SYNTHESIS OF FEO AND ZNO NANOPARTICLES USING FLOWER EXTRACT OF *Hibiscus rosa sinesis*

Mridusmita Gogoi, Gautam Deori, Probin Phanjom*
Department of Applied Biology, School of Biological Sciences
University of Science & Technology,Meghalaya, Baridua-793101, India
*Author for Correspondence: phanjon@gmail.com

ABSTRACT:

Nanoparticle research is a new dimension of research work in the area of physical and biological science. Nanoparticles have been found to be of utmost importance in the area of pharmacological research, food industry, electronics industry etc. In the present work investigation were carried out using the aqueous extract obtained from the flower Hibiscus rosa sinensis for obtaining nanoparticles from Zinc sulphate ($ZnSO_4$), ferrous sulphate ($FeS0_4$). The nanoparticles obtained were characterized using different biophysical techniques.

Keyword: *Hibiscus rosa sinensis,* nanoparticle, Zinc sulphate, ferrous sulphate, TEM

INTRODUCTION:

Nanoparticles are ultrafine particles with their size ranging from 1-100nm. Nanoparticles (NP) have attracted considerable attraction due to their unusual and fascinating properties, with various applications, over their bulk counterparts (Daniel and Astruc, 2004; Kato, 2011). Although chemical and physical methods may successfully produce pure, well-defined nanoparticles but these are quite expensive and potentially dangerous to the environment. Nanometer-sized particles offer novel structural, optical and electronic properties that are not attainable with individual molecules or bulk solids (Siromani et al., 2011). Nanoparticles interaction with biological materials and established a series of nanoparticle/ biological interfaces that depend on colloidal forces as well as dynamic biophysicochemical interactions. This interactions lead to the formation of new nanomaterial with control size shape, surface chemistry, roughness and surface coatings (Nel *et al.,* 2009). Nanoparticles present a higher surface area to volume ratio with decrease in size, distribution and morphology of the particles (Awwad *et al*., 2012).Since the synthesis of nanoparticles using physical and chemical methods involves use of high pressure, temperature and toxic chemicals, synthesis using biological methods has been prove to be more economic and environment friendly, since the use of harmful and toxic chemical are not involved. Prelimenary investigation on biological synthesis of iron and zinc nanoparticles using flower extract of *Hibiscus rosa sinesis* was carried out in the present study.

MATERIALS AND METHODS

Materials

Zinc sulphate ($ZnSO_4$), ferrous sulphate ($FeS0_4$) and glassware was supplied from laboratory. All glassware were washed with sterile distilled water and dried in hot air oven before use. The flower of *Hibiscus rosa sinensis* was collected from USTM campus for the study.

Biosynthesis of Nanoparticles using flower extract

20 g of flower *Hibiscus rosa sinensis* were thoroughly washed with running water to remove dust particles on the flower and then with distilled water. They were dried using blotting paper, cut into fine pieces using a sterilized blade. The flower materials were then boiled in 100 ml distilled water at 80°C for 15 mins. The flower extract was cooled to room temperature and filtered using Whatman No. 1 filter paper.

Preparation of zinc oxide nanoparticles

For the synthesis of zinc oxide nanoparticles, 5ml of *Hibiscus rosa sinensis* flower extract was taken and mixed with 25ml of 1mM zinc sulphate solution. The sample was incubated in the dark for 24hrs. The colour of the sample changes from pink to light grey.

Preparation of iron oxide nanoparticles

For the synthesis of iron oxide nanoparticles, 5ml of *Hibiscus rosa sinensis* flower extract was taken and mixed with 35ml of 1mM iron sulphate solution. The sample was incubated in the dark for 24hrs. The colour of the sample changes from pink to dark grey.

Characterization of zinc and ferrous oxide Nanoparticles

a) UV-Visible spectroscopy
b) Transmission electron microscopy (TEM)
c) Energy dispersive spectroscopy (EDS)
d) Fourier transform infrared spectroscopy (FTIR)

UV-Visible Spectroscopy-

The reduction of zinc oxide ions (Zn^+) and iron oxide (Fe^+) to zinc and iron nanoparticles was spectrometerically identified by UV-Vis spectrophotometer (PD-303 UV) at different wavelength (200-700nm). The color change in the reaction mixture was recorded spectrometerically.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) of the sample was done using PHILIPS- CM 200 instrument operated at an accelerating voltage of 200 kV with resolution of 5 nm and 200 nm. A drop of solution was placed on a carbon coated copper grid and later exposed to infrared light (45 minutes) for solvent to be evaporated.

Energy Dispersive Spectroscopy (EDS)

The EDX analysis was carried out using *JEOL JSM 7600F* which can provide information about the composition and the electronic structure of nanoparticles and presence of metals in the sample.

Fourier Transform Infrared Spectroscopy (FTIR)

The characterization of functional groups on the surface of zinc oxide and iron oxide nanoparticles synthesized using flower extracts were investigated by FT-IR (IRTRACER-100 Model). The spectrum was scanned in the range of 370-4000 cm^{-1}.

Phytochemical Tests-

Flavonoid test-

Few drops of dilute sodium hydroxide (NaOH) solution were added to 2ml of test solution in a test tube. An intense yellow color appeared in the test tube. It becomes colorless with addition of few drops of dilute amino acid, which indicates the presence of flavonoids.

Saponin test-

2ml of test solution are diluted with 2 ml distilled water and shake. Formation of foamy and lather indicates presence of saponins.

Steroid test-

0.5 ml of flower extract was dissolved in 3ml of chloroform ($CHCl_3$) and filtered. When few drops of conc. H_2SO_4 were added to the filtrate, appearance of reddish brown color ring showed the positive presence for steroids.

Alkaloid test-

2ml of test solution are taken with 2N HCl. Aqueous layer formed was decanted and then 1 or few drops of Mayer's reagent was added. Formation of white precipitates indicated the presence of alkaloids.

Mayer's reagent- Mercuric chloride(1.36g) and potassium iodide (5g) in 100ml of distilled water was taken and the solution was made.

Tannin test-

2ml of the aqueous extract was added to 2ml of water; 1 to 2 drops of diluted ferric chloride solution were added. Development of brownish- green or blue black color indicates presence of tannins.

Terpenoids test-

Salkowski test was applied for the detection of terpenoids. The the extract was mixed with 2ml of $CHCl_3$ (Chloroform), followed by addition of 3ml of conc. H_2SO_4. A reddish brown color at the interface confirmed the presence of terpenoids.

RESULTS AND DISCUSSIONS

UV- Visible spectroscopy analysis

UV-Visible spectrophotometer analysis was performed for confirming the formation of Zinc oxide and ferrous oxide nanoparticles. For the synthesis of zinc oxide nanoparticles, 5ml of *Hibiscus rosa-sinensis* flower extract was taken and mixed with 25ml of 1mM zinc sulphate solution and incubated in dark condition for 24 hrs. The colour changes from pink to light grey as shown in the Fig.1.The absorbance peak of surface Plasmon resonance was observed at around 280 nm as shown in the Fig.2. For the synthesis of iron oxide nanoparticles, 5ml of *Hibiscus rosa-sinensis* flower extract was taken and mixed with 35ml of 1mM iron sulphate solution and incubated in dark condition for 24 hrs. The colour changes from pink to dark grey as shown in the Fig.3. The absorbance peak of surface Plasmon resonance was observed at around 275nm as shown in the Fig 4. The colour changes arise from the excitation of the surface Plasmon resonance (SPR) phenomenon (Valentine et al). The optical absorption spectrum of metal nanoparticles depends on the particle size, shape, state of aggregation and the surrounding dielectric medium.

Fig 1- Digital photographs of (A) Flower extract, (B)1mM $ZnSO_4$, (C) ZnO nanoparticles with *H. rosa-*

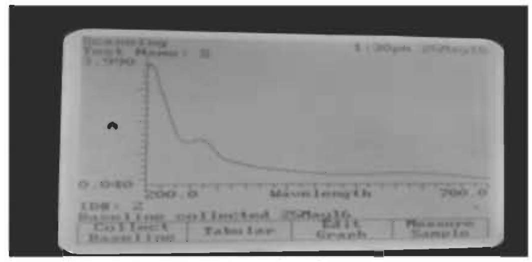

Fig 2- UV-Visible absorption peak of *H. rosa-sinensis* synthesized Zn O nanoparticles at 280 nm

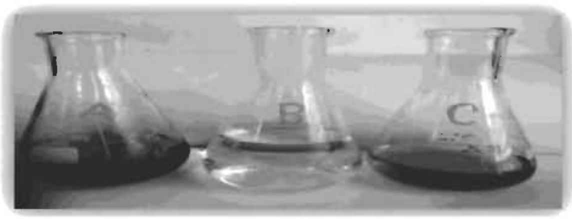

Fig 3- Digital photographs of (A)Flower extract, (B) 1 mM $FeSO_4$, (C) FeO nanoparticles with *H. rosa-sinensis* after 24 hrs of incubation.

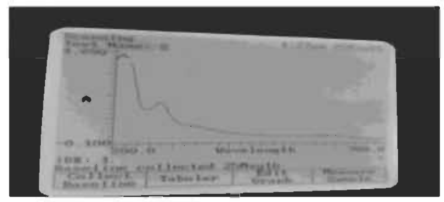

Fig 4- UV-Visible absorption peak of *H. rosa-sinensis* synthesized Fe O nanoparticles at 275 nm

Transmission Electron Microscopy (TEM) analysis

Transmission electron microscopy (TEM) of the sample was done using PHILIPS- CM 200 instrument operated at an accelerating voltage of 200 kV with resolution of 5 nm and 200 nm. A drop of solution was placed on a carbon coated copper grid and later exposed to infrared light (45 minutes) for solvent to be evaporated Transmission electron microscopy (TEM) has provided further insight in the morphology and size details of the zinc and iron nanoparticles. TEM analysis indicates that the particles size of zinc oxide nanoparticles is found to be 4-7nm. Rod shaped iron oxide nanoparticles having length of about 100-200nm with breadth 5nm width was observed as shown in Fig.5 and Fig. 6 respectively.

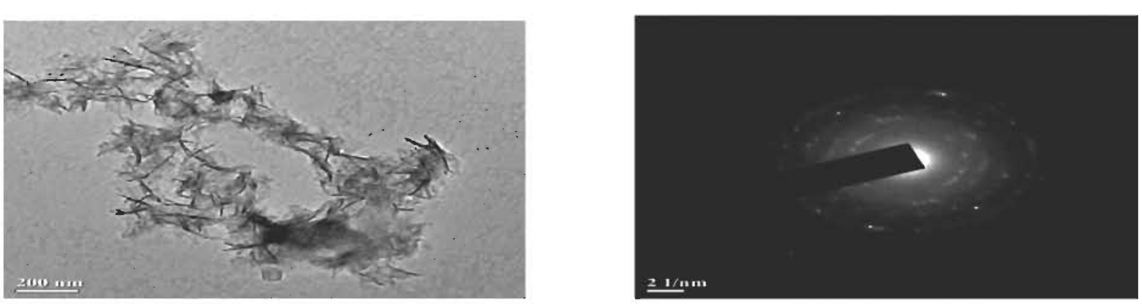

Fig 5- TEM micrograph of rod shape ZnO NPs of 100-200nm synthesized using *H. rosa-sinensis* extract

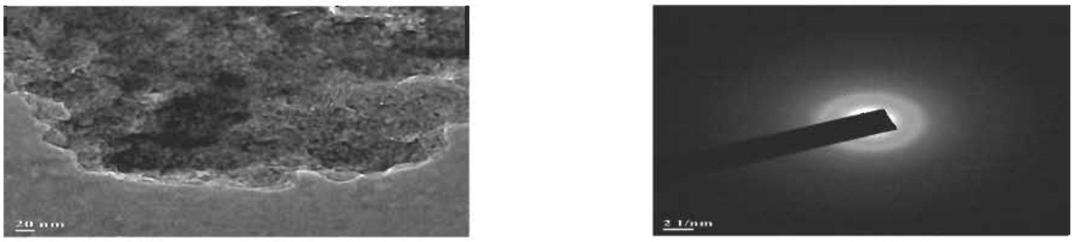

Fig 6- TEM micrograph of spherical FeO NPs of 4-7nm synthesized using *H. rosa sinensis* extract

Energy Dispersive Spectroscopy (EDS) analysis

The EDX analysis was carried out using *JEOL JSM 7600F* which can provide information about the composition and the electronic structure of nanoparticles and presence of metals in the sample. To find out the purity of the metal particles synthesized, EDX spectrum was obtained which showed along with zinc and iron nanoparticles as shown in the Fig.7 and Fig.8 respectively. Thus, the aqueous extract of flowers of *Hibiscus rosa-sinensis* found to be a potential source bio-reductant to reduce metal salts into their nanoparticles.

Fourier transform infrared spectroscopy (FTIR) analysis

The characterization of functional groups on the surface of zinc oxide and iron oxide nanoparticles synthesized using flower extracts were investigated by FT-IR (IRTRACER-100 Model). The spectrum was scanned in the range of 370-4000 cm^{-1}. FTIR studies confirmed the biofabrication of the ZnO nanoparticles and FeO nanoparticles by the action of different phytochemicals with its different functional groups present in the extract solution as shown in the Fig.9 and Fig.10.

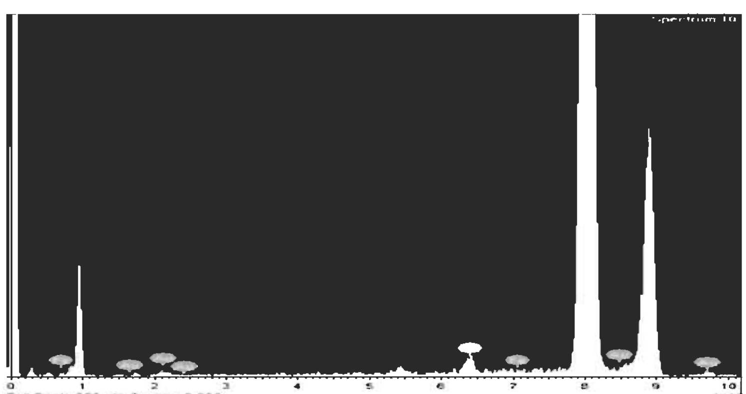

Fig 7- EDX spectra of Fe NPs synthesized using *H. rosa-sinensis* extract

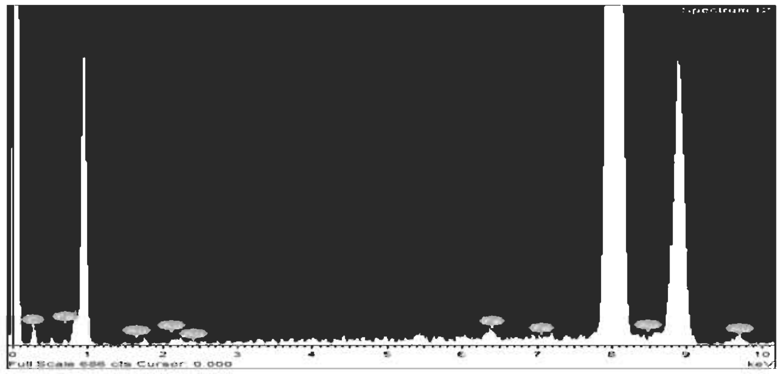

Fig 8- EDX spectra of ZnO NPs synthesized using *H. rosa-sinensis* flower extract

Phytochemical Analysis

The percentage yields of extracts and the phytochemical constituents of *Hibiscus rosa sinensis* were studied. Preliminary phytochemical analysis revealed the presence of flavonoids, steroids, tannins, and terpenoids, while the test for alkaloids and saponins were found to be negative as shown in the Table 1.

Table: 1 Qualitative phytochemical screening of *H. rosa-sinensis* flower extract (+: positive; - : negative)

Chemical constituent	**Presence(+) / Absence(-)**
Flavonoids	+
Saponin	-
Steroids	+
Alkaloids	-
Tannin	+
Terpenoids	+

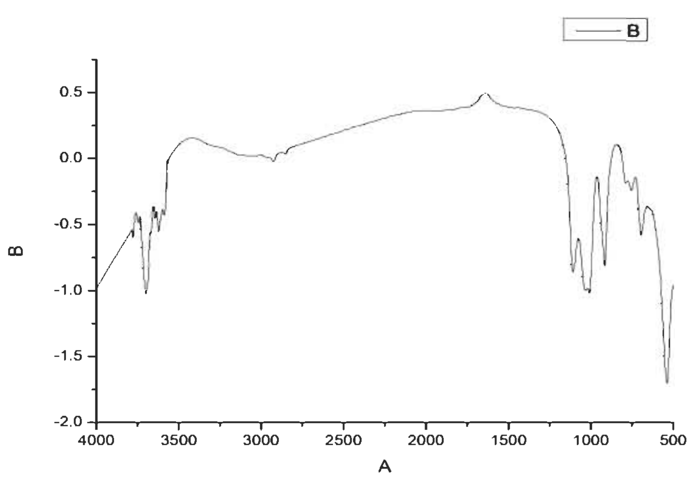

Fig 9- FTIR Spectra for ZnO NPs synthesized using *H. rosa-sinensis* flower extract

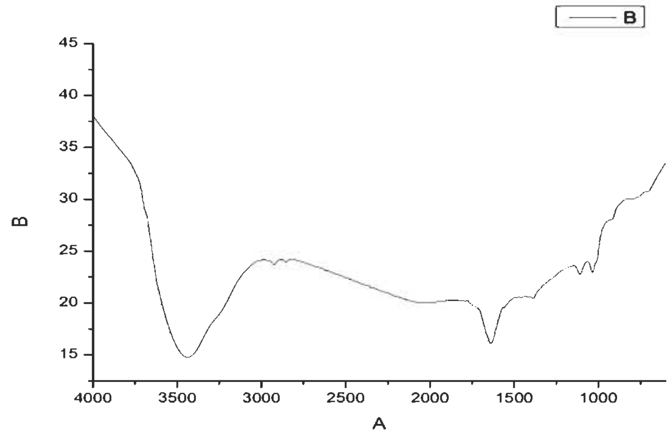

Fig 10 - FTIR Spectra for FeO NPs synthesized using *H. rosa-sinensis* flower extract

CONCLUSION

Synthesis of Zinc oxide nanoparticles and Iron oxide nanoparticles using *Hibiscus rosa sinensis* flower extract was demonstrated in the present study. Zinc oxide nanoparticles with an average size of nm and iron oxide nanoparticles with an average size of nm were synthesized. The synthesized Zn NPs and Fe nanoparticles were characterized by UV-Visible spectroscopy, TEM, EDS and FTIR measurements. The flower extract could be used for the synthesis of two different nanoparticles; it could be exploitation as an easy source for nanoparticles productions. Moreover, it provides as an alternatives to chemical method, since it is cheap, pollutant free and eco-friendly.

ACKNOWLEDGEMENT

The authors would like to thank SAIF,NEHU for TEM and EDX facilities, IASST,Guwahati for FTIR analysis. The authors would also like to thank the Department of Applied Biology,University of Science and Technology,Meghalaya for providing all the research facilities to carry out the work.

REFERENCES

Awwad AM, Salem NM, Abdeen A (2012): *Nanoscience and Nanotechnology* 2(6):164-170.

Daniel MC and Astruc D (2004): *Chem Rev.*104: 293-346

Kato H (2011): In vitro assays: Tracking nanoparticles inside cells. *Nat Nanotechnol* 6;139-140

Mahdavi M, Namvar F, Ahmad MB, Mohamad R (2013): *Molecules* 18(5):5954-5964

Makarov VV, Makarova SS, Love AJ (2014): Langmuir, ACS Publication

Nel AE, Maidler L, Velegol D, Xia T, Hoek EMV, Somasundaram PL, Klaessig F, Castranova V, Thompson M (2009): *Natural Material* 8: 543-548

Siromani A, Daniel K, Singhal G, Kunalkasaraiya RB, Sharma AR, Singh RP (2011): *Journal of Nanoparticle Research* 13: 2981-2988