Raman Spectroscopic Study of Nickel Oxide Nanoparticles and Its Antibacterial Activity

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Abstract. This paper illustrates the Raman Spectroscopic study of Nickel Oxide nanoparticles synthesized using ASH supported method [1]. Synthesized nanoparticles were isochronally annealed for 2 hrs at 300°C & 700°C. These particles are characterized using Raman spectroscopy & Scanning Electron Microscopy. Raman analysis is in good agreement of XRD results reported in our previous paper [2]. It confirms the formation of pure NiO cubic phase at 700°C annealing temperature. Scanning Electron Microscopy (SEM) elucidates the morphology of the particles & its size was found to be in between 40-90 nm. The antibacterial activity of the NiO nanoparticles was investigated by cup & well diffusion method using pathogenic strains including three gram negative bacteria i.e. Pseudomonas aeruginosa, Salmonella typhi, E.coli and one gram positive species i.e. methicillin-resistant Staphylococcus` aureus. Antibacterial activities were studied by measuring the diameter of zone of inhibition. The Staphylococcus aureus was found to be susceptible towards the NiO nanoparticles with diameter of zone inhibition of 26mm.

INTRODUCTION

Nickel oxide is one of the transitional metal oxides with cubic lattice structure. NiO nanoparticles have a large surface area and unique properties in comparison to the bulk. They have low toxicity [3] and have good biocompatibility [4]. It has lot of applications in gas sensor, energetic materials, electrochemical super capacitor, solar cell battery cathodes [5] etc. Nickel oxide nanoparticles exhibit a higher antibacterial activity against a gram-positive and gram-negative pathogen [6]. Antibacterial activity destroys a bacterium or suppresses its growth or their ability to reproduce. E. coli induces microbial spoilage in milk, which is resent in the digestive tract of the humans, animals, faecal contaminated foods and water. [7]. Enterobacter species causes bone illness, nosocomial infections etc [8]. Bacillus species which produce subtilin and subtilosin will cause two types of illness like diarrhoea, vomiting. [9]. Hence, there is an urgent need to use the antibacterial activity to restrain the growth of bacteria. One of the methods is to use the Nickel oxide nanoparticles to prevent and control these issues. As the properties of the nanoparticles varies with the method of synthesis, it is necessary to have an idea of the particle size of synthesized nanoparticles. The fabrication techniques decide the phase and particle size of nanoparticles.

The present study deduce the structural and morphological properties of Nickel oxide nanoparticles synthesized using a novel, very simple, eco-friendly and cost effective ASH SUPPORTED [1] method. The antibacterial activity of the NiO nanoparticles were investigated by cup & well diffusion method using pathogenic strains including gram negative and gram positive bacteria.
EXPERIMENTAL

Nickel oxide nanoparticles have been synthesized using ASH Supported method [1]. Obtained nanoparticles (As prepared sample) were further annealed in air at 300°C (sample #1) & 700°C (sample #2). The sample so formed, were analyzed by X-Ray Diffraction (XRD) using angle dispersive X-ray diffraction (ADXRD) beamline (BL-12) on Indus-2 synchrotron radiation source, India [2]. For further studies Raman Spectroscopic measurement has been performed on Micro Raman system from Jobin Yvon Horibra LABRAM-HR 800 visible (400 - 1100 nm). Raman spectra were recorded from 50 cm⁻¹ to 1500 cm⁻¹ in the present study. He-Ne of wavelength 632.8 nm source was used as excitation Laser Source. The morphological features such as size and shape of the nanoparticles were examined through JEOL Scanning Electron Microscopy installed at UGC-DAE-CSR Indore. And as far as its application part is concerned, the antibacterial activity of the NiO nanoparticles was investigated by cup & well diffusion method using pathogenic strains including three gram negative bacteria i.e. Pseudomonas aeruginosa, Salmonella typhi, E.coli and one gram positive species i.e. methicillin-resistant Staphylococcus aureus which were procured from microbial culture collection unit, CHRC, Indore. Bacterial cultures were prepared to 0.5 McFarland standards prior to the assay in LB broth medium. Pure bacterial cultures were uniformly swabbed on Mueller Hinton agar medium plate. Wells were bored with the help of sterile borer. These wells were filled with sonicated suspension of nanoparticles of NiO (1mg/0.1ml sterile distilled water). The plates were incubated at 37°C for 24 hrs and 48 hrs, respectively. Antibacterial activities were studied by measuring the diameter of zone of inhibition.

RESULTS & DISCUSSIONS

Raman Spectroscopy

The five vibrational bands of Raman Spectra of Nickel Oxide emanates from one-phonon (1P) TO at 400-440 cm⁻¹ and LO at 530-560 cm⁻¹, two-phonon (2P) 2TO at 740 cm⁻¹, TO+LO at 925 cm⁻¹ and 2LO at ~1100 cm⁻¹ along with one magnon, two magnon and four magnon excitation at 40 cm⁻¹, 1500 cm⁻¹ and 2800 cm⁻¹ respectively [10,11]. The present article delineates the vibrational analysis of Nickel Oxide Nanoparticles at two temperatures namely 300°C and 700°C as illustrated in Fig.1. At 300°C at is in mixed phase (NiO and Ni) while at 700°C it is pure phase (only NiO). It is observed that the mixed phase (NiO +Ni) at 300°C nanopowder exhibits peaks at 372 cm⁻¹, 554 cm⁻¹, 698 cm⁻¹ and 1085 cm⁻¹ ascribed to one-phonon (1P) transverse optical (TO), (1P) longitudinal optical (LO), two-phonon (2P) TO and 2P LO of vibrational origin respectively.

FIGURE 1. Raman Spectra of NiO nanoparticles annealed at 300°C (Sample #1) &700°C (Sample #2)

In the case of pure phase at 700°C, peaks are at 372 cm⁻¹, 526 cm⁻¹, 698 cm⁻¹, 1074 cm⁻¹ and 1345 cm⁻¹corresponding to at one-phonon (1P) transverse optical (TO), (1P) longitudinal optical (LO), two-phonon (2P) TO and 2P LO of vibrational origin and two-magnon (2M) band associated with Ni²⁺-O²⁻-Ni²⁺ super exchange interaction, respectively. 2M band is associated only with 700°C as it depends on size of the Nanoparticles. It is
observed for large Nanoparticles [12]. With increase in temperature from 300°C to 700°C, red shift is observed in phonon vibrations due to defects and surface defects with increase in crystallite size at 1P (LO) and 2P (LO). Vibrational properties show the enhancement in the intensity with rise in temperature due to increase in grain size. Two phonon (2LO) peak is quite intense (1074-1084 cm\(^{-1}\)) while the 2-phonon band (TO + LO) become so broadened that it disappears at all.

**Scanning Electron Microscopy**

The surface morphological features of synthesized nanoparticles were studied by Scanning Electron Microscope. Fig. 2 shows the SEM image of NiO nanoparticles annealed at 700°C (Sample #2) with magnification of 500.

![FIGURE 2. SEM image of NiO nanoparticles annealed at 700°C (sample #2)](image)

It shows all parameters such as accelerating voltage, spot size and working distances. Monograph shows that obtained nanoparticles of NiO are crystalline in nature. The shape of NiO nanoparticles are like irregular flowers. It is clearly observed that all the particles are agglomerated. It is reported in literature that NiO nanoparticles have the tendency to agglomerate due to their high surface energy and high surface tension of the nanoparticles. The fine particle size results in a large surface area that in turn, enhances many of the applications of nanoparticles. So it is clear from SEM micrograph that prepared NiO particles are in nanometer range and the aggregate size increases on increasing the annealing temperature from 300°C to 700°C, this agglomeration resulted in an increase of the crystallite size of the particles [13].

**APPLICATION OF NIO NANOPARTICLES: ANTIBACTERIAL ACTIVITY**

The antibacterial activity of the NiO nanoparticles was investigated by cup & well diffusion method using pathogenic strains including three gram negative bacteria i.e. Pseudomonas aeruginosa, Salmonella typhi, E.coli and one gram positive species i.e. methicillin-resistant Staphylococcus aureus. The nanoparticles of NiO (1mg/0.1ml) nanoparticles prepared at 700°C were found to inhibit the growth of Staphylococcus aureus with diameter of zone of inhibition of 26 mm.
FIGURE 3. Effect of Antibacterial activity of NiO annealed at 700°C (sample #5) (a) Pseudomonas aeruginosa (b) Salmonella typhi (c) E.coli (d) Staphylococcus aureus

While they were not found to be susceptible against gram negative bacteria. Nanoparticles of different sizes prepared at 100°C, 200°C, 300°C, 400°C, 500°C, 600°C, 800°C, 900°C and 1000°C did not exhibit antimicrobial activity (Fig. 4.).

FIGURE 4. Effect of antibacterial activity of different sample of NiO nanoparticles of different sizes annealed at different temperatures (as shown) on Staphylococcus aureus pathogenic strain. Antibacterial effect was not exhibited.

CONCLUSIONS

This study delineates the antibacterial activity using NiO nanoparticles. These nanoparticles were fabricated through novel, simple, echo friendly and cost effective ASH SUPPORTED method. Raman study reveals that pure NiO (major phase) is obtained at 700°C and at this temperature particles are in nanometer range confirmed by SEM. It is also concluded that NiO nanoparticles prepared at 700°C, only were found to inhibit the growth of Staphylococcus aureus with diameter of zone of inhibition of 26mm. And they were not found to be susceptible against gram negative bacteria.
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REFERENCES