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Bio-reductive Synthesis and Characterization of Plant Protein Coated Magnetite Nanoparticles

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Abstract. Over the past two decades, there have been increased emphases on the topic of green chemistry and chemical processes. Utilization of non toxic chemicals, environmentally benign solvents, and renewable materials are some of the key issues that merit important consideration in a green synthetic strategy. The *Datura Inoxia* leaves possesses biomolecules such as cardiac glycosides, proteins, phenolic compounds, flavonoids and sugar, which could be used as reducing agent to react with ferrous and ferric ions and as scaffolds to direct the formation of Fe_3O_4 NPs in solution. To the best of our knowledge, the use of Dhatura innoxia plant extract at room temperature for the bio-reductive synthesis of Fe_3O_4 nanoparticles has not been reported. The formation of the Fe_3O_4 magnetic nano particles was first monitored using UV-Vis absorption spectroscopy. FT-IR spectroscopy and TGA/DTG analysis further confirms the formation of plant protein coated magnetite nano-bio hybrid. The dried form of synthesized nanoparticles was further characterized using XRD, TEM.

Introduction

Magnetic nanosized particles have already been known for over 50 years, but research into their potential use in medicine and pharmaceutics is now the hot topic in this domain [1, 2, 3]. The unique combination of high magnetization and paramagnetic behaviour opens these materials to a

very wide range of applications. Particularly, the possibilities of nanoparticle modification by biologically active compounds to use them in controlled drug delivery systems, as agents in magnetic resonance imaging and for magnetic-induced tumor treatment via hyperthermia are very interesting [4]. Iron oxide based nanoparticles belong to the most widely used materials in this field, although they have worse magnetic properties, lower saturation magnetization, and lower specific loss of power than Fe and Co nanoparticles which have just started to gain attention for biomedical purposes, too [5]. However, iron oxides have several advantages over Fe and Co nanoparticles, e.g., better oxidative stability, compatibility in nonaqueous systems, and nontoxicity. Among the four well-known crystalline polymorphs of iron(III) oxide (α-Fe_2O_3 as hematite, β-Fe2O3, γ-Fe_2O_3 as maghemite and ε-Fe_2O_3), maghemite has gained the greatest interest in above mentioned applications [6]. Moreover, magnetite Fe_3O_4 is also very promising candidate as it is biocompatible and biodegradable [7, 8]. Several methods are generally employed for iron oxide nanoparticle preparation, including co precipitation [9], which is preferred due to its simplicity. On the other hand, thermal decomposition [10] seems to give the best control of nanoparticles size and morphology. The resulting physico-chemical properties of nanosized magnetic product obviously depend strongly on the fabrication conditions, especially on material origin, concentration and pH of solution as well as on the mode of thermal treatment used (annealing temperature, atmosphere and rate of heating/cooling). It was found that ferromagnetic low temperature phase γ-Fe_2O_3 can be easily transformed into the antiferromagnetic more stable phase α-Fe_2O_3 when the temperature exceeds 500 °C [11]. Thus it is extremely important to optimize the preparation procedure in order to prevent formation of undesired product(s). The particle size also plays a crucial role. Typical particle sizes for the ferro- to superparamagnetic phase transformation are between 10 and 20 nm for oxides and 1–3 nm for metals [12]. Morales et al. observed that the use of polymers in the material synthesis limits the particle size [13]. Ultrasmall magnetic iron oxide nanoparticles (<5 nm) with very uniform size distribution can be also synthesized using the water-in-oil microemulsion method [14].

Currently, a large number of physical, chemical, biological, and hybrid methods are available to synthesize different types of nanoparticles [15]. The nanoparticles formed using each method show specific properties. However, biosynthesis of metal nanoparticles by plants is currently under development. Green nanotechnology has attracted a lot of attention and includes a wide range of processes that reduce or eliminate toxic substances to restore the environment. The synthesis of metal nanoparticles using inactivated plant tissue [16], plant extracts [17], exudates [18], and other parts of living plants [19] is a modern alternative for their production. Green synthesis of nanoparticles makes use of environmental friendly, non-toxic and safe reagents [20].

The green method of synthesis of nano particles is easy, efficient, and eco-friendly in comparison to chemical-mediated synthesis [21, 22, 23, 24]. The chemical synthesis involves toxic solvents, high pressure, energy and high temperature conversion and microbe involved synthesis is not feasible industrially due to its lab maintenance. Since, green synthesis is the best option to opt for the synthesis of nanoparticles, therefore the magnetite nanoparticles are synthesized by using aqueous extract of Datura inoxia and ferrous and ferric ions. Magnetite was of particular interest due to its unique magnetic and electrical properties. Datura inoxia leaf extract was selected as it is of high medicinal value and it does not require any sample preparation and hence is cost-effective. To the best of our knowledge, the use of Datura inoxia plant extract at room temperature for the green synthesis of protein coated magnetite nano-bio hybrid has not been reported.

Datura inoxia (family Solanaceae) is a common hallucinogenic alkaloid containing toxic annual weed, found in India. It is also use as an ornamental plant for its large leaves and large white flower. The native range of *Datura inoxia* appears to have Mexico and the U.S. Southwest, India, and China [25]. Further Datura species are widely distributed in tropical and temperate regions of both hemispheres [26]. The plant has been used to treat asthma, diarrhea, as an analgesic, to control fever, kill parasites, and as a drug for clinical purposes [27].

In this present study it has been found that the proteins available in the plant extract have been found to be very effective stabilizing agent by forming a coating on the surface of the nano particle besides being acting as reducing agent for the formation of magnetite nano particles. These green-synthesized nano particles were examined by ultraviolet-visible spectroscopy; transmission electron microscopy (TEM), and X-ray analysis (XRD) to determine their size and shape; TGA and FTIR analysis were also performed to confirm the protein coating on nano particle forming protein coated magnetite nano-bio hybrid.

Materials and Methods

Preparation of Aqueous Leaf Extract of *Datura Inoxia*. Fresh leaves of *Datura inoxia* were washed under running tap water to remove any debris and dust attached to the leaves and subsequently with Millipore water 3–4 times [28]. Leaves were air dried for two weeks at room temperature (25 °C). The dried leaves were finely powdered through grinding using Lumix grinder. The extract was prepared by taking 40 g of powdered leaves in a 500 mL round flask with 300 mL of sterile Millipore water. Then the above was boiled for 10 min and sieved and filtered twice by using Watman filter paper No 42. The filtrate was collected and stored at 4 °C and used within a week. Small amount of filtrate was dried at 80 °C and analyzed by FT-IR techniques [29].

Phytochemical Analysis. Qualitative phytochemical analysis of the leaf extract was performed following standard procedure available in the literature [30, 31, 32]. The result of all the analysis has been tabulated in the result and discussion section.

Nanoparticles Synthesis. Ferric (III) chloride, Ferrous (II) chloride and NaOH were purchased from CDH, and the aqueous leaves extract of *Datura inoxia* was used for the bioreduction process. To synthesize nanoparticles from *Datura inoxia*, 0.53 gm of Ferrous chloride tetra hydrate ($FeCl_2.4H_2O$, AR) and 1.11 gm of Ferric chloride hexa hydrate ($FeCl_3.6H_2O$, AR) after weighing is dissolved in 100 ml of sterile deionised water in a 250 ml beaker. The mixture is heated at 80 oC

under mild stirring. After 10 minutes when the plate of stirrer gets heartened up, 5 mL of the aqueous solution of leaf extract was added to the above solution drop wise. After few minutes the initial colour of the mixture becomes darker. Further 20 ml of 1M NaOH (0.8 gms) was measured and dissolved with sterile deionized water in a beaker and added drop wise to the solution. A change in color of the colloidal solutions and precipitation occurred, confirming green synthesis of Ferric oxide (Fe3O4) nanoparticles.

Characterization of Nanoparticles. Magnetite (Fe3O4) nanoparticles synthesized by this green method were initially examined using Carry 60 Agilent UV – vis spectrophotometer. FT-IR spectroscopy of *Datura inoxia* leaf extract and magnetite nanoparticles was carried out in the range 4000-400 cm^{-1} by Perkin Elmer FT-IR spectrophotometer which confirmed that the protein present in the extract has the ability to act as reducing agent and stabilizer for Fe3O4 nano particles forming protein coated magnetic nano-bio hybrid. X-ray diffraction and TEM images were also taken to identify the nature of crystal and particle size respectively. Thermogravimetric analysis (TGA) was performed under nitrogen atmosphere at a heating rate of 10 °C/min from room temperature up to 700 °C which further confirms the protein coating on the magnetite surface.

Result and Discussion

The *Datura inoxia* leaves material was collected from the location Latitude: 27N 48’ 15.64 and Longitude: 75E 01’ 51.36” (FET, MUST University, Lakshmangarh, Sikar district of Rajasthan province of India). The leaves were dried and later finely powdered for extraction of phytochemicals present in it (Fig. 1).

(a) (b) (c)

Fig. 1. (a) *Datura inoxia* naturally growing near the vicinity of FET, Lakshmangarh, Sikar district of Rajasthan province of India, (b) Millipore washed leaves were room dried for two weeks, (c) Leaves extract revealed brown color.

Bio-reductive green-synthesized Ferric oxide (Fe3O4) nano-bio hybrid was produced by treating ferric ions with the leaves extract of Datura inoxia. Ferric chloride was taken as the metal precursor in the present experiments whereas leaves extract act as a reducing as well as a stabilizing agent. The color change was noted by visual observation in the Schott Duran beaker which contains Ferric chloride solution with Datura inoxia leaves extract. The color of the Ferric chloride / leaves extract solution changed from light brown to dark brown after 5 min. This color change indicates the formation of Fe3O4 magnetic nanoparticles in the solution. The initial pH of the leaf extract was 5.03, whereas that of Ferric and Ferrous chloride was 3. The final pH after the completion of reaction was observed as 10.

The plant was tested for the presence of phytochemicals. The aqueous extract of Datura inoxia was evaluated for the presence of various phyto constituents by performing a series of qualitative chemical tests. Ten phytochemicals test were performed, out of which five were present in the leaf extracts of Datura inoxia (Table 1).

Table 1. Presence or absence of phytochemicals in leaves extract of Datura inoxia.

S.No.	Phytochemicals	Occurrence
1	Tannins	No
2	Saponin	No
3	Cardiac Glycosides	Yes
4	Phenolic compounds	Yes
5	Flavonoids	Yes
6	Alkaloids	No
7	Sugars	Yes
8	Proteins	Yes
9	Starch	No
10	Phytosterol	No

The phytochemicals present in the leaf extracts act as reducing agents, which include protein, ardiac Glycosides, Phenolic compounds, Flavanoids and Sugars. The aqueous Datura inoxia leaf extracts was found to have these contents, suggesting they are the most favorable starting material for preparation of protein coated Fe_3O_4 magnetic nano-bio hybrid.

The formation of Fe_3O_4 magnetic nanoparticles was further confirmed by using UV-visible spectroscopy (UV-vis), Fourier-Transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and transmission electron microscopy (TEM). The formation of the Fe3O4 nanoparticles was first monitored using UV-Vis absorption spectroscopy. The UV-Vis spectroscopy revealed the formation of Fe_3O_4 nanoparticles by exhibiting the typical surface plasmon absorption maxima at 290 nm [33]. The characteristic peak around 290 nm was obtained (Fig. 2) ; confirming the synthesis of Fe_3O_4 nanoparticles. No other peak was observed in the spectrum which confirms that the synthesized products are magnetic Fe_3O_4 only. Generally, biosynthetic methods are considered as

time consuming when compared with chemical methods. To the best of our knowledge, reaction time of 15 mins is required to prepare plant protein mediated nano-bio hybrid.

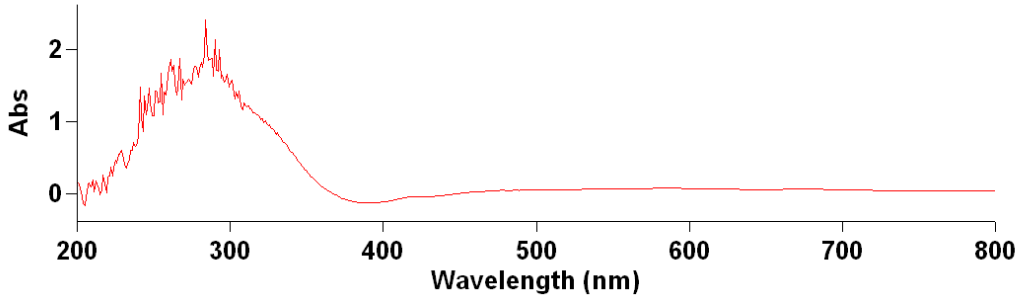

Fig. 2. UV – visible spectrum of solution after treatment with leaf extracts.

The colloidal solutions were dried in watch glasses to analyze the samples. The dried form of synthesized Fe_3O_4 magnetic nanoparticles as well dried form of leaves extract of Datura inoxia was further characterized using Fourier transform infrared (FTIR) spectroscopy. The FTIR measurement was carried out to identify the possible biomolecules responsible for capping and reducing agent for the Fe_3O_4 magnetic nanoparticles synthesized by leaves extract of Datura inoxia. The FTIR study confirms the protein coating on nano particle forming protein coated magnetite nano-bio hybrid. FTIR spectroscopy was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation (Table 2 and Table 3). Fig. 3, shows FTIR spectra of Datura inoxia powder and Fe3O4 magnetic nanoparticles. The shift in band from 3417.44 cm-1 to 3437.14 cm-1 shows the involvement of the -OH group in the stabilization process. The strong band at 1640.11 cm^{-1} and the shoulder peak at 1411.06 cm-1 are identified as the amide I and amide II of the protein, which arise due to -C=O and -NH stretching vibrations in the amide linkage of the protein. The shift of the band from 1,640.11 cm-1 to 1,639.21 cm^{-1} was attributed to the binding of a -C=O group with the nanoparticles.

Table 2. FTIR bands and functional groups of dried extracts of Datura inoxia.

BANDS(cm^{-1})	FUNCTIONAL GROUP	TYPE OF VIBRATION	INTENSITY
3417.44	Alcohol OH	Stretch, H-bonded	Strong broad
1640.11	Amide C=O	Stretch	Medium-weak,multiple bonds
1411.06	NH	Stretch	Medium-weak,multiple bonds
1385.65	Alkyl halide C-F	Stretch	Strong
1321.45	Alkyl halide C-F	Stretch	Strong
1110.26	Ether C-O	Stretch	Strong
837.45	Alkene =C-H	Bending	Strong
765.09	Alkyl halide C-Cl	Stretch	Strong
617.97	Alkyl halide C-Cl	Stretch	Strong
521.92	Alkyl halide C-Br	Stretch	Strong

Table 3. FTIR bands and functional groups of magnetite nanoparticles.

BANDS(cm^{-1})	FUNCTIONAL GROUP	TYPE OF VIBRATION	INTENSITY
3437.14	Alcohol OH	Stretch, H-bonded	Strong broad
1639.21	Amide C=O	Stretch	Strong
1564.26	Aromatic C=C	Stretch	Medium-weak, multiple bonds
1414.04	Assymetric COO-	Stretch	Strong
1020.48	Symmetric COO-	Stretch	Strong
583.45	Fe-O	Stretch	Strong

FT-IR spectroscopy confirmed that the leaf extract has the ability to act as reducing agent and stabilizer for magnetite nanoparticles. The peaks at 1414.04 and 1020.48 cm^{-1} are attributed to the asymmetric and symmetric stretching vibration of COO-. The FTIR spectrum of DI powder has a characteristic stretching vibration band at 1321.45 cm^{-1} denoting the asymmetric stretching vibration of the Amine -C-N group, which disappeared after synthesis of Fe_3O_4 magnetic nanoparticles. The band at 1110.26 cm^{-1} can be assigned to the symmetric C–O vibration associated with a C–O–CN group. The FTIR study confirms the protein coating on nano particle forming protein coated magnetite nano-bio hybrid.

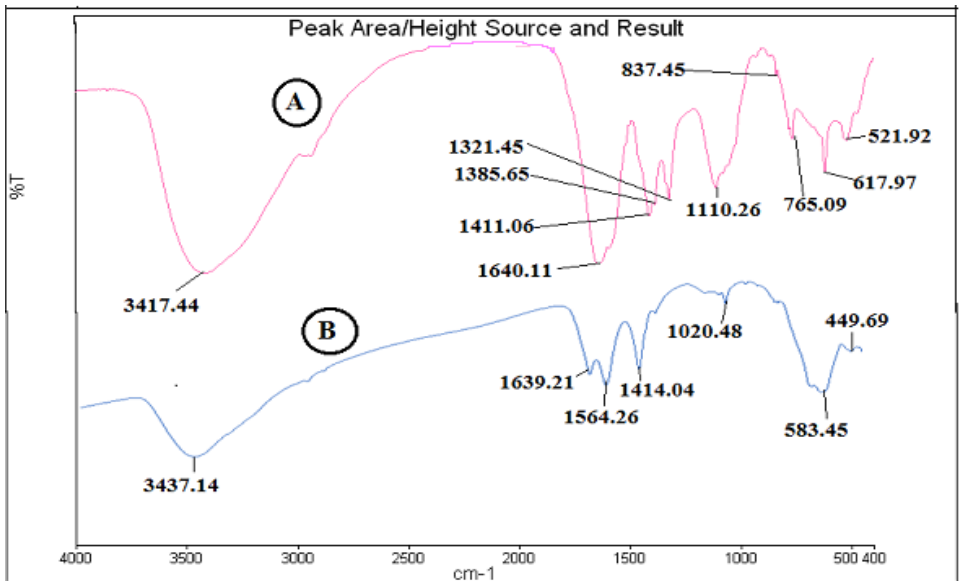

Fig. 3. (A) FTIR spectrum of dried aqueous extract of *Datura inoxia* leaves, (B) FTIR spectrum of synthesize Fe_3O_4 magnetic nanoparticles.

The presence of magnetite nanoparticles can be seen by two strong absorption bands at around 583.45 and 449.69 cm^{-1} which, corresponding to the Fe-O stretching band of bulk magnetite (Fe_3O_4). These results revealed that the C=O groups were bonded on the magnetite particle surface. Overall the observation confirms the presence of protein in leaf extract, which acts as a reducing agent and stabilizer for magnetite nanoparticles.

Ferric chloride $FeCl_3.6H_2O$ and ferrous chloride $FeCl_2.4H_2O$ and Datura inoxia leaf extract are in one aqueous phase in the reaction system. The C=O of amide group in Datura inoxia leaf extract

chelated with Fe^{3+} and Fe^{2+} to form ferric and ferrous Protein. With heating, OH- of NaOH would be involved in the reaction. A competition between of C=O….Fe^{3+} and C=O....Fe^{2+} bonds and the formation of HO^{-}…Fe^{3+} and OH-…Fe^{2+} …bonds and a result of formation of ferric hydroxide, Fe(OH)3 and ferrous hydroxide, $Fe(OH)_2$. The formation of ferric hydroxide and ferrous hydroxide form a shell core structure with Protein chain of *Datura inoxia* leaf extract as core. Ferric hydroxide and ferrous hydroxide in core dehydrated (-H_2O) forming protein coated magnetite (Fe_3O_4) nano-bio hybrid crystals. The shell of Protein of *Datura inoxia* leaf extract chains attached on Fe_3O_4 surface through chelation of C=O…Fe^{3+} and C=O…Fe^{2+} at the end of the reaction, Fe_3O_4 nano-bio hybrid crystals were capped and stabilized by Protein chain of *Datura inoxia* leaf extract. The formation mechanism has been discussed on the light of the discussion available in reference [21].

XRD is an effective characterization to confirm the crystal structure of the synthesized protein coated Fe3O4 nano-bio hybrid. Magnetite (Fe3O4) nano particles synthesized by this green method were examined by X-ray powder diffraction equipped with CuKα radiation source using Ni as filter at a setting of 45 kV/40 mA. All XRD data were collected under the experimental conditions in the angular range $5^{o} \leq 2\theta \leq 90^{o}$. Ten characteristic peaks at 27.5, 30.3, 31.8, 35.7, 43.5, 45.5, 57.4, 63.0, 75.6 and 84.1 were corresponding to the (210), (211), (211), (220), (311), (222), (331), (422), (440) and (442) crystal planes of a pure Fe3O4 matched well with that of it JCPDS file No. 82-1533 indicating that sample has a cubic crystal system with no characteristics peaks of impurities were observed (Fig. 4).

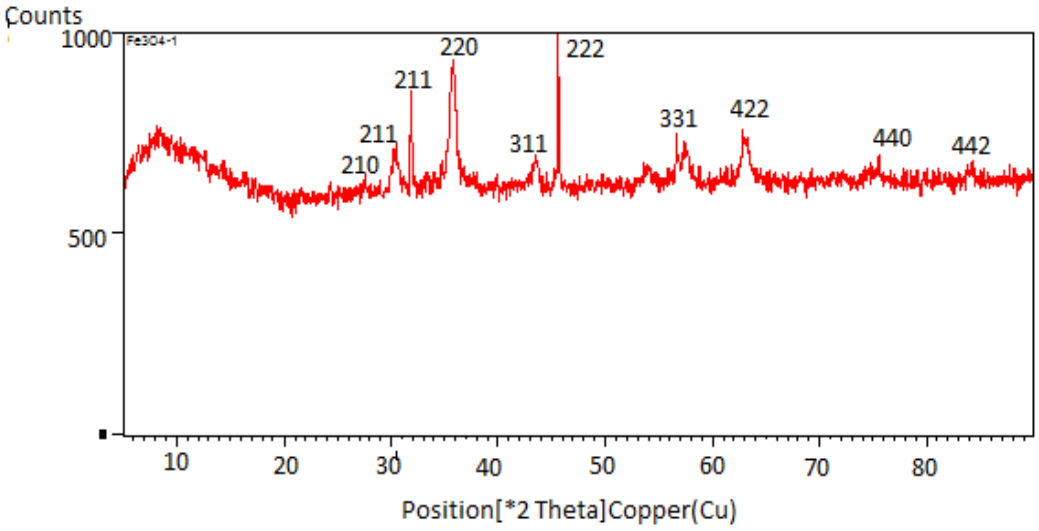

Fig. 4. XRD pattern of the synthesized protein coated Fe_3O_4 nano-bio hybrid by bioreductive method.

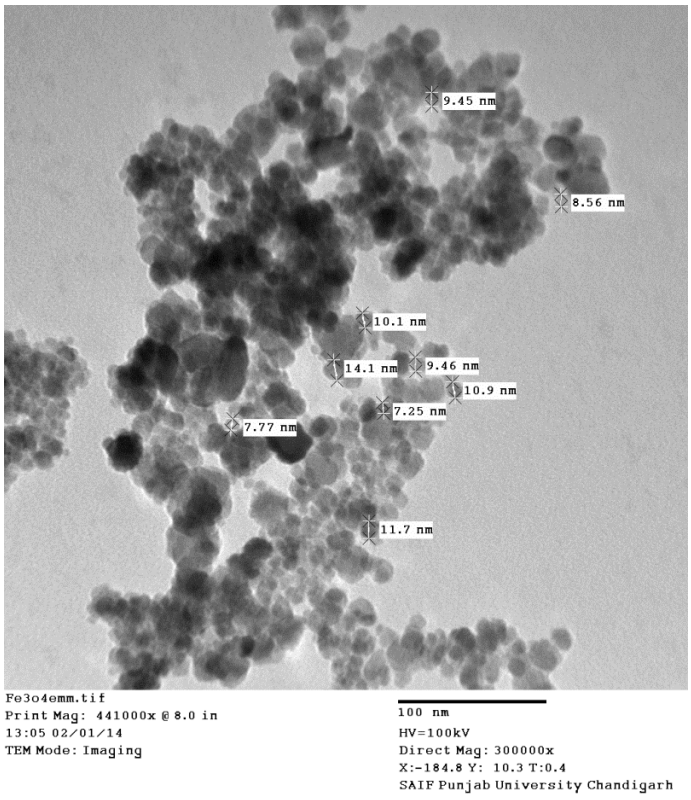

Fig. 5. TEM image of Fe_3O_4 nanoparticles (uniform size distribution and most of Fe_3O_4 nanoparticles are approximately cubic, with mean diameters ranging from 7-14 nm).

The transmission electron microscopy (TEM) micrograph for synthesized protein coated Fe3O4 nano-bio hybrid is shown in Fig. 5. The particles had a rather narrow size distribution, where most of the Fe3O4 particles were within 7-14 nm. Therefore the Fe3O4 nanoparticles were successfully synthesized by this green method using Datura inoxia leaf extract as reducing agent and stabilizer forming protein coated magnetic nano-bio hybrid.

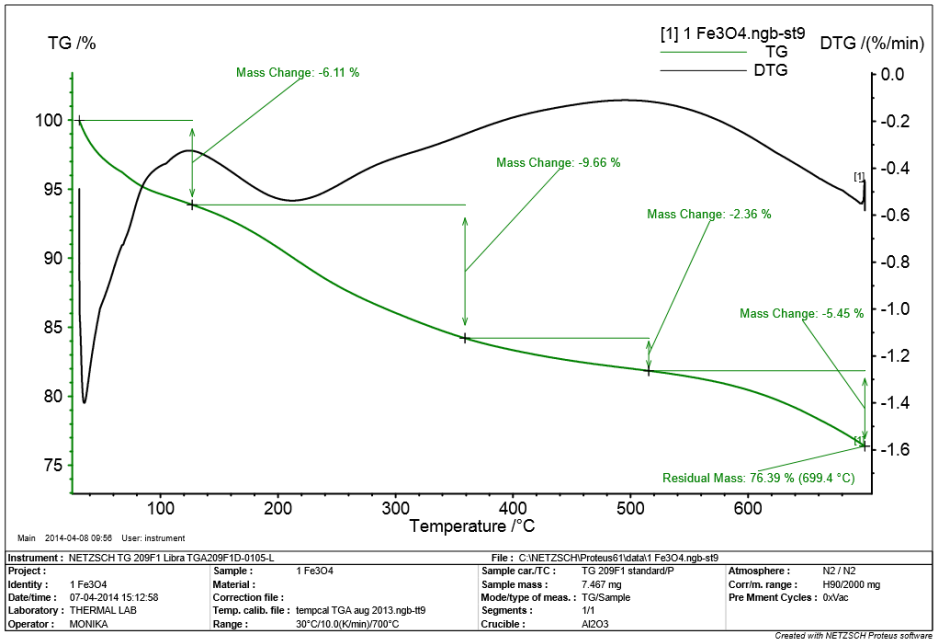

Fig. 6. Thermogravimetric analysis (TGA) results of magnetic nanoparticles.

Fig. 6 revealed the TGA results for these magnetic nanoparticles. An initial weight loss of 6.11% around 120 °C was occurred, followed by 9.66%, 2.36% and 5.45% at 340 °C, 510 °C and 699 °C respectively. The final residual left was 76.39%, thus confirming that the protein was conjugated to the magnetic naoparticles forming the nano-protein hybrid. The initial weight loss of magnetite nanoparticles powder under 100 °C is likely to be caused by the contained water. The protein of Datura inoxia leaf extract decomposes completely at temperature higher than 600 °C and the residual weight of magnetite nanoparticles is 76.39 % at 700 °C. The results of TGA illustrated that there is amide I and amide II in Datura inoxia leaf extract in the magnetite nanoparticles with

weight is around 34%. Overall the TGA demonstrated that Datura inoxia leaf extract existed on the surface of magnetite nanoparticles.

Conclusion

In the present study we report a green approach for the synthesis of protein coated Fe3O4 magnetic nano-bio hybrid using leaves extracts of Datura inoxia containing protein which have been found to be very effective stabilizing agent by forming a coating on the surface of the nano particle besides being acting as reducing agent for the formation of magnetite nano bio-hybrid. The Datura inoxia aqueous leaves extract appears to be environmentally friendly, so that this protocol could be used for rapid production of Fe3O4 magnetic nanoparticles. This is a simple, green and efficient method to synthesize Fe3O4 magnetic nanoparticles at room temperature without using any harmful reducing agents and any capping or dispersing agent. In the future, selection of such plants may create a new platform for realizing the potential of herbal medicines in nano science for drug delivery and biomedical application. This is a preliminary study of the biological mechanism for biosynthesis of nanoparticles.

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