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GREEN TECHNOLOGY TO SYNTHESIS AND CHARACTERIZATION OF ZERO VALENT IRON NANOPARTICLES FROM EXTRACT OF *CLITORIA TERNATEA*

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Abstract – Nanoparticles are solid colloidal particles ranging from 1 to 100 nm in size, they consist of macromolecular materials in which the active ingredients is dissolved, entrapped, or encapsuled, or absorbed. For environmental remediation the recent studies indicate the potential of iron nanoparticles. Biological resources such as bacteria, algae, fungi and plants have been used for the production of low-cost, energy-efficient and nontoxic environmentally friendly products. Nano scaled irons were synthesized from the plant *Clitoria ternatea* extract of under environmental condition at room temperature by using 0.01M $FeCl_3$(ferric chloride) at 30 °C in 30 min. The zero valent iron nanoparticles are synthesized in oxidation state. Box Behnken design optimization tool was used to predict the nanoparticles yield with three variables. The reductants present in the plant extracts act as reducing and stabilizing agent. A systematic characterization of nano scaled iron particles was performed using UV-Vis, pH, FT-IR and SEM studies. The diameter of iron nanoparticles was found within the range 50-100 nm. In the green nanotechnology the valuable approach of synthesis of nanoparticles by the exploitation of different biomaterials. This study focuses on various studies outlining the novel routes for biosynthesis of these nanoparticles by plant resources along with outlining the future scope of work in this area.

INTRODUCTION

"Nano" is derived from the Greek word for dwarf. A particle size which is 1-100 nanometers is called nanoparticles. The physical, biological and chemical property of nanoparticle differs from that of bulk materials due to the variation in size, shape and distribution of atoms (Jagathesan and Rajiv, 2018). The green synthesis of nanoparticles has grabbed the attention of scientists since it has several merits such as simple, inexpensive, good stability of nanoparticles, less time consuming, nontoxic byproduct and larges calesynthesis (Aisida *et al.*, 2020; Igwe., 2008). The greener environmentally friendly processes in chemistry and chemical technology are becoming increasingly popular (Iraivani, 2011; Herrera *et al.*, 2008). Magnetic nanoparticles have attracted a great attention due to their unique physical, chemical and structural properties when the particle sizes approach to nanoscale (Abilash *et al.*, 2011; Arroyo *et al.*, 2016). It is these unique features that endow magnetic nanoparticles with wide applications, such as magnetic storage, catalysis, microwave absorption, magnetic resonance contrast, cancer hyperthermia, cell separation and drug delivery (Bystrzejewska-Piotrowska *et al.*, 2012; Priya Banerjee *et al.*, 2014). The bioreduction of metal nanoparticles by combination of bimolecular found in plant extracts such as enzymes, proteins, amino acids, vitamins, polysaccharides, typically obtained by contact of a plant broth with metal salts, has been investigated in recent years (Awwad *et al.*, 2013; Phumying *et al.*, 2013). Iron plays a significant role in metabolic reactions of all living entities (Bibi *et al.*, 2019). In general, Fe_3O_4 has created interest in scientific and technological levels due to its unique electric and magnetic properties along with itshigh chemical reaction property (Anchan *et al.*, 2019; Vanaja *et al.*, 2013). The synthesis of nanoparticles is evolving into an important branch of nano technology and developing of green technology, because these methods are considered safe and ecologically sound the nanomaterial fabrication has been found to be an alternative to the conventional methods (Malabdi *et al.*, 2012; Machado *et al.*, 2013). Here in the present work we have reported for the synthesis of green iron nanoparticles using the leaf and flower extract of the plant.

PLANT DESCRIPTION

Binomial name: *Clitoriaternatea,* Common name: Butterfly pea, Order: Fabales, Family : Fabaceae, Plant part taken: leaves, flower

MATERIALS AND METHODS

Reagents and Chemicals: Ferric Chloride ($FeCl_3$) was taken as the reducing agent for the synthesis of iron nanoparticles. Commonly 0.1M or 0.001M $FeCl_3$ solution are used for the synthesis of the iron nanoparticles but we prepared the 0.01M $FeCl_3$ for this project in order to increase the exposure of the nanoparticles and tends to shorting the incubation time, high up the level of rate of reduction.

Collection and Extraction of plant: ***Clitoria ternatea*** Leaves and flowers were collected from Tiruvannamalai region. They were washed and cleaned with triple distilled water and dried with water absorbent paper. Then it was cut into small pieces with an ethanol sterilized knife and crushed with mortar and pestle dispensed in 100 ml of sterile distilled water and heated for 15 minutes at 80 °C. The extract was then filtered using filter paper. The filtrate was collected in a clean and dried conical flask by standard sterilized filtration method and was stored.

Synthesis of Nanoparticles: During the synthesis of iron Nanoparticles both the precursor and the reducing agent were mixed in a clean sterilized flask in 1:1 proportion. For the reduction of Fe ions, 5ml of filtered *Clitoriaternatea* plant Leaves extract was mixed to 5 ml of freshly prepared 0.001 M aqueous $FeCl_3$ solution with constant stirring at 50-60°C. Within a particular time change in colour from Light Green to Black colour obtained by nanoparticles synthesis.

Optimization

Statistical regression model, Box-Behnken design, was used to optimize the nanoparticle synthesis process. Box-Behnken design carried out involving 3 variables and 2 levels. The statease Design-Expert 11 statistical software assesses the experimental runs. The response equation is shown eq.1

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + \varepsilon$$

Eq.1

Where Y is the response that is nanoparticle yield (%), n is the number of variables, β_0 is the model intercept term and β_i is the liner effect term, β_0 is the square effect term, β_{ij} is the interaction effect term, X_i and X_{ij} is the level of the independent variables and ε is the random error. The equation Eq. (2) represents the design equation of nanoparticles yield in terms of actual values of independent variables.

Characterization of Nanoparticle

UV-Vis Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy in UV-Visible spectral region. This means it uses light in the visible and adjacent (near-UV and near infrared (NIR)) ranges. The absorption in the visible ranges directly affects the perceived colour of the chemicals involved. In the region of the electromagnetic spectrum, molecules undergo electronic transitions. UV-Vis spectroscopy is used to confirm the synthesized particles are iron nanoparticles in order to show the peaks between optimized region.

pH Analysis

The pH was determined by using digital pH meter Systronics. The pH of the reduced solution with nanoparticles synthesized was found to be acidic. After reduction the pH of sample was found to be decrease and move towards the acidic range. Majorly synthesized nanoparticles solution was shown in the pH range between 2.16± (0.7). according to this nanoparticles solution which is synthesized in this project are evaluated and compared with standard sample.

FT-IR Analysis

FT-IR enables the in-situ analysis of interfaces to investigate the surface adsorption of functional groups on nanoparticles. An advantage of FT-IR is that it enables uses to analyse a layer of nanoparticles coated on the ATR element, while also altering the overlying phase. They are mainly used to measure light absorption of so called midinfrared light, light in the wave number range of 4000 to 400 cm^{-1}(wavelength 2.5 -25µm).

SEM Analysis

Scanning Electron Microscope (SEM) analysis is to confirm the morphology of the iron nanoparticles and ensure the shape and structure of iron nanoparticles. The signal that derived from electron sample interaction reveal information about the sample including the surface morphology and chemical composition of the sample.

RESULTS AND DISCUSSION

The plant extract of *Clitoria ternatea* was found to be suitable for generation of iron nanoparticles. The size distribution and average size particle of iron nanoparticles were obtained by using particle size analyzer. The time required for the bioreduction of metal ion varied from few minutes. Colour changes was observed from Light Green to Black colour indicating the formation of iron nanoparticles. Ferric acid is formed by reduction of ferric chloride solution of 0.01M concentration and gets precipitated in the plant extract. Detection of presence of functional moieties on the iron nanoparticles using FTIR. Sample was scanned in the wavelength ranging between 4000-400cm^{-1}. The structure of the nanoparticles was determined by scanning electron microscopy analysis. Biocompatible synthesis of nanoparticles is the centralized area of the study in the field of nano technology. Biological synthesis of nanoparticles occur due to various reasons. The unreacted component can lead to the formation of undesired products with varied property making the method non-reproducible. It is still a challenging process for a researcher to work with this green synthesis even though immediate recovery of the product resolves the problem.

Optimization

The Box-Behnken method of RSM evaluated the effect of Fecl$_3$, time and plant extract on nanoparticles production. In the Box-Behnken design, three variables with three levels were used for modeling and optimization of the nanoparticle synthesis. The responses of the variables were shown in diagram and the random experimental runs minimized response variability. The value of regression coefficient was determined using least square to give a polynomial model of nanoparticle synthesis. The yield equation of predicted model is given below:

$$\text{Yield}=-0.042969-0.024500\ X_1+0.046750\ X_2+0.001300\ X_3+0.004875\ X_1 * X_2-0.000250\ X_1 * X_3+4.14779E\text{-}20\ X_2 * X_3-0.002125\ X_1^2-0.004906\ X_2^2-0.000024\ X_3^2 \quad \text{Eq.5}$$

Model is significant with the F-Value of 45.38. There is only 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, B² are significant model terms. Model terms with the value 0.1000 indicte that the model terms are significant. Model reduction can be used for improving the model if there are more insignificant model terms (not counting those required to support hierarchy). Lack of fit is not significant relative to pure error if lack of fit, F-value of 0.46. There is a 72.37% chance that a Lack of Fit F-value this large could occur due to noise.Non-significant lack of fit is good.

U-V Vis Spectroscopy

Ultraviolet visible spectroscopy (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. This means is uses light in the visible and adjacent (near-UV and near infrared (NIR)) ranges. The bioreduction of Fe+3 in aqueous solution was monitored by periodic sampling of aliquots of the mixture and subsequently measuring UV-Vis spectra. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer Systronics 118 at the range of 200-500 nm and observed the absorption peaks at 216-265 nm regions due to the excitation of surface Plasmon vibrations in the iron nanoparticles, which are identical to the characteristics UV-Visible spectrum of metallic iron.

pH Analysis

The pH values of the iron nanoparticles are acidic. The ranges of the solution before the reduction are in medium acidic condition but after the reduction are in too acidic range. High acidic condition is generally referred for the synthesis of the iron nanoparticles in order to the high acidic profile giving accurate of iron nanoparticles. The nanoparticles solution *Clitoria ternatea* before the reaction the pH is 4.20 and after the reaction the pH is 2.18.

BBD Optimization

The contour plot and response surface graphs were shown in Fig. 1 (a-f). The variable interaction was assessed by using model equations. These designs

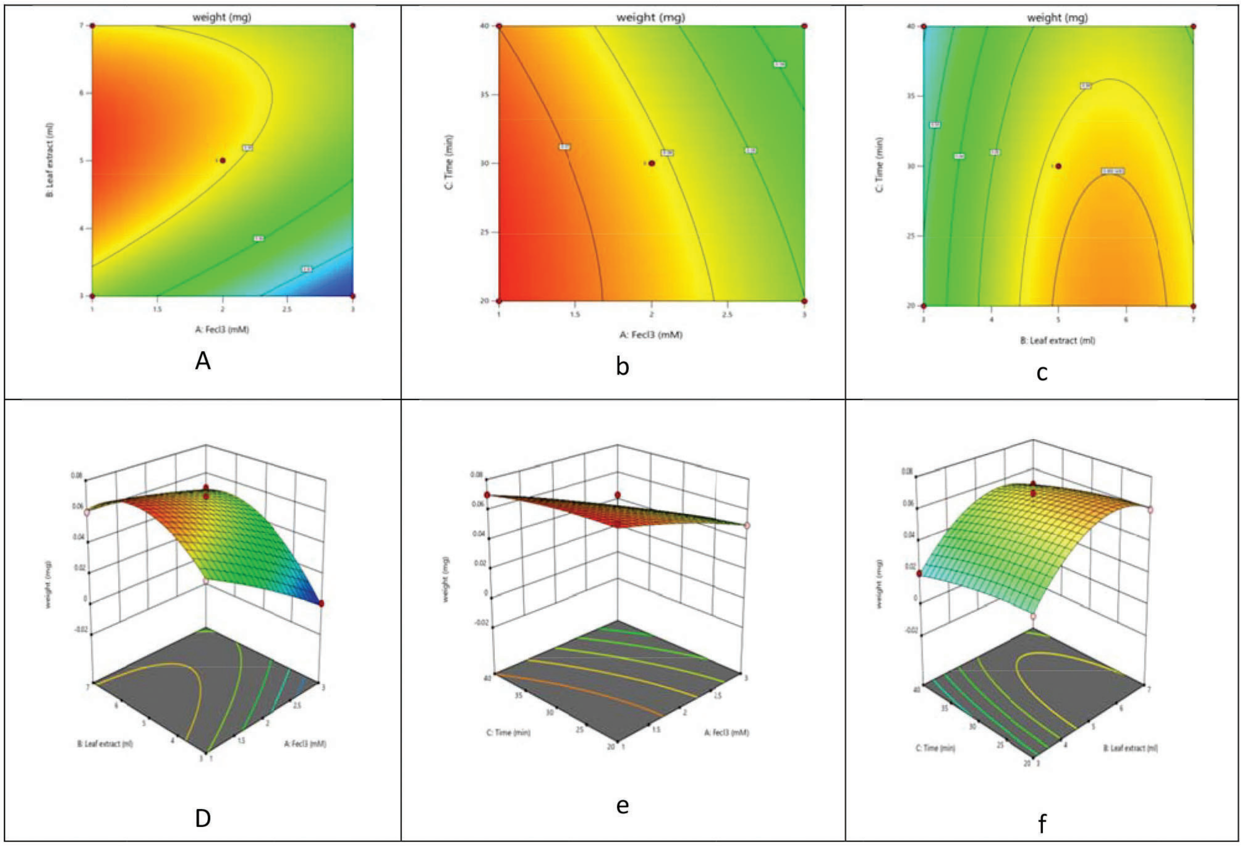

Fig. 1. Contour plot(a-c) and 3D graph(d-f)

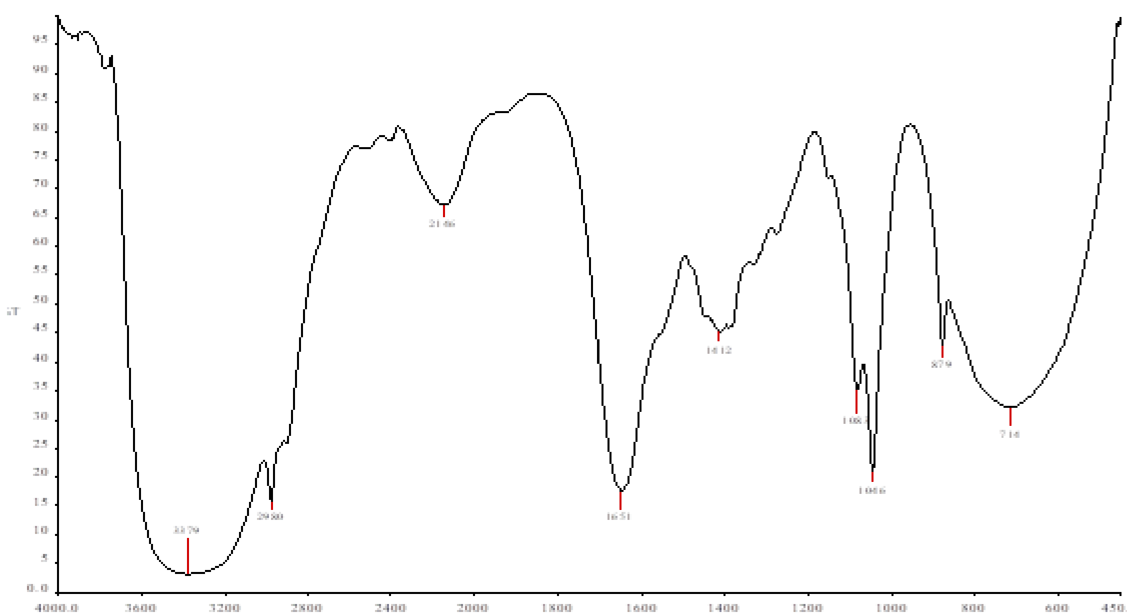

Fig. 2. FTIR spectra

were made by plotting the process response on the z-axis against other two independent variables on x and y axes, respectively and others were kept at its center level. From these response graphs, it clearly indicates that there was strong interactions between the independent process variables. The nanoparticle yield was increased with increasing in plant extract, time and $FeCl_3$ up to its middle level.

FTIR

FTIR spectroscopy analysis reveals the presence of different functional groups of the bioactive compounds present in the leaf and flower extracts, when the extract passed in the FT-IR region, in the functional groups where separated based on its bonding position, the resulting peaks confirm the presence of wide range of functional groups of bioactive compounds. The result of FT-IR spectrum of leaf and flower extract of *Clitoria ternatea* confirmed the presence of phenols and alcohols with a peak at 3379 cm^{-1} corresponded to hydroxyl and O-H bonding frequency respectively. The peak at 2980 cm^{-1} and 2146 cm^{-1} assigned to the C-H stretching which means that some alkene compounds are present. The peak value at 1651 cm^{-1} confirms primary amines. The peak value at 1412 cm^{-1} confirms aromatic amines. The peak value at 1046 cm^{-1}confirms aliphatic amines. The peak value at 879 cm^{-1} confirms carboxylic acids, and the peak value at 714 cm^{-1} confirms primary and secondary amines. In the present study, a wide range of functional groups of bioactive compounds such as alcohols, phenol, alkanes, primary, secondary, aromatic, aliphatic amines, carboxylic acids where found in both leaf and flower extract. Further, these functional group can be isolated and tested in future for different kinds of biological activities depending on their therapeutics uses.

Sem Analysis

In order to test the analytical capabilities of high resolution of SEM of various material containing nanoparticles of known size and chemical composition have been analyzed. The first example focuses on particle size distribution measurements of iron nanoparticles which were prepared on plant extract. The superior material of contrast of SEM imaging well suited to dimensional measurement can be observed in Figure 2. In addition, it reveals the presence of iron nanoparticles. The validity of SEM results has been demonstrated elsewhere by the good agreement with results obtained by UV-Vis, pH and FT-IR analysis of the same material. The magnification ranges of all images are 1280×960 and 20.0 [kv], SP=13.0, WD=16.6. It can be summarized that without need of accurate elemental qualification, the qualitative evolution of the chemical composition of iron nanoparticles of different inner morphologies by means of SEM automated analysis is possible. The range of the application of SEM is extended to further example of complex nanoparticles shape and composition

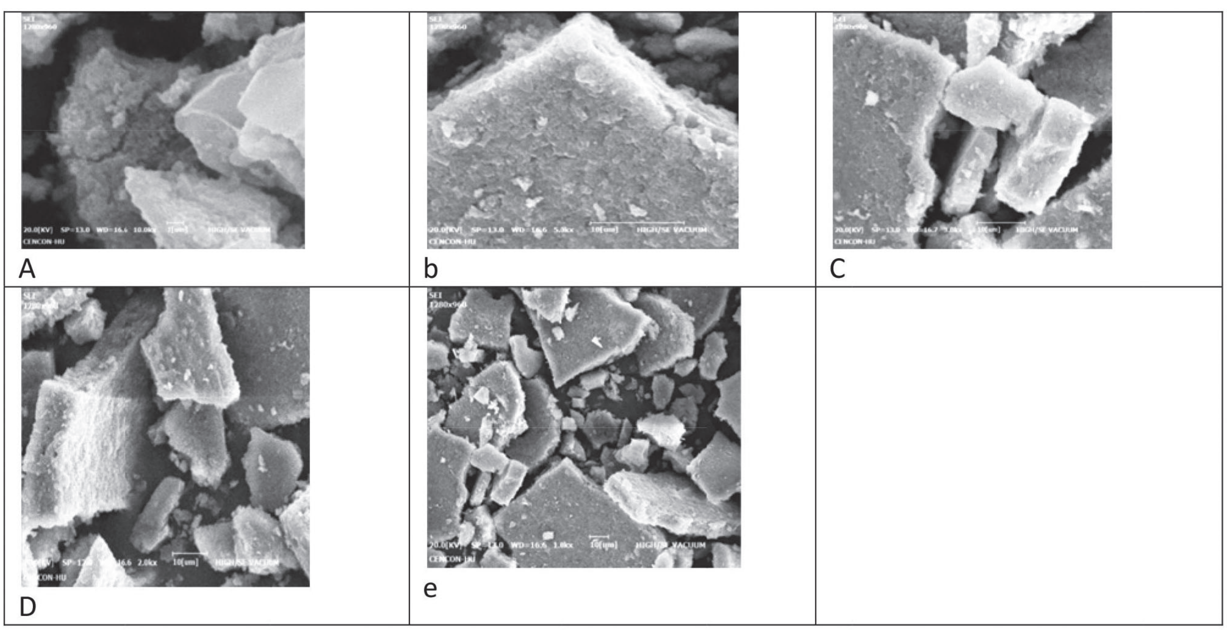

Fig. 3. SEM analysis of nanoparticles(a-e)

such as core-shell structures.

CONCLUSION

The extracts of plants and spices may be capable of producing Iron Nanoparticles under the UV-Visible wavelength. Nanoparticles showed quite good surface Plasmon resonance behavior. The colour change was also remarkable when Ferric chloride was mixed with reducing agent, i.e., plants and flower extract. As and when reduction occurred the colour changed with concerned change in pH of solution. A new kind of approach was applied in this project having good efficacy then other green synthesis method. The nanoparticle is an alternative to chemical synthesis protocolos and low cost reductant in the approach has a success of rapid time sacale for synthesizing Iron Nanoparticles. For more confirmation, we can go for higher characterization techniques such as UV-Vis, pH, FTIR, SEM. This study focuses the production of iron nanoparticles via various green methods and their potential for remediation of environmental pollution. The effect is made to highlight the various green agents for the synthesis of iron nanoparticles like plant extracts, and they are reaction pathways to some extents. Moreover, this study discuses that particle size, morphology and other properties relates with the properties of materials procedures and protocols. The synthesis of iron nanoparticles, which proved to be a good catalyst for widespread environmental application. Thus, plant materials look more feasible as agents for production of iron nanomaterial's due to its environmentally friendly characteristics and economic value as an alternative to the large-scale production of nanoparticles.

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