IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Grass like CO_3O_4 nanoparticle for application in vitro anti-bacterial efficacy by CBD method

*Shinde S.A. and Kote J. R.

School of Life Sciences Swami Ramanand Teerth Marathwada University Nanded

Sharad Chandra Arts, commerce and Science College Naigaon Dist- Nanded

Abstract

We developed Co_3O_4 to by CBD (Chemical particle bath deposition) method for using the cobalt nitrate as precursor. With mixture as urea, NH_4F, hydrothermal heat at 90ºc of 17 hrs to produced cobalt hydroxide Co $(OH)_2$ after more heat at 350ºc1hrs to from the nanosheet assemblyCo_3O_4 . The synthesized nanoparticles were characterized by FTIR spectroscopy and X-ray diffraction studies. Co_3O_4 are high-quality mono-dispersed, more stable as other nanoparticles and defect-free nanoparticles. The surface morphology of these nanoparticles was revealed by scanning electron microscopy. XRD pattern Co_3O_4 nanoparticles have cubic structure and size ranging from 32 nm. The particle size and microstructure were studied by transmission electron microscopy (TEM) images. Raman spectra confirmed that developed compounds as. $Co3O_4$ nanoparticles are combat the growth of two *E. coli*. This nanoparticle shows the good antioxidant agent and also studied cytotoxicity effect against human blood cells by hemolytic assay.

Key Words : Antibacterial, XRD, SEM, Co_3O_4 Nanaoparticles, TEM

Introduction

Nanomaterials world becomes highest attraction due to their small size, effective, physical and chemical properties[1-4].The different area cover by the Co_3O_4 like circulating angiogenic cells,[5] magnetic nanomaterials,[6-7] polar semiconductor,[8] gas sensor,[9-10] spintronics,[11-15] catalysis,[16-21] atomic layer deposition[22-24] (ALD), chemical vapor deposition(CVD),[25–28] pulsed laser deposition (PLD),[29] also developed a simple pyrolytic technique to prepare metal oxide nano particles employing coordination polymers or coordination complexes as sole precursors.[30–32,6]. At room temperature and the most stable form of cobalt oxide. Development of Co_3O_4 NPs by CBD method recently; several studies have been reported for the synthesis of Co_3O_4 nanostructures, including nanowires and nanotubes, using chemical bath deposition (CBD) [17]. However, synthesis of transition metal oxide nano structure with their different properties and having environmentally stability is very difficult. Moreover the available preparative above mentioned methods are costly and time consuming. We have developed Co_3O_4 to by CBD (Chemical particle bath deposition) method for using the cobalt nitrate as precursor. With mixture as urea, NH_4F, hydrothermal heat at 90ºc of 17 hrs to produced cobalt hydroxide Co $(OH)_2$ after more heat at 350ºc 1hrs to from the nanosheet assembly Co_3O_4.

The present work we carry out Co_3O_4 as antimycobacterial agent no one use these biomaterials as tubercle agent and focus on Co_3O_4 as cytotoxic effect on human blood cells, an also study Co_3O_4 potential

antioxidant agent by DPPH method. The Co_3O_4 powder sample was characterized by means of XPS, X-ray diffraction (XRD), Raman shift, SEM, and TEM. The main objective of this study is to develop the bactericidal effect of cobalt oxide nanoparticles using various mycobacterium strains. Such a type of investigation would expose better utilization of nanoparticles use as more application in different field like life science, physical science and also medical science.

Experimental

Materials

The all chemicals were obtained from used in this experiment are analytical grade Himedia Latur (Maharashtra) India. Cobalt Nitrate Co $(NO_3)_2$, $6H_2O$, urea $(CO\ (NH_2)_2)$ and ammonium fluoride (NH_4F) were used as received. Double distilled water was used the experiment. The synthesis of $Co3O_4$by using, 0.1M Cobalt nitrate, 0.5M urea, 0.2M ammonium fluoride was dissolved in 50 ml of deionized water under magnetic stirring for 30 minutes. The obtained pink solution transferred into falcons tubes and then tubes were sealed and kept into water bath maintained temperature at 90^0 c for 17 hours afterward completion of reaction pinkish colour cobalt hydroxide are achieved. The precipitate washed with distilled water and ethanol then dried at 60^0 c. The pink colour cobalt hydroxide samples converted into black colour Co_3O_4 by annealing 350^0 c in a muffle furnace for 1 hour in air. The colour changed pink to dark black after annealing that the formation of the Co_3O_4 phase

Physical measurements

To determine the structural features of the samples, Fourier transform infrared (FTIR) spectroscopy was carried out at 25ºC using a PerkinElmer spectrum rxift-ir system FT-IR spectrometer with 64 scans for wave numbers ranging from 400 to 4000 cm^1 and resolution 4 cm^1. The KBr pellet method was used to prepare the samples. The morphology and size of Co_3O_4 particles have been characterized by scanning electron microscopy (SEM) (Hitachi S-3400N) operating at 15 kV. The particle size and microstructure were studied by transmission electron microscopy (TEM) with a JEOL (Japan) JEM2100. For size measurement, sonicated stock solution of all nanoparticles (0.5 mg ml1) was diluted 20 times. The powder XRD measurements were carried out using Bruker D8 Advance X-ray Diffractometer. The X-rays were produced using a sealed tube and the wavelength of X-ray was 0.154 nm (Cu-Ka). Figure 1. To analysis with all charecterstic

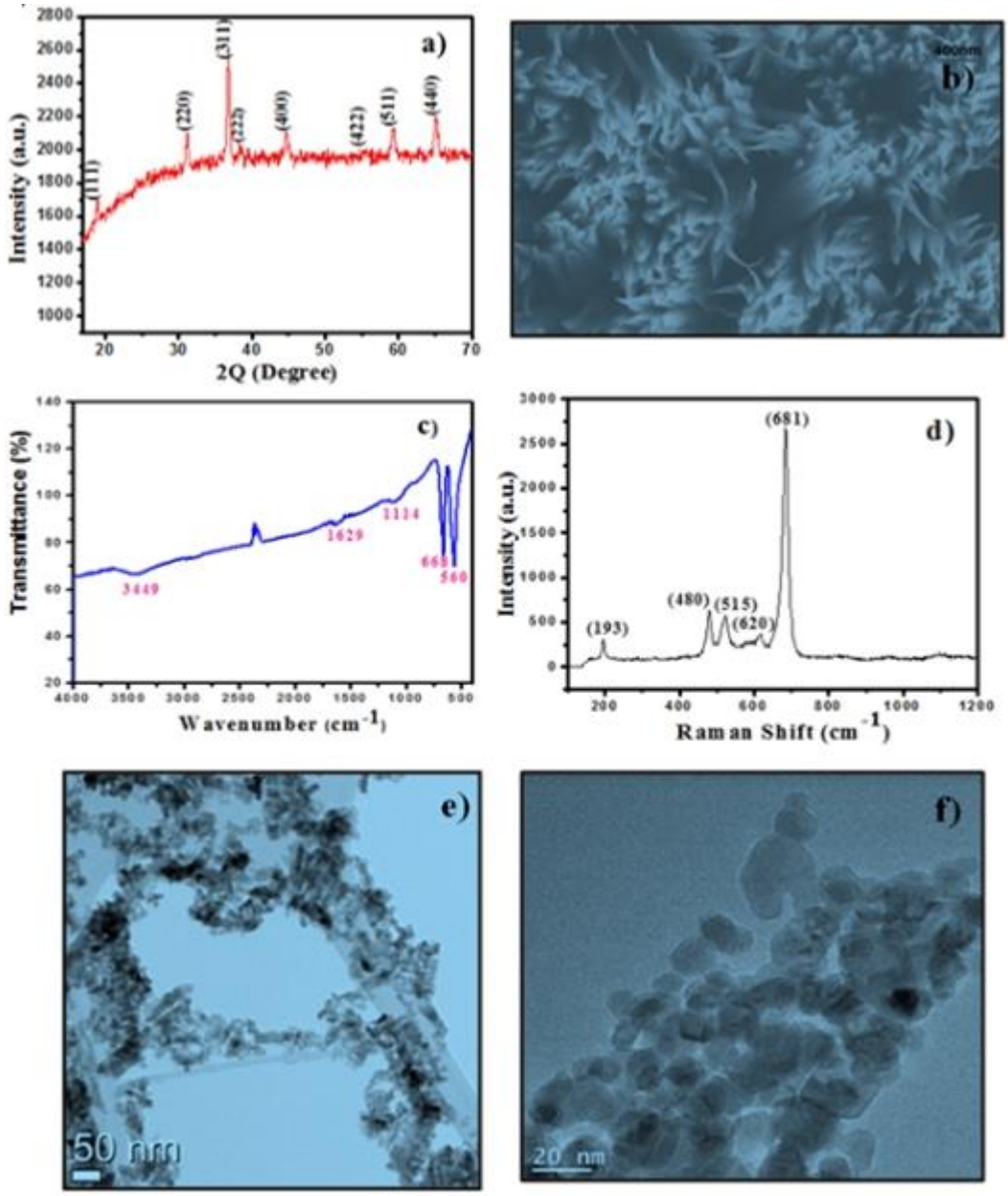

Figure:- 1 a) XRD pattern of Co_3O_4.b) SEM image of Co_3O_4. c) IR spectrum of (a) Co_3O_4 nanoparticle. d) Raman shift of Co_3O_4.e) and f) TEM image of Co_3O_4.

Biological measurements

Isolation of microorganism

The *E.coli* was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh (PB), and India was sub cultured and maintained into Lowenstein Jensen media.

Determine cobalt oxide nanoparticles for antibacterial study

The *E.coli* As per of experimental standardization, initially 1mg/ml; concentration of Co_3O_4 nanoparticles was used for antimycobacterial analysis and it was further take upto only 70ug/ml add into the well, however the clear zone of inhibition was observed under the experimental condition. In short; a sterile cork borer of 7mm diameter was used to bore holes into the inoculums seeded solidified LJ medium [03]. A 70µl volume of each of (1mg/ml) the Co_3O_4 nanoparticles was loaded into the well using sterile pipette. The plates were kept in refrigerator for prediffusion of the sample and incubated at 37ºc for 48 hours. Growth of *M.tuberculosis* and *Mycobacterium avim* was observed after the diameter of inhibition zone was measured subtracting the well size Streptomycin (10µg/ml) was used as reference standard.

Table 1 Result of agar cup assay method

Name of Compounds	Average zone of inhibition in (mm)
Co_3O_4 NPs (PG1, PG)	33.23± 0.122
Streptomycin	42.45± 0.163

CBD (Chemical particle bath deposition)

Co_3O_4 to by CBD (Chemical particle bath deposition) method for using the cobalt nitrate as precursor. With mixture as urea, NH_4F, hydrothermal heat at 90ºc of 17 hrs to produced cobalt hydroxide Co (OH) 2 after more heat at 350ºc1hrs to from the nanosheet assembly Co_3O_4.

Infrared spectroscopy analysis of metal oxide nano particles

FT-IR spectra of the thermally stable end product of the species Co_3O_4 show strong bands at about 570 cm_1 and 665 cm_1 and Co3O4-II show strong bands at about 567 cm_1 and 665 cm_1 (Fig. 2(a) and (b)). The small band obtained at about 3422 cm_1 and 3434 cm_1 respectively due to presence of moisture in KBr. The Ⅱrst band is associated with the Co_3+ vibration in the octahedral hole and the second band is attributed to the Co2+ vibration in the tetrahedral hole 33 which confirms the formation of the Co3O4 spinals.

X-ray diffraction analysis

The X-ray powder diffraction (XRD) patterns of both Co_3O_4 nanoparticles (Fig. 3(a) and (b)) were produced well defined diffraction patterns, indicating that they are crystalline and the visible peaks can be well indexed to the $Co3O_4$ phase [34 All six diffraction peaks can be assigned undisputedly to (111), (220), (400), (311), (511) and (440) lattice planes, which are in good agreement with those of the bulk Co3O4. There is no evidence for parasitic phases like CoO or metallic Co.

Scanning electron microscopy analysis

The morphology and particle size of the metal oxide nanoparticles were examined by SEM (Fig. 4(a) and (b)). Square and been visualized in their respective SEM micrographs with the

size ranges from 10–25 nm and 100–150 nm respectively.

Transmission electron microscopy

The transmission electron microscopy of Co3O4 NPs shows nearly spherical geometry with a mean size of 30 _ 10 and 100 _ 20 nm respectively. The result is represented in Fig. 6(a) and (b). The observed size of the NPs was approximately

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