IJPSR (2022), Volume 13, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 April 2022; received in revised form, 10 May 2022; accepted, 27 May 2022; published 01 December 2022

# NICKEL OXIDE NANOPARTICLES SYNTHESIS BY CHEMICAL REDUCTION TECHNIQUE AND IT'S APPLICATION AS ANTIMICROBIAL AND DYE DEGRADING AGENT

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**Keywords:**

Chemical Reduction Method (CRM), Nickel oxide nanoparticles (NiO NPs), Characterization, Photocatalytic efficacy, Congo Red Dye, Antimicrobial activity

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**ABSTRACT:** Nanoparticles are fascinating materials that find many applications in basic and applied research. The chemical reduction technique is one of the easiest methods for synthesizing Nickel Oxide Nanoparticles (NiO NPs) in low-temperature ranges. Nickel Nitrate Hexahydrate ($Ni(NO_3)_2.6H_2O$), Ascorbic acid, NaOH, and $NaBH_4$ were applied as starting reagents to synthesize NiO NPs. Noble metal oxide nanoparticles have been extensively utilized for many applications in the present scenario. In the present study, attention has been focused on the fabrication of NiO NPs synthesized by the Chemical Reduction Method (CRM). The synthesized nanoparticles were characterized by UV-Vis spectroscopy, FTIR spectroscopy, X-ray diffraction, EDAX, and SEM Techniques. The optical band gap energy value has also been determined as 2.59eV from the UV-Vis spectrum. The nanometal oxide is subjected to photocatalytic activity on Congo red dye under solar irradiation and exhibits very good degrading ability within a few minutes of exposure. Its pharmacological activity is also analyzed using pathogenic organisms like *Staphylococcus aureus, Bacillus subtilis, Escheria coli,* and *Pseudomonas aeruginosa* bacteria and found to possess very good antimicrobial efficiency.

**INTRODUCTION:** Nanoparticles exhibit novel material properties due to their small size that are significantly different from those of their bulk counterparts. Nanoparticles with uniform size and well dispersion are desirable for many applications in designing ceramic, magnetic, electrochromic and heterogeneous catalytic materials [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(12).4934-41

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(12).4934-41

Several workers have investigated the transition metal oxide nanoparticles in the last few years. Nanoscale oxide particles of transition metals are gaining continuous importance for various applications as catalysts, passive electronic components, and ceramic materials [2].

Several researchers have prepared various transition metal oxides NPs by various methods like sol-gel [3], surfactant-mediated synthesis [4], thermal decomposition [5], polymer-matrix assisted synthesis [6, 7,] and spray-pyrolysis [8]. Ultrasonic radiation, hydrothermal synthesis, carbonyl method, laser chemical method, pyrolysis by microwave, precipitation-calcination, micro

emulsion method and combustion [9-14]. However, to the best of our knowledge, most of the reported experimental techniques for synthesizing nanopowders are still limited in laboratory scale due to some unresolved problems, such as special conditions, tedious processes, complex apparatus, low yield, and high cost [15-17]. However, the main drawback of such approaches is the creation of a more concentrated pollutant-containing phase.

Recently, NiO NPs have been investigated as an important inorganic material. The NiO NPs are crucial materials that can be grown and used in various applications, such as solar cell, capacitor, and rechargeable lithium-ion batteries. In addition, NiO NPs have attracted great attention because of their potential applications and their specific physical and chemical properties [18].

Recent developments support Nickel oxide is considered a good adsorbent due to its chemical and magnetic properties [19, 20]. Various adsorbents, such as NiO NPs -modified diatomite [21-25] and polyvinyl alcohol/titanium oxide [26-28] were applied to adsorb dyes, aromatic compounds, and heavy metals from the aqueous solutions are studied extensively by the researchers. Discharges of hazardous dyes and chemicals from textile and tannery industries into water bodies are worrying for both toxicological and esthetical reasons [29-32]. NiO NPs-modified diatomite was reported as a good choice for removal of basic red 46 dye from aqueous solution [33-36].

Among many metal oxide nanoparticles, the synthesis and study of NiO NPs seem to be especially interesting because they exhibit high catalytic activity and selectivity as a catalyst in hydrogenation reactions. This prompted us to synthesize Nickel oxide nanoparticles by chemical reduction method [37, 38].

The innate properties of NiO NPs depend on their size, shape, and structure [39]. However, NiO NPs are easy to aggregate and oxidize during the synthetic process due to their deficiency of surface ligands. Therefore, it is necessary to develop various approaches to prepare high-quality NiO NPs [40]. In the present study, we try to synthesize high-quality NiO nano particles and analyze their antimicrobial and dye degradation efficiency, which is also cost-effective.

**Experimental:**

**Collection of Chemicals:** All the chemicals used in the experiment are of Analytical Grade and used as such purchased from the National scientific company, Madurai. Nickel Nitrate HexahydrateNi$(NO_3)_2$. $6H_2O$ of 98% purity is used. Deionized water is used as a solvent. Sodium borohydride ($NaBH_4$) is used as a reducing agent, while sodium hydroxide (NaOH) is used to adjust the pH, and ascorbic acid is used as the antioxidant for colloidal Nickel Oxide Nanoparticles.

**Synthesis of Nickel Oxide Nanoparticles:** Ascorbic acid solution (0.02 M) was prepared in deionized water. A 0.01 M solution of Nickel Nitrate HexahydrateNi$(NO_3)_2$. $6H_2O$ prepared in deionized water was added to the ascorbic acid solution under continuous magnetic stirring. To adjust the pH, 1 M solution of NaOH in deionized water was added. After stirring for 30 minutes at room temperature, 0.1 M solution of $NaBH_4$ in deionized water was added under continuous stirring. The stirring was continued for 15 min an ambient atmosphere to complete the reaction. The green color of the initial reaction mixture turned into ash color colloidal form.

**Characterization Techniques:** The absorption optical spectrum of NiO NPs was recorded using Shimadzu UV-2450 PC double beam spectrophotometer from the range between 200-800 nm at (25+1) °C. The FTIR spectrum of NiO NPs was recorded with Shimadu IR-Affinity-I FTIR spectrometer. The sample was prepared at 0.25 mm thickness as KBr pellets (1mg in 100 mg KBr) and stabilized under reactive humidity before acquiring the spectrum. The spectrum was measured between 400 and 4000 $cm^{-1}$ for 70 scans.

The XRD measurement on powder samples of synthesized NiO NPs was carried out using a diffractometer system (XPERT – PRO, PAN analytical). The size was calculated from the width of the XRD peaks using the Debye Scherrer formula. EDAX spectrum was obtained on an EDAX GENESIS 4000 equipment at an accelerating voltage of 20 kV. SEM performed a morphological examination of NiO NPs. The sample was coated on a copper grid, and the microscopic analysis was conducted using a Quanta FEI 250, SEM operated at 20 KV.

**Degradation Studies on Congo Red Using NiO NPs as a Function of Time:** 50 mg of NiO NPs was added to each beaker containing dye solution and allowed to stir in darkness for 30 minutes before the commencement of the experiment. This is to maintain equilibrium between adsorption and desorption process between dye molecules and nanoparticles surface. After 30 minutes, the nanoparticles' dye solutions were exposed to sunlight irradiation. At every regular time interval, aliquot of dye sample was withdrawn and sent to a centrifuge machine (eppendorf, centrifuge 5430, operate at maximum revolutions per minute: 780 rpm) for centrifugation. Centrifugation is to separate NiO NPs from degraded dye solution to prevent the dispersion of nanoparticles. Extra care must be taken when withdrawing the sample from the centrifuge tube to avoid disturbing the deposition of nanoparticles on the centrifuge tube wall. A slight movement can cause resuspension of nanoparticles in dye solution. By doing this, a clean supernatant solution can be obtained, making sure that there are no traces of nanoparticles in the sample, which could lead to inaccurate results. The presence of nanoparticles can contribute to the amount of light scattering during the absorbance measurement. After centrifugation, the sample was subjected to UV-Vis double Beam Spectrophotometer (Shimadzu UV-2450 PC) scanning at 200 to 800 nm.

**Antimicrobial Activity Test:**

**Preparation of Sample with Copper Oxide Nanoparticles:** The antimicrobial activity was determined by the agar well plate method (modified producer of Thakur *et al*., 2007). Antibacterial activities were tested against different organisms, such as *Staphylococcus aureus, Bacillus subtilis, Escheria coli,* and *Pseudomonas aeruginosa,* by agar well plate method. The bacterial cultures were inoculated into the Nutrient broth and incubated at 37°C for 24 h.

The culture suspension was compared with McFarland Nephelometric standard 0.5 tubes and adjusted with saline. (Baron and Finegold, 1990). These target isolates were swabbed over the surface of Muller Hinton agar plates. The wells were made on the agar plate using a cork borer. The pre-prepared samples (50 µl) and normal saline (control) were added separately to the wells. The plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured along with the diameter of the well. A required amount of agar medium was prepared along with copper oxide nanoparticles of concentration 100µg/ml. Another agar media without nickel oxide nanoparticles was also prepared which was used for culturing strains without nanoparticles. Both were autoclaved and poured into different petriplates. The above strains were cultured to the petriplated by spread plate methods. The cultures without nanoparticles were kept as a control for a comparison study [41-44].

**RESULTS AND DISCUSSION:** The presence of NiO NPs was checked by the following methods. These methods strongly support the formation of NiO NPs.

**By Colour Change:** The formation of nanoparticles was visibly evident from the colour change. The colour was changed from green to ash colour as the time increased. This colour change indicates the formation of stable NiO NPs in **FIG. 1.**

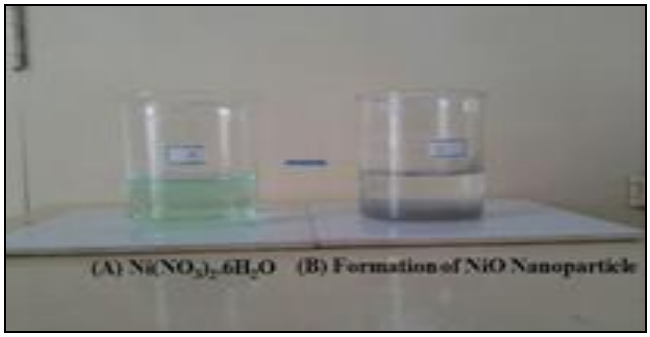


**FIG. 1: COLOUR CHANGE INDICATING SYNTHESIS OF NiO NPs**

**Characterization of Nickel Oxide Nanoparticles:**

**UV- Visible Spectroscopy:** The absorption of NiO NPs was measured by UV-visible spectroscopy. The absorption band of NiO NPsis was observed in the range of 200-300 nm. The UV spectra of NiO NPs synthesized by the chemical reduction method (CRM) are shown in **FIG. 2.** This spectrum was recorded immediately after the synthesis of particles. The figure shows the absorption peaks at 225 nm, which proves the formation of the NiO NPs in the solution. The initial green color changes to ash color colloidal form are due to the surface plasmon resonance of Nickel Oxide Nanoparticles [45].

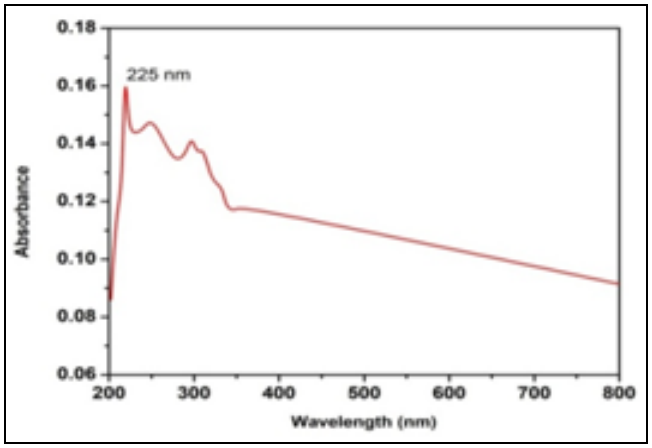


**FIG. 2: UV-VISIBLE SPECTRA OF SYNTHESIZED NiO NPs**

The bandgap energy can be determined using the Tauc relation. The dependence of the optical band gap ($E_g$) on the absorption coefficient and the incident photon energy (hν) is given by [46].

$$\alpha h\nu = A(h\nu - E_g)^n$$

Where A is the optical constant, α is the absorption coefficient, h is the incident light, ν is the frequency of the incident photon, and n is the number characterizing the nature of the transition process; n=2 for direct transition and n=1/2 for indirect transition.

The value of $E_g$ is obtained by plotting $(\alpha h\nu)^2$ versus hν and then extrapolating the linear region on the energy axis as in **FIG. 3.**

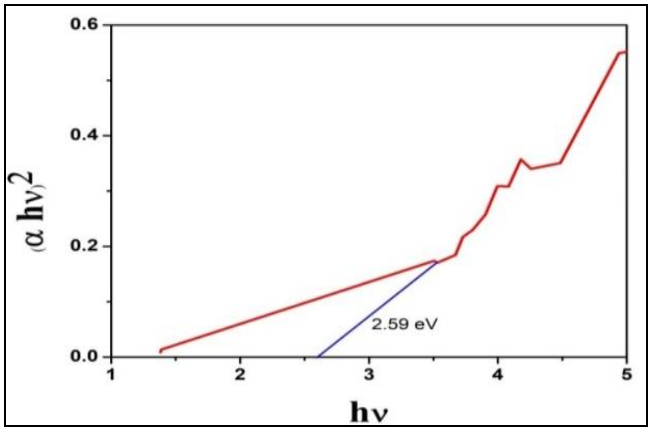


**FIG. 3: TAUC PLOTS OF NiO NPs**

The value of $E_g$ is 2.59 eV for 225 nm NiO NPs. The decreasing of $E_g$ with increasing thickness may be due to the possibility of structural defects, which in their rule, lead to the formation of donor levels within the energy gap. These results are identical to those in reference [47].

**Fourier Transforms Infrared Spectroscopy: FIG. 4** shows Fourier transformed spectrum of NiO NPs at room temperature. The spectrum was recorded in the range of 4000–400cm$^{-1}$.

The FTIR spectrum shows the characteristics peaks at 378 cm$^{-1}$, at 640cm$^{-1}$, 1627 cm$^{-1}$, 2376 cm$^{-1}$, 3464 cm$^{-1}$. The band at 378 cm$^{-1}$ reveals the presence of NiO NPs.

The 2376 cm$^{-1}$ corresponds to C-H Stretching of alkanes and 1627 cm$^{-1}$ C=O stretching.

The broad and strong absorption bands were 3464 cm$^{-1}$ corresponding to O-H Stretching [48].

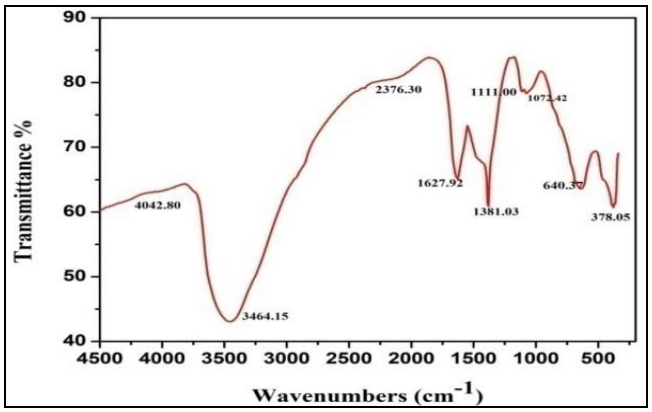


**FIG. 4: FT-IR SPECTRA OF SYNTHESIZED NiO NPs**

**X-Ray Diffraction Studies: FIG. 5** shows the XRD pattern of synthesized NiO NPs. XRD pattern shows that most of the diffraction peaks at 2θ values 38.23°, 34.80°, 36.80° were assigned to (111), (202), and (311) crystal planes.

A similar result was obtained by Wu, *et al.*, 2010. The XRD patterns of synthesized NiO NPs were closely matched with the standard data JCPDS card no. 4-835.

The obtained XRD pattern strongly supports indicated formation of NiO NPs. The crystal size can be calculated according to the Debye-Scherrer formula [49].

$$D = K\lambda / \beta \cos\theta$$

Where k= 0.9, λ is X-ray wavelength, β is the full width at half maximum, and θ is the angle obtained from 2θ values corresponding to the maximum intensity peak in the XRD pattern. The mean crystal size of NiO NPs is 46 nm.

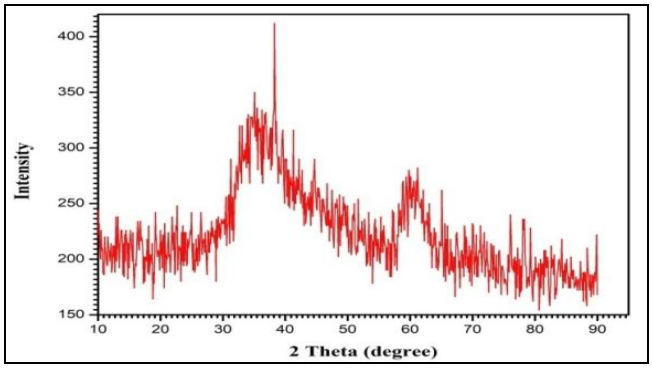


**FIG. 5: XRD PATTERN OF NiO NPs**

**Energy-Dispersive X-Ray Spectroscopy: FIG. 6** shows the EDAX of NiO NPs Concentration of elements Ni and O varies periodically along with the size of the atom being accompanied by maxima of Ni along 79.18 % and O along 20.82% respectively.

There is no evidence of impurity in the composition. Fine particles tend to form agglomerates. The fine crystal shape of powdered particles is frequently seen on the surface of coarser powders.

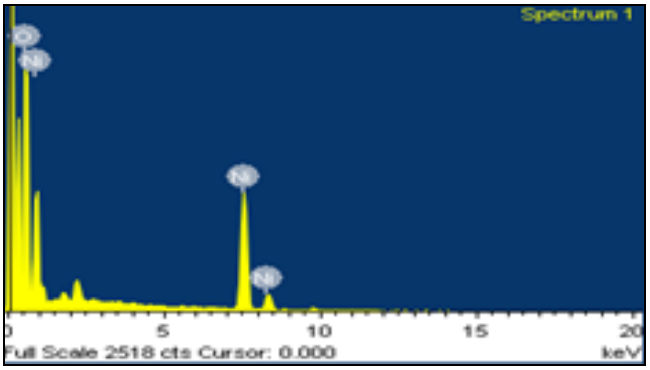


**FIG. 6: ENERGY-DISPERSIVE X-RAY SPECTROSCOPY OF NiO NPs**

**Scanning Electron Microscopy:** The Scanning Electron Microscopy (SEM) nanoparticle analysis was done using the Hitachi model S-3400N SEM machine.

The magnification was done at 5 µm, 2 µm. From the image, we conclude that the nanoparticles are clustered. The surfaces conclude that the nanoparticles are clustered.

The surface of the aggregates is rough. The particles are more or less sponge and range from 40-60 nm in size. The SEM images in **FIG. 7** indicate that the NiO NPs are crystalline and size 46 nm.

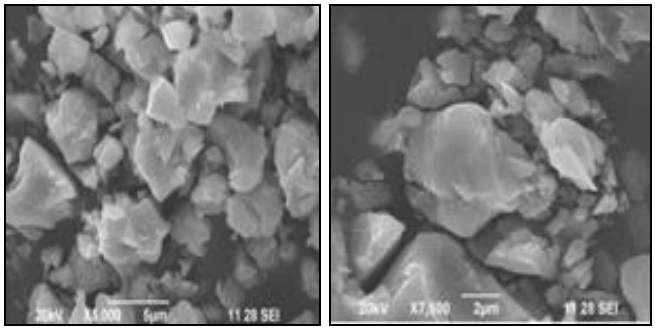

**FIG. 7: SEM ANALYSIS OF SYNTHESIZED NiO NPs**

**Degradation of dye Solution by Nickel Oxide Nanoparticle:** In this study, degradation of Congo red dye solution was performed to study photocatalytic activity of NiO NPs. UV-Visible Spectrophotometer was used in this research study to measure the absorbance of dye solution before degradation and after degradation at the time of exposure under sunlight radiation [50-52].

**Analysis of Photocatalytic Activity:** The photocatalytic activity of the synthesized NiO NPs was evaluated by taking congo red dye, and it is exposed to solar irradiation. Furthermore, the dye degradation can also be visually detected by the gradual colour change from red to almost colourless as shown in **FIG. 8.** The characteristic absorption peak for congo red was noticed at 500 nm. The control exhibited no change in coloration during exposure to sunlight. The degradation of the dye in the presence of NiO NPs was verified based on the UV spectra. It was seen that the intensity of absorption peak at λ max decreases as reaction time increases from 0 minutes to 150 minutes of exposure to sunlight, as shown in **FIG. 8.**

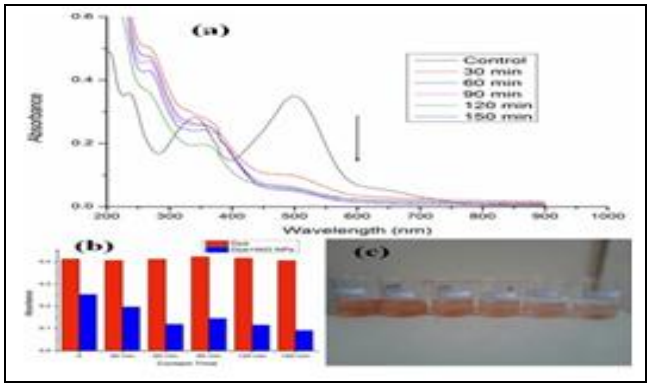


**FIG. 8: (A) UV-VIS SPECTRA OF CONGO RED DYE DEGRADATION AS A FUNCTION OF TIME, (B) VARIATION OF CONGO RED DYE DEGRADATION USING WITH AND WITHOUT NiO NPs AT DIFFERENT TIME INTERVALS AND (C) DECOLOURIZATION IMAGE**

**Antimicrobial Potential of Nickel Oxide Nanoparticles:** Antimicrobial activity of biologically synthesized nickel oxide nanoparticles was seen against Gram-negative (*Pseudomonas aeruginosa and E. coli)* and Gram-positive (*Staphylococcus aureus and Bacillus* subtilis*)* bacteria. Further, the zone of inhibition of nickel oxide nanoparticles against Gram-negative and Gram-positive bacteria was measured.

The results indicated that NiO nanoparticles synthesized from *Erythrina variegata leaf* extract showed effective activity in Gram-negative and Gram-positive bacteria. The Antimicrobial effect of the biosynthesized NiO NPs was examined using the disc diffusion assay, which is mainly used to test the sensitivity of bacterial strains towards antibiotics with a clear zone around the well, which reflects the bacterial sensitivity towards antibiotics [53, 54].

The mean diameters of inhibition zones obtained in the present study are given in **FIG. 9.** The results showed that NiO NPs biosynthesized using *Erythrina variegata* leaves extract showed good inhibition against the four studied bacterial strains.

**TABLE 1: ANTIMICROBIAL ACTIVITY OF TITANIUM DIOXIDE NANOPARTICLES USING *ERYTHRINA VARIEGATA* LEAVES EXTRACT AGAINST PATHOGENS**

| Organisms | Diameter of Zone of Inhibition (mm) |
|---|---|
| *Bacillus subtilis* | 5 mm |
| *Staphylococcus aureus* | 10 mm |
| *Escherichia coli* | 8 mm |
| *Pseudomonas Aeruginosa* | 12 mm |

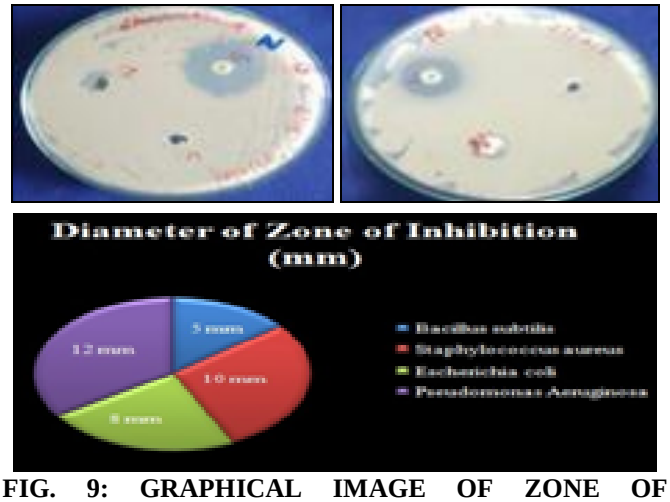


**FIG. 9: GRAPHICAL IMAGE OF ZONE OF INHIBITION OBTAINED BY WELL DIFFUSION METHOD**

The antimicrobial activity of NiO nanoparticles concludes that the NiO nanoparticles show significant antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Escheria coli,* and *Pseudomonas aeruginosa.* The *Pseudomonas aeruginosa* was more susceptable than others.

**CONCLUSION:** In the present work, the chemical reduction method (CRM) has been synthesized; the Nickel oxide nanoparticles (NiONPs) have been carried out by the chemical reduction method (CRM). The average size of the synthesized NiO NPs is 46 nm. The absorption peak that appeared at 225 nm confirms the formation of NiO NPs. The observed XRD peaks for nickel oxide nanoparticles are ascribed to the growth of nanoparticles along different crystallographic planes. The photocatalytic results conclude that these synthesised NiO NPs have good efficiency in degrading Congo red under solar irradiation. Hence, they can find applications in the textile industry and water treatment plants. They also act as an efficient bactericidal agent against gram-positive and negative bacterial strains.

**ACKNOWLEDGMENT:** We are happy to acknowledge the Science Instrumentation Centre (SIC) of The Standard Fireworks Rajarathinam College for Women, Sivakasi, for the analysis. Research Centre Department of Chemistry, VHNSN College, Virudhunagar, for providing the instrumental support for analyzing the samples; we also thank the International Research Centre of Kalasalingam Academy of Research and education, Krishnankoil for providing analytical facilities.

**CONFLICTS OF INTEREST:** The authors declared that they have no conflict of interest.

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**How to cite this article:**
Selvi JM, Murugalakshmi M and Gnanaprakash M: Nickel oxide nanoparticles synthesis by chemical reduction technique and its application as antimicrobial and dye degrading agent. Int J Pharm Sci & Res 2022; 13(12): 4934-41. doi: 10.13040/IJPSR.0975-8232.13(12).4934-41.