Green synthesis of Nickel-doped zinc oxide using Pineapple (*Ananas comosus*) leaf extract and Evaluation of their antimicrobial activity

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Abstract
This study explores green synthesis as an environmentally friendly and cost-effective method employing selective plant extracts as catalysts for nanoparticle formation. Ananas comosus, a tropical plant rich in essential nutrients and known for its non-toxic, antioxidant and potentially anticancer properties, was utilized for the synthesis of zinc oxide and nickel-doped zinc oxide nanoparticles. Characterization techniques including UV-Vis spectrometry, X-Ray diffraction and Scanning electron microscopy were employed to analyze the optical, structural and morphological properties of the nanoparticles.

Results showed a reduction in the optical band gap of Ni-ZnO compared to pure ZnO, possibly due to absorption of photons by nickel dopant ions in the ZnO lattice. XRD analysis revealed a hexagonal Wurtzite structure with predominant diffraction peaks in the (1 0 1) plane and SEM-EDX confirmed the purity and irregular nanosized shapes (ranging from 21 to 60 nm) of the nanoparticles. Antimicrobial testing using the agar well diffusion method demonstrated promising antimicrobial activity, suggesting potential applications of these nanoparticles as antimicrobial agents in pharmaceutical industries. Overall, this study underscores the feasibility and potential benefits of utilizing Ananas comosus leaf extracts in green synthesis for nanoparticle production with valuable properties for various industrial and medical applications.

Keywords: Green Synthesis, Optical Properties, Structural Properties, Antimicrobial efficiency.

Introduction

Metal oxide-doped nanostructures have emerged as pivotal materials in both renewable energy and biomedical applications, owing to their promising optical, electrical and magnetic properties. Among these, zinc oxide (ZnO), a II-VI group semiconductor with a direct optical band gap of 3.37 eV, stands out for its suitability in optoelectronic and piezoelectric devices[4,16,24].

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The incorporation of transition metal elements into ZnO further enhances its properties, potentially extending its applications to spintronics and photovoltaic devices. Various synthetic methods have been employed to fabricate pure and doped zinc oxide nanoparticles, each influencing the material properties and applications[3,21,25,27]. Hydrothermal synthesis, photo catalyst techniques, co-precipitation, auto-combustion processes, magnetron sputtering and sol-gel processes represent some of the methodologies used to tailor the characteristics of ZnO nanoparticles[2,7,15,17,20,22,26,27]. Of these, green synthesis has gained significant traction due to its eco-friendly and cost-effective nature[12]. This approach utilizes plant extracts rich in bio-organic compounds such as terpenoids, alkaloids and amino acids which act as effective stabilizers and size-controlling agents during nanoparticle synthesis.

Ananas comosus, commonly known as pineapple, is a tropical plant used not only for its nutritional richness boasting vitamins C, A and potassium K but also for its antioxidant, anticancer and non-toxic properties[10,13]. The presence of vitamin C in *A. comosus* leaves suggests its potential utility as a size-control agent in nanoparticle synthesis. Chemical analyses have identified a range of bioactive compounds in *A. comosus* leaves including alkaloids, flavonoids, tannins, phytosterols, glycosides and phenols[1]. Notably, flavonoids and phenols have demonstrated efficacy in reducing nanoparticle size and stabilizing metal and metal oxide nanoparticles.

Previous research has successfully employed green synthesis approaches utilizing *A. comosus* extracts for synthesizing silver oxide and zinc oxide nanoparticles, highlighting the versatility of this botanical source[6,18] investigating green synthesis of nickel-doped zinc oxide nanoparticles. This study focuses on the green synthesis of both pure and nickel-doped zinc oxide nanoparticles using *A. comosus* leaf extract. The synthesis process was meticulously characterized using several advanced techniques to elucidate the structural, optical and antibacterial properties of the synthesized nanoparticles.

Material and Methods

The synthesis of zinc oxide nanoparticles involved the extraction of bioactive compounds from *A. comosus* leaves which act as reducing and capping agents. The process ensures minimal environmental impact and utilizes sustainable resources.

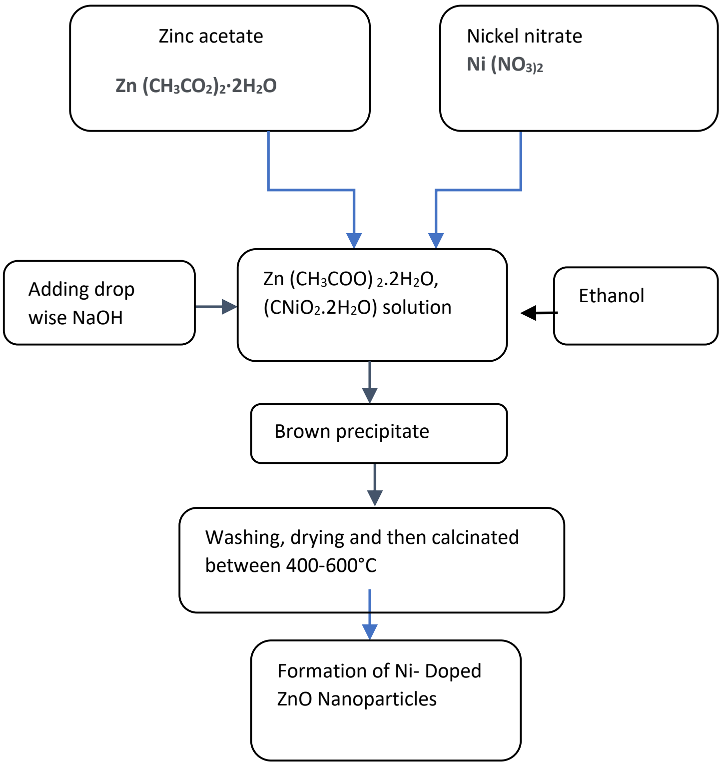

Figure 1: Synthesis process of $Zn_{1-x}Ni_xO$ Nanoparticles

Nickel doping was achieved by introducing nickel salts during the nanoparticle synthesis phase, aiming to modify and enhance the material's magnetic and optical properties.

Green Synthesis: Green synthesis method was employed in this study to fabricate nanocomposites from *A. comosus* leaf powder. The compounds zinc acetate and nickel nitrate, were directly sourced from Fisher Scientific Pvt. Limited and used without further purification. Throughout the experiments, only deionized water ws utilized to ensure purity.

Initially, 20 grams of shade-dried *A. comosus* leaf powder underwent reflux in 100 mL of deionized water at 100°C for two hours followed by gradual cooling to room temperature. The resulting solution was then centrifuged and filtered through Whatmann filter paper to obtain a clear extract, which was stored at 4°C for subsequent use.

For the green synthesis of the NiO/ZnO nanocomposite (NCs), 20 mL each of 0.01 M nickel nitrate and 0.01 M zinc acetate solutions were combined in a 250 mL round bottom flask. The mixture was gently stirred and heated to 80°C on a magnetic stirrer followed by the addition of 20 mL of the prepared leaf extract, once the desired temperature was achieved. The solution's alkalinity was adjusted by carefully adding sodium hydroxide solution. After one hour of continuous stirring, the solution gradually transformed into a distinct brown hue, accompanied by the formation of a blackish-brown precipitate, indicating successful formation of NiO/ZnO NCs using environmentally friendly methods.

To enhance the crystallinity of the NCs, the resulting suspension underwent calcination at temperatures ranging between 400-600°C as the final step. After calcination, the suspension was centrifuged at 8000 rpm for 5 minutes to isolate and purify the synthesized nanocomposites. Additionally, pure ZnO nanoparticles were synthesized as a comparative measure using the same procedure, but excluding nickel nitrate. Figure 1 illustrates the step-by-step synthesis process for clarity and reference.

In conclusion, the green synthesis method employed in this study represents a conscientious approach to nanomaterial fabrication, leveraging natural plant extracts and minimizing the use of hazardous chemicals. This approach aligns with principles of sustainability and eco-efficiency, contributing not only to advancements in materials science but also to the reduction of environmental impact in scientific research and development efforts. The chemical reactions involved in the green synthesis of NiO/ZnO nanocomposites and pure ZnO naoparticles can be summarized as follows:

Step 1: Preparation of leaf extract
Comosus leaf powder+ Deionized water → Leaf extract

Step 2: Green synthesis of NiO/ZnO NCs
$Ni(NO_3)_2+Zn(CH_3COO)_2$+Leaf extract+NaOH→NiO/Zn nanocomposite+By-products

These reactions exemplify the utilization of green chemistry principles, where natural plant extracts serve as eco-friendly reducing agents and stabilizers, minimizing the environmental footprint associated with traditional chemical synthesis methods. The resulting nanomaterials exhibit potential applications in various fields, driven by their enhanced properties and sustainable production methods.

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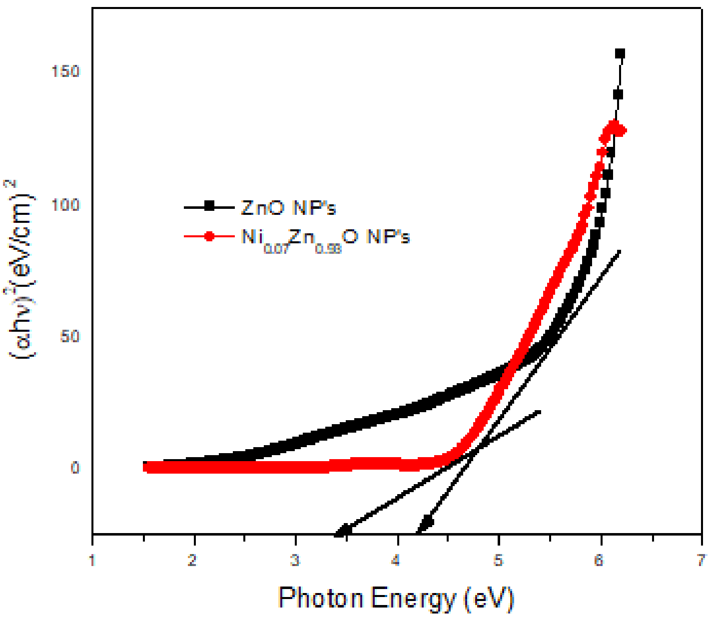

Figure 2: Optical band Gap of $Zn_{1-x}Ni_xO$ Nanoparticles

UV-Vis Spectroscopy: UV-Vis spectroscopy, based on the Beer-Lambert law, was employed to determine various optical properties of the synthesized samples. These properties include the absorbance coefficient, optical band gap, optical conductivity, refractive index and extinction coefficient. The optical band gap is particularly crucial as it dictates the potential electron transitions from the valence band to the conduction band within the semiconductor.

The optical band gap of the synthesized sample was determined using the Tauc method[5] where a tangent line was extrapolated on the x-axis of the plot of hν and $(\alpha h\nu)^2$ [Figure 2].

The equation used for this determination is:

$$(\alpha h\nu) = (h\nu - Eg)^n \tag{1}$$

where (α) represents the absorption coefficient, (hν) is the photon energy, (Eg) is the band gap energy and (n) is the exponent specific to semiconductors [in this case, (n = 1/2)].

The spectrophotometer Lambda 750 (Perkin Elmer) was employed to measure absorption spectra across the range of 200-800 nm. Pure ZnO was previously reported to have an optical band gap of 3.69 eV whereas Ni-doped ZnO exhibited a slightly lower value of 3.37 eV. This reduction indicates an enhanced electron transition rate from the conduction to valence band in the doped sample. The absorption spectra also displayed a flattening effect, attributed to photon absorption by the dopant element.

Various optical parameters were calculated and are summarized in table 1. Previous studies have identified an absorption peak at 335 nm for the Ni-doped ZnO sample. This revision aims to clarify the key principles and findings of UV-Vis spectroscopy in characterizing the synthesized materials, emphasizing the significance of optical band gap determination and its implications.

X-Ray Diffraction: X-ray diffraction (XRD) analysis revealed consistent hexagonal Wurtzite structure and Bragg peaks at specific planes, confirming the structural integrity of both pure and nickel-doped ZnO[8]. The lattice parameter measurements provided in table 1 further corroborate this observation. The peaks observed in the XRD spectrum indicate the absence of any additional phases or impurities in the synthesized nanoparticles.

The Debye-Scherrer equation is:

$$D = \frac{k\lambda}{\beta_{hkl}\cos\theta} \tag{2}$$

where k=0.9k, λ is the wavelength of X-rays, β is the full width at half maximum (FWHM) and θ is the Bragg angle. The average grain size D of the nanoparticles was determined to be 46.34 nm for pure ZnO and 43.77 nm for nickel-doped ZnO nanoparticles as in figure 3.

SEM-EDX: SEM-EDX analysis is a robust technique utilized to examine surface morphology, grain size and elemental composition of materials. The SEM instrument captures energy signals from scattered electron beams passing through the sample, generating detailed images[11,23]. Furthermore, the EDX spectrum derived from this analysis offers critical insights into the elemental composition of the synthesized materia[9,19]. These techniques are pivotal for comprehending both the structural and chemical attributes of synthesized materials.

The findings indicate successful synthesis of materials with desired structural and chemical properties. Presence of nickel, zinc and oxygen elements in the synthesized sample confirms the successful incorporation of targeted dopants. Moreover, the absence of organic chelating agents suggests efficient removal of residual impurities during the synthesis process [Figures 4 and 5]. This revision aims to clearly communicate the capabilities and outcomes of SEM-EDX analysis, highlighting its role in characterizing synthesized materials and confirming the efficacy of the synthesis process.

Antimicrobial Activities: To assess the antibacterial effectiveness of green-synthesized ZnO NPs and Ni-doped ZnO NPs, two bacterial strains were studied: *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) using the well diffusion method[14]. Muller Hinton agar medium (MHA) from HiMedia Laboratory Pvt. Ltd. was utilized for *in vitro* cultivation of the bacterial strains. The MHA medium was prepared using deionized water and autoclaved for 15 minutes at 121°C, maintaining a final pH of 7.2

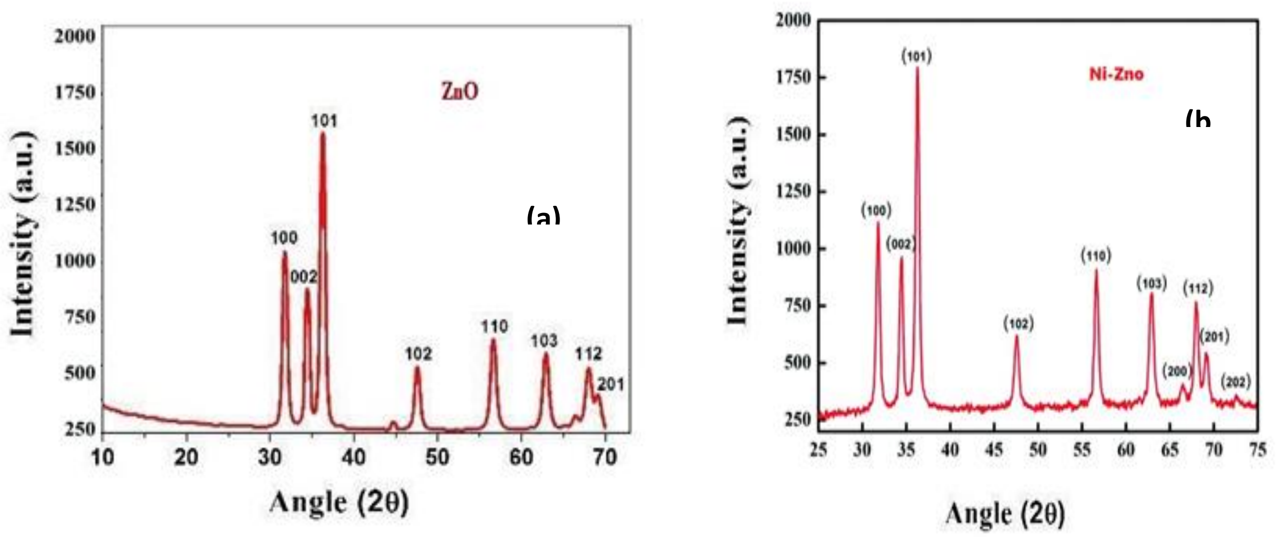

Figure 3 (a, b): XRD Spectrum of $Zn_{1-x}Ni_xO$ Nanoparticles

Table 1
Structural and Optical Parameter of Zn_{1-x} Nix O(x= 0, 0.07) nanoparticles

Synthesized Sample	ZnO	Ni doped ZnO
Lattice Constant	a=b=3.250Å c=5.20 Å	a=b=3.25 Å , c=5.20 Å
Grain Size	46.34nm	43.77nm
XRD Diffraction Plane[8,9,11,25]	(100) (002) (101) (102) (110) (103) (112) (201)	(100) (002) (101) (102) (110) (103) (112) (200) (201) (202)
SEM	150nm	60nm
SEM-EDX	Intrinsic	Zn=0.93% Ni=0.07%
Optical Band Gap[9,11,25]	3.69 eV	3.37eV

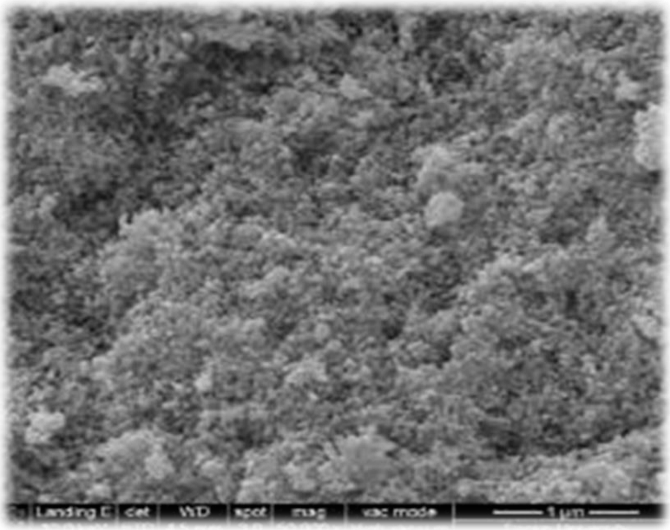

Figure 4: SEM Image of s of Ni doped ZnO

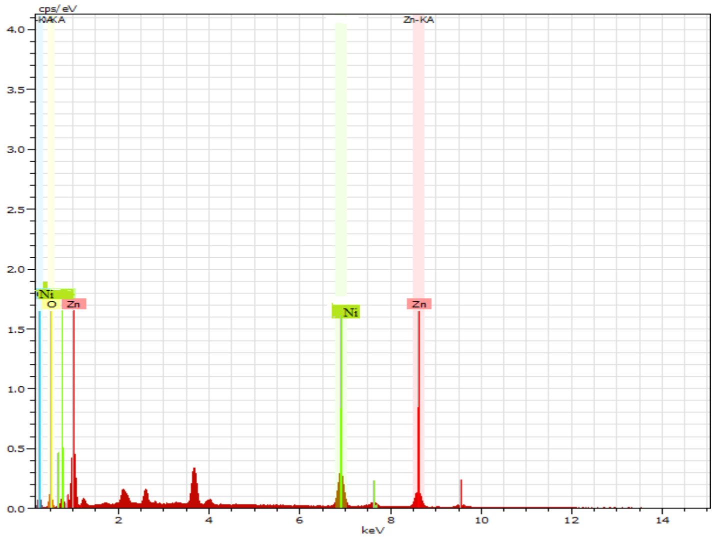

Figure 5: Elemental analysis of Ni doped ZnO

Table 2
Antimicrobial Assessment

Bacteria	**Conc. (in ppm)**	**Zone of Inhibition (ZnO NPs) in mm**			
E. coli	100	11	10	11	10.66
	500	16	15	15	15.66
B. subtilis	100	12	11	11	11.33
	500	17	16	16	16.33
		Zone of Inhibition (Ni-doped ZnO NPs) in mm			
E. coli	100	13	12	13	12.66
	500	19	18	18	18.33
B. subtilis	100	16	17	16	16.33
	500	21	21	20	20.66

Results and Discussion

To evaluate the dose-dependent antibacterial potential of the synthesized nanoparticles, concentrations of 100 mg/L and 500 mg/L were incorporated into the wells. Bacteria isolated from a 5-day culture were inoculated into freshly prepared nutrient medium. Each inoculated Petri dish was incubated at 37°C for 24 hours. The experiment was conducted in triplicate and average values were recorded. Ampicillin served as the control antibiotic. Following incubation, the diameter of the inhibition zones around the wells was measured and recorded.

The inhibition results against the Gram-negative bacterium *E. coli* and Gram-positive bacterium *B. subtilis* are summarized in table 2. Inhibition zones ranged from 10 to 21 mm for both ZnO NPs and Ni-doped ZnO NPs. The most significant inhibitory effect was observed with 500 ppm Ni-doped ZnO NPs against *B. subtilis*, followed by 500 ppm Ni-doped ZnO NPs against *E. coli*. Notably, Ni-doped ZnO NPs exhibited superior antibacterial activity against both *E. coli* and *B. subtilis* compared to pure ZnO NPs. These findings show the potential of both ZnO NPs and Ni-doped ZnO NPs as alternative antimicrobial agents, particularly in pharmaceutical applications.

Conclusion

This study employed a green synthesis method utilizing *Ananas comosus* (pineapple) leaf extract to fabricate nanocomposites consisting of NiO/ZnO and pure ZnO nanoparticles. Zinc acetate and nickel nitrate were utilized as precursor compounds.

The green synthesis method employed in this study represents a sustainable approach to nanomaterial fabrication, utilizing natural plant extracts to minimize environmental impact and to reduce reliance on hazardous chemicals. The synthesized NiO/ZnO nanocomposites and pure ZnO nanoparticles exhibited enhanced properties suitable for various applications in materials science and biomedical fields.

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References

1. Ahmad N. and Sharma S., Green Synthesis of Silver Nanoparticles Using Extracts of Ananas comosus, *Green and Sustainable Chemistry*, **2(4)**, 141-147 **(2012)**

2. Anbuselvan D., Nilavazhagan S., Santhanam A., Chidhambaram N., Gunavathy K.V., Ahamad T. and Alshehri S.M., Room temperature ferromagnetic behavior of nickel-doped zinc oxide dilute magnetic semiconductor for spintronics applications, *Physica E: Low-Dimensional Systems and Nanostructures*, **129**, 114665 **(2021)**

3. Badawi A., Althobaiti M.G., Ali E.E., Alharthi S.S. and Alharbi A.N., A comparative study of the structural and optical properties of transition metals (M = Fe, Co, Mn, Ni) doped ZnO films deposited by spray-pyrolysis technique for optoelectronic applications, *Optical Materials*, **124**, 112055 **(2022)**

4. Borysiewicz M.A., ZnO as a Functional Material, a Review, *Crystals*, **9(10)**, 505 **(2019)**

5. Bunaciu A.A., Udriştioiu E.G. and Aboul-Enein H.Y., X-Ray Diffraction: Instrumentation and Applications, *Critical Reviews in Analytical Chemistry*, **45(4)**, 289-**299 (2015)**

6. Campbell D. and White P.R.A., Ultraviolet – visible spectroscopy, Polymer Characterization, CRC Press, 58-66 **(2017)**

7. Dasari M., Godavarti U. and Mote V., Structural, morphological, magnetic and electrical properties of Ni-doped ZnO nanoparticles synthesized by co-precipitation method, *Processing and Application of Ceramics*, **12(2)**, 100-110 **(2018)**

8. Erdman N., Bell D.C. and Reichelt R., Scanning Electron Microscopy, Scanning Electron Microscopy, Springer, 229-318 **(2019)**

9. Fabbiyola S. et al, Optical and magnetic properties of Ni-doped ZnO nanoparticles, *Journal of Alloys and Compounds*, **694**, 522-531 **(2017)**

10. Hikal W.M., Mahmoud A.A., Said-Al Ahl H.A.H., Bratovcic A., Tkachenko K.G., Kačániová M. and Rodriguez R.A.A., Pineapple: Waste Streams, Characterisation and Valorisation: An Overview, *Open Journal of Ecology*, **11(09)**, 610-634 **(2021)**

11. Hodoroaba V.D., Energy-dispersive X-ray spectroscopy (EDS), Characterization of Nanoparticles, Elsevier, 397-417 **(2020)**

12. Khan M.I. et al, Investigation of in-vitro antibacterial and seed germination properties of green synthesized pure and nickel doped ZnO nanoparticles. *Physica B: Condensed Matter*, **601**, 412563 **(2021)**

13. Kulkami S.R., Borse D.B., Agarwal A.D. and Saptale S.P., Green synthesis of Ag nanoparticles using Vitamin C (Ascorbic Acid) in a batch process, International Conference on Nanoscience, Engineering and Technology (ICONSET 2011), 88-90 **(2011)**

14. Kumer A. and Khan M.W., The effect of alkyl chain and electronegative atoms in anion on biological activity of anilinium carboxylate bioactive ionic liquids and computational approaches by DFT functional and molecular docking, *Heliyon*, **7(7)**, e07666 **(2021)**

15. Muktaridha O., Adlim M., Suhendrayatna S. and Ismail I., Progress of 3d metal-doped zinc oxide nanoparticles and the photocatalytic properties, *Arabian Journal of Chemistry*, **14(6)**, 103175-103175 **(2021)**

16. Nasrollahzadeh M., Sajadi S.M., Sajjadi M. and Issaabadi Z., Applications of Nanotechnology in Daily Life, *Nanotechnology in Health Care*, **4(12)**, 113-143 **(2019)**

17. Prerna, Arya S., Sharma A., Singh B., Tomar A., Singh S. and Sharma R., Morphological and Optical Characterization of Sol-Gel Synthesized Ni-Doped ZnO Nanoparticles, *Integrated Ferroelectrics*, **205(1)**, 1-13 **(2020)**

18. Raja Ahmad R.A. et al, Biosynthesis of zinc oxide nanoparticles by using fruits extracts of Ananas comosus and its antibacterial activity, *Malaysian Journal of Fundamental and Applied Sciences*, **15(2)**, 268-273 **(2019)**

19. Raja K., Ramesh P.S. and Geetha D., Synthesis, structural and optical properties of ZnO and Ni-doped ZnO hexagonal nanorods by Co-precipitation method, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **120**, 19-24 **(2014)**

20. Romeiro F.C. et al, Rapid synthesis of Co, Ni co-doped ZnO nanoparticles: Optical and electrochemical properties, *Journal of Solid State Chemistry*, **230**, 343-349 **(2015)**

21. Sajjad M., Ullah I., Khan M.I., Khan J., Khan M.Y. and Qureshi M.T., Structural and optical properties of pure and copper doped zinc oxide nanoparticles, *Results in Physics*, **9**, 1301-1309 **(2018)**

22. Saleem M., Atiq S., Naseem S. and Siddiqi S.A., Structural and magnetic studies of Ni-doped ZnO synthesized with auto-combustion and co-precipitation techniques, *Journal of the Korean Physical Society*, **60(10)**, 1772-1775 **(2012)**

23. Siddique M.N., Ali T., Ahmed A. and Tripathi P., Enhanced electrical and thermal properties of pure and Ni substituted ZnO Nanoparticles, *Nano-Structures & Nano-Objects*, **16**, 156-166 **(2018)**

24. Straumal B.B., Protasova S.G., Mazilkin A.A., Goering E., Schütz G., Straumal P.B. and Baretzky B., Ferromagnetic behaviour of ZnO: the role of grain boundaries, *Beilstein Journal of Nanotechnology*, **7**, 1936-1947 **(2016)**

25. Tiwari A. and Sahay P.P., Modification in the physical properties of nanocrystalline ZnO thin films by Sn/Ni co-doping for transparent conductive oxide applications, *Physica B: Condensed Matter*, **629**, 413638 **(2022)**

26. Torquato R., Sagar E.S., Kiminami R.H.G.A. and de Melo Costa A.C.F., Structural characterization and magnetic morphological study of Ni doped ZnO synthesized by combustion reaction application for DMS, *Materials Science Forum*, **727-728**, 511-515 **(2012)**

27. Water W., Li C.J. and Ji L.W., Application of Ni-doped ZnO nanorods in surface acoustic wave ultraviolet photodetectors, *Modern Physics Letters B*, **36(10)**, 2250131-2250131 **(2022)**.

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