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Synthesis of Nickel Hydroxide Nanoparticles by Reverse Micelle Method and its Antimicrobial Activity

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Abstract

Rod shaped $Ni(OH)_2$ nanoparticles of average size 10-20 nm were synthesized in Triton/water/cyclohexane/Polyvinyl pyrolidone quaternary microemulsion by reverse micelle method in different water to surfactant ratio. Nanoparticles were characterized by XRD, TEM, FTIR and DSC techniques. Antibacterial activity of $Ni(OH)_2$ nanoparticles at different concentrations (0.05 to 1.2 mg/ml) were tested on three bacterial strains i.e. E. coli, P. aerugenosa and S. aureus and further compared with seven well known antibiotics. A significant increase in the antibacterial activity of nickel hydroxide nanoparticles have been observed with the increase in concentration of nanoparticles. Optimum concentration for inhibiting growth of bacterial cell was found to be 0.4 mg/ml. It is believed that $Ni(OH)_2$ nanoparticles due to its small size penetrated the bacterial cell membrane and binds to functional groups of proteins, resulting in denaturation of protein and also believed to have caused damage to the bacterial cell by interacting with phosphorous and sulphur containing compounds such as DNA causing death of bacterial cell.

Keywords: Nickel hydroxide nanoparticles, nanotechnology, antibacterial activity, reverse micelle synthesis.

Introduction

Nanoparticles due to their smaller size and large surface to volume ratio, exhibit interesting novel properties which include nonlinear optical behavior, increased mechanical strength, enhanced diffusivity, high specific heat, magnetic behavior and electric resistivity, etc.[1] Compare to other methods, the reverse micelle method is one of the most promising wet chemistry synthesis approaches. This method provides a favorable microenvironment for controlling the chemical reaction. As such the reaction rate can be easily controlled and it is possible to obtain a narrow nanoparticle size distribution[2]. The size of the core of the reverse micelle can also be controlled by changing water to surfactant ratio[3]. Reverse micelle solutions are a transparent, isotropic and thermodynamically stable, water-in-oil type microemulsion in which the aqueous phase is dispersed as nano-sized droplets surrounded by a monolayer of surfactant molecules in a continuous polar organic phase[4, 5]. As compared to other noble metals, like silver[6, 7], gold[8], palladium[9] and platinum[10], nickel nanoparticles have been less frequently studied as antibacterial agent probably because they are relatively difficult to be reduced and difficult to avoid aggregation, as well as their easy tendency toward oxidation.

The antibacterial effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to attach closely with microbial membranes and is not merely due to the release of metal ions in solution[11]. The antibacterial effects of Ag and Cu nanoparticles using single representative strains of *E. coli* and *B. subtilis*, where the Cu nanoparticles demonstrated superior antibacterial activity compared to silver nanoparticles[12]. Nickel nanoparticles have been shown good antibacterial activity against *E. coli, L. cassie, S. aureus, P. aerugenosa* and *B. subtilis*[13]. Antibacterial activity of nickel hydroxide nanoparticles have been rarely reported, especially with investigated bacterial cultures.

In continuation of our earlier work on nickel nanoparticles[13], in the present work, we have reported the synthesis of nickel hydroxide nanoparticles in quaternary microemulsion by reverse micelle method in a favorable microenvironment with different water to surfactant (W/S) ratio. Furthermore, we also investigated the antimicrobial effect of nickel hydroxide nanoparticles against three bacterial strains at different concentrations and compared its antibacterial activity against representative microorganisms of public concern.

Material and Methods

Microemulsion was prepared from cyclohexane, Triton X-100 as a surfactant, hydrazine hydrate and tetrahydrofuran (THF) (Sigma-Aldrich, USA), poly vinyl pyrolidone K-90 (PVP) (Hi-Media Pvt. Ltd.). Aqueous solution of nickel

chloride hexahydrate (Qualigen Chem. Pvt. Ltd.) was prepared by using triple distilled water (conductivity less than 1×10^{-6} ohm^{-1} cm^{-1}). Beef extract, yeast extract and agar-agar type-1 (Hi Media Pvt. Ltd. Bombay), peptone (Qualigen Chem. Pvt. Ltd.) were used for preparing medium for bacterial culture. Ampicillin (10 mcg), Penicillin G (2 unit), Fluconazole (25 mcg), Clotrimazole (10 mcg), Gentamicine (30 mcg), Straptomycin (25 mcg) and Tetracycline (30 mcg) antibiotics procured from Hi-Media Pvt. Ltd. Bombay. Bacterial cultures *viz*., *Escherichia coli* (gram negative) (MTCC 294), *Pseudomonas aeruginosa* (gram negative) (MTCC 1035) and *Staphylococcus aureus* (gram positive) (MTCC 3160) were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh (India).

Synthesis of nickel Hydroxide Nanoparticles: The microemulsion was prepared by using PVP, cyclohaxane, 0.5 M of $NiCl_2.6H_2O$ solution. The average size of nanoparticles powder was controlled by using different water/surfactant (W/S) ratio of 5.0, 10.0 and 15.0. The microemulsion was mixed rapidly with continuous stirring, and after half an hour of equilibration, 2.0 M hydrazine hydrate solution was added with continuous stirring. The reverse micelles were broken by adding THF. The nickel nanoparticles thus released were separated and washed with triply distilled water to remove residual PVP in the sample.

Characterization of Nickel Hydroxide Nanoparticles: Size, shape and morphology of nickel hydroxide nanoparticles were evaluated by using TEM (Hitachi -H-7500, Resolution: 2 Å) and X-ray diffractometer (Rikagu Mini-2 using Cu Kα1, λ = 0.15406 nm), at a constant applied voltage of 30 kV and current 20 mA. The scanning range was 10-80 degree at a scanning rate of 0.01-100 degree min^{-1} with a step angle of 0.02°. The infra red analyses were performed with FT-IR spectrophotometer (Thermo-USA, FTIR-380) in the wavelength range of 400-4000 cm^{-1}. Thermal analysis was carried out by using Differential Scanning Calorimeter (TA Instruments USA, DSC Q10) in the range 50-600 ^{0}C.

Determination of Antibacterial Activity: Antibacterial activity of the nickel hydroxide nanoparticles was determined using agar well diffusion method[14]. 25 ml of molten and cooled nutrient agar media were poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination. All the bacterial test organisms were grown in nutrient broth for 24 hr. A 100 µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawns in petri dishes. Agar wells (8 mm) were prepared with the help of a sterilized stainless steel cork borer. The wells in each plate were loaded with 100 µl of different concentrations of nickel hydroxide nanoparticles. The inoculated plates were incubated at 25°C. Seven antibiotics were used as positive control for all the three investigated bacterial cultures and their growth inhibition of bacteria was compared with nickel hydroxide nanoparticles at different concentrations. All the tests were carried out in triplicates to check the reproducibility of results.

Results and Discussion

After addition of aqueous $NiCl_2.6H_2O$ (0.5 m) in double distilled water, microemulsion showed a gradual change in colour at room temperature with time from dark green to pale blue.

X-Ray Diffraction analysis: Cu $K_\alpha1$ (1.5406 A^0) radiation was used for characterization of nickel hydroxide nanoparticles at a temperature of 298 K. Figure-1 shows XRD patterns of nickel hydroxide nanoparticles in W/S ratio of 5.0, 10.0 and 15.0 respectively.

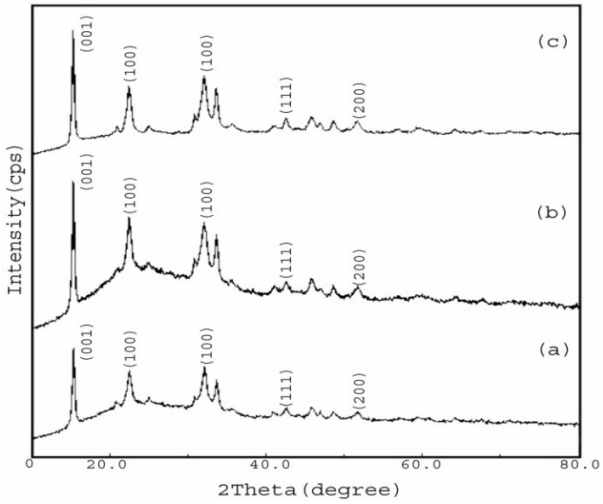

Figure-1
XRD pattern of the nickel hydroxide nanoparticles prepared in W/S ratio of 5.0 (a), 10.0 (b) and 15.0 (c)

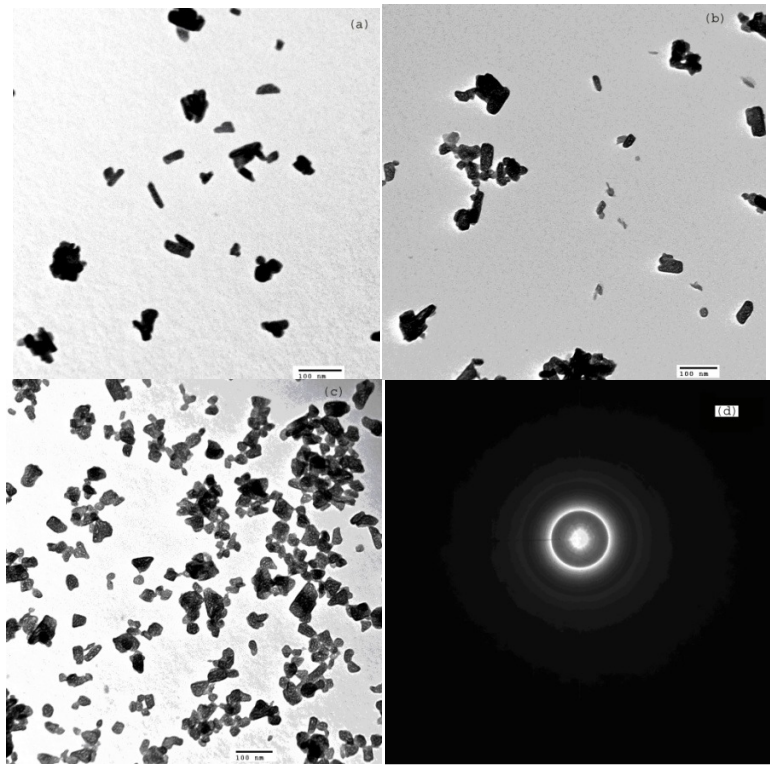

Figure-2
TEM images of the nickel hydroxide nanoparticles synthesized in W/S ratio of 5.0 (a), 10.0 (b), 15.0 (c) and Selected Area Electron Diffraction (SAED) (d)

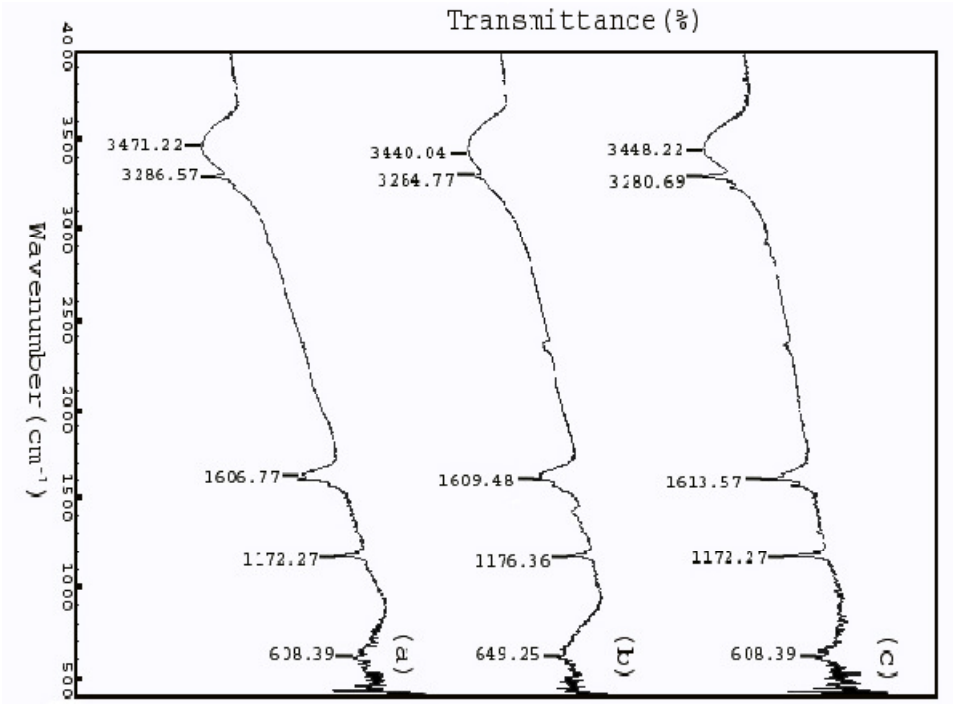

Figure-3
FTIR spectra of the nickel hydroxide nanoparticles synthesized in W/S ratio of 5.0 (a), 10.0 (b) and 15.0 (c)

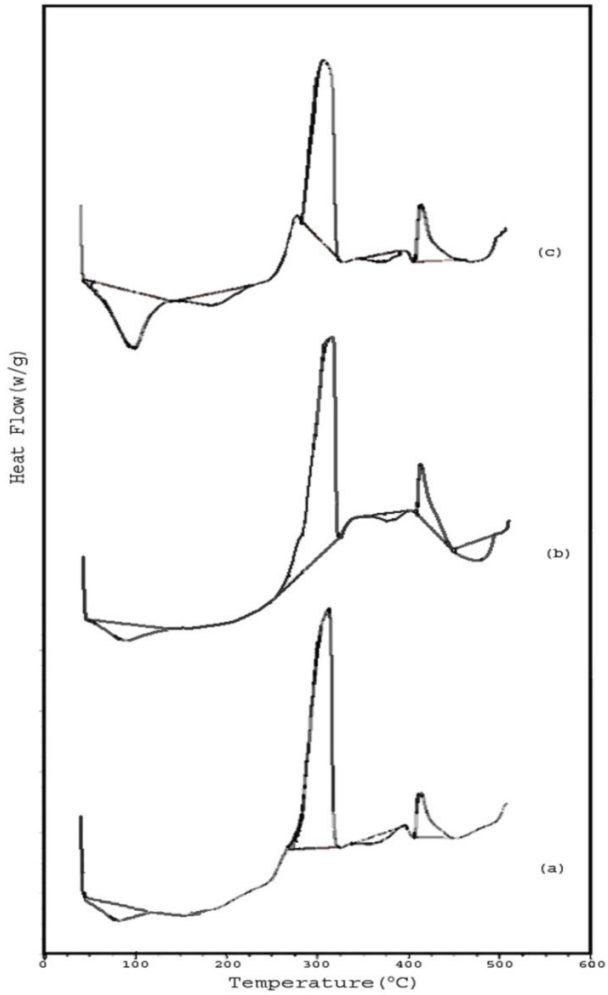

Figure-4
DSC curves of nickel hydroxide nanoparticles synthesized in W/S ratio 5.0 (a), 10.0 (b) and 15.0 (c)

Average particle size of nickel hydroxide nanoparticles calculated using Scherer equation[15] in different W/S ratio i.e. 5.0, 10.0 and 15.0 was 15, 10 and 12 nm respectively. It is observed that the size of nanoparticles depends upon the W/S ratio. The peaks observed at an angles of 15^0 (001), 32^0 (100), 44.5^0 (111) and 51.8^0 (200) are characteristic of FCC nickel phase[16]. Additionally, one peak of α phase $Ni(OH)_2$ at 22.4^0 corresponding to (100) is also found and the respective "JCPDS" (Joint Committee on Powder Diffraction Standards) card no. 74-2075[17].

Transmission Electron Microscopy: Samples of aqueous suspension of $Ni(OH)_2$ nanoparticles were prepared by placing a drop of suspension on copper grids and allowing the water to evaporate. Figure-2 shows TEM images of Nickel hydroxide nanoparticles in different W/S ratio. TEM observations revealed the formation of rod shape nickel hydroxide nanoparticles of average particle size 15, 12 and 22 nm in W/S ratio of 5.0, 10.0 and 15.0 respectively. This may be due to nucleation and seedling processes occurring inside the micellar core. The results of TEM were supported by X-ray diffraction study.

Fourier Transform Infra-Red (FTIR) spectroscopy: FTIR spectroscopy is a sensitive method to investigate especially nanoscaled materials surface atom state[18-23]. Infrared spectroscopic characterization was performed with the help of KBr disk and recorded with a wavelength range of 400-4000 cm^{-1} (figure-3). FTIR spectrum gives information about the synthesis conditions of nanoparticles. The broad absorption band centered at around 3400-3500 cm^{-1} may be due to the O–H stretching vibration, which is the characteristic of $Ni(OH)_2$. The sharp band centered around 1700-1600 cm^{-1} is due to the bending mode of H–O–H vibration of water molecules[24]. Some other peaks are also observed in FTIR spectrum such as medium absorption band at 3250-3300 cm^{-1} may be due to ≡C–H stretching vibration and sharp absorption band at around 1172 cm^{-1} may be due to ≡C–H bending overtone vibration. The absorption band characteristics of Ni–O centered at around 600-650 cm^{-1} was

also observed. It is observed that some acetylene groups of the surfactant molecules are also present at the surface of $Ni(OH)_2$ nanoparticles.

Differential Scanning Calorimeter (DSC) Technique: The isothermal oxidation behavior and the oxidized structure of nickel hydroxide nanoparticles have been investigated over a temperature range 50–600°C in ambient air. The oxidation began at about 313.11°C for W/S ratio of 5.0 and at 308.52°C for W/S ratio of 10.0 and 15.0 and the weight gain increased linearly as the isothermal time lengthened. The isothermal oxidation kinetics becomes parabolic as the temperature was raised above 450°C (figure-4). The experiments revealed unique oxidation behavior of nickel at the nanometer scale, such as early oxidation and melting phenomena, variable activation energies and different oxidation kinetics between low and high conversion ratios. Unlike its bulk counterpart where the activation energy is a constant, the activation energy of nickel nanoparticles depended on the conversion ratio, ranging between 1.4 and 1.8 eV.

Figure-5 (a-v) illustrates the inhibition of bacterial growth on agar plates caused by nickel hydroxide nanoparticles. The zone of inhibition in case of *E. coli, P. aerugenosa* and *S. aureus,* was observed at 400 ppm. The antibacterial activity was evaluated on the basis of diameter of zone of inhibition which was measured at cross-angles after 24 hr of incubation and the average of three readings is shown in table-1.

On comparing the values of zone of inhibition with seven different well known antibiotics, i.e., Ampicillin (10 µg), Penicillin G (2unit), Fluconazole (25 µg), Clotrimazole (10 µg), Gentamicine (30 µg), Straptomycin (25 µg) and Tetracycline (30 µg), it was found that nickel hydroxide nanoparticle are good inhibitors of bacterial growth and show almost similar antibacterial activity as that of standard antibiotics towards all the three strains of bacteria at different concentrations (table-2).

It was also observed that the zone of inhibition increases significantly on increasing concentration of $Ni(OH)_2$ nanoparticles from 0.4 to 1.2 mg/ml. It was observed that 0.4 mg/ml is the optimum concentration of $Ni(OH)_2$ nanoparticles for inhibiting growth of bacterial test organism. It is observed that $Ni(OH)_2$ nanoparticles are very effective antibacterial agent for both gram positive and gram negative strains (table-2).

Nanoparticles have large surface area available for interactions that enhances bactericidal effect compared to large sized particles; hence they impart increased cytotoxicity to the microorganisms[25].

Sensitivity of bacterial strains to standard antibiotics:

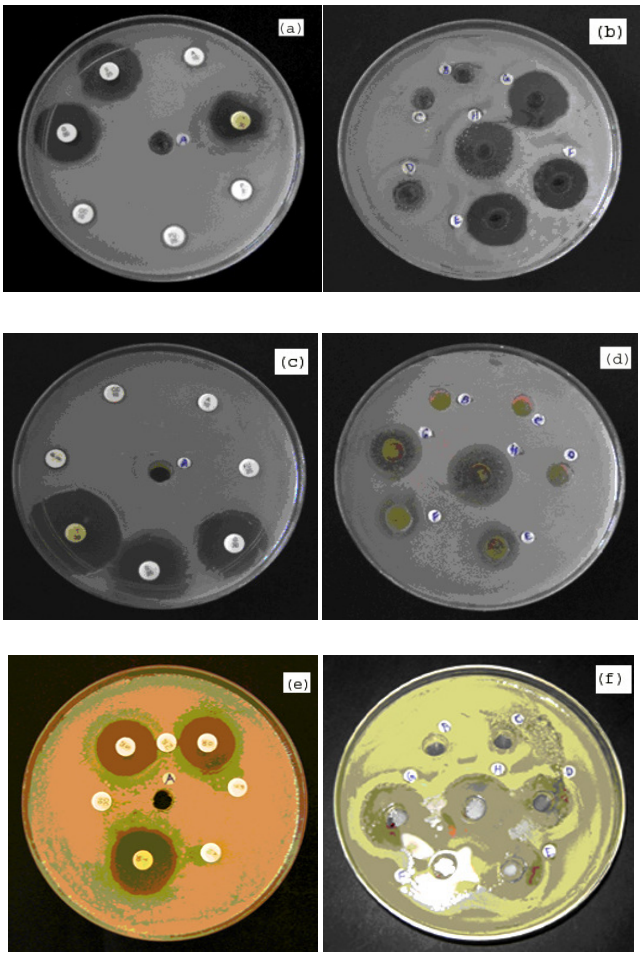

Figure-5

Photographs of agar plate containing seven standard antibiotics (a, c and e) and different concentration of nickel hydroxide nanoparticles i.e. 0.05 mg/ml (A), 0.1 mg/ml (B), 0.2 mg/ml (C), 0.4 mg/ml (D), 0.6 mg/ml (E), 0.8 mg/ml (F), 1.0 mg/ml (G) and 1.2 mg/ml (b, d and f) on *E. coli.* (a and b), *P. aerugenosa* (c and d) and *S. aureus* (e and f)

The mechanism by which the nanoparticles penetrates into bacteria is not understood completely, but studies suggest that when bacterial culture were treated with nanoparticles, changes takes place in its membrane morphology and cause a significant increase in its permeability affecting proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death[26].

Table-1
Antibacterial activity of nickel hydroxide nanoparticles (mm) excluded well diameter at different concentrations investigated on three bacterial strains

S. No.	Bacteria	200 mg/ml	400 mg/ml	600 mg/ml	800 mg/ml	1000 mg/ml	1200 mg/ml
1.	***E. coli***	00	3.7±0.4	7.7±0.4	9.7±0.4	12.3±0.4	14.3±0.4
2.	***P. aerugenosa***	00	3.0±0.8	6.7±0.9	8.7±0.9	10.7±0.9	13.3±0.9
3.	***S. aureus***	00	9.7±0.4	11.7±0.4	14.0±0.0	16.0±0.0	18.0±0.0

Table-2
Antibacterial activity of seven antibiotics (mm) investigated on three bacterial strains

S.No.	Bacteria	Ampicilline	Penicillin-G	Flucanazole	Clotrimazole	Gentamicin	Streptomycine	Tetracycline
1.	*E. coli-*	-	-	-	-	18.0±0.0	18.0±0.0	18.0±0.0
2.	*P. aerugenosa*	-	-	-	-	18.0±0.0	20.0±0.0	24.0±0.0
3.	*S. aureus*	-	-	-	-	13.3±0.4	15.7±0.0	17.0±0.0

It is believed that nickel hydroxide nanoparticles due to its small size penetrated the bacterial cell membrane and binds to functional groups of proteins, resulting in protein denaturation[27] and also believed to have caused damage to the bacterial cell by interacting with phosphorous and sulphur containing compounds such as DNA[11] *etc*. causing bacterial cell death[28]. Complete bacterial inhibition depends upon the concentrations of nickel hydroxide nanoparticles and on the number of bacterial cells. It reflects that nickel hydroxide nanoparticles have significant bactericidal effect in reducing bacterial growth for practical applications.

Conclusion

The $Ni(OH)_2$ nanoparticles were synthesized by reverse micelle method in different W/S ratio. The characterization of nanoparticles was performed by XRD, TEM, FTIR and DSC technique. Antibacterial activity of nickel hydroxide nanoparticles were tested at different concentrations i.e. 0.5 to 1.2 mg/ml and further compared with seven well known antibiotics. Antibacterial activity of nickel hydroxide nanoparticles is almost comparable to the seven investigated antibiotics. A significant increase in the antibacterial activity of nickel hydroxide nanoparticles have been observed with the increase in concentration. $Ni(OH)_2$ nanoparticles showed excellent antimicrobial activity against all the three investigated bacterial strains. It is believed that when bacterial culture were treated with nanoparticles, changes takes place in its membrane morphology and cause a significant increase in its permeability affecting proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death. Hence, $Ni(OH)_2$ nanoparticles can have immense use as water soluble metallic catalysts in chemical reactions, biolabelling and as antibacterials.

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