SYNTHESIS OF FLUORESCENT METAL OXIDE NANOPARTILES BY POLYMER AND ITS BIOLOGICAL ACTIVITY

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New synthetic protocols are extensively being explored for the synthesis of mono disperse metal NCs, because of their promising applications in the field of optoelectronics, bio-imaging, biological sensing, and catalysis. Due to the increasing popularity of green methods, different works had been done to synthesize metal oxide NPs using different sources. In this present study natural gum contains a water soluble polysaccharide as L-arabinose and D-galactose was used to evaluate to synthesis metal oxide nanoparticle. The objectives are

KEYWORDS: *Moringa oleifera,* L-arabinose,

INTRODUCTION OF NANOPARTICLES

Nanotechnology is a known field of research since last century. Nanoparticles (NPs) are wide class of materials that include particulate substances, which have one dimension less than 100 nm at least. They are ultrafine unit with dimensions measured in nanometres (nm; 1 nm = 10^{-9} metre). NPs are not simple molecules composed of three layers such as surface layer, shell layer, and the core, which is essentially the central portion of the NP and usually refers the NP itself. NPs are broadly divided into various categories depending on their morphology, size and Chemical properties. Based on physical and chemical characteristics, some of the well- known classes of NPs are given as Carbon-based NPs, Metal NPs, Ceramics NPs, Semiconductor NPs, Polymeric NPs, Lipid-based NPs[1]

Different types of nanoparticles

Nanoparticles can be classified into different types according to the size, morphology, physical and chemical properties. Some of them are carbon-based nanoparticles, ceramic

nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymeric nanoparticles and lipid-based nanoparticles.

MATRIALS AND METHDOS

Materials

All graded analytical chemicals and reagents used in this study were purchased from SRL and Himedia, Mumbai, India.

Reducing agent

Gum was collected from an injured site of tree. The fresh Moringaoleifera gum was collected from the garden of perambalur, Dhanalakshmi agriculture college campus. Solubility and Ph were determined at different concentration(1-5%)

Preparation of Gum Extract

Colloidal extract of gum freshly collected gum of *Moringaoleifera* which were taken and dried for 5 days. From that 1 g of weighed gum were solubilized in 100 ml distilled water under magnetic stirring for 3 h along with 50° C. After stirring it, the gum was precipitated at the proportion of 1:1 v/v was filtered through Whatman No 1 filter paper and used for further experimental studies.[42]

Estimation of sugar by DNS method

Reducing sugars have the property to reduce many of the reagents. One such reagent is 3,5-dinitrosalicylic acid (DNS). 3,5-DNS in alkaline solution is reduced to 3 amino 5 nitro salicylic acid. 5% of bagasses extract was taken and used as sample. 3 mL of standard sugar with distilled water concentrations ranging from 0 -10 mg/ml was prepared.1 mL DNS reagent to all the test tubes and test tubes kept in a boiling water bath for 5 minute (table 1). the tubes allowed cool to room temperature and readed extinction at 540 mm against the blank and ploted on graph.

Table 1.preparation of standard for DNS method

TEST TUBE	**GIUCOSE**	**H_2O**	**DNS**
Blank	Water	2	1ml
Standard 1	0.2	1.8	1
Standard 2	0.4	1.6	1
Standard 3	0.6	1.4	1
Standard 4	0.8	1.2	1
Standard 5	1.0	1.0	1
Sample	0.2	1.8	1

Synthesis of metal nanoparticle by KOH[43].

Briefly, 100 mL of 3Mm $ZnSO_4$, $CuSo_4$ and $FeSO_4$ was Prepared and heated under 60 °C with constant stirring. 1N KOH was also dissolved separately in water . After both solutions have dissolved completely, drop wise, KOH was added into metal solutions independently under 60 °C with vigorous stirring. The mixture solution was left for 3 hours until the reaction was completed. A white precipitate (ZnO) was formed and collected by centrifugation at 4000 rpm for 10 minutes, washed with acetone twice and ultrapure water once to remove all the impurities. The obtained product was then dried at room temperature and ground to form powder.

Standardization of reduction

1 % Gum extract was added to 3mM $CuSO_4$ at different ratio (1:9, 2:8. 3:7. 4:6, 5:5) in a final volume of 10 ml. Changes of colour and precipitation versus time were noted. Similar reaction mixture was prepared by using 1N KOH alone

Synthesis of polymer metal Nanoparticles

Gum extract (10 ml) was added to 90 ml of 3mM aqueous solution of $CuSo_4$ Zn $So_4$and $FeSo_4$ kept under constant shaking in dark condition. The colour change and formation of precipitation was observed. The nanoparticles were collected by centrifugation at 10,000 rpm for 30 min.

Synthesis of polymer-KOH mediated metal Nanoparticles

Gum extract (10 ml) was added to 90 ml of 3mM aqueous metal solution and kept under constant shaking for 1 h . after 1ml of 1N KOH were added drop wise under constant stirrering. The colour change and formation of precipitation was observed. The nanoparticles were collected by centrifiuagtion at 15,000 rpm and kept in oven under 200° C for 2 h to burn organic impurities.

Characterization Studies

Formation of metal nano particles were characterized by UV-vis spectrophotometer. 5 ml of reaction mixture was taken and subjected to UV- visible spectrum recorded between 200-700 nm.

Detection of fluorescence

Fluorescent ability of metal NP was characterized under trans illumination. Metal nanoparticle was placed on whatmann filter paper and irradiation was carried out using a spectroline handheld lamp consisting of a 4W UV-A lamp by placing it 5 cm above an uncovered 25 mL glass beaker filled with 10 mL of the above prepared solution. The irradiation was carried out for 5 min.

Antibacterial activity

Sterile Mueller Hinton agar plat was prepared and the test pathogen Escherichia coli and *Staphylococcus aureus* were swabbed on agar plates. 100µL of Pure metal nanoparticles (1mg/ml) were loaded on sterile disc and placed over the surface of agar plate. Plates

were incubated for 24h and zone of inhibition was recorded and compared with positive control.

Blood clotting time measurement

In vitro Clotting time measurement was carried out using a modified method s reported by Osoniyi and Onajobi. Clotting tubes containing 0.1ml each of crude extract and ZnO nanoparticle suspended in Phosphate Buffered Saline (PBS) and Acid citrate dextrose (anticoagulant) were incubated in a water bath at 37 °C. Freshly drawn blood (0.5ml) was carefully transferred by running it down the side of the tube into the contents of each of the incubated tubes, while simultaneously starting a stopwatch. At 30s interval, the tubes were gently slanted to an angle of 45° to check for blood clot formation. The time for the first observation of clot was recorded, and the slanting at interval continued until the tubes could be inverted without blood flowing. The stopwatch was stopped instantly, and the time was recorded as the final clotting time

SEM analysis

The biosynthesized bioactive metal nanoparticles size were analyzed using SEM EDAX techniques .SEM images were recorded using a JEOL JSM 6390 system. An energy dispersive spectroscopy (JED 2300, JEOL) analysis was applied to the prepared samples for qualitative elemental analysis.

RESULT AND DISCUSSION

Reducing agent extraction

The collected gum of *M. oleifera* was found to be hydrophilic in nature and thus can be used for preparation of emulsion. Highly soluble in water up to 3%, sparingly soluble at 4% and least soluble at 5%. The gum material highly soluble in ethanol and acetone up to 2% and become colloid at 3%. It is sparingly soluble in water forming viscous solution at 4% and insoluble in ethanol and acetone at 3%. Preliminary experiments performed for

evaluation of total sugar of *M. oleifera* gave an idea of the concentrations of gum to be used in the study. Emulsions were prepared with *M. oleifera* gum with 1 per cent w/v (plate 1 a). The pH of the aqueous extract was determined using a digital pH meter. The effect of pH was observed with emulsion prepared at different concentration and found to be increased from 6.8 to 7.8. The total polysaccharide was estimated as 3.8 mg/g (fig1).Polysaccharide gums derived from plants have been reported to be useful as suspending agents, emulsifying agents, binders, disintegrants etc., in different pharmaceutical formulations [44]

Table 1: solubility and pH of gum material used in study

Sample Percentage	pH	Total sugar mg/g	Water	Ethanol	Acetone
1	6.8	3.8	✓	✓	✓
2	7.4		✓	✓	✓
3	7.5		✓	☒	☒
4	7.6		✓	☒	☒
5	7.8		☒	☒	☒

Reduction of metal nanoparticle

Plate 2 reveals that the polymeric gum substance found to be effective reducing agent at % compare to KOH. More precipitation was recorded in gum treated Zn than KOH reduction. The mixture contained 90 ml 0f 3mM concentrations of metal solutions and 10 ml of gum (with and without KOH mediated nanoparticles were directly observed by changes of colour (plate 3) synthesized, purified and dried to obtain different metal nanoparticles (plate 4). The effect of KOH and reduction influence the yield of metal

nanoparticle productivity was recorded and given in table 2. Reduction of metal by *M.olefera* gum alone shows poor yield of metal nanoparticle precipitation ranges between 0.06-0.008 mg. It was noted addition of KOH enhance formation of precipitation. The Zinc reduction by KOH was cloudy white and the yield of precipitate was 0.24 mg. reduction of $CuSO_4$ gave greenish blue precipitate and the yield is 0.32 mg. the reduction of ferric sulphate changes colour from light green to blue and the yiel of precipitate is 0.4 mg.

Table 2. Effect of KOH on reduction.

3 mM Metal solution	**Initial Colour**	**KOH Added**	**Yield**	***M.olefera* gum added**	**Yield mg**
$ZnSo_4$	Colourless	White Colour	0.24	Colourless	0.08
$CuSo_4$	Light blue	Greenish Blue Precipitate	0.32	Light blue	0.06
$FeSo_4$	Light green	Dark blue	0.4	Light green	0.08

Characterization of ZnO nanoparticle

UV-Vis spectroscopy was also performed to further confirm the formation of metal NPs. The absorption spectrum of synthesized ZnO NPs by gum was shown in Figure 2b and KOH alone in Fig 2a. The absorption peak was observed at 357 nm, which attribute to the intrinsic band-gap of Zn-O absorption found on Gum mediated reduction. Similar result of absorption band that represent ZnO NPs was also obtained from previous

research in which the range of absorption band were from 355 to 380 nm as summarized in Table 3. These supporting data confirm the presence of ZnO NPs as the absorption band obtained are similar. Wang et al(45). also obtained similar findings which deduced that the obtained peak showed a better UV absorption for ZnO NP. Furthermore, the absorption peak of ZnO NPs also confirmed the properties of ZnO NPs, which is known for UV protections in sunscreens products.

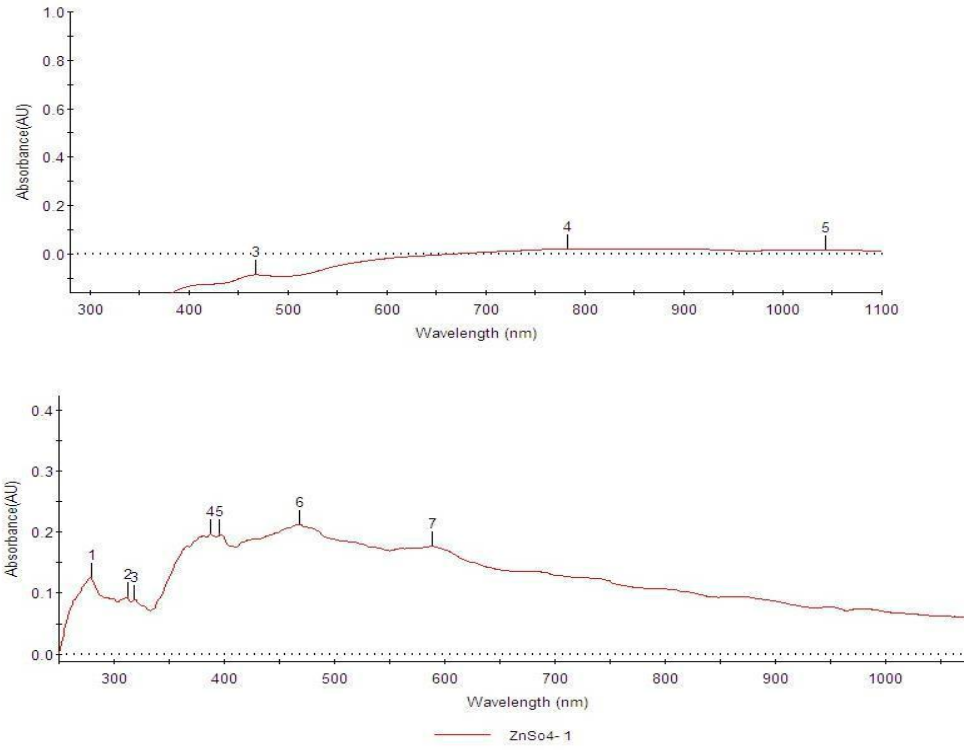

Figure 2. UV spectrum of $ZnSO_4$ reduction

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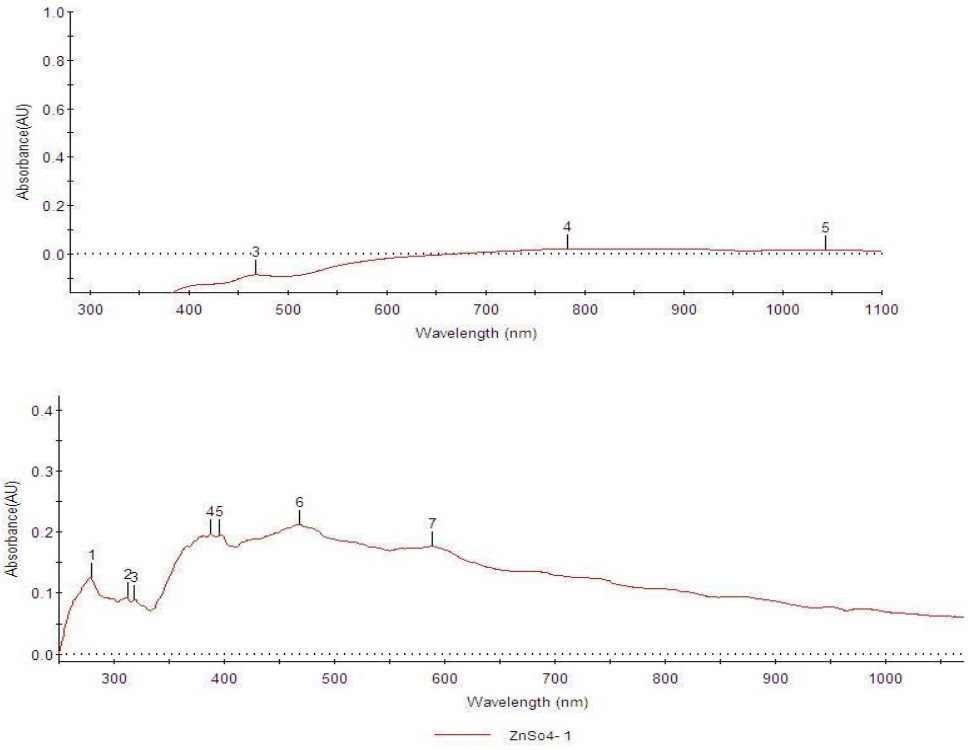

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Characterization of FeO nanoparticle

UV Visible spectrum (figure 4) of the solution involved in this biosynthesis of iron oxide nanoparticles has its characteristic absorption peak at 315 nm wavelength corresponding to ferric resonance. On the other hand, the $FeCl_3$.with KOH solution has its characteristic absorption peak at 257 nm wavelength which is disappeared by the addition of polymeric compound. After experiencing biosynthesis, UV Visible spectrum of sample (FE+GUM) showed 6 different peaks

indicates presences of other capping agents, which is the mixture of extract solution and the precursor, have characteristic absorption different peak(table 5). Results show that all absorption curves exhibit an intense absorption in the range of 500– 700 nm wavelength due to polymeric compounds. absorbance spectral ranges between 250 and 300 nm is corresponding to iron oxide nanoparticles was recorded in this study closer to the already reported absorbance near 250 nm maxima for magnetic nanoparticles by Samrot et al.(47).

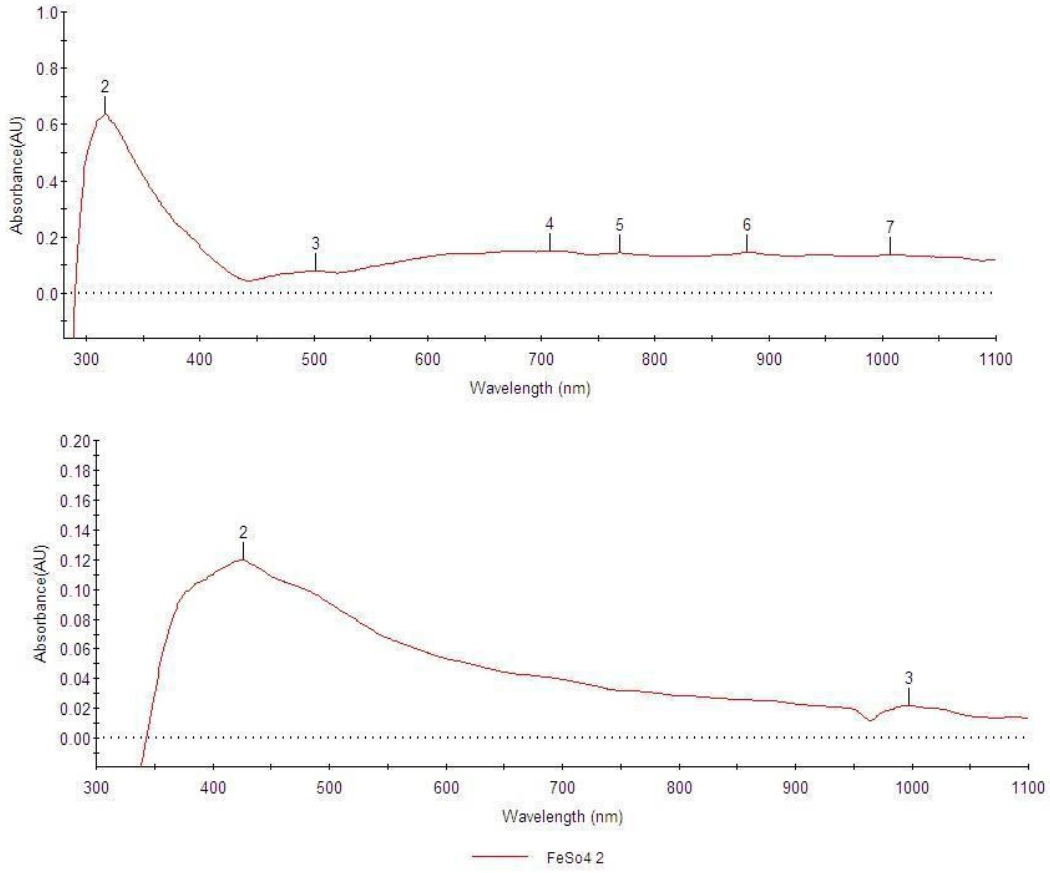

Figure 4. UV spectrum of $FeCl_3$ reduction

CONCLUSION

Fluorescent metal nanoparticles (F-NPs) have received great attention due to their attractive features, such as water solubility, wide availability, ease of fictionalization and

good biocompatibility, and considerable efforts have been devoted to the preparation and

applications. In this present study natural gum contains a water soluble polysaccharide as L-arabinose and D-galactose was used to evaluate to synthesis metal oxide nanoparticle such and its characterization studies with Copper, Zinc and Ferric into oxides.

The characterisation studies concludes that the reduction of Ferric, Zinc and Copper is greatly enhanced by KOH in addition to polymeric reducing agent. M.olifera is found to be

good capping agent and also an Eco-friendly and Cost-effective approach in biosynthesis of

stable metal oxide nanoparticles. The produced metal oxides are characterized by UV and

confirmed the formation of nanoparticles .Among the three Cuo and ZnO were Fluorescent

and anticoagulant in nature. CuO act as promising antibacterial. Highly pure CuO NPs was

prepared by a simple precipitation method. EDAX spectrum revealed that CuO NPs were

monoclinic crystals and pure.

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