor condenser and the magnetic stirrer. Leaching process was carried for 6 hours at 80 °C the solution and the insoluble residue obtained are separated by filtration by using membrane filter paper and washed with distilled water yields a pink filtrate and a black Residue. The filtrate was split into two parts for further preparations.

c) Preparation of cobalt oxide nanoparticles:

To the filtrate capping agent PVA was added and NaOH solution was added until precipitate was complete, the precipitate formed was separated from the solution using centrifugation process. The solid material obtained is cobalt oxide nanoparticles, which is filtered, dried and sintered at 400 °C and yield was 0.82 g.

d) Preparation of Ag doped cobalt oxide nanoparticles:

To the iltrate, silver nitrate solution (3% weight of cathode material), PVA capping agent was added. To this NaOH solution added until precipitate was complete, the precipitate formed was separated from the solution using centrifugation process. The

solid material obtained is Ag doped cobalt oxide nanoparticles, which is filtered, dried and sintered at 400 °C and yield was 0.81 g.
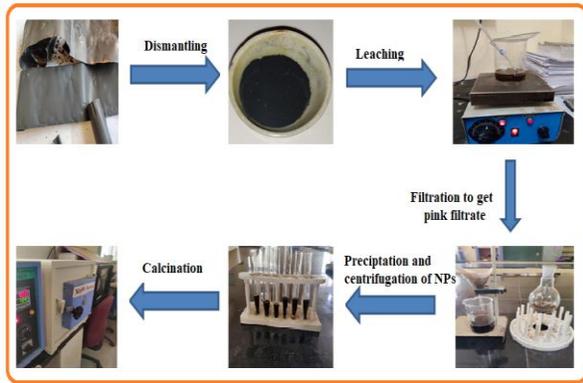
Figure 1: Flow chart of preparation of nanoparticle.

III. RESULTS AND DISCUSSION
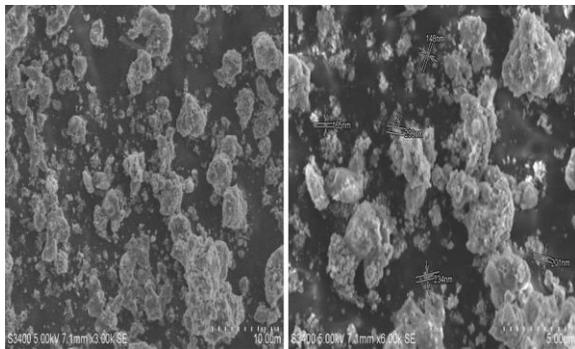
Figure 2: Shows SEM image of cobalt oxide alone.
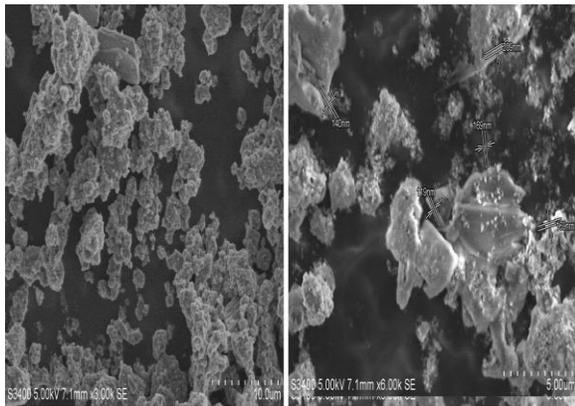
Figure 3: Shows the SEM images of Ag doped cobalt oxide nanoparticle.

SEM analysis of Co_3O_4 and Ag doped Co_3O_4 is shown in figure 4 and 5 the SEM micrographs clearly reveal the formation of agglomerates consisting of small smooth spherical nanoparticles around a much larger central NPs. From the SEM image, it can be seen that increasing agglomeration of nanoparticles. This is due to the kinetic equilibrium process, where the high concentration of carbonic species results in accelerated nucleation of cobalt atom with oxygen ion and carbon atoms from the capping agent, which led to formation of larger particle.
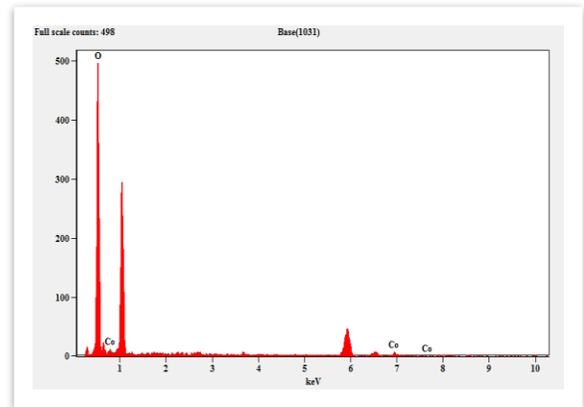
Figure 4: Shows the EDS Spectra of Co_3O_4-nanoparticles alone.

An elemental analysis of cobalt oxide and Ag doped Co_3O_4 samples was performed with energy dispersive X ray (EDS) spectroscopy , using a scanning Electron microscope (SEM). EDS analysis was conducted to determine the elemental composition Of a synthesized nanoparticles figure 6 and 7 shows the results of EDS analysis. Analysis with EDS shows that Cobalt oxide consist of oxygen and cobalt atom on the weight basis as 90.30%, 9.70% respectively .The EDS spectra of the Co_3O_4 sample reveal prominent EDS peaks at 0.9keV, 7keV, 7.8keV, 0.7keV. EDS spectra of silver doped cobalt oxide nanoparticles figures consist of Ag, Co and O atoms on weight basis as 95.02%, 2.54%,2.45% respectively . From this we observe doping has been successfully and sample being pure the EDS spectra of Cobalt oxide samples reveal prominent EDS peaks at 0.9keV, 7keV, 7.8keV 0.7keV.

Table 1: EDS analysis of Co_3O_4 Nanoparticles.

Element Line	Weight %	Weight % Error	Atom %
O K	90.30	± 1.63	97.17
Co K	9.70	± 2.07	2.83
Co L	---	---	---
Total	100.00		100.00
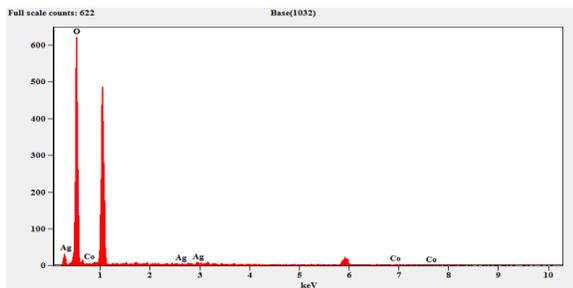
Figure 5: Shows the EDS spectra of Ag dopedCo_3O_4 Nanoparticles.

Table 2: EDS analysis of Ag doped Co_3O_4 Nanoparticles.

Element Line	Weight %	Weight % Error	Atom %
O K	95.02	± 1.55	98.90
Co K	2.54	± 1.62	0.72
Co L	---	---	---
Ag L	2.45	± 1.11	0.38
Ag M	---	---	---
Total	100.00		100.00
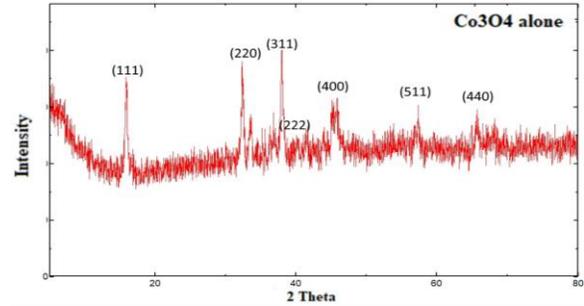
Figure 6: Shows the XRD of Co_3O_4 Nanoparticles.
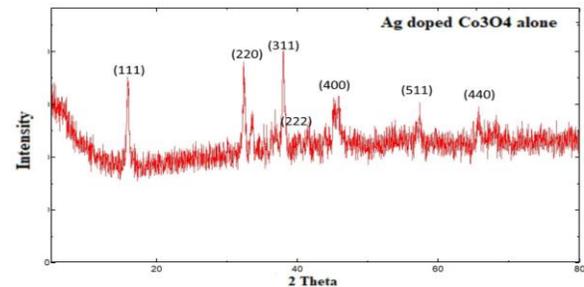
Figure 7: Shows the image of Ag doped Co_3O_4 Nanoparticles.

X ray diffraction studies confirmed that cobalt nano particles were synthesized from cobalt oxide with a size ranging from 40m to60nm.The nanoparticle showed a crystalline structure with a round shape and smooth surface.

The XRD pattern of the Co_3O_4 nanoparticles after calcined at 45 °C the intensity and definition of the diffraction peaks associated with Co_3O_4 increase with increasing heat treated temperature.

The variation of XRD crystal size Co_3O_4 nanoparticles prepared by the thermal treatment of precursor at different temperature. It can be indicated the crystal size rapidly increases from approximately 13nm at 300 ºC to ~ 38nm at 600ºC.

Powder XRD was a rapid analytical technique primarily used for phase identification of a crystallite material and can provide information on unit cell dimension. The XRD pattern of the CuO NPs was in monoclinic phase was shown in figure 8 and 9.The average crystallite size was calculated using Debye Scherer formula.

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

Where D is the crystallite size, is the wavelength(1.5406A° for Cu Kα) of the x-ray

radiation ,β is the full width at half maximum of the peaks at the diffracting angle theta crystallite size was calculated to be 20.09 nm for Co_3O_4 alone and 13.57 nm for Ag doped Co_3O_4 as shown below in table 1.

XRD patterns of the Co_3O_4 nanoparticles calcined at 450 °C, is shown in Figure 1, which indicates the cobalt oxide has cubic phase structure. The peak positions (2θ = 17.7º(111), 33.88º(220), 39.59º(311), 40.85º(222), 43.2(400), 59.2(511), 63.5(400) and relative intensities obtained for the Co_3O_4 match with the JCPDS card No: 073-1701 file, identifying it as Co_3O_4 with a cubic structure of Nanoparticles.

Table 3: Shows size of Nanoparticles calculated.

Sample	2 θ	θ	cos θ	sin θ	FWHM (degree)	FWHM (radians)	β cos θ	Size (nm)	d-spacing
Co_3O_4 alone	37.9194	18.9597	0.9993	0.1099	0.3985	0.00695	0.00690	20.09	
Ag doped Co_3O_4	37.9194	18.9597	0.9993	0.1099	0.2244	0.00391	0.00388	13.573	0.0049
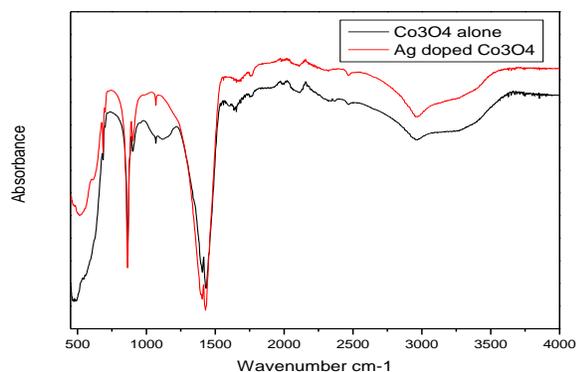
Figure 8: Shows the IR spectrum of Co_3O_4 and Ag doped Co_3O_4 Nanoparticles.

Fourier transform IR spectroscopy is useful in meaning the absorption of IR radiation by a sample, and the results were shown by means of a wavelength. The evaluation of the IR spectrum includes the correlation of the absorption bands (vibrational bands) and the chemical compounds in the sample. The FTIR spectrum of Co_3O_4 and Ag doped Co_3O_4 nanoparticles powder was shown in figure 8. The Co_3O_4 Nanoparticles exhibited vibrational mode at $500cm^{-1}$, $900cm^{-1}$, $1100cm^{-1}$, $1480cm^{-1}$, $2900cm^{-1}$ were assigned for Co_3O_4 stretching vibration , rocking vibrational mode of water molecules at $850cm^{-1}$.

ANTIMICROBAL ACTIVITY:

a) Bacterial strains:

The newly synthesized compounds have been screened for their antibacterial activity by agar well diffusion method. Gram-positive bacteria, a) Bacillus, Staphylococcus aureus and Gram-negative bacteria b) Escherichia coli, Pseudomonas aeruginosa were used for antibacterial activity. All bacterial strains were maintained on nutrient agar medium at ±37°C.

b) Antibacterial activity:

The antibacterial activity of newly synthesized compounds Co_3O_4 and Ag doped Co_3O_4 was evaluated using agar disc diffusion assay . Briefly, a 24 and 48 hours old culture of selected bacteria was mixed with sterile broth (0.9%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 (106 colony forming units (CFU) per ml). Petri plates containing 20 ml of Mueller Hinton Agar and Sabouraud dextrose agar was used for antibacterial activity. The inoculum was spread on the surface of the solidified media and a 4 small well has made by using cork borer at 1st well standard antibacterial agent Ampicillin 5mM 50 μL was taken and in the other 3 wells different concentration 5mM, 10mM and 20mM of synthesized compounds were taken which are dissolved in DMSO dimethyl sulfoxide was used as negative control. Plates inoculated with the bacteria were incubated for 24 hour at 37 °C. The diameters of zone of inhibition were measured in millimeters by using antibiotic zone scale procured from Hi-media.

All the tests were performed in triplicate and the average was taken as final reading.

Table 4: Anti-bacterial activity of synthesized compounds.

Tested samples	Bacillus	S. Aures	E-coli	Pseudomoas
Std	2.6±0.2	2.8±0.2	2.8±0.2	1.8±0.2
Co_3O_4 NPs	1.4±0.2	1.4±0.2	1.2±0.2	1.5±0.2
Ag doped Co_3O_4 NPs	1.2±0.2	1.6±0.2	1.8±0.2	1.4±0.2

Although the specific mechanism of the antimicrobial effect related with the use of Co_3O_4 nanoparticles is not known, several mechanisms of action on bacterial cells have been discussed. Even if not specific to Co_3O_4, but for most nanoparticles oxide nanoparticles, Zhang et al. reported that the generation of reactive oxygen species (ROS) within bacterial cells is enhanced when using Co_3O_4-water suspensions. The antibacterial activity of Co_3O_4 nanoparticles seems to be different depending on the type of bacteria cells. The results in table for 5 mM concentration of standard and nanoparticles showed that the Ampicillin standard showed highest activity when compared nanoparticles, In case Bacilus and pseudomanas the activity is higher for Co_3O_4 nanoparticles, in case S.Aures, E.coli the activity is highest for Ag doped Co_3O_4 nanoparticles. Thefore it can be concluded that both Co_3O_4 Ag doped Co_3O_4 nanoparticles presented higher antibacterial activity.

It has been reported that the antimicrobial properties depend on the surface morphology, size, phobicity and shape of nanoparticles. It seems that small particles with a large surface area have better antibacterial activity, as compared with larger ones.

Co_3O_4 nanoparticles appear to be twenty times more toxic to the protozoan as compared with their bulk material. It has been shown that the toxicity depends on the exposure time. Also, in many studies the toxicity of Co_3O_4 nanoparticles was shown to be higher than their bulk form. Even though the molecular toxicity mechanisms of Co_3O_4 nanoparticles on eukaryote models are not fully understood, many studies have shown that these nanoparticles promote mitochondrial damage, DNA damage and oxidative DNA damage. In vitro assays revealed that toxicity of Co_3O_4 nanoparticles depended on the size and shape of the particles. Results showed that Co_3O_4 nanoparticles were more toxic than Cu^{2+}, due to the increase in the generation of ROS, DNA damage and decrease levels of reduced glutathione (GSH) compared to control.

IV. CONCLUSION

In the present work, we witnessed an increase in antimicrobial behavior of Ag doped Co_3O_4 nanoparticles compared to the undoped Co_3O_4 nanoparticles. The synthesized Co_3O_4 and Ag doped Co_3O_4 nanoparticles the crystallite sizes were measured to be 148nm and 119nm respectively. From SEM a analysis the morphology of nanoparticles was found to be spherical smooth shape. Doping was confirmed from EDS spectra. Formation of Co_3O_4 nanoparticles is confirmed with FT-IR spectroscopy which showed band at 1425 and 990 cm-1. Ag doped nanoparticles exhibit excellent antibacterial effect against S.Aures, E.coli bacteria with zone of inhibition value of 1.6 mg/ml and 1.8mg/ml. Ag doped Co_3O_4 nanoparticles exhibit excellent antibacterial effect compared to undoped Co_3O_4 nanoparticles.

V. REFERENCE

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