International Journal of Recent Trends in Science And Technology, P-ISSN 2277-2812 E-ISSN 2249-8109
Special Issue, ICRAFHN 2018 pp 60-64

Original Research Article

Cytotoxicity studies of cobalt oxide nanoparticles from one precursor

Jivan R. Kote[1], Ambadas S. Kadam[2], Paresh S. Gaiker[3], Shivraj Nile[4] , Rajaram S. Mane[5], B. M. Kareppa[1*]

[1,1*] Department of Biotechnology, DSM College Parbhani, Maharashtra, INDIA.
[2] DSM College, Jintur, Maharashtra, INDIA.
[3]School of Life Science, [5]School of Physical Sciences, SRTM, University, Nanded, Maharashtra, INDIA.
[4] Konkuk University, Seoul, KOREA.
Email: 7588570955kote@gmail.com

Abstract We developed Co3O4 by CBD (Chemical particle bath deposition) method for using the cobalt nitrate as precursor. With mixture as urea, NH4F, hydrothermal heat at 90ºc of 17 hrs to produced cobalt hydroxide Co (OH) 2 after more heat at 350ºc for 1hrs to from the nanosheet assembly Co3O4. The synthesized nanoparticles were characterized by FTIR spectroscopy and X-ray diffraction studies. Co3O4 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 Co3O4 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 crystalline cubic phase of Co_3O_4 nanoparticle. Co3O4 nanoparticles are combat the growth of one *Mycobacterium* species like *Mycobacterium tuberculosis* (MTCC-300). This nanoparticle shows the good cytotoxicity effect against human blood cells by hemolytic assay.
Key Words: Co_3O_4 Nanoparticle, XRD, SEM, TEM, hemolytic assay *Mycobacterium tuberculosis.*

***Address for Correspondence:**
Dr. B.M.Kareppa, Konkuk University, Seoul, KOREA.
Email: kareppabm@gmail.com

Access this article online	
Quick Response Code:	Website: www.statperson.com
	Accessed Date: 10 March 2018

INTRODUCTION

Nanomaterials world becomes highest attraction due to their small size, effective, physical and chemical properties (Sun *et al*. 2000; Fernandez *et al*. 2004; Bell *et al*.2003; Yuvakkumar *et al*. 2014) The different area cover by the Co3O4 like circulating angiogenic cells (Spigoni *et al*. 2015), magnetic nanomaterials (Ghosh *et al*. 2014; Dar *et al*.2015), polar semiconductor (Kormondy *et al*. 2014), gas sensor (Eranna *et al*. 2005; Wu at al. 2010), spintronics (Moyer *et al*. 2011; Chen *et al*. 2004; Gonzalez *et al*. 2008; Posadas *et al*.2011; Liu *et al*. 2003), catalysis (Wollenstein *et al*. 2003; Natile and Glisenti *et al*. 2002; Sun *et al*. 2013; Xie *et al*. 2009; Jiao and H. Frei *et al*. 2009; Agiral *et al*. 2013), atomic layer deposition (ALD) (Klepper *et al*. 2007; Donders *et al*. 2011; Ding *et al*. 2008), chemical vapor deposition(CVD) (Shalini *et al*. 2001; Barreca *et al*. 2011; Fujii *et al*. 1995; Mane *et al*. 2002), pulsed laser deposition (PLD) (Kennedy *et al*. 1995), also developed a simple pyrolytic technique to prepare metal oxide nano particles employing coordination polymers or coordination complexes as sole precursors (Ghosh *et al*. 2011). At room temperature and the most stable form of cobalt oxide. Development of Co3O4 NPs by CBD method recently; several studies have been reported for the synthesis of Co3O4nanostructures, including nanowires and nanotubes, using chemical bath deposition (CBD) (Natile and Glisenti *et al*. 2002)[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 Co3O4 to by CBD (Chemical particle bath deposition) method for using the cobalt nitrate as precursor. With mixture as urea, NH4F, 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 Co3O4. The present work we carry out Co3O4 as antimycobacterial agent no one use these biomaterials as tubercle agent and focus on Co3O4 as cytotoxic effect on human blood cells. The Co3O4 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.

MATERIALS AND METHODS

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 Co3O4 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 Co3O4 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 cm1 and resolution 4 cm1. The KBr pellet method was used to prepare the samples. The morphology and size of Co3O4 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).

Biological measurements Isolation of microorganism: The *Mycobacterium tuberculosis* (MTCC 300) 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.

The cytotoxicity effect of Co_3O_4 nanoparticles with human blood cells: The hemolytic activities of the test compounds were determined using human red blood cells (Ahmad *et al*. 2010; Rajput *et al*. 2013) Human erythrocytes from healthy persons were collected in tubes containing EDTA (1–2 mg/ml) as anti-coagulant. The erythrocytes were harvested by centrifugation (Heraeus Megafuge 40, Thermo Fisher Scientific Inc., MA) for 10 min at 634 × g at 20°C, and washed three times in PBS. PBS was added to the pellet to yield a 10% (v/v) erythrocytes/PBS suspension. The 10% suspension was diluted 1:10 in PBS. From each suspension, 100 µl was added in triplicate to 100 µl of a different dilution series of test compounds (or Rifampicin as a standard antitubercular agent) in the same buffer in Eppendorf tubes. Total hemolysis was achieved using 1% Triton X-100. The tubes were incubated for 1 h at 37°C and centrifuged for 10 min at 634 × g at 20°C. From the supernatant fluid, 150 µl was transferred to a flat-bottomed microtitre plate (Tarson India Ltd., India), and the absorbance.

RESULT AND DISCUSSION

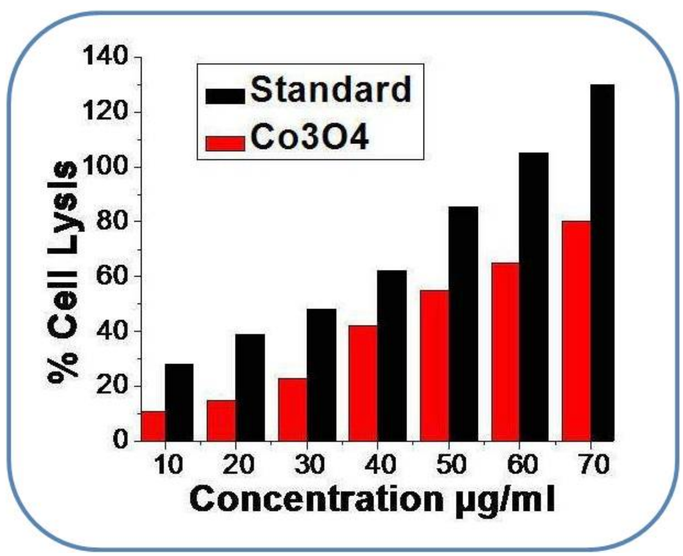

Figure 1: Haemolytic assay

CBD (Chemical particle bath deposition): Co3O4 to by CBD (Chemical particle bath deposition) method for using the cobalt nitrate as precursor. With mixture as urea, NH4F, hydrothermal heat at 90ºc of 17 hrs to produced cobalt hydroxide Co (OH) $_2$ after more heat at 350ºc1hrs to from the nanosheet assembly

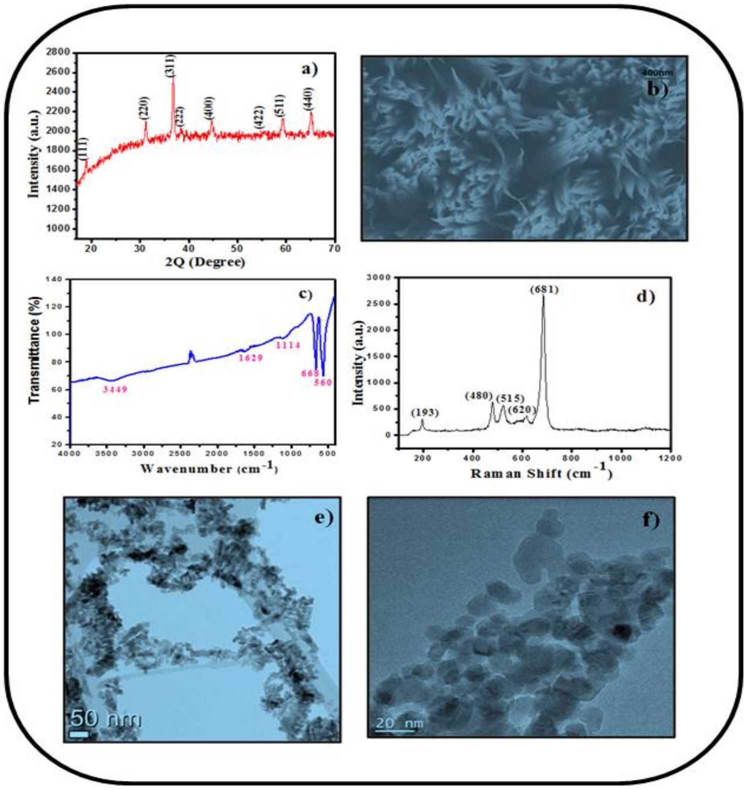

Figure 3.a) XRD pattern of Co3O4.**b)** SEM image of Co3O4. **c)** IR spectrum of (a) Co3O4 nanoparticles. **d)** Raman shift of Co3O4.**e)** and **f)** TEM image of Co3O4.

Cytotoxicity effect of Co_3O_4 nanoparticles with human blood cells: The in vitro hemolytic assay is a screening tool for gauging in vivo toxicity toward host cells (Christie *et al*. 2007). The four compounds tested showed no significant toxicity to human erythrocytes at minimum growth inhibitory concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40µg/ml, 50 µg/ml, 60µg/ml, 70 µg/ml, Co_3O_4 nanoparticles concentration), whereas they had profound effects on inhibition *M. tuberculosis* (fig. 2). Only 0–12% hemolysis was observed at the tested concentrations, while Rifampicin showed 100% hemolysis at 0.5 mg/ml.

Infrared spectroscopy analysis of metal oxide nano particles: Fig 3c) illustrates that FTIR spectrum of Co_3O_4 nanoparticle with five distinct bands. Fourier transform infrared (FTIR) ab- sorption spectrum of the Co_3O_4 nanoparticle In the studied region 4000–400 cm^{-1}, two absorption bands originate from stretching active vibrations of the Co-O bonds in the spinel structure. The first band ν_1 at 560 cm^{-1} is associated with the OB_3 vibrations in the spinel lattice where B denotes the Co^{3+} ions in octahedral coordination (Co_3-O_6) t2g. The second band ν_2 at 668 cm^{-1} is attributed to the ABO_3 vibration where A denotes the Co^{2+} ions in tetrahedral coordination (Co_2-O_4)eg. Confirming the Co^{2+} $[Co^{3+}]_2$ O_4 spinel structure.

X-ray diffraction analysis: The X-ray powder diffraction (XRD) patterns of both Co3O4 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 Co3O4 phase. 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. For the structural study of the nanoparticle of Co_3O_4 was the carried out by the XRD study. The XRD pattern for the Co_3O_4 as shown in fig (1a).The reflection peaks at 2Ɵ of 19.07^o, 31.30^o, 36.83, 38.51^o, 44.81^o, 55.63 ^{o}, 59.33 ^{o} and 65.22^o can be easilly indexed as (111), (220),(311), (222),(400),(422),(511) and (440) planes respectively. Which is matched with the standard spectrum of (Joint Committee on Powder Diffraction Standards file, no: 43-1003) with a space group Fd3m (227). The existence of sharp diffraction peaks was an pointing of the polycrystalline nature of the as-synthesized for cubic Co_3O_4.The average particle of the Co_3O_4 nanoparticle was calculated by 32 nm by Debye–Scherrer's equation using full-width at half maximum (FWHM) value for the (311) plane.

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 50 nm and 20 nm respectively. The result is represented in (fig 3 e and f) observed size of the NPs was approximately
Raman Spectroscopy: The Raman spectra of cobalt oxide which contain five Raman active phonon vibration mode at 193(F_{2g}), 480(E_g), 515(F_{2g}), 621(F_{2g}), and 681(A_{1g}) cm^{-1} in (fig.3 d). These peaks confirmed that crystalline cubic phase of Co_3O_4 nanoparticles.

CONCLUSION

By using green approach, the Co3O4 NPs were synthesized by CBD (Chemical particle bath deposition) method for using the cobalt nitrate as precursor which is a rapid method and gives excellent reproducible when compared to other synthetic processes.Using Co3O4 nanoparticles use for biological application maintaining the human blood cells, *in-Vitro* condition. Thus the field Co3O4 NPs this one ability to kill the particular concentration and also anti-TB agent. Co3O4 NPs. Co3O4 NPs materials are new way for nanotechnology and biotechnology. CBD (Chemical particle bath deposition) method has shown to be less toxic against human blood cells. Co3O4 NPs are used for biomedical and sensor application.

REFERENCES

1. Agiral A, Soo HS, Frei H (2013) Visible Light Induced Hole Transport from Sensitizer to Co3O4 Water Oxidation Catalyst across Nanoscale Silica Barrier with Embedded Molecular Wires. Chem Mater 25: 2264–2273.
2. Ahmad A, Khan A, Manzoor N, Khan LA (2010) Evolution of ergosterol biosynthesis inhibitors as fungicidal against Candida. Microb Pathog 48:35–41.
3. Anargyros, Astill DSJ, Lim ISL (1990) Comparison of Improved BACTEC and Lowenstein-Jensen Media for Culture of Mycobacteria from Clinical Specimens. Journal of Clinical Microbiology 28:1288-1291.
4. Barreca D, Devi A, Fischer R, Bekermann AD, Gasparotto A, Gavagnin M, Maccato C, Tondello E, Bontempi E, Depero LE, Sada C (2011) Strongly oriented Co3O4 thin films on MgO(100) and MgAl2O4(100) substrates by PE-CVD. Cryst Eng Comm 13:3670-3673.
5. Bell AT (2003) The impact of nanoscience on heterogeneous catalysis. Science 299:1688–1691.
6. Chien HP, Yu MC, Wu MH, Lin TP, Luh KT (2000) Taiwan Provincial Chronic Disease Control Bureau; Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan Comparison of the BACTEC MGIT 960 with Lowenstein-Jensen medium for recovery of mycobacteria from clinical specimens. The International Journal of Tuberculosis and Lung Disea 4:866-870.
7. Christie MS, Kenneth LR, David BW (2007) Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to in vivo pulmonary toxicity profiles. Toxicological Sci 97:163–80.
8. Dar MA, Nam SH, Kim JY, Ahmad I, Cho BK, Kim WB (2015) Magnetic Response of Hydrothermally Prepared Self-Assembled Co3O4 Nano-platelets. Journal of Electronic Materials 1:1-5.
9. Ding Y, Xu L, Chen C, Shen X, Suib SL (2008) Syntheses of Nanostructures of Cobalt Hydrotalcite Like Compounds and Co3O4 via a Microwave-Assisted Reflux Method. J. Phys. Chem C 22: 8177–8183.
10. Donders ME, Knoops HCM, Van MCM, Kessels WMM, Notten PHL (2011) Remote Plasma Atomic Layer Deposition of Co3O4 Thin Films. J Electrochem Soc 4:G92-G96.
11. Eranna G, Joshi BC, Runthala DP, Gupta RP (2004) Oxide Materials for Development of Integrated Gas Sensors-A Comprehensive Review. Critical Reviews in Solid State and Materials Sciences 29:111-188.
12. Fernandez MG, Martinez AA, Hanson JC, Rodriguez JA (2004) Nanostructured oxides in chemistry: characterization and properties. Chem. Rev 104:4063–104.doi: 10.1021/cr030032f
13. Yuvakkumar AR, Nathanael AJ, Hong SI (2014) Inorganic complex intermediate Co3O4 nanostructures using green ligation from natural waste resources. RSC Adv 4:44495–44499.
14. Fujii E, Torii H, Tomozawa A, Takayama R, Hirao T (1995) Preparation of cobalt oxide films by plasma-enhanced metalorganic chemical vapour deposition. J Mater Sci 30:6013.
15. Ghosh T, Dash SK, Chakraborty P, Guha A, Kawaguchi K, Roy S, Chattopadhyay T, Das D (2014) Preparation of antiferromagnetic Co3O4 nanoparticles from two different precursors by pyrolytic method: in vitro antimicrobial activity. RSC Adv 4:15022–15029.
16. Ghosh T, Chattopadhyay T, Das S, Mondal S, Suresh E, Zangrando E, Das D (2011) Thiocyanate and Dicyanamide Anion Controlled Nuclearity in Mn, Co, Ni, Cu, and Zn Metal Complexes with Hemilabile Ligand 2-Benzoylpyridine. Cryst Growth Des 11:3198–3205.
17. Gonzalez MSM, Fernandez JF, Rubio-Marcos F, Lorite I, Costa-Kraumer JL, Quesada A, Banares MA, Fierro JLG (2008) Insights into the room temperature magnetism of ZnO⁄Co3O4ZnO⁄Co3O4 mixtures. J Appl Phys 103:083905.
18. He T, Chen D, Jiao X, Yanyan Xu, Yuanxiang Gu (2004) Surfactant-Assisted Solvothermal Synthesis of Co3O4 Hollow Spheres with Oriented-Aggregation Nanostructures and Tunable Particle Size. Langmuir 19: 8404–8408.
19. Jiao F, Frei H (2009) Nanostructured Cobalt Oxide Clusters in Mesoporous Silica as Efficient Oxygen-Evolving Catalysts. Angew Chem 10:1873–1876.

20. Kennedy RJ (1995) The growth of iron oxide, nickel oxide and cobalt oxide thin films by laser ablation from metal targets. IEEE Trans Magn 31:3829-3831.
21. Klepper KB, Nilsen O, Fjellvag H (2007) Growth of Thin Films of Co3O4 by Atomic Layer Deposition. Thin Solid Films 515:7772-7781.
22. Kormondy KJ, Posadas AB, Slepko A, Dhamdhere A, Smith DJ, Mitchell KN, Willett-Gies TI, Zollner S, Marshall LG, Zhou J, Demkov AA (2014) Epitaxy of polar semiconductor Co3O4 (110): Growth, structure, and characterization. Journal of Applied Physics 115:243708.
23. Liu Y, Wang G, Xu C, Wang W (2002) Fabrication of Co3O4 nanorods by calcination of precursor powders prepared in a novel inverse microemulsion. Chem Commun 14:1486-1487.
24. Mane AU, Shalini K, Wohlfart A, Devib A, Shivashankar SA (2002) Strongly oriented thin films of Co3O4 deposited on single-crystal MgO (100) by low-pressure, low-temperature MOCVD. J Cryst Growth 240:157–163.
25. Mondal S, Chattopadhyay T, Neogi SK, Ghosh T, Banerjee A, Das D (2011) Na 2 Cd 2 I 6 L 2 (H 2 O) 6 [L= Urotropine]: An interesting precursor for synthesizing CdO particles. Mater Lett 65:783–785.
26. Mondal S, Chattopadhyay T, Das S, Maulik SR, Neogi S, Das D (2012) CdO and CdS nanoparticles from pyrolytic method: Preparation, characterization and photocatalytic activity. Indian J Chem Sect A: Inorg Bio-inorg Phys Theor Anal Chem 51:807–811.
27. Moyer JA, Vaz CAF, Arena DA, Kumah D, Negusse E, Henrich VE (2011) Magnetic structure of Fe-doped CoFe2O4 probed by x-ray magnetic spectroscopies Phys Rev B 84:054447.
28. Natile MM, Glisenti A (2002) Study of Surface Reactivity of Cobalt Oxides: Interaction with Methanol. Chem Mater 7:3090–3099.
29. Posadas A, Berg M, Seo H, Smith DJ, Kirk AP, Zhernokletov D, Wallace RM, Lozanne Ade, Demkov A.A (2011) Microelectronic Engineering 7:1444-1447.
30. Rajput SB, Shinde RB, Routh MM, Karuppayil SM (2013) Anti-Candida properties of asaronaldehyde of Acorus gramineus rhizome and three structural isomers. Chinese Medicine 8:1-8.
31. Shalini K, Mane AU, Shivashankar SA, Rajeswari M, Choopun S (2001) Epitaxial growth of Co3O4 films by low temperature, low pressure chemical vapour deposition. J Cryst Grow 1–2: 242–247.
32. Spigoni V, Cito M, Alinovi R, Pinelli S, Passeri G, Zavaroni I, Goldoni M, Campanini M, Aliatis I, Mutti A, Bonadonna RC, Cas AD (2015) Effects of TiO2 and Co3O4 Nanoparticles on Circulating Angiogenic Cells. Plos One 3:e0119310.
33. Sun S, Murry CB, Weller D, Folks L, Moser A (2000) Monodisperse FePt Nanoparticles and Ferromagnetic FePt Nanocrystal Superlattices. Science 287:1989-1992.
34. Sun H, Ang HM, Tade MO, Wang S (2013) Co3O4 Nanocrystals with Predominantly Exposed Facets:Synthesis, Environmental and Energy Applications. J Mater Chem A 1:14427.
35. Wollenstein J, Burgmair M, Plescher G, Sulima T, Hildenbrand J, Bottner H, Eisele I (2003) Cobalt oxide based gas sensors on silicon substrate foroperation at low temperatures. Sens Actuators B 93:442-448.
36. Wu ZS, Ren W, Wen L, Gao L, Zhao J, Chen Z, Zhou G, Li F, Cheng HM (2010) Graphene Anchored with Co3O4 Nanoparticles as Anode of Lithium Ion Batteries with Enhanced Reversible Capacity and Cyclic Performance. ACS Nano 6:3187–3194.
37. Xie X, Li Y, Liu Z-Q, Haruta M, Shen W (2009) Low-temperature oxidation of CO catalysed by Co3O4 nanorods. Nature 458:746-749.

Source of Support: None Declared
Conflict of Interest: None Declared