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STUDY ON THE EFFECT OF *OCIMUM GRATISSIMUM* AND *VERNONIA AMYGDALINA* PLANT LEAF EXTRACTS AND PH IN THE SYNTHESIS OF ZINC OXIDE NANOPARTICLES.

Rebecca Emmanuel Mfon [1,3*], Simon R. Hall, [2] and Andrei Sarua [1]

[1]School of Physics, H.H Wills Physics Laboratory Tyndall Avenue, University of Bristol, Bristol BS8 1TL United Kingdom
[2]School of Chemistry, Cantock's close, University of Bristol, Bristol BS8 1TS United Kingdom
[3]Department of Physics Federal University Lafia, P.M.B 146 Lafia, Nasarawa State, Nigeria
* Correspondence E-mail: rebecca.mfon@science.fulafia.edu.ng

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ABSTRACT

Zinc oxide nanoparticles were synthesized at three different pH levels in the presence of Ocimum gratissimum (Og) and Vernonia amygdalina (Va) plant leaf extracts. The nanoparticles were characterized using optical spectroscopy and electron microscopy and their optical response, sizes and optical energy band gap values were found to be pH and leaf extract dependent. While the Og nanoparticles had mean sizes in the range 38 nm – 63 nm, the Va nanoparticles were slightly larger with mean sizes ranging from 44 nm – 62 nm. The peak absorbance for the Og and Va zinc oxide nanoparticles occurred at 360 nm and 355 nm respectively. The SEM images of the nanoparticles showed them to be spherical and in clusters, with sizes which were pH dependent and decreased in the order pH 12 > pH 10 > pH 8. The FTIR scans showed that phenols and flavonoids among others were the phytochemicals that took part in the synthesis of the derived ZnO nanostructures. The Photoluminescence spectra of the ZnO nanoparticles showed two emission peaks namely a UV emission peak at 2.2 -2.3 eV and a green emission peak at 3.2 eV and the nanoparticles showed band edge photoluminescence, with spectral intensities which were also leaf extract and synthesis pH dependent. While the optical energy band gap(E_g) values of the Og zinc oxide nanoparticles increased with synthesis pH with obtained values in the range 3.22 – 3.24 eV that of the Va zinc oxide nanoparticles decreased with increase in pH of synthesis and was in the range 3.29 – 3.20 eV

Keywords: *plant leaf extracts, optical energy bandgap, optical spectroscopy, electron microscopy, photoluminescence*

INTRODUCTION

Nanoparticles have been put into varied uses to improve man's quality of life and revolutionise healthcare delivery (Marchiol, 2012). While they can be synthesized physically or chemically, their biosynthesis using plants and various parts of it is gaining popularity (Malik *et al*., 2014) because not only is the process fast, it is cheap and minimally toxic to the environment (Keat *et al*., 2015, Ahamed *et*

al., 2016). Used plant parts are reported to contain phytochemicals such as flavonoids, phenols reducing sugars or polysaccharides which are said to have reductive abilities and can cap as well as stabilise the nanoparticles produced (Ahamed *et al*., 2010). Different parts of plants such as their stem, roots, leaves, fruits and even flowers (Shah *et al*., 2015; Emeka *et al*., 2014) have successfully been used for synthesizing nanoparticles and the nature of the resulting nanoparticles in each case has been reported. Though nanoparticles have been used in the field of medicine for drug delivery to targeted cells of the body, they have also been used for bio-imaging, as well as for the destruction of tumours (Li *et al*., 2011, Singh *et al*., 2016). Furthermore, the fact that some nanoparticles have antibacterial and antimicrobial properties, have led to their use for the treatment of various diseases (Mohammad *et al*., 2010) and more recently some nanoparticles have been used to enhance the efficacy of antibiotics (Singh *et al*., 2016). There are various types of nanoparticles, but of particular interest in this work are zinc oxide nanoparticles.

Zinc oxide nanoparticles have been put into a wide range of applications in the fields of medicine and electronics (Mohammad *et al*., 2010, Patil *et al*., 2012). They are presently considered by some industries for food preservation and packaging (Espitia *et al*., 2012). Furthermore, there are speculations that they could be effective as Nano fertilizer for growing crops (Marchiol, 2012). In this present research, zinc oxide nanoparticles were synthesized from solutions of three different pH values and in the presence of the leaf extracts of *Ocimum gratissimum* (Og) and *Vernonia amygdalina* (Va) plants (Fig. 1). The produced nanoparticles were characterized using UV-Vis spectrometer, scanning electron microscope (SEM), Fourier transform infra-red and photoluminescence analysis.

Ocimum gratissimum also known as African basil or clove basil is called Ntoong, Nchanwu or Efirin in Nigeria and belongs to the plant family Lamiaceae. It is a shrub common to West Africa though also popular in India as well as the Caribbean. Grown mostly to extract oil, Indonesians use its leaves as tea (Rabelo *et al*., 2003). The plant, an aromatic perennial herb, has also been used in folklore medicine for treating headaches, cough, high fever and convulsion, as well as for keeping wound surfaces sterile (Prabhu *et al*., 2009).

Vernonia amygdalina plant on the other hand is a perennial shrub which belongs to the family of plants called Asteraceae. Also known as bitter leaf because of its bitter taste and as Etidot by some natives of the South-Eastern region of Nigeria, *Vernonia amygdalina* is very widely eaten in Nigeria and used in treating many diseases. Its leaf extract has been reported to be effective as an antimalarial and is also said to stop hiccups and tackle kidney problems (Udochukwu *et al*., 2015; Ugwoke *et al*., 2010). It is considered an effective laxative (Inyang, 2003), used for treating bacterial infections (Momoh *et al*., 2010), and its leaves even serve as fodder for animals. Most recently, *Vernonia amygdalina* is reported to have anti-inflammatory properties and can be used by diabetics for controlling high sugar levels (Bukar *et al*., 2013; Ebong *et al*., 2008).

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The effect of these two plant leaf extracts in the synthesis of Zinc oxide nanoparticles was studied. The morphology and optical properties of the produced ZnO nanoparticles were explored using optical spectroscopy and electron microscopy.

Fig. 1: (a) Ocimum gratissimum and (b) Vernonia amygdalina plants

MATERIALS AND METHODS

Chemicals and plant leaf extracts

All the chemicals used were purchased from Sigma Aldrich and were of analytical grade and milli-Q water was used for preparing all the solutions required. Zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$ of molecular weight 219.50 and purity > 99 % as well as sodium hydroxide pellets (NaOH) of molecular weight 40 g were used for this work. *Ocimum gratissimum* (Og) and *Vernonia amygdalina* (Va) shade - dried leaves (leaves dried in a shade, away from direct sunlight) were used for preparing the needed plant leaf extracts.

Preparation of chemicals and plant leaf extracts

Zinc acetate solution (0.2 M) was prepared by dissolving 43.9 g of the salt in 1000 cm^3 of milli-Q water and two concentrations of NaOH solution were also made. Sodium hydroxide of concentration 2 M was prepared by dissolving 40 g of the pellets in 500 cm^3 of water, while 0.2 M NaOH solution was prepared by dissolving 8 g of the pellets in 1000 cm^3 of water. These two NaOH solutions were used for the experiment with the former used for adjusting the pH of synthesis of the ZnO nanoparticles.

To prepare 100 ml of the Og or Va plant leaf extract, 130 ml of milli-Q water was added to 15 grams of each of the ground shade dried *Ocimum gratissimum* or *Vernonia amygdalina* leaves (Fig. 2) and the mixture was boiled for one hour. Thereafter, 100 ml of each leaf extract was filtered and stored in a refrigerator for use.

Experimental Method

70 ml each of 0.2 M zinc acetate dihydrate solution and 0.2 M sodium hydroxide were mixed together and 30 ml of *Ocimum gratissimum* or *Vernonia amygdalina* leaf extract was added. While stirring this mixture, 2 M NaOH was added dropwise to

adjust the pH of the resulting solution to pH 8, 10 or 12. With continuous stirring for 2 hrs, a cloudy precipitate of zinc hydroxide ($Zn(OH)_2$) was produced. This was centrifuged at 5000 rpm for 60 mins and the resulting sample was dried in an oven set at 70 ℃ to produce zinc oxide nanoparticles at these three different pH levels.

The reaction (Alias *et al*., 2010; Oskam, 2006) for the production of ZnO nanoparticles are as shown (1-3):

$$Zn\ (CH_3COO)_2.2H_2O + 2NaOH \rightarrow Zn(OH)_2 + 2CH_3COONa + 2H_2O \quad (1)$$

The zinc hydroxide $Zn(OH)_2$ reacts with water to give the growth unit $Zn(OH)_4^{2-}$ and hydrogen ions:

$$Zn(OH)_2 + 2H_2O \rightarrow Zn(OH)_4^{2-} + 2H^+ \quad (2)$$

When centrifuged, $Zn(OH)_4^{2-}$ changes to ZnO:

$$Zn(OH)_4^{2-} \rightarrow ZnO + H_2O + 2OH^- \quad (3)$$

The Block diagram for the synthesis pathway of the ZnO nanoparticles and the resulting powder is as shown in Fig. 2.

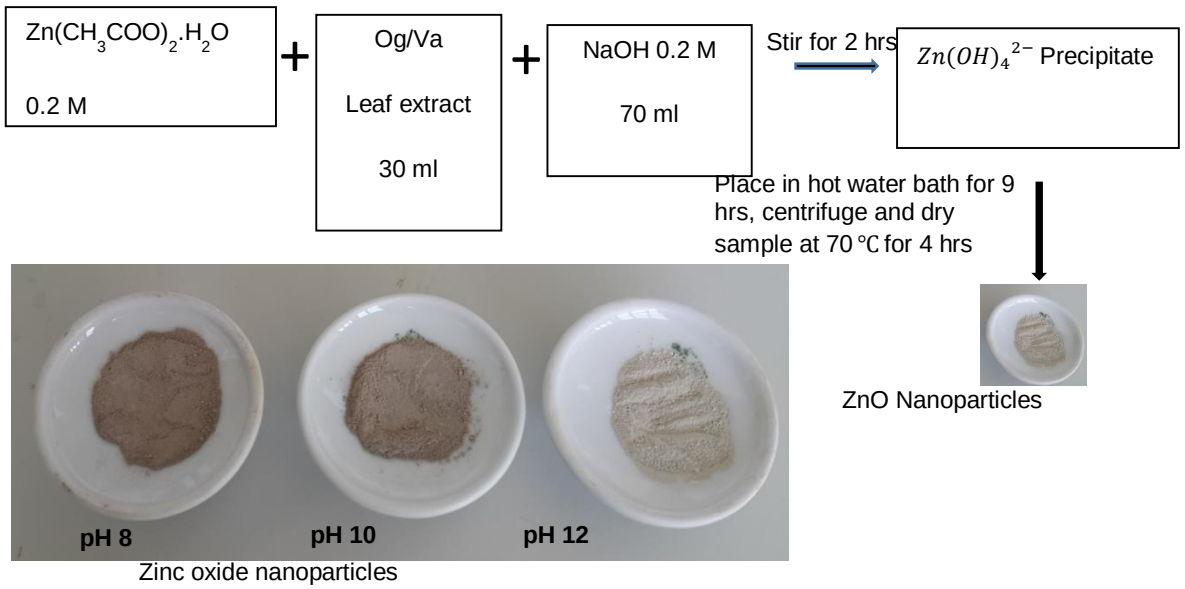

Fig. 2: Schematic illustration of zinc oxide nanoparticles synthesis pathway.

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Characterisation of ZnO nanoparticles

The resulting ZnO nanoparticles were characterised using UV-Vis spectrometer, scanning electron microscope (SEM), photoluminescence (PL) and Fourier transform infrared (FTIR) techniques. The UV-Vis spectroscopy was done with Shimadzu UV-2600 spectrophotometer which is equipped with an ISR-2600 integrating sphere attachment and the scans were in the range 220 - 1400 nm. This instrument was also used for the diffuse reflectance spectra (of the nanoparticles) which provided data for Tauc plots from which the optical energy bandgap values of the ZnO nanoparticles were derived. The SEM model JOEL JSM 6330F field emission scanning electron microscope which uses accelerating voltage of about 10.0 kV and current in the range 2 $-10.5\ \mu A$ with a working distance (WD) of about 7 mm was used to take images of the ZnO nanoparticles samples. The obtained images revealed shapes of the nanoparticles and with the Gatan micrographs, the sizes of the produced nanoparticles were measured. The photoluminescence (PL) instrument uses the 325 nm line of the helium-cadmium (He-Cd) laser with a Renishaw RM-2000 spectrometer and Peltier cooled Charge Couple Device (CCD) array for measuring the luminescence spectra of samples. The objective lens chosen for this analysis was OFR × 40 NUV quartz lens with NA = 0.4 and with a working distance of 2 mm and 3600 nm grating, a spot size of about 1-2 μm was produced on the sample surface. The FTIR instrument used for identifying the functional groups on the nanoparticles surface due to the plant leaf extracts was Perkin Elmer 100 FTIR Spectrometer. The instrument with an attenuated total reflectance (ATR) module was used for scanning the ZnO nanoparticles samples to display their spectra and the wave number range selected was from 600 to 4000 cm^{-1}.

The optical energy bandgap E_g of the nanoparticles was determined using R values derived from the diffuse reflectance spectra (Fig. 4) and Kubelka-Munk function given as (Mallika *et al*., 2013):

$$F(R) = \frac{(1-R)^2}{2R} \quad (4)$$

And the Tauc relation given as:

$$(h\upsilon\alpha)^{\frac{1}{n}} = A(h\upsilon - E_g) \quad (5)$$

where $E = \frac{1240}{\lambda}$ in electron volts, λ is in nm, h is a Planck constant, υ is the frequency of the light (Hz) shone on the sample, $\alpha = F(R)$ and $n = \frac{1}{2}$ for zinc oxide (Mallika *et al.*, 2013) which is a direct band gap semiconductor, Tauc plots (Fig. 5a and 5b) with a linear fit to the region of maximum change gave the optical energy bandgap of the nanoparticles.

RESULTS

The characteristics of the synthesized ZnO nanoparticles were found to be plant leaf extract and synthesis pH dependent. The absorbance and diffuse reflectance spectra of the ZnO nanoparticles are as shown in Fig 3 and 4. The absorbance spectra had intensities which changed with synthesis pH. For the Va ZnO nanoparticles the peak absorbance was at 355 nm while for the Og ZnO nanoparticles it was at 360 nm. While for the Og zinc oxide nanoparticles their optical energy band gap values E_g

increased with synthesis pH and in the range 3.22 – 3.24 eV, E_g for the Va zinc oxide nanoparticles decreased with an increase in pH at synthesis and was in the range 3.29 – 3.20 eV(Fig 5 a and 5 b). The SEM images (Fig 6 – 7) showed the synthesized ZnO nanoparticles to be spherical and in clusters with sizes which changed with synthesis pH. For both the Og and Va zinc oxide nanoparticles, their sizes increased with pH of synthesis as shown in Table 1 in the order pH 12 > pH 10 > pH 8. The mean size of the Og pH 12 nanoparticles was 63 $\pm$ 0.8 nm, that of its pH 10 nanoparticles was 43 $\pm$ 0.6 nm while its pH 8 nanoparticles had a mean size of 38 $\pm$ 0.4 nm. Conversely the pH 8 Va nanoparticles had a mean size of 44 $\pm$ 0.8 nm, those made from pH 10 solution had a mean size of 57 $\pm$ 0.5 nm and those made from pH 12 solution had a mean size of 62 $\pm$ 0.6 nm. The FTIR scans (Fig 8) identified phenols, flavonoids, Amines as carboxylic acids as biomolecules present in the plant leaf extracts used for the synthesis. The peaks due to the Og ZnO nanoparticles were sharper than those of the Va ZnO nanoparticles. The O-H bend at 1429.68cm^{-1} and O-H stretch peak at 3312.03 cm^{-1} denote the presence of phenols and flavonoids. Other functional groups identified on the nanoparticles surface were the N-H bend at 1572.43 cm^{-1} due to Amines, the O-H stretch at 2980.49 cm^{-1} for carboxylic acid and some polysaccharides. The photoluminescence spectra (Fig 9) show enhanced PL intensity with two emission peaks. A UV emission peak at 2.2 -2.3 eV due to singly ionized oxygen vacancies and a green emission peak at 3.2 eV due to the near bandgap excitonic emission (Sangeetha et al., 2011) probably due to minor defect states in the nanoparticles. The PL spectral intensities for both the Og and Va zinc oxide nanoparticles decreased with increased synthesis pH.

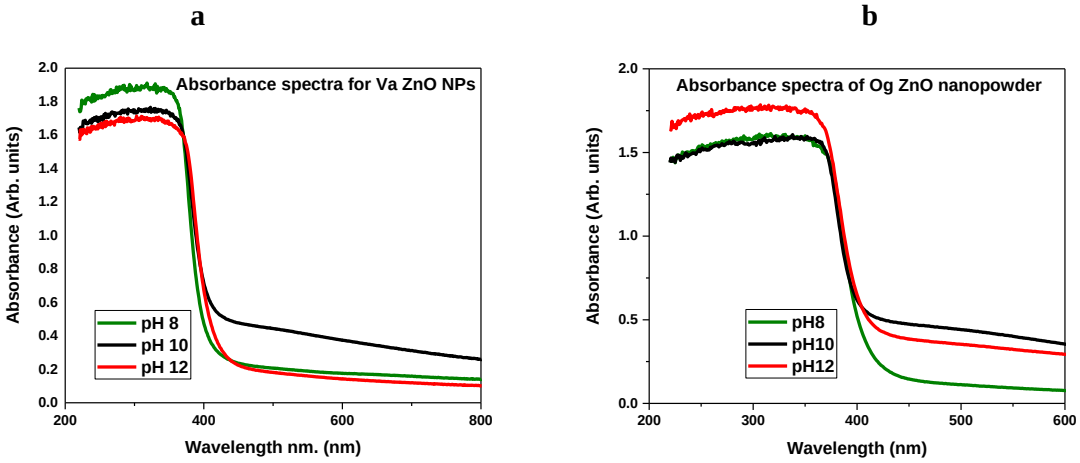

Fig. 3: UV-vis absorption spectra of the synthesized ZnO nanoparticles.

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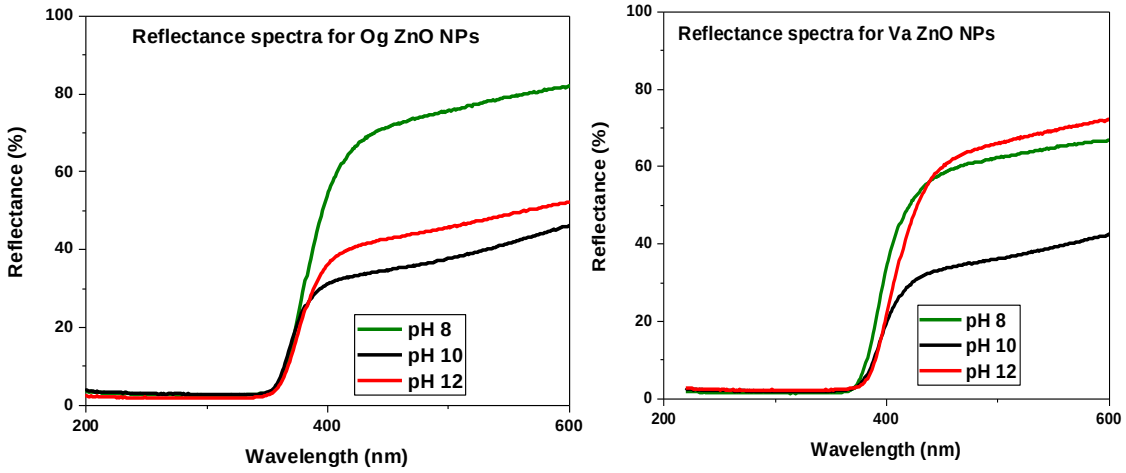


Fig. 4: Diffuse reflectance spectra of the synthesized ZnO nanoparticles

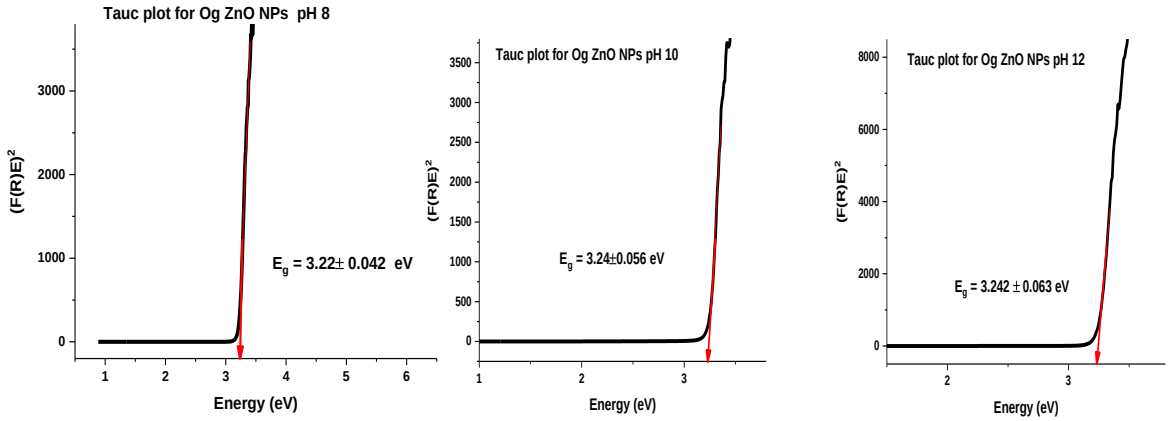


Fig 5a: Tauc plot of the Og pH 8, 10 and 12 ZnO nanoparticles showing their determined optical energy bandgap values.

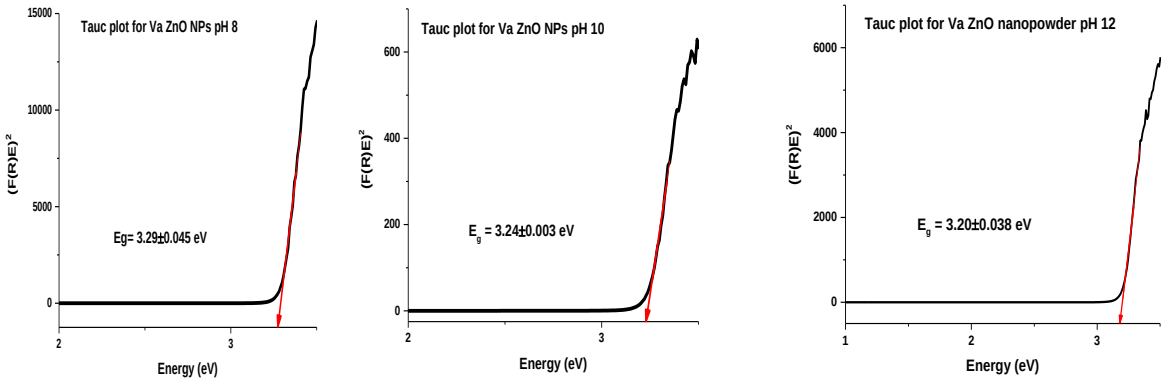
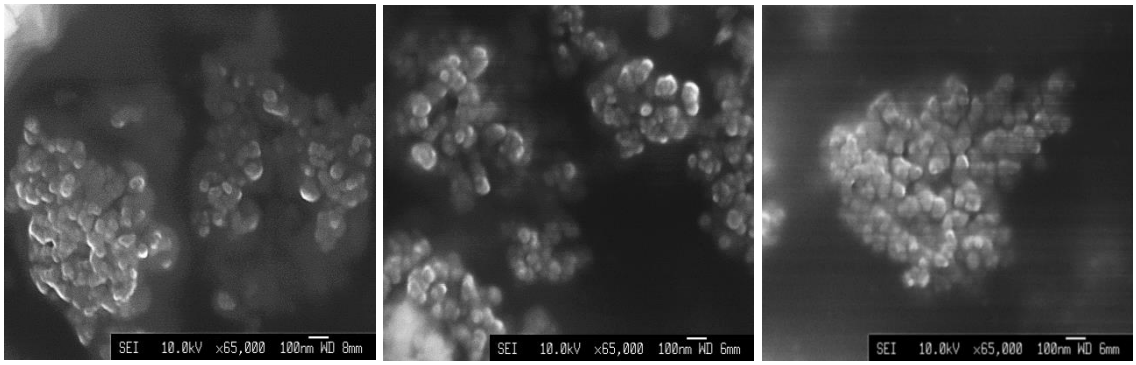
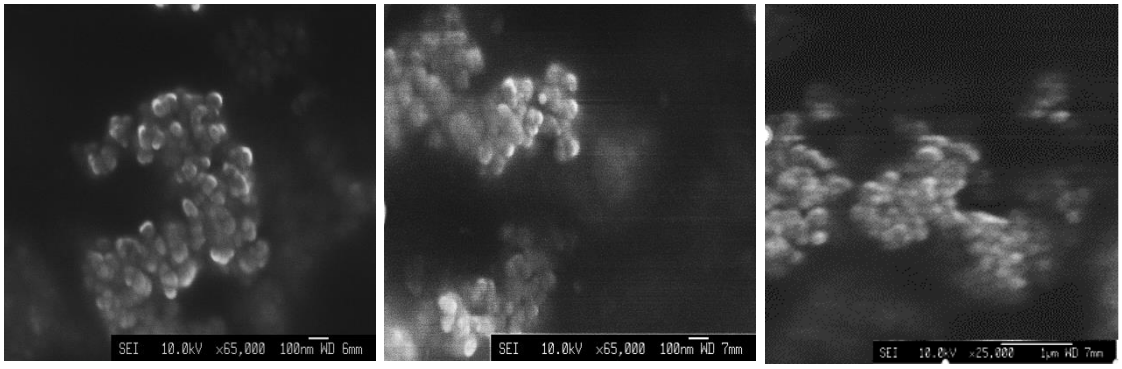


Fig. 5b: Tauc plot of the Va pH 8, 10 and 12 ZnO nanoparticles showing their determined optical energy band gap values



a **b** **c**

Fig.6: SEM images of (a) pH 8 (b) pH 10, (c) pH 12 Og zinc oxide nanoparticles



a **b** **c**

Fig.7: SEM images of (a) pH 8 (b) pH 10, (c) pH 12 Va zinc oxide nanoparticles

DISCUSSION

The observed peak absorbance for the Og and Va zinc oxide nanoparticles respectively are less than the value for bulk ZnO given as 380 nm (Liqiao *et al*., 2011) and show a blue shift in excitonic absorption which could maintain a quantum confinement effect (Koch *et al*., 1985). For the Og ZnO nanoparticles, a low synthesis pH produced nanoparticles of low optical energy band gap while for the Va ZnO nanoparticles, the reverse was the case. These results agree with the range of values given elsewhere by Pholnak (*et al*. 2016). The Og zinc oxide nanoparticles synthesized at a raised synthesis pH were larger and had high E_g values while the Va ZnO nanoparticles made from a high synthesis pH were large though with lower optical energy bandgap values. This trend agrees with reports by some authors (Alias *et al*., 2010; Debanath *et al*., 2013, and Pholnak *et al*., 2016) and shows that pH adjustment is key to nanoparticles size control. The spherical ZnO nanoparticles which were in clusters according to Ikono (Ikono *et al*., 2012) indicate that the ZnO nanoparticles were pure. The increase in size of the ZnO nanoparticles with pH of

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synthesis agrees with reports by Koao (Koao *et al*., 2015) and shows that probably some ZnOH got dissolved when the synthesis pH was raised. Phenols, amines, and flavonoids are some of the biomolecules present in the plant leaf extracts used. The PL spectra show UV emission peaks which being less than 400 nm, are ascribed to a recombination of free excitons (Chithra *et al*., 2015) and show that the nanoparticles exhibit band edge photoluminescence. While the UV emission peaks are due to singly ionized oxygen vacancies, the green emission peaks above 500 nm could be due to some minor defect states in the nanoparticles.

Table 1: Showing the sizes and optical energy band gap $(E_g)$of Og and Va zinc oxide nanoparticles (ZnO Nps) synthesized at various pH values.

pH of synthesis of ZnO Nps	Og ZnO nanoparticles		Va ZnO nanoparticles	
	Size of Nps (nm)	E_g of Nps (eV)	Size of Nps (nm)	E_g of Nps (eV)
pH 8	38 ± 0.4	3.22 ± 0.042	44 ± 0.8	3.290 ± 0.045
pH 10	43 ± 0.6	3.240 ± 0.056	57 ± 0.5	3.240 ± 0.003
pH 12	63 ± 0.8	3.242 ± 0.063	62 ± 0.6	3.200 ± 0.038

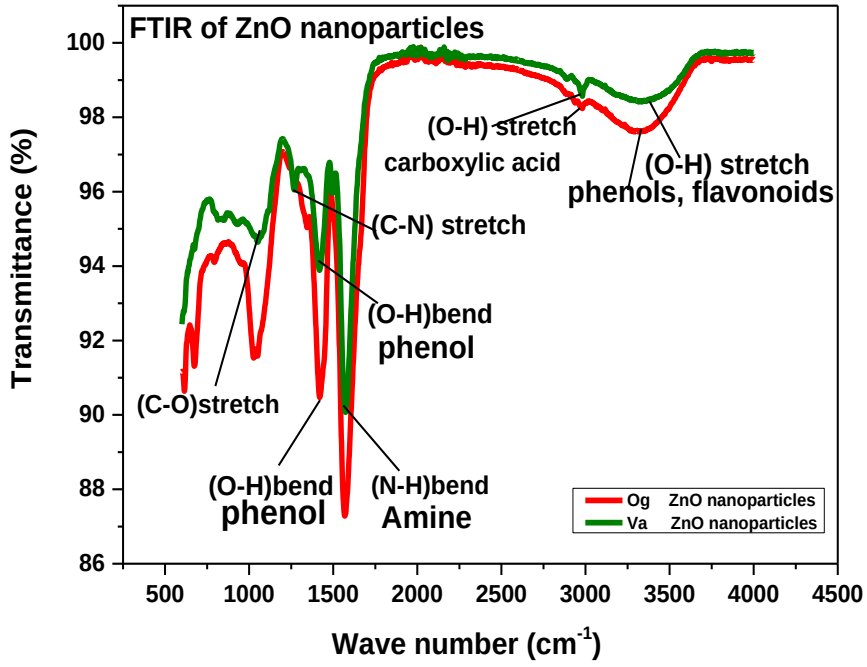

Fig.8: FTIR scan results of the ZnO nanoparticles

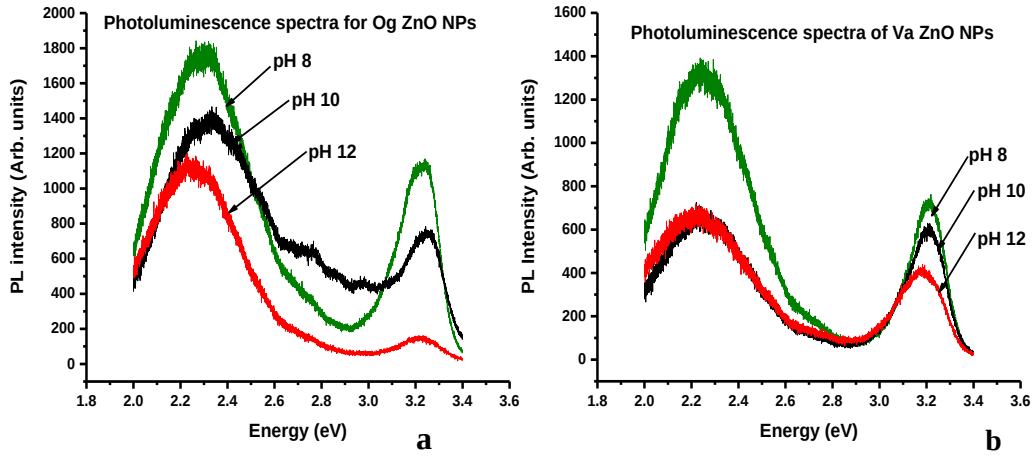

Fig.9: (a) Photoluminescence spectra for (a) Og and (b) Va ZnO nanoparticles

CONCLUSION

Zinc oxide nanoparticles were synthesized in the presence of *Ocimum gratissimum* and *Vernonia amygdalina* plant leaf extracts at three different pH levels. The nanoparticles which were spherical in shape and in clusters had absorbance spectra energy bandgap and sizes which were pH and leaf extract dependent though with opposite pH versus size dependence. While the Og nanoparticles had sizes and energy band gap values which increased with pH of synthesis, the Va nanoparticles though bigger at higher synthesis pH had optical energy bandgap values which decreased with higher pH of synthesis indicating that pH of synthesis is key to the nanoparticles size control. The photoluminescence spectral intensities were pH dependent and increased for lower pH synthesis and the nanoparticles were found to exhibit band edge photoluminescence. It is clear from the obtained results that the two plant leaf extracts used for the nanoparticles synthesis affected the structure of the produced nanoparticles and this will add to their already known food and medicinal values.

Potential Conflicts of Interest

"The authors declare that there is no conflict of interest in this work and article"

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