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Research Article

Green Synthesis of Iron Oxide nanoparticles using Neem leaf (Azadirachta indica) extract & itâ€™s Characterisation via UV-Vis Spectroscopy

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ABSTRACT

Nanotechnology has come into limelight few years back and is following the same trend by the virtue of its opportune and multifarious diligence that it shows in the field of Biomedical (Biomarker Mapping, Targeted Therapy, Detection and Diagnosis etc.) etc. Azadirachta indica aka Neem Plant is highly rich in antioxidant and phytochemical properties which ultimately fabricate it to be a good way for synthesising remarkably stable Iron Oxide nanoparticles (Fe3O4-NP's). This review approaches to the procedure and characterisation via UV-Vis Spectrometer of Fe3O4-NP's. The nanoparticles were chosen for research because of its more antimicrobial and antioxidant properties comparatively to the normal plant extract. At last the comparison can be made on the basis of the results which come after the synthesis using UV-Vis Spectroscopy.

Keywords: Nanoparticles; UV-Vis Spectroscopy; Centrifugation

INTRODUCTION

"Nano" comes from a word in Greek which sufficiently stands for "Dwarf". Thus the study of Nano means extremely minute particles is phoned as *NANOTECHNOLOGY*. In past few decades the use of this science has been advancing and coming up with all the more new techniques day by day. In simpler words, the science which seems well related to the study, of structures, systems, devices etc. by manoeuvring a particular atom or molecule at such an ultrafine stage which is equal to 1 Billionth of a meter (10^{-9}m) [1].

[2] The paramount vindication of such technology is that it can upgrade the productiveness of consumption of energy, useful in keeping the environment clean and most importantly can serve as the cure of hundreds of health related issues. In near future it is expected that by the use of this technology objects can harvest energy from their environment as well.

The particles of the size too ultrafine to notice by naked eye (1nm=10^{-9}m) are called Nanoparticles. Nanotechnology is directly linked to the synthesis of these particles; determination of their shapes and size, chemical and physical properties, characteristics etc. There are ample of existing application of nanoparticles as discussed above also [3]. The most common and very well used is in the [4] Targeted Therapy for Cancer treatment. Cancer cells, as are well known that they grow quite unusually or abnormally inside the body of an organism and hence it becomes all the more adverse to either completely kill that particular growth and even the cells as well. But if the functioning needs to be stopped for those particular cells in internally infected region nanoparticles play a vital role because of their ultrafine surface area that they take. They can coherently be injected in the affected part and accordingly they start working inside the body. This is majorly the most important application of Nanoparticles.

Playing a vital role in diverse fields of Chemistry, physics and material science as well [5], Metal Oxides have recently come in the limelight zone of many researchers and scientists. Metal have huge capacity to form a varied classes of oxides compounds. And in turn these formed compounds exhibit ample of structural geometries including electronic structure which have semiconducting, conducting, metallic properties in whole [6]. Its application part is also so diverse in the fields of technology which can be listed as:

- Prevarication of microelectronic circuits, censors
- Fabrication of piezoelectric devices, fuel cells
- Used as coating for preserving the surface from corrosion
- Can also acts as catalysts

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Since it is well known fact that along with incipient science of Nanotechnology, nanoparticles are emerging as a perfect alternative to those of bulk or single particle species. Due to such a microscopic or minute size along with corner sites with quite high density, Oxide nanoparticles flaunt extraordinary physical and chemical properties [7]. Fundamentally, there are three important groups influencing the particle size of any material.

The Lattice symmetry (related to structural characteristics) being the first one. Since bulk oxides have tangible crystallographic structure therefore they are said to be more stable and lusty [8]. The maturation of prominence of surface free energy and stress along with lowering size of the particle need to be considered: since stability (thermodynamic) is directly associated with amendment of cell parameters or transformations in structure as well. The best part of nanoparticles is that they get disappeared in really very extreme cases which takes place because of its environment and high surface free energy at the same time. But for a mechanical object to be stable it should have less surface energy. The nanoparticles which consequently pass this test- their bulk materials are said to be highly stable. This phenomenon can be seen in many Metal Oxides Nanoparticles. Lowering the size of particles increases the interface strain as well as the surface strain which leads to concomitant structural perturbations.

Secondly [9], electronic properties of the oxides are directly connected to the effect of size. The carriage of discontinuous, atom-like states (electronic) is responsible for producing quantum size confinement effects in any material made of nanostructures. The aspects which need to be dealt with while tackling electronic properties of surface of oxides in bulk are long-effects of the Made lung field which are usually limited in a nanostructured oxide.

It is quite obvious with the above points that structure and electronic properties obviously influence the properties of a solid. There are many oxides which have quite a [10] huge band gap and low reactivity in their bulk state.

Nano science is a huge branch of Science which deals with the study, synthesis, applications, and characterisation etc. of nanoparticles [11, 12]. There exists 2 top most methods for producing the product as per one desires in terms of size, shape and functionalities as well. These 2 are named as TOP-DOWN and BOTTOM-UP method [13, 14] (Figure1).

Nanoparticles are of manifold types and can be prepared using heterogeneous methods. One of the most commonly used method is [15], Co-precipitation using Chemicals as stabilising/capping agents. But since technology has shifted to [16], greener approach a little more so scientists came up with Green Methods for preparation of Nanoparticles because it would be: More Eco-friendly (Most Importantly), Pocket Friendly, Efficient Method, Reliable, Sustainable, Rapid process means less time taking, Non-pathogenic etc [17]. The conclusion to Why Green method seems quite easy but if the same question shifts to- Why metal Oxide nanoparticles? The simplest answer can be given on the grounds of following reasons:

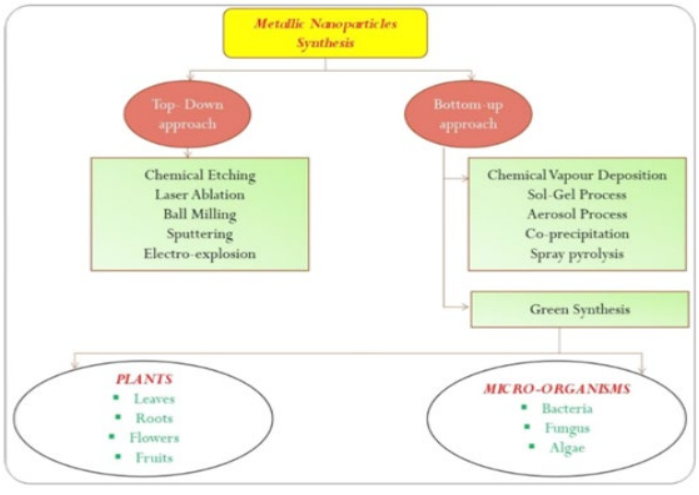

Figure 1: Metallic Nanoparticles synthesis.

- Chemically Stable
- Have no adverse effect inside body
- Ample of applications like photo catalytic, antibacterial, antifungal etc.

Metal Oxides can also be synthesised keeping in view the demand and the needs but here only *Iron Oxide NP's* are considered. This is by the virtue of the entreaties that they serve [18]. Some of them are herein discussed:

Applications

- Used in MRI's (magnetic Resonance Imaging) as contrasting agents
- Used in Cancer treatment as carriers in Targeted drug delivery
- Used in Gene therapy
- Used in hyperthermia cure
- Used as a sensor in IVD (In-Vitro Diagnostics)
- Can also produce antibodies and hence act as Nano adjuvant etc.

These NP's are highly super magnetic and these magnetic properties can be easily classified on the basis of its size [19, 20] (Table1).

Description of the plant studied

This particular paper is all about the use of Neem plant extract in synthesising NP's of Iron Oxide and then size and shape description as mentioned.

METHODS AND MATERIAL

If the results are to be compared with actual existing method with that of the Green adopted approach then initially the need of the hour is to have a reference by which results can be compared and hence to know a little about the most commonly used techniques for preparation of Fe_3O_4-NP's. The following is the one of the increasingly used procedure or technique named as "Co-precipitation" [21].

Co-precipitation method (chemical method)

In simple words, very superficial and effortless method for the

Table 1: Description of the plant studied.

	Neem
Scientific Name	Azadirachta indica
Family Name	Meliaceae
Part of the plant to be used	Leaves
Form in which they were used	Extract

synthesis of Iron Oxide nanoparticles from any aqueous salts of Fe^{3+} solutions followed by the adding a base either at room temperature or most commonly at certain inflated temperature is Co-precipitation method. Since the Iron salts are acidic in nature therefore in order to increase pH of the solution a base is added [22].

From this method it becomes more concise and easier to produce nanoparticles of controlled size and magnetic properties.

MATERIALS

Anhydrous $FeCl_3$ – Any Fe^{3+} salt can be used instead of Ferric Chloride as the main aim is to get Ferric ions. For example- Ferric Sulphate etc. can also be used. But since Iron Oxide is being prepared so Fe_3O_4 should not be used quite obviously, any base can be used like NaOH, $NaBH_4$ etc. Most commonly Sodium Hydroxide is used, Acetone (for washing the apparatus), Deionised Water – Tap water needs to be avoided as it may contain interfering chemicals which can disrupt the simple proceedings, Whitman Filter Paper , Weighing Machine for weighing the sample, Microwave or Oven- Used for drying the sample, Centrifuge Machine [23], Sonicator (Optional) – Used to carry out precipitates by applying sound waves, Centrifuge Tubes , Beakers, Titration Flask, Magnetic Stirrer Along With Hot plate, Glass Stirrer, Pipette, Dropper [24, 25].

Preparation of Fe^{3+} solution

The amount control of the sample is in the hands of the performer. Prepared 0.25M sample of $FeCl_3$. Using Molarity equation the sample to be weighed was thus calculated and hence weighed by tarring the weighing balance to zero. A very little volume of Sulphuric acid was added in order for it to dissolve perfectly. Then, the pipetted out a calculated volume of this sample and added it to Titration flask and volume is made to the marked point. The sample is hence prepared. Any other salt can also be taken no as such boundation is there. The colour of prepared solution is Light orange. (All the solutions used must be freshly prepared and if somehow the sample is prepared earlier it needs to be kept in refrigerator only after properly covering it with aluminium foil.)

Preparation of base

Prepared 1M NaOH Sodium Hydroxide solution in a similar way and kept it aside. (It is very important to use freshly prepared solutions.)

Experimental procedure

Properly measured amount of freshly prepared $FeCl_3$ was taken in a conical flask and then a Stirring magnet was put to this conical and solution was heated till certain temperature (60°C) along with continuous magnetic stirring for approximately 20 minutes. Initially the colour of the solution was noted and which seemed like Light Orange in shade. Then came the turn to add base to it in order to increase the pH because $FeCl_3$ became quite acidic due to addition of a little amount of acid to it. So in order to increase the pH one has to make sure the addition of base or OH^- ions. On addition of NaOH to $FeCl_3$ the following reaction takes place.

$$\underset{\text{Ferric Chloride}}{FeCl_3(aq)} + 3\underset{\text{Sodium Hydroxide}}{NaOH(aq)} \longrightarrow \underset{\text{Ferric Hydroxide}}{Fe(OH)_3(s)} + 3\underset{\text{Sodium Chloride}}{NaCl(aq)}$$

Generous drop-wise addition of NaOH to conical flask is to be done along with continuous mild stirring through magnetic stirrer and heating. Gradual addition of Sodium Hydroxide will led the solution to start becoming darker and darker in shade. After sometime the initial orange colour ultimately changed to reddish brown shade solution along with some settled down precipitates at the bottom of the conical flask. At that particular time addition of base was terminated as the precipitation was quite completed at this point. The settled down stuff are **The Precipitates of Ferric Hydroxide**

(Sometimes it happens that the precipitates are not easily separable for which SONICATOR is used in which sound waves are sent in order to disunite the precipitates although this proceeding is optional and more often not used) (Figure 2).

As the preparation part seemed completed then forwarded to the collection of precipitates and further drying them in order to obtain Iron Oxide NP's.

The contents in the conical flask were shifted to centrifuge tubes and then applied in the centrifugation machine in such a pattern that- each one has one centrifuge tube opposite to the other one. (If this pattern is not followed then the contents may spill out of the tubes and NP's may be lost) (Figure 3).

Deionised water and acetone were used for washing purpose and washing procedure was repeated 3-4 times in order to avoid the loss of product from the centrifuge tubes. These centrifuged NP's were transferred to a crucible and were put in the preheated oven for about 2 hours.

After that when water molecules got evaporated due to high temperature the actual product formed were Iron (III) Oxide NP's. (Figure 4)

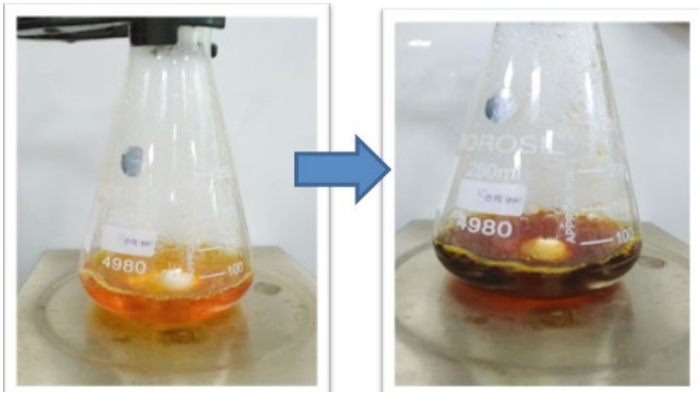

Figure 2: Colour change when NaOH was added to $FeCl_3$ solution.

Figure 3: Centrifugation machine.

Figure 4: Dried sample in crucible.

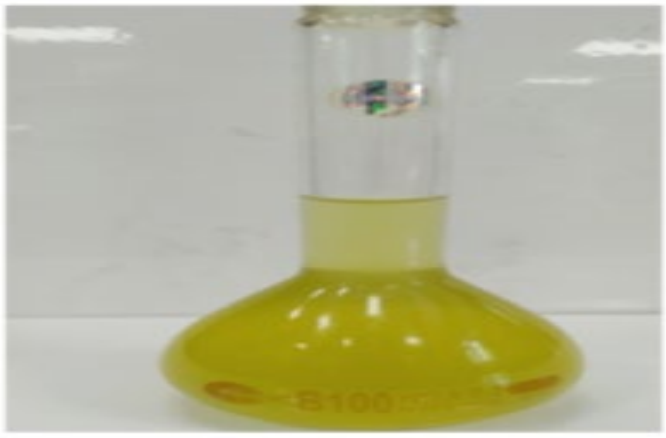

Figure 5: Neem extract.

PREPARATION USING NEEM EXTRACT (GREEN METHOD)

MATERIALS

Anhydrous $FeCl_3$ – Any Fe^{3+} salt can be used instead of Ferric Chloride as the main aim is to get Ferric ions. For example- Ferric Sulphate etc. can also be used. But since Iron Oxide is being prepared so Fe_3O_4 should not be used quite obviously, being a Green Approach obviously base must not be used in this rather finding a stabilising agent can serve the purpose as an alternative. {The Green extract of any antioxidant rich plant can be used. Here particularly the Neem Extract had been studied.}, Acetone (for washing the apparatus), Deionised Water – Tap water needs to be avoided as it may contain interfering chemicals which can disrupt the simple proceedings, Whitman Filter Paper , Weighing Machine for weighing the sample, Microwave or Oven- Used for drying the sample, Centrifuge Machine, Sonicator (Optional) – Used to carry out precipitates by applying sound waves, Centrifuge Tubes, Beakers, Titration Flask, Magnetic Stirrer Along With Hot plate, Glass Stirrer, Pipette, Dropper.

Preparation of plant extract

The research was done using leaves extract although any other part like stem, roots can also be used but particularly this procedure relates with the extract of leaves portion. Leaves of certain plants like Neem, Tulsi, etc. are very rich in antioxidant properties and hence can be used as precipitating and stabilising agents in metal oxide nanoparticles synthesis [26].

Since here Neem is being talked about so it is generally composed of ample of bioactive molecules and some of them are Saponins, Flavonoids, Carbohydrates, Glycosides, Alkaloids, Tannin, Phenolic compounds etc. These all play a vital role in synthesis part.

Now, as far as preparation is concerned, the leaves were plucked freshly and then initially washed with tap water for removing dirt particles followed by cleaning with Deionised water. Leaves need to be dried completely before crushing by putting them on a clean surface. Properly pristine and naïve leaves were shifted to either Mortar Piston or a Grinder and grinded to paste by addition of gradual amount of Deionised Water. Through Whitman's Filter paper and funnel the extract was poured to Titration flask and volume was made up till the mark.

The colour of the extract came out to be Light Green. (Figure **5**)

Experimental procedure

Properly measured amount of freshly prepared $FeCl_3$ was taken in a conical flask and then a Stirring magnet was put to this conical and solution was heated till certain temperature (60°C) along with magnetic stirring for approximately 20 minutes. Initially the colour of the solution was noted and which seemed like Light Orange in shade [27].

pH of Neem extract was found to be 8.2 and hence it would be pretty good alternative of base which is a chemical because of Neem extract being natural and chemical free. In order to increase the pH of $FeCl_3$ addition of Neem extract can serve the purpose because $FeCl_3$ is quite acidic in nature and for making the precipitates slightly alkaline medium is required.

Gradual drop wise addition of prepared Neem extract to the conical was done until and unless the colour of the solution started becoming dark (light yellow to dark brownish) and precipitates began to separate out. The solution was taken off and then let it cool for some time.

(Sometimes it happens that the precipitates are not easily separable for which SONICATOR is used in which sound waves are sent in order to disunite the precipitates although this proceeding is optional and more often not used.)

As the preparation part seemed complete then forwarding to the collection of precipitates and further drying them in order to obtain Iron Oxide NP's.

The contents in the conical flask were to be shifted to centrifuge tubes and then applied in the centrifugation machine in such a pattern that- each one has one centrifuge tube opposite to the other one. (If this pattern is not followed then the contents may spill out of the tubes and NP's may be lost.)

Deionised water and acetone were used for washing purpose and this procedure was repeated 3-4 times so that there is no left over product in the tube and not even a single particle gets lost. These centrifuged NP's were transferred to a crucible and put in the preheated oven for about 2 hours. After this when water molecules got evaporated due to high temperature the actual product formed were *Iron (III) Oxide NP's.*

CHARACTERISATION

Characterisation can be defined as a way which is useful to get more information regarding the particular properties of a substance (may

be nanoparticles or any other). This provides accurate rapid results which are often said to be reliable to get an idea about the actual results and compare them with already existing ones. Here Iron Oxide Nanoparticles when and how are subjected is mentioned [28].

UV-VIS Spectroscopy

It may be defined as an analytical method through which can detection as well measurement of the amount of wavelength of UV or visible light which would have been either absorbed or emitted from a sample comparatively to the blank sample can be done. Through this sample concentration, composition etc. can be found out easily. The instrument which is used for this purpose is baptized as UV-VIS Spectrophotometer [29].

Consistent with the Einstein theory, wavelength is inversely

$$Energy = \frac{Plank's\ Constant * Speed\ of\ Light}{Wavlength}$$

Light has certain amount of energy associated with it and since this whole method revolves around the light therefore it can be said that- smaller wavelengths light wave will be associated with more energy and vice versa is also true. As it is quite clear already that a particular amount of energy is absorbed by a certain substance in order to elevate an electron from lower energy state to the higher one and same amount of energy gets emitted when it reverts back to its original state. Varied environments with respect to bonding in an exclusive substance necessitate different amounts of energies to promote or demote the electrons simultaneously [30].

Human's range of visible region is in between 380nm-780nm which corresponds to violet and red colour respectively. Since the wavelength of UV light is shorter than that of visible i.e. near about 100nm hence UV-Vis spectrophotometer can easily analyse different substances by specific wavelengths with respect to the maximum wavelengths. As it is well known fact that optical characteristics are present in nanoparticles which makes them eligible in order to respond to size, shape, concentration and refractive index at its surface hence UV-Spectroscopy becomes quite a necessary method in order to find whether the particles produced are stable or not. (Figure 6)

Procedure for understanding the working of UV-Spectrophotometer

Basically a comparison is to be brought about between the intensity of light reflected from a sample and intensity of light reflected from a reference point. Here Nanoparticles which were prepared through chemical method can be considered as the reference and those by green synthesis can be later on called the sample whose absorbance is to be find out. Initially in order to remove all the air from inside the instrument a calibration technique was adopted in which Deionised or else Distilled water was filled in cuvette Cuvette needs to be handled with utmost care only the rough sides can be touched that too with tissue paper in order to prevent the fingerprints because UV-Spectrophotometer can absorb these fingerprints which can ultimately lead to variations in the results]. After that its absorbance is to be noted and that is known to be the Blank reading which means calibration of the instrument is done.

Chemical method (When NaOH was used as stabilising agent)

The nanoparticle liquid which was prepared after stirring was used because the solution was added to the cuvette. So the cuvette is to be filled with the liquor prepared and then fitted in the column according to the mandatory precautions adopted. Then connected Laptop or Desktop is used to record and save the graph plotted between Absorbance (y-axis) v/s Wavelength (x-axis). In order to check the stability the solution was kept for 24 hours or so and then again the absorbance was noted down. If it came out to be the same or nearly equal then it is relatively said that the nanoparticles prepared are stable and if some changes are there then the nanoparticles were asked to be less stable and the method is known to be less relevant because the main aim of the procedure is to find the best suited as well as stable product.

The graph is as follows: (Figure7)

The display of the graph showed the Maxima to be at 416nm i.e. 2.283 Absorbance. Now if the stability of Iron Oxide Nanoparticles synthesised is to be seen the same procedure is to be repeated with the solution after letting the mixture to stand for certain time intervals which can be around 24 hours or so. If this method is to be used then the absorbance comes out to be almost equal even after 24 hours. This proves that Chemical Method is quite a good method for synthesising Iron Oxide NP's.

Also the Band Gap in energy can be calculated on the basis of Maximum Absorption band of IONP's according to the following equation:

$$E_{bg} = \frac{1240}{Wavlength}\ eV$$

Where $E_{bg} = Band\ Gap\ Energy$

Green method (When Neem extract was used as stabilising agent) (Figure 8)

The IONP's prepared through green method were treated through the UV-Spectrophotometer the same way that of chemical one and

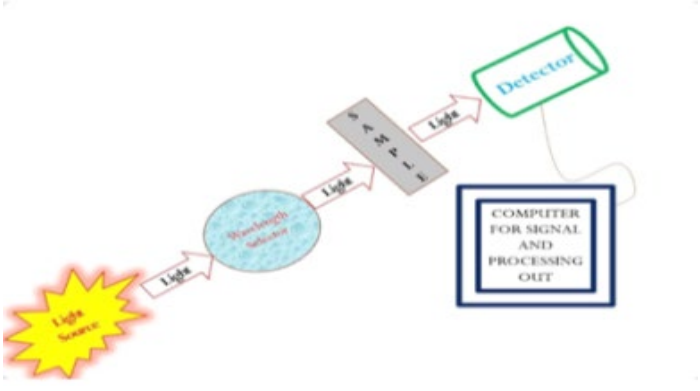

Figure 6: Working of UV-Vis Spectrophotometer.

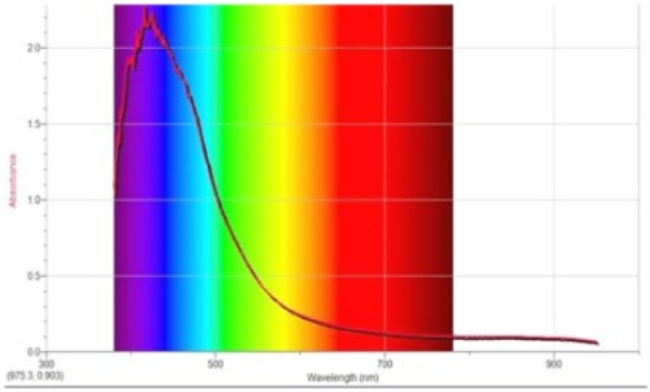

Figure7: Absorption spectrum through chemical method.

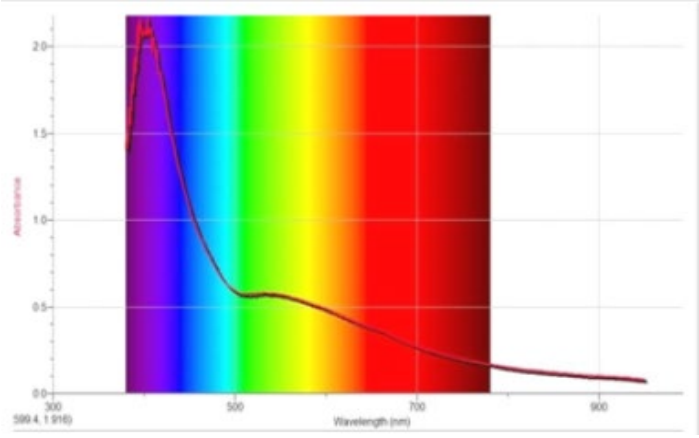

Figure 8: Absorption spectrum through Green Method.

absorbance was noted down using the attached computer graphical image as shown above.

Here the Absorption Maxima came nearly about 396nm i.e. 2.157 Absorbance which was pretty close to the characteristic lambda maxima of ION's.

When the absorbance was noted after certain hours in order to see the stability of Nanoparticles then also it came similar to former one.

DISCUSSION

Comparison between chemical & green Method

If both Green as well as Chemical methods are to be considered they are said to be extensively used for preparing Metal Oxides Nanoparticles. Here are the following points in order to find or choose that which one is more efficient and can be adopted more often:

The use of Chemicals as Capping & Stabilising Agents like Sodium Hydroxide, Sodium Boron Hydride, Hydrazine Hydrate, etc. is found to be quite hazardous to the environment as well as the living organisms. When the results came out to be almost similar if UV Spectroscopy results are considered and the process is almost same so *"The green method proves out to be more efficient as compared to the chemical one."* The flavonoids and the Terpenoids present in Neem Extract proved to be the capping agents and stabilising agents as well which makes them perfect method for synthesising the IONP's as it will not at all harm the surroundings.

- The rate of dispersion also came out to be faster while using Green extract because the produced Nanoparticles from Bio-materials seemed to be Mono-dispersed.
- The dry leaves produced more stable extract in collation to wet leaves.

Concluding all the Green method comes out to be extremely feasible leaving behind the Chemical one.

Role of acidity and basicity

As far as the acidity and basicity is concerned the rate of formation of nanoparticles fasten up when basic medium is present and also it effects the size of each nanoparticle means the reaction time will increase and it may cause aggregation of the particles due to which it will look bigger in size. In contrast the rate is quite less when the medium seems acidic.

The $FeCl_3$ is certainly acidic in nature which means a base needs to be added. Through chemical method NaOH or any other base mentioned above can be used but in Neem Extract the basic properties is served by Terpenoids and Flavonoids present in the extract and hence fastening the rate of reaction up.

Change of colour

In both the process the initial colour of $FeCl_3$ solution can be observed to be orangish which became darken on addition of basic medium to it and gets converted to Brownish-Blackish product with the adduct formation at the bottom of the flask and the precipitates came out to be Reddish black in colour.

Effect of concentration of $FeCl_3$

Concentration of dehydrating agent FeCl3 also plays major role in production of iron oxide nanoparticles. At first when 0.1M FeCl3 was prepared 1.68 g of it is added to 100ml of distilled water. As concentration of FeCl3 was very less, extract was able to precipitate it out but yield of nanoparticles formed was not good. In order to produce a good yield, 0.5 M FeCl3 and 30 g of extract is prepared but precipitate was not formed as concentration of fecl3 was more for which extract was not enough to precipitate it out. When concentration of FeCl3 was decreased to 0.25M, it resulted in the formation of nanoparticles in good yield.

CONCLUSION

Ample of varied ways can be adopted (i.e. bio components kinds like fungi, bacteria, yeast, plant extract etc.) in order to synthesis Metals Oxides NP's. Out of them the most effective and efficient method is to use plants extract as capping and stabilising agents for nanoparticles. Most of the scientist gets truly fascinated by thinking and researching on this topic as a part of future scope. This particular paper was about to encompass the research on Green Synthesis. Over all view of this was to focus more on biological ways which are safe, environment friendly, economical, fastens the rate of reaction up rather than Chemical ones.

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