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Spectroscopic Investigations of Phyto-Synthesized ZnO NPs Using Calendula officinalis L. leaf Extract

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Abstract

In current decade, zinc oxide nanoparticles (ZnO NPs) widely exploring owing to its versatile properties, prodigious demands in energy, beauty, healthcare sector. Therefore, the current work opted environmentally benign method to prepare ZnO NPs. In this process Aqueous *Calendula officinalis L* leaf extract was prepared for the reducing metal ions. The aqueous solution of zinc acetate (0.45M) were dropped at the rate of 5 ml/10 minute in the prepared extract. The ZnO NPs were successfully manufactured using a 50 ml and the aqueous solution (5 g) of Calendula leaves under constant stirring (1200 rpm) at a temperature of 600C for 2 hours. Brown coloured dried powder was obtained after three times washing. Furthermore, it characterized using different physico-chemical techniques. The spectroscopic study of synthesized ZnO NPs in distilled water, a peak observed at 355 nm. After initial confirmation of desired NPs, the FESEM generally apply to know their external assembly, which found small and big agglomerated form. The median crystalline particle size was estimated approx. ~24 nm using an X-ray diffraction (XRD) pattern. Fourier transform infrared spectroscopy (FT-IR) showed an absorption peak of the Zn–O bond between 4000 and 500 cm^{-1}. This phyto-synthesis method for preparing the ZnO NPs using the leaf extract of *Calendula officinalis L*. evidenced to be superior, and environmental friendly, and these nano products shows the various applications.

1. INTRODUCTION

Nanoparticles (NPs) have opened a new horizon in the field of medicine delivery, gene delivery, nanomedicine, and biosensing in the 21st century [1, 2]. NPs are also applicable in environmental protection, sensing, communication, cosmetics, medical industry, etc. [3]. The magnetic and catalytic properties of NPs, along with their improved surface-to-volume ratio, can be enhanced by some unique modified processes [4, 5]. NPs can be synthesized by using an array of physical, chemical and biological methods. The hydrothermal processes, physical vapor deposition, microemulsion, precipitation, ultrasonic irradiation, chemical reduction, plasma, and sol–gel methods are the best example of the above methods [6]. These methods required a variety of hazardous chemicals, and a huge amount of energy to synthesize the NPs. The above methods also utilized a very high temperature and pressure which is very harmful to the environment. Accordingly, the product cast is very high due to the higher order of the equipment used in these methods [7]. As a result, an environmentally friendly method is required to synthesize the NPs without utilizing any toxic components. Recently, many scholars are taking interest to prepared the NPs via herbal rout. In medical science, especially in diagnosis and treatment of various decease the biological method of the synthesis of NPs is comparatively more advantageous than the physical/chemical methods. Thus, the biological method has replaced the physical and chemical method for the synthesis of NPs due to the high energy consumption, low cost, environmental impact and production of more stable and biocompatible NPs [8, 9]. Microorganisms [10], enzymes [11], fungi [12], and plant extracts [13] are used to manufacture metal and metal oxide NPs by green route. Nevertheless, it is challenging to develop effective environmentally friendly methods to generate NPs with absolute

size, shape, and composition. Nontoxic and environmentally friendly NPs are prepared by a variable phytochemistry-based process using biological entities [14, 15].

ZnO NPs are one of the cost-effective metal oxide NPs with minimal toxicity and highest biocompatibility, which are used in photothermal therapy, antibacterial and anticancer drug distribution, cell imaging, and biosensing, making them a promising tool in the field of biomedical sciences [16, 17]. Various synthesis techniques for NPs improve both the crystal size and their properties [18]. Phyto-synthesis is widely accepted due to its nontoxicity, environmental friendliness, and wide range of applications such as antimicrobial properties, antioxidants, anti-aging, anticancer, and so on. In the phyto-synthesis method, secondary metabolites trap the metal ions and act as capping agents that help stabilize the NPs in terms of electrostatic, steric, hydration forces, depletion, and Vander-Waals forces [19, 20]. The interactions of ZnO NPs with microbial cells are possible due to the smaller surface area and higher charge density of ZnO NPs [21, 22]. The *Calendula officinalis* L. is a flowering plant which belongs to the family Asteraceaeis. A wide rage of pharmacological effects can be observed in the extract of *Calendua officinalis L.* [23] due to which it can be unitilized as antiseptic, stimulant, diaphoretic, antispasmodic and anti-pyretic agents [24, 25]. It has been observed that the leaf extracts of the calendula officinalis have anti-cancerous, antiviral anti-genotoxic, anti-oxidant, anti-microbial and anti-inflammatory properties [26, 27].

The present study focuses on the synthesis of ZnO NPs via the green route using *Calendula officinalis L*. The prepared ZnO NPs were characterized by XRD, FE-SEM, UV-Vis and FT-IR spectroscopy for the size, shape, optical properties and stability of the ZnO NPs.

2. REAGENTS AND METHODOLOGY

2.1 Chemi- products:

Sodium hydroxide (NaOH) and Zinc acetate dehydrate ($C_4H_6O_4Zn.2H_2O$) of Merck life science Pvt. limited Mumbai, was purchased from Winner scientific store Naini, Prayagraj, Uttar Pradesh. All the chemicals utilized in this investigation.

2.2 Leaves procurement and Processing

The fresh *Calendula* leaveswere plucked from the Rox burgh Botanical Garden, University of Allahabad, India. Wash thrice to remove the dust and impurities. Leaves are kept in shade for a weak then cut it into fine pieces.

2.3 Preparation of the Leaf Extract

25 gm *Calendula leaves, cut in very fine pieces* were added in 0.1 L ionized H_2O and rotate on magnetic stirrer for 6 hours at 60^0C and 1200 rpm [28]. The aqueous solution was filtered using filter paper and kept for 24 hours at 4 ^{0}C. The zinc ions which are supportive to form the ZnO NPs, were reduced from the fused aqueous extract and zinc acetate.

2.4 ZnO NPs Phytosynthesis

An aqueous of *Calendula* leaves (50 ml) was maintained at a temperature of 60 ^{0}C with regular stirring (1200 rpm) for 2 hours and 0.45M aqueous solution of $ZnC_4H_6O_4.2H_20$ (50 ml) was slowly dropped in aqueous solution of *Calendula* leaves. The precipitate thus formed was centrifugated for 15 minutes at 6000 rpm. Washed by double ionized water two times and left for the whole night at 100^{0}C [29].

2.5 Spectroscopic Investigation of ZnO NPs

2.5.1 X-ray Powdered Diffraction

ZnO Nanomaterials was analyzed via XRD (Rikagu, Ultima IV Japan) and found to be crystalline powder with Cu-Kα radiation (λ = 1.54020 Å).

2.5.2 Field Emission-Scanning Electron Microscopy

These nanomaterials were further analyzed using FE-SEM (JEOL India Pvt. Ltd., Delhi India, for superficial phenotype. Furthermore, also evaluated with energy dispersive X-ray spectrometer (Bruker, Germany), to examined its elemental composition. A very small amount of nano-powder kept on the carbon coated copper grid, and then FE-SEM grid was dried with a fine layer of sample and fixed with gold for 300 seconds.

2.5.3 Fourier Transform Infra-Red Spectroscopy

The Perkin-Elmer Bucking hamashire, UK FTIR spectroscopy is used to investigate bond and functional groups existing in the phyto synthesized ZnO NPs. During the analysis, the band and fictional group are identified by using FTIR spectra with a resolution of 4.0 cm^{-1} in order to 4000 – 450 cm^{-1}.

2.5.4 UV-Vis Spectroscopy

The optical properties of the phyto-synthesized ZnO NPs were investigated by using UV-Vis Spectroscopy. The shape and size of the synthesized ZnO NP, temperature even synthesis technique also affect the sharpness of the absorption peek. In this experiment to check the optical properties of phyto-synthesized ZnO NPs, 0.05gm ZnO NPs was dispersed in 4 mL of double ionized water. The absorption spectrum was recorded by using an ultraviolet visible (UV-Vis) spectrophotometer (Spectra Max, San Jose, CA, USA) in between a wavelength range of 300–500 nm.

3. RESULTS AND DISCUSSION

3.1 X-ray Diffraction Analysis of Phyto-synthesized ZnO NPs

Powdered XRD pattern of zinc oxide NPs is investigated and depicted in the photograph 2, that illustrate peaks at various diffraction angle 2θ value, depicted in Table1, equivalent to lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201) respectively, furthermore analyzed that these phyto-

synthesized NPs are crystalline wurtzite structure, after matched with the XRD database (JCPDS Card no: 80 – 0074). Yet, ZnO sample Powder X-ray diffraction pattern does not show any extra diffraction peaks of impurity, provide evidence that the synthesized sample is extremely pure. The NPs diameter further evaluated with Debye-Scherer equation-

$D = K\lambda/\beta Cos\theta$ (1) [30]

Where: D = Average crystallite size of the particles, K = Debye-Scherer's constant (= 0.94), λ = Wavelength of the Cu Kα-radiation (= 0.1540 nm), β = Full width half maximum (FWHM) of the peak, θ = Bragg's angle.

Table 1
XRD analysis at various diffraction angles

S. No.	2 Theta	FWHM (β)	Miller indices	Particle size (D)
1	31.75°,	0.38586	(100)	22.35
2	34.41°	0.38586	(002)	22.50
3	36.23°	0.38586	(101)	22.62
4	47.51°	0.38586	(102)	23.49
5	56.57°	0.38586	(110)	24.41
6	62.84°	0.38586	(103)	25.19
7	66.37°	0.38586	(200)	25.69
8	67.91°	0.38586	(112)	25.92
9	69.06°	0.38586	(201)	26.10

The mean crystallite diameter of the NPs was calculated as ~ 24 nm, evaluated from the FWHM of the most intense peak corresponding to (101) plane localized at 36.23°. ZnO NPs were obtaining in hexagonal wurtzite crystal structure with well crystalline nature. The lattice has been calculated and found to be (a = b) equal to 3.2535 Å and c equal to 5.2151 Å.

The change in crystallographic properties can also be understood with the help of W-H plot between βCosθ versus 4Sinθ. Figure 3 depict W-H plots from mathematical relations

$\beta Cos\theta = k\lambda/D + 4\mu_s Sin\theta$ (2)

Strain μ_s is determined from the slope of straight line. The values of micro-strain have been calculated and found to be 0.00451. The reciprocal of intercept reveals the grain size of the sample. The variations of grain sizes calculated from both Debye-Scherer's as well as W-H plot are approximately same.

3.2. FE-SEM Analysis of ZnO NPs

Figure 4 showed the FE-SEM images of the ZnO NPs. The agglomerated NPs are shown at the surface morphology. The calculated particles size of ZnO NPs using FE-SEM micrographs shows different from the particle size calculated from XRD because FE-SEM gives the particle size whereas XRD gives the crystallite size of the sample. The formation of NPs in their agglomerated form is attested by surface morphology [31]. The FE-SEM image (Fig. 4) showed that the particles are of different shape and size.

Histogram of FE-SEM image is form by IMAGE-J. The histogram gives the statistics about the distribution of pixel values in the whole image Histogram of FE-SEM images by IMAGEJ. The software program Image J (version 1.46r) was used for FE-SEM image analysis. The observed standard deviations and mean deviations of ZnO NPs using Image-J software and found to decreases with increasing the magnification from x200 to x1100 (Fig. 5, Table 2). The standard deviation (51.287) revealed that pixels spread out around the mean of the crystallite of ZnO NPs. The mean deviation (127.148) is measured the closest alternative to standard deviation.

Thus, the instability of the FE-SEM micrograph is expressed as a significance of standard deviation. The observed irregular shape nanoparticles in SEM images indicate that synthesized nanoparticles containing large volume to surface ratio. The histogram of FE-SEM image which exhibits that ZnO sample for different magnification have uniform particles size distribution on the surface of the cluster.

3.3 FT-IR Analysis of phyto-synthesized ZnO NPs

Figure 6 showed FT-IR spectrum of compositional and fictional group present in the phyto-synthesized ZnO NPs. The FTIR spectra were recorded in the form of pallet utilizing spectroscopic grade KBr in wave number range of 4000 – 500 cm^{-1} in the different reflectance mode. FT-IR spectra resulted in numerous bands at various absorption peaks (Table 2, Fig. 6) [32, 33]. To be more precise, an extensive band at 3401 cm^{-1} is assigned to the O-H stretching mode of hydroxyl group present in the prepared sample. Peaks of 3080 cm^{-1} are due to C-H stretching vibration of alkane functional groups. The peaks detected at 2345 cm^{-1} is assigned to the C = C alkane / C = N stretching.

Table 2
Bond and functional group present in FTIR spectra

S.N.	Absorption Peak (cm^{-1}) of ZnO NPs	Bond and functional Groups
1	3401	O-H stretch of alcohol phenol
2	3180	C-H stretching of alken
3	2345	C = C/alken-C = N stretching
4	1569	C-H stretching of amine
5	1423	(C-O), C-F, alkyl halide strong
6	1022	C-N Stretching amine or Phenols
7	948	C-N Stretching amine
8	773	C-N Stretching amine
9	688	C-N Stretching amine
10	614	C-N Stretching amine

However, the content of the 1569 cm^{-1} is assigned to the (N-H) starching of the amine functional group and 1423 cm^{-1} is assigned to the (C-O), C-F, alkyl halide strong groups present in the sample. The probably 1022 cm^{-1}, 948 cm^{-1}, 773 cm^{-1}, 688 cm^{-1} is assigned comes from C-N stretching amine functional group present in the sample [34]. The FTIR spectra confirm the formation of chemical bonding due to functional group present in the sample.

3.4 UV–Visible Analysis of Phyto-synthesized ZnO NPs

The leaves extract not only acts as a reducing agent but also a stabilizing agent. The zinc ions are reduced in the zinc oxide solution by the secondary metabolite of the plants. This was studied by taking the UV visible spectrum in between 280 nm-800 nm. The Fig. 7 showed the absorption spectrum of phyto-synthesized ZnO NPs. The absorption peak was visualized at 355 nm which confirm the formation of particles at nano scale [35]. For the formation of ZnO NPs, the absorbance peak was found in the previous report in range of 300–360 nm [36]. The smaller size particles were also visualized at FE-SEM image (Fig. 4) that also support the above statement and bump around 370 nm. This is because of more interaction of Zn ions with secondary metabolites. Bioactive constituents of plant extract interacted with the Zn ions and act as stabilizing as well as abbreviating agents in reduction of the metal size [37, 38]. The band gap energy was evaluated by equation-

$E_g = 1240/\lambda\ eV$ (3)

The optical band gap energy of phyto-synthesized ZnO NPs was observed 3.986 eV (Fig. 8) which is comparable to the previously reported [39.40].

4. CONCLUSION

Several methods are introduced to synthesized NPs but recently the biological methods advanced over physico-chemical approaches due to lesser energy consumption, cost effective and eco-friendly nature. Present work enlightened the ZnO NPs using aqueous greenery extract of *Calendula officinalis* L. The secondary metabolites of plant extracts provide stabilizing agents for the synthesis. The spectroscopic technique such as XRD FE-SEM, FT-IR, UV–Vis were utilized to observed the structural characteristics, morphological, stability and the optical prosperities of the phyto-synthesized ZnO NPs. The FE-SEM micrograph showed that the synthesized ZnO NPs were crystalline, cubic, sphere and other shape. The average crystalline sizes of the synthesized ZnO NPs were evaluated ~ 24nm. The optical band gap energy of the phyto-synthesized ZnO NPs is found 3.986 eV. Thus, the phyto-synthesized ZnO NPs using *Calendula officinalis* L. can be used in nano herbal drug preparation for antioxidant, and analytical sensing also.

Thus, the results provide the clear view to adopt the phyto-synthesis of ZnO NPs which can be utilized in various applications such as bio-medicals, cosmetics, cleaning of waste/sewagewater, bio-sensing, pharmaceuticals and other relevant field.

Declarations

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Data availability statement: The experimental data and the results that support the findings of this study are available.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

1. Ajay Kumar Tiwari has written the manuscript 2. Saket Jha has done the correction in the manuscript 3. Rohit Shukla has made the work flow diagram of the synthesis of the ZnO NPs.4. Ram Raseele Awasthi has provided the X- Ray diffractometer for the characterization of the sample 5. Abhishek Kumar bhardwaj has check the manuscript and made the correction in manuscript6. Abhimanyu Kumar Singh has check all the characterization part of the manuscript 7. Rudra Prakash Ojha has checked the grammars and spelling 8. Anupam Dikshit has made the final check up of the manuscript and also drafting part of the manuscript

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Figures

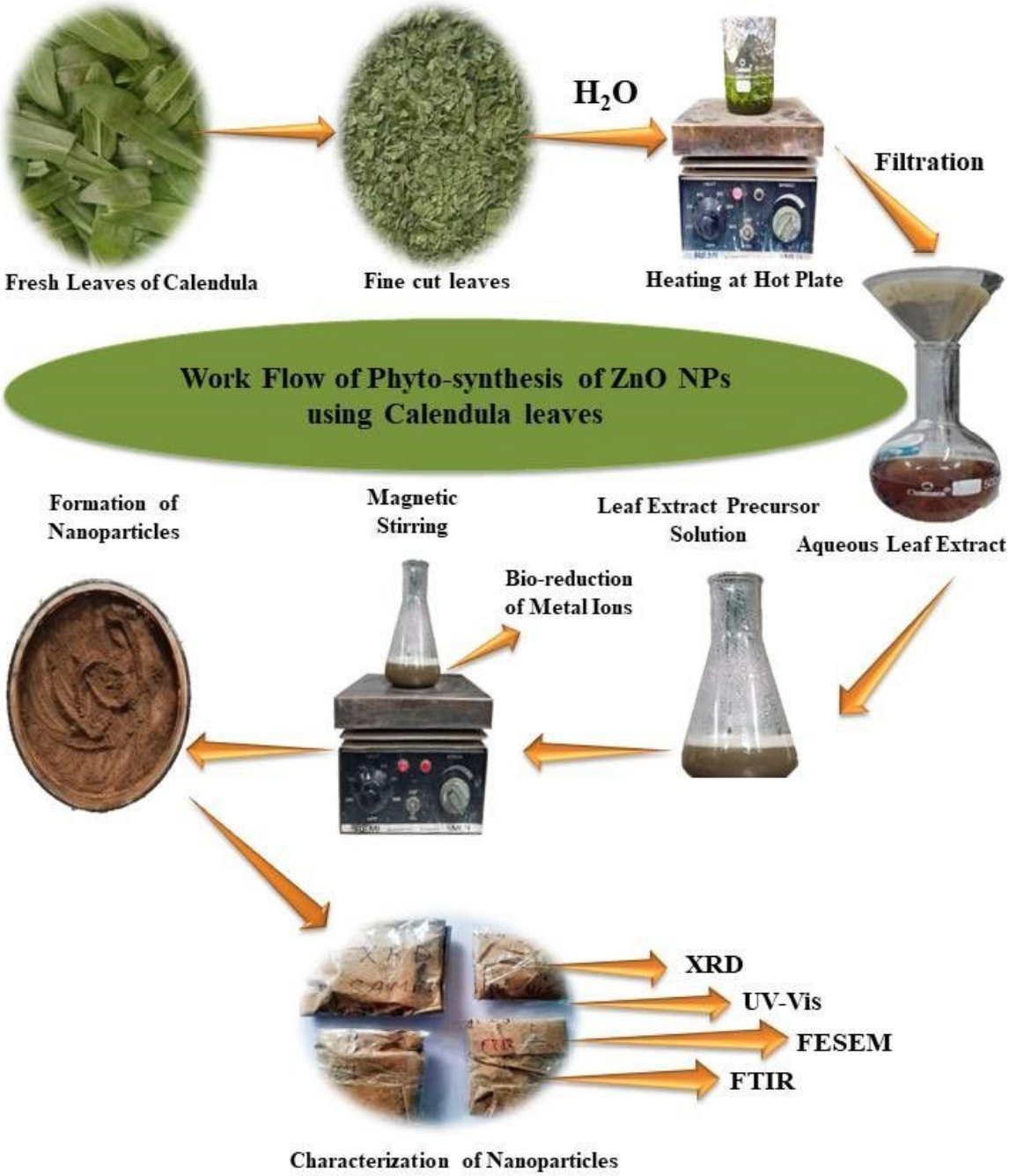


Figure 1

Work flow for the phytosynthesis of ZnO NPs using greenery extract of *Calendula officinalis* L.

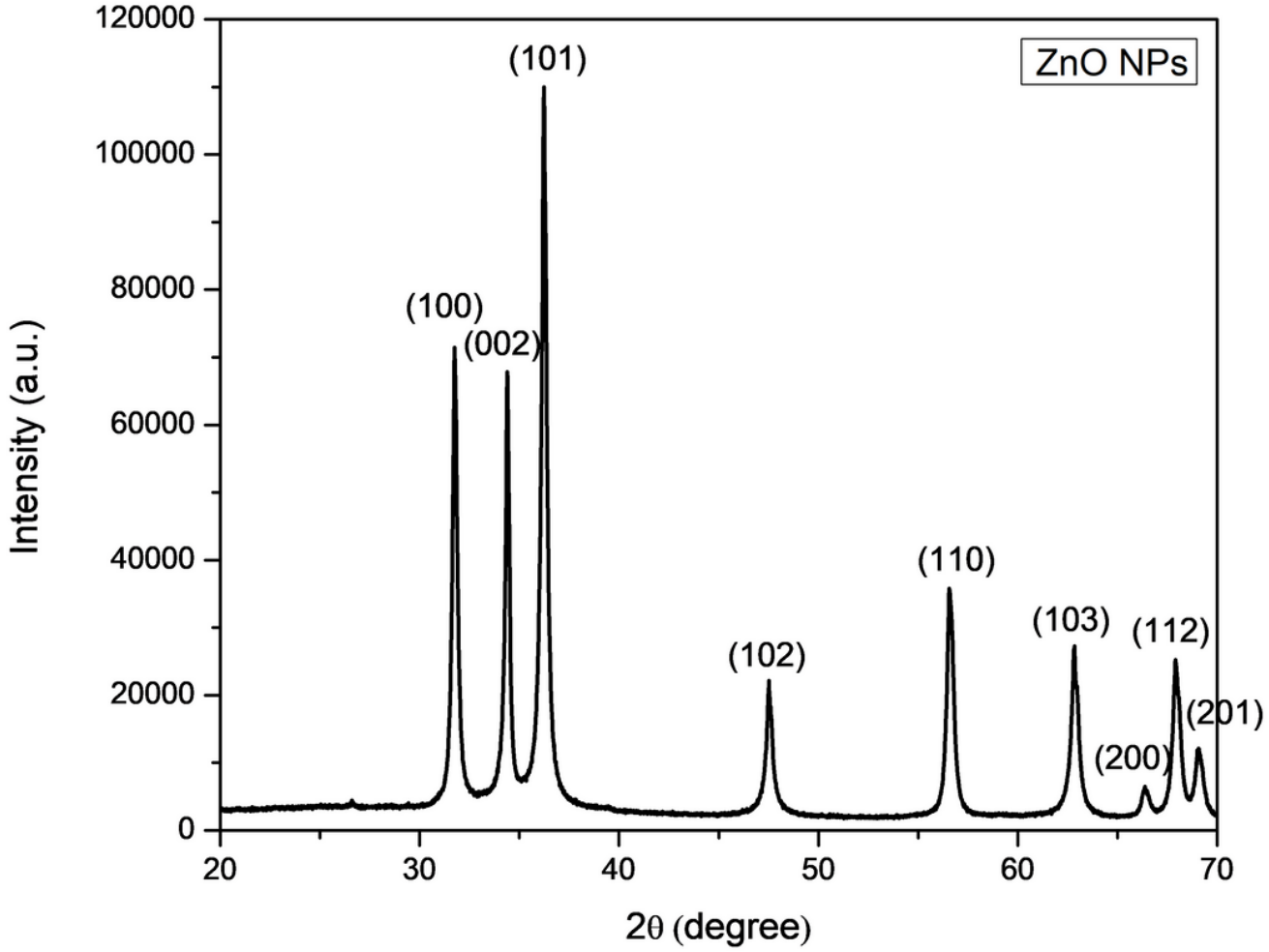


Figure 2

XRD pattern of ZnONPs

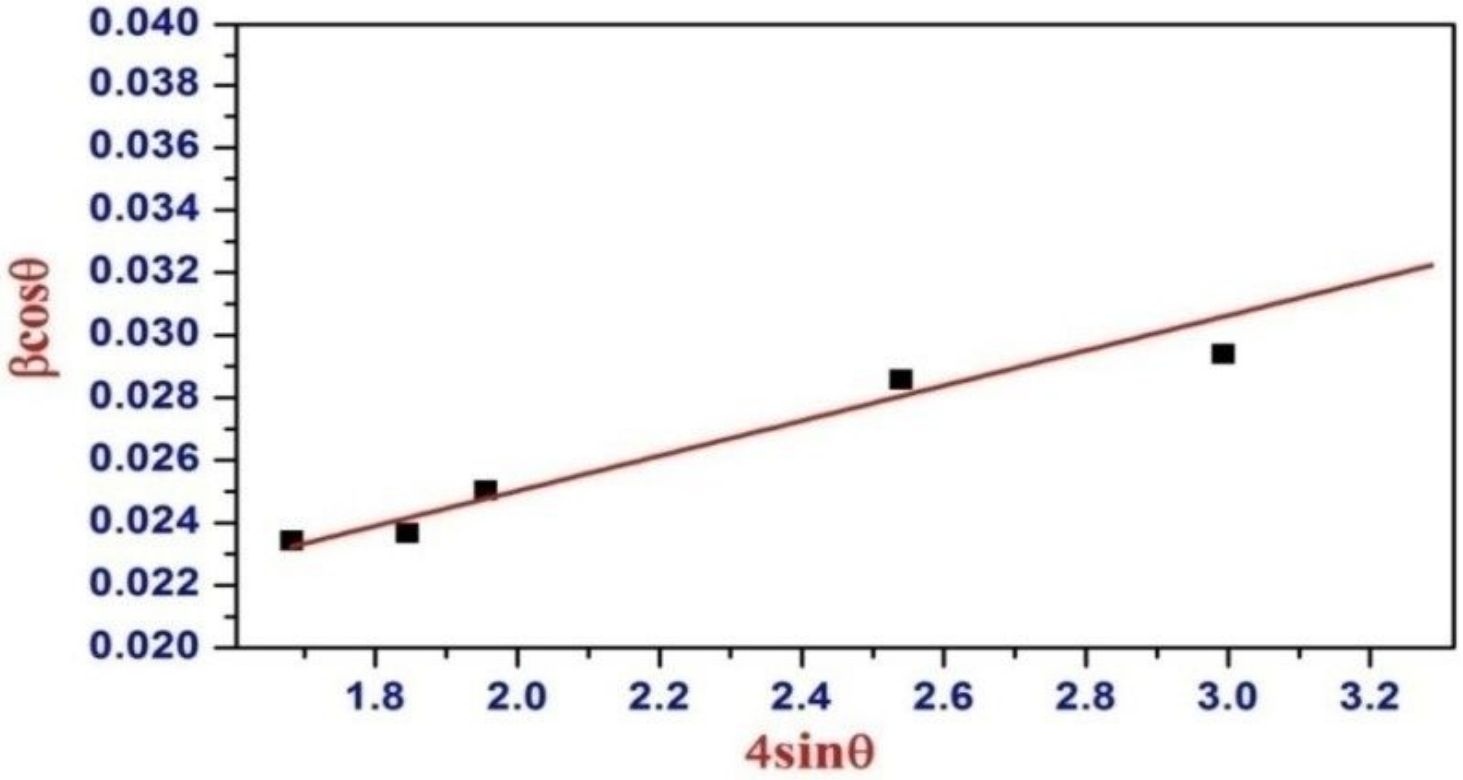


Figure 3

Williamson-Hall (W-H) Plot for ZnO NPs

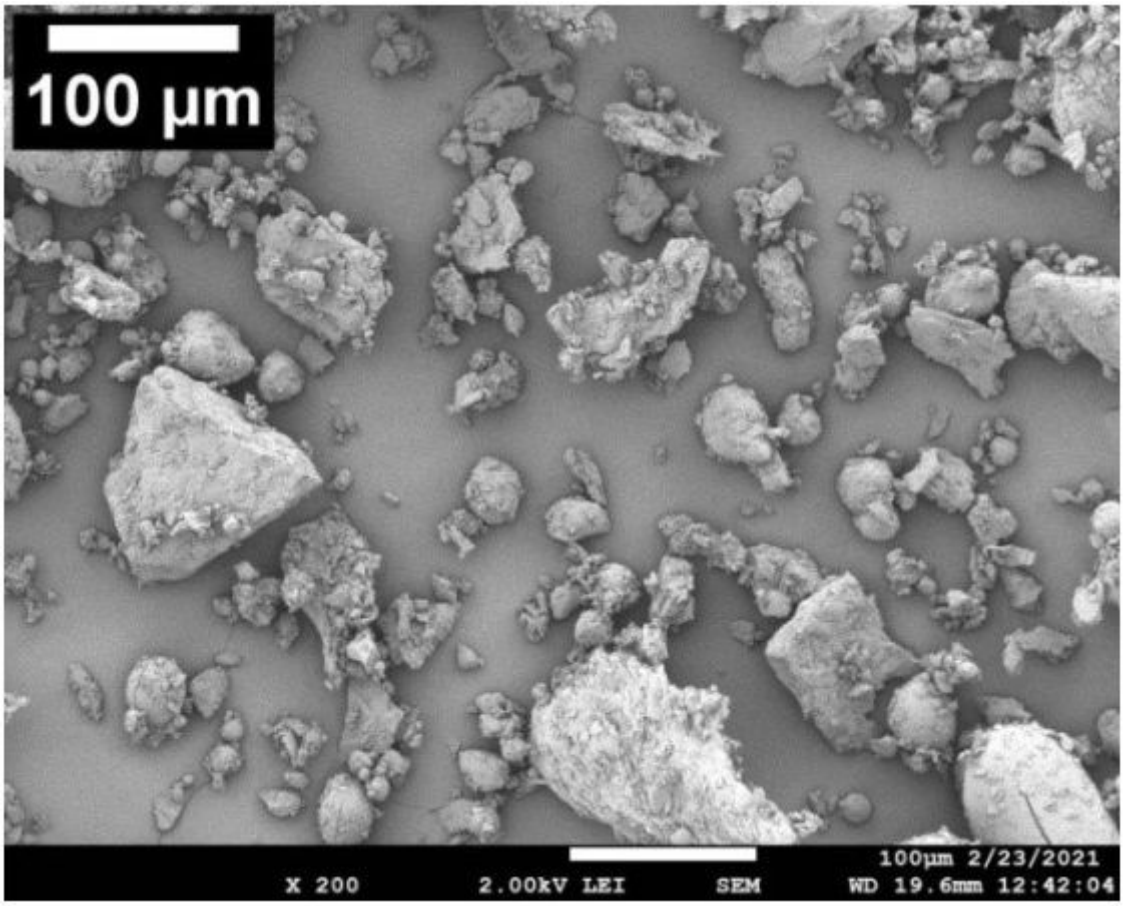


Figure 4

FE-SEM micrographs of ZnO NPs at different magnification

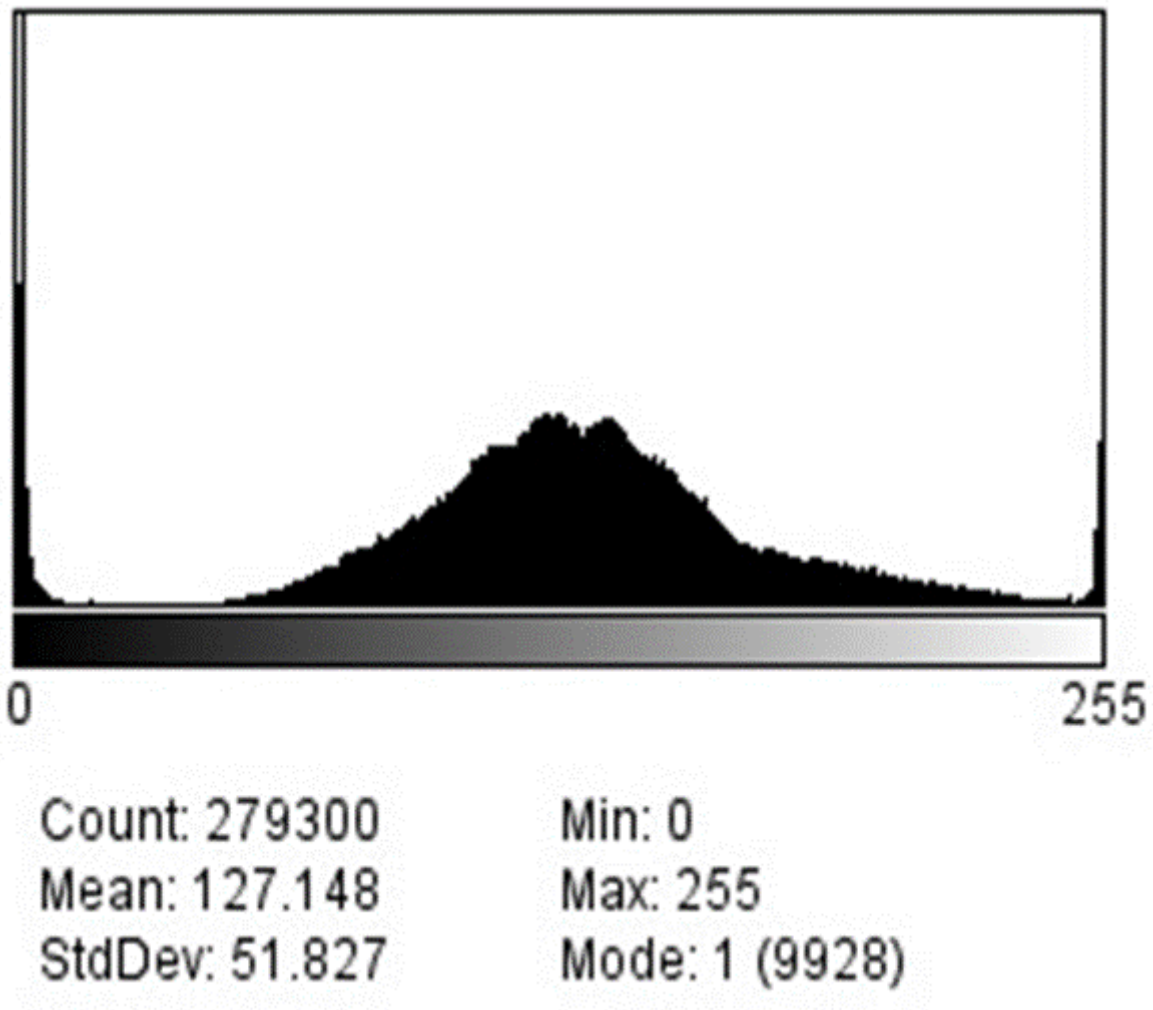


Figure 5

Histogram of SEM images by IMAGEJ gives the information of particle distribution in the pixel values.

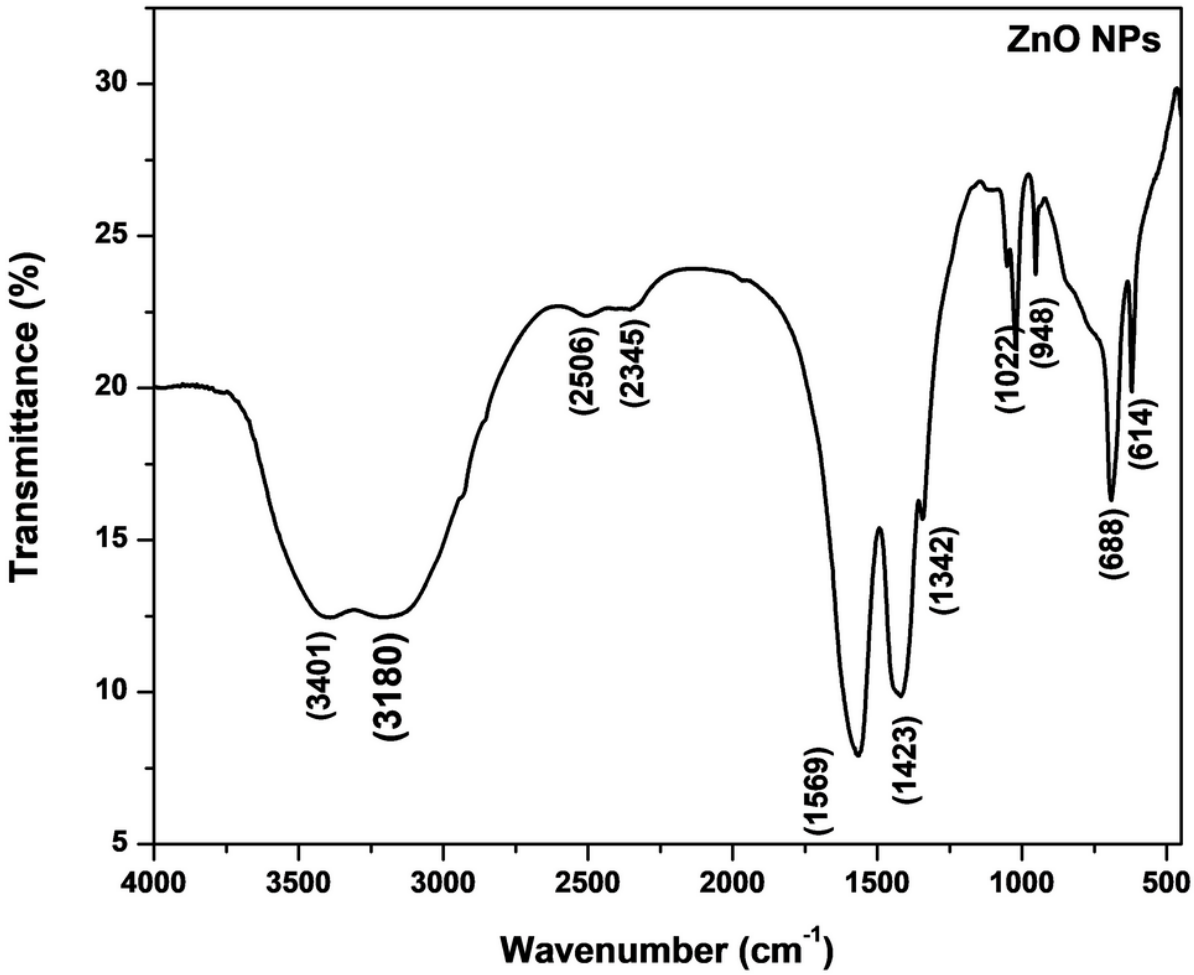


Figure 6

FT-IR spectra of phyto-synthesized ZnO NPs

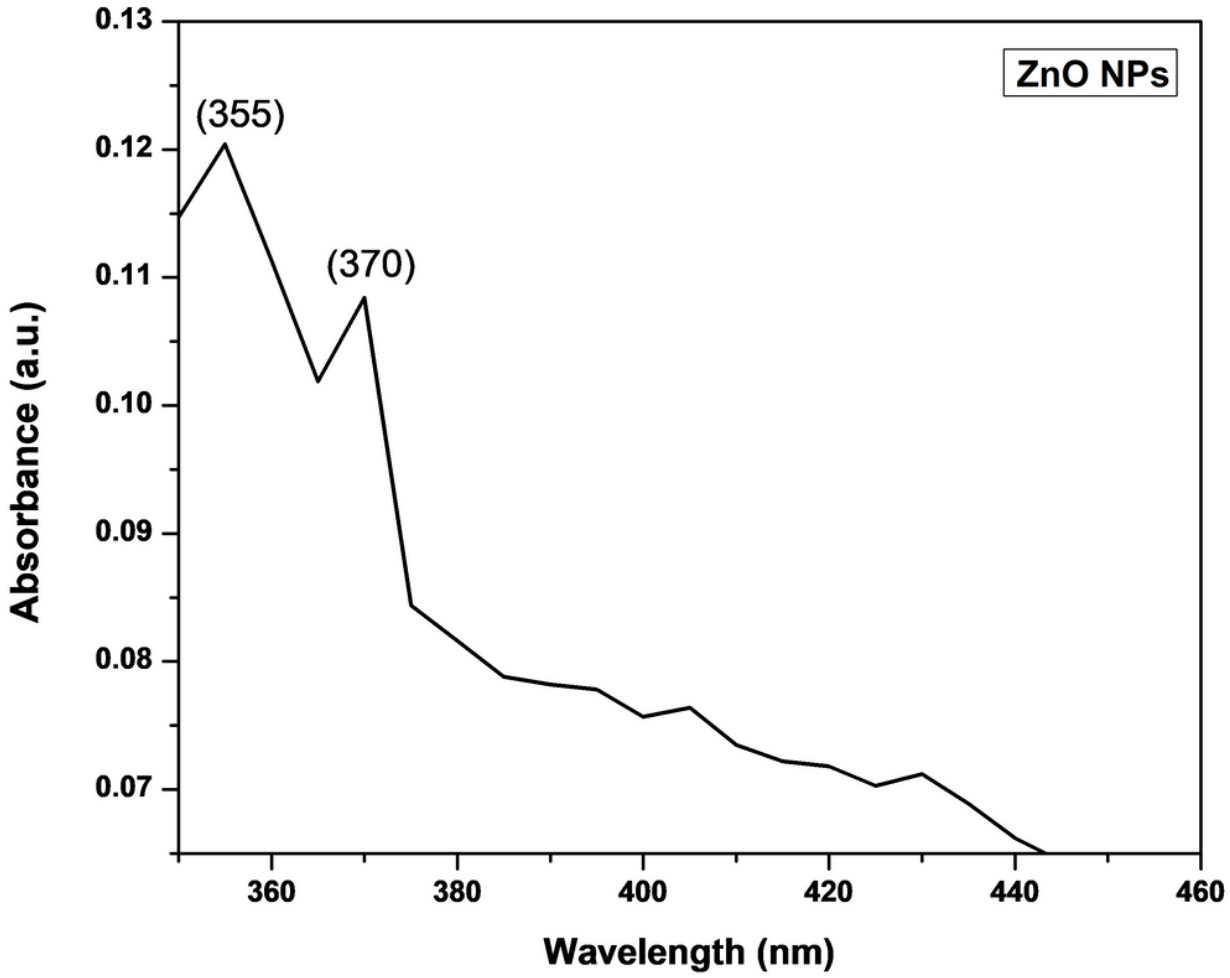


Figure 7

UV-Visible Spectrum of phyto-synthesized ZnO NPs.

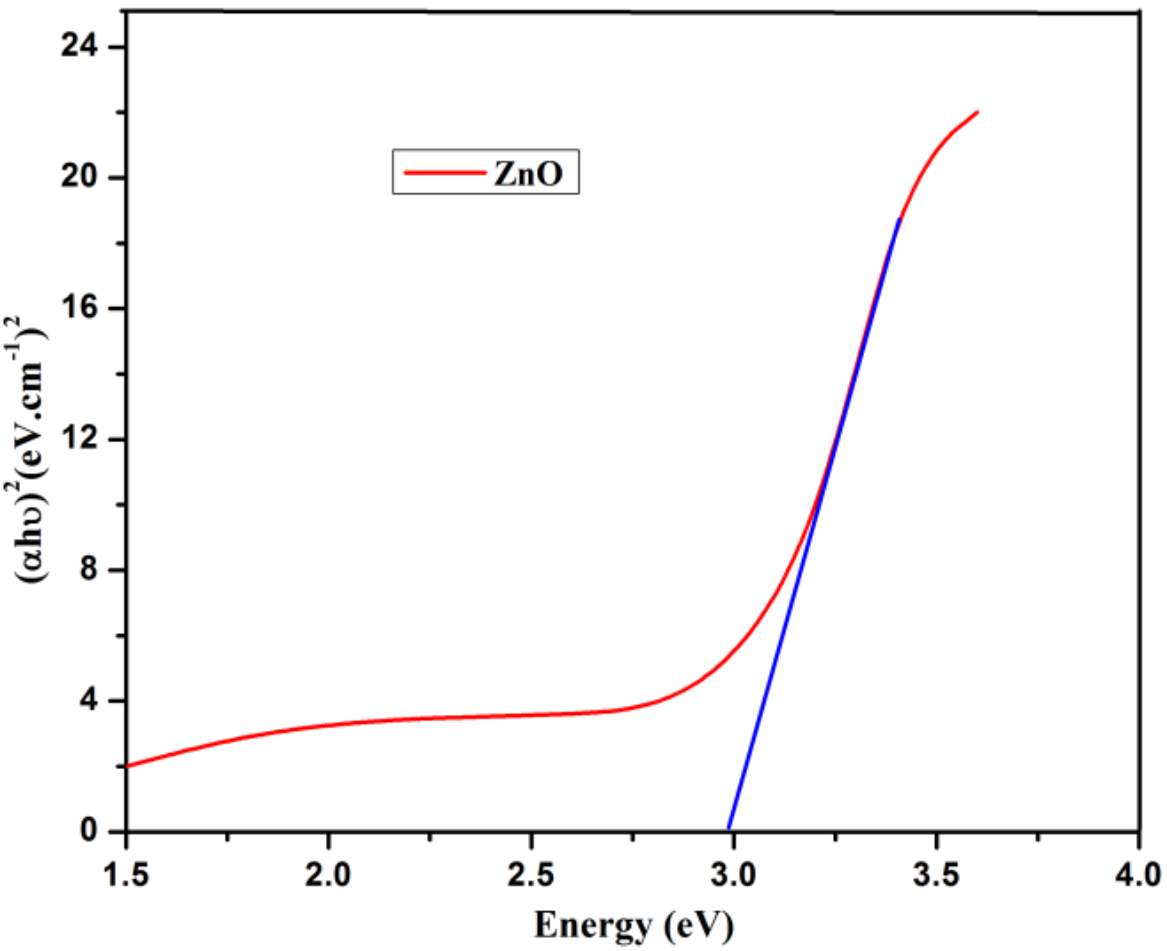


Figure 8

Optical band gap of phyto synthesized ZnO NPs