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ORIGINAL RESEARCH

Azadirachta indica influenced biosynthesis of super-paramagnetic iron-oxide nanoparticles and their applications in tannery water treatment and X-ray imaging

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Abstract

Super-paramagnetic iron-oxide nanoparticles (SPIONs) have been exploited from a very long time and are researched profoundly due to their unique properties. In this study, SPIONs were synthesized using environmentally accepted green synthesis process where *Azadirachta indica* leaf extract was used as one of the reducing agents along with basic metal precursors used in chemical processes. The synthesized SPIONs were characterised using microscopic analysis (SEM, TEM, and AFM), spectroscopic analysis (FT-IR, UV–Vis, XRD, Raman, and zeta potential), and magnetometry (SQUID). The SPIONs were then tested for its application in the removal of heavy metals from tannery waste water and also X-ray imaging.

Keywords SPIONs · X ray imaging · Heavy metal removal

Introduction

Nanoparticles (NPs) of size ranged between 1 and 50 nm have tremendous impact on various disciplines of science [1, 2]. These particles possess unique characteristics when compared to its precursor such as optical, magnetic, electrical, electrocatalytic, hardness, strength, etc., due to its relatively smaller surface-to-volume ratio [3], and being applied in face creams, cosmetics, sports equipments, textiles, cell phones, and many other industrially important products [4]. Nanotechnology is exploited in the field of biomedical application for imaging, targeted drug delivery, biosensors, treatment, etc. [5, 6]. Apart from these, it has also found applications in environmental for cleaning up environmental pollutants [7], dye removal [8–10], waste water treatment [11], heavy metal removal [12], etc. Among the nanoparticles, specifically metal-derived nanoparticles like iron-oxide nanoparticles have pinned a lot of interest among the research community. Super-paramagnetic iron-oxide nanoparticles (SPIONs) can be of different chemical makeup, such as magnetite (Fe_3O_4) or α-Fe_2O_3 (hematite) and γ-Fe_2O_3 (maghemite), and they have high magnetization effect under the magnetic field. However, this super-paramagnetic effect of SPIONs is size-dependent [13].

SPIONs are synthesized using chemical co precipitation method [14] which is known as one of the simplest methods than the other procedures like thermal decomposition, microemulsion, hydrothermal synthesis, sonochemical synthesis [15], pyrolysis, template-assisted synthesis, and sol–gel method [16], and is one of the easiest procedures. Usually, in chemical synthesis, the iron precursors are mixed together and reduced using a reducing agent like NaOH, TMAOH (tetramethylammonium hydroxide), NH_3, etc. [17, 18]. Use of such chemicals in the synthesis of nanoparticles may be one of the many reasons for high toxicity of iron-oxide nanoparticles towards the environment [19]. Hence, plant extracts are used as reducing agents in the synthesis process, called green synthesis [20], since they are easily available, nontoxic, and have a broad variety of metabolites that help in reduction of ions [21]. In this study, *Azadirachta indica* was used as one of reducing agents along with other chemicals in producing SPIONs. Using the plant extract as one of the reducing agents is expected to reduce the toxicity of iron oxides as well as increases its application. Iron-oxide nanoparticles are efficient absorber of heavy metals such as

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Cd, Pb, Cu, Zn, Ni, and Cr [11, 22]. Tannery waste water contains heavy metals like lead (Pb), cadmium (Cd), copper (Cu), chromium (Cr), etc. [23]. Even there are reports there for the presence of chromium in drinking water [24]. Thus, the produced nanoparticles were used for heavy metal removal and also as a contrasting agent in X-ray imaging of chicken egg.

Materials and methods

Chemicals used

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and iron(II) chloride heptahydrate ($FeCl_2 \cdot 7H_2O$) purchased from LOBA-CHEMIE and sodium hydroxide purchased from SRL, India were used in this study. Fifty Gauss strength magnets were utilised for removal of SPIONs.

Synthesis of iron-oxide nanoparticles

Preparation of *Azadirachta indica* leaf extract

Azadirachta indica leaves were collected from Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India, and washed thoroughly using distilled water. They were shade dried and ground to powder. 10 g of powdered *Azadirachta indica* leaves were subjected to aqueous extraction by adding 100 ml of deionized sterile water. The mixture was kept in shaker for 24 h and then filtered using Whatman No. 1 filter paper. The resultant filtrate was used in the synthesis of iron-oxide nanoparticles.

Synthesis of SPIONs by coprecipitation method

0.12 g of $FeCl_3 \cdot 6H_2O$ and 0.6 g of $FeCl_2 \cdot 7H_2O$ were taken separately in 50 ml of nitrogenized MilliQ water. These two solutions were mixed together in the ratio of 2:1 and heated at 60 °C for 5 min under continuous stirring. Solution was allowed to cool and 10 ml of prepared *Azadirachta indica* leaf extract was added slowly with continuous stirring; following that, 25 ml of 0.1 N NaOH was added dropwise as stirring was continued [25]. The solution was left undisturbed until the black-coloured precipitate was obtained, which was separated using a magnetic bar, washed thrice with MilliQ water, and lyophilized to obtain powdered particles. These particles were used for further studies.

Characterization of SPIONs

The produced SPIONs were subjected for UV–visible spectroscopy analysis (UV 3600, Shimadzu), Fourier transform infrared spectroscopy (FT-IR) analysis (IR Affinity-1s, Shimadzu), scanning electron microscopy (FeSEM) (Zeiss Ultra55), transmission electron microscopy (TEM) (TEECNAI G2 Spirit Biotwin), atomic force microscopy (AFM) (Bruker), Raman spectroscopic analysis (LabRam HR 800), X-ray diffraction spectroscopy (XRD) (Smart lab, Rigaku), X-ray photon spectroscopy (XPS) (Ultra DLD-Kratos), zeta potential analysis (Brookhaven ZetaPALS), and superconducting quantum interference device (SQUID) analysis.

Heavy metal removal analysis

50 ml of raw heavy metal water from tannery waste was collected. 25 ml was digested with 10 ml nitric acid, and after digestion, the sample was made up to 50 ml with MilliQ water and water sample was given for ICP-OES analysis (Perkin Elmer 2000DV) to detect the initial concentrations of lead, copper, and cadmium heavy metals present in the polluted water.

The other 25 ml collected polluted water added with 0.01 g of SPIONs and mixed continuously for 10 min and allowed to react with water for 1 h. Later, these SPIONs were removed using a magnetic bar and the water was processed again as described earlier and given for ICP-OES analysis (Perkin Elmer 2000DV) to detect heavy metals.

X-ray imaging using chicken eggs

Chicken eggs from local shop were obtained and cleaned. About 1.5 ml of egg's white matter was carefully sucked out. 1 mg/ml of synthesized SPIONs in distilled water was injected inside the eggs and carefully shaken to disperse the SPIONs. The eggs were visualized under X-ray machine (Wipro G machine) with the capacity of 300 mA and the film was taken using the computerized radiography (CR) of Fujifilm FCR Prima T. The magnetic property of SPIONs was also tested by placing the magnet near the egg.

Results and discussion

Synthesis of SPIONs

Black-coloured precipitate was obtained within 15 min. SPIONs were separated using magnet, and it was washed and used for further study.

UV–visible spectroscopy analysis

The maximum absorbance was found to be around 250 nm from the graph (Fig. 1), which was the characteristic peak of SPIONs as per earlier reports [19, 26]. There was absorbance maximum near 390 nm which might be conferred by plant extract.

Fourier transform infrared spectroscopy (FT-IR)

Prominent bands in the IR range less than 569 cm^{-1} were considered as the finger print region for iron-oxide nanoparticles depicting the Fe–O bonds in SPIONs [27]. Bands for O–H stretching were for adsorbed water [28, 29] (Fig. 2). Other peaks might be assumed for the polyphenols in the *Azadirachta indica* leaf extract which may function as reducing agent as well as capping agent.

Scanning electron microscopy (FeSEM)

SPIONs produced were in the range of 25 nm (Fig. 3). Awwad and Salem [25] successfully produced 5–8 nm-sized magnetite using Carob leaves extract.

Transmission electron microscopy (TEM)

Iron-oxide nanoparticles were found to be on par with the SEM results (Fig. 4).

Atomic force microscopy (AFM)

The SPIONs' size range was recorded to be around 25 nm, which was supporting the TEM and SEM image (Fig. 5).

Raman spectroscopy analysis

The following peaks at 240 rcm^{-1}, 260 rcm^{-1}, and 500 rcm^{-1} were assigned for E_g mode where the peak at 600 rcm^{-1} was for A_{1g} mode which stood for Fe_3O_4 within verse-spinel crystal structure (Fig. 6) as reported earlier [28, 30, 31].

X-ray diffraction analysis (XRD)

The sites and intensity of the diffraction peaks at 220, 311, 400, 511, and 440 were for cubic spinel structure of Fe_3O_4 (JCPDS 85-1436) [32] (Fig. 7).

X-ray photon spectroscopy (XPS)

From the obtained XPS images, binding energy of $Fe_2p_{3/2}$ was occurring at 711.8 eV, where the binding energy for FeO and Fe was seen at 709.6 and 706.7 eV, respectively. The peak at 531 was suggestive of metal oxide [28]. Binding energy for C–C was observed at 285.8, where it was 289.5 eV for O–C=O, thus, suggesting the molecular carbon's presence (Fig. 8).

Zeta potential analysis

Zeta potential of the plant-derived SPIONs was found to be −38.62 mV (Fig. 9). Particles with value between −30 and +30 mV are stable [17]. The value obtained for SPIONs in this study did not fall in between the desired range, it could

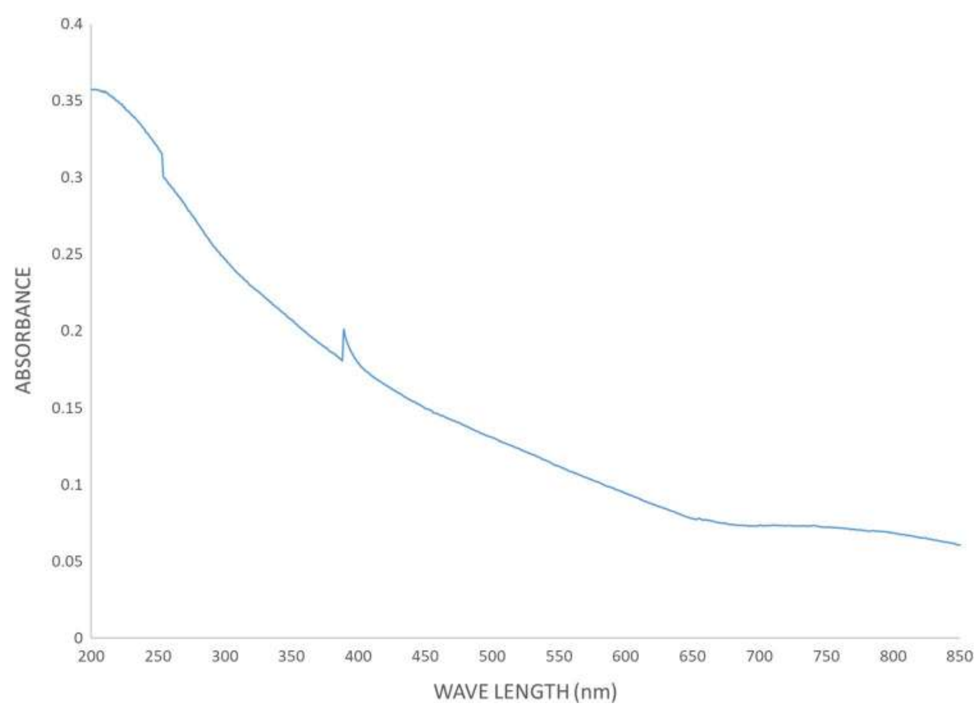
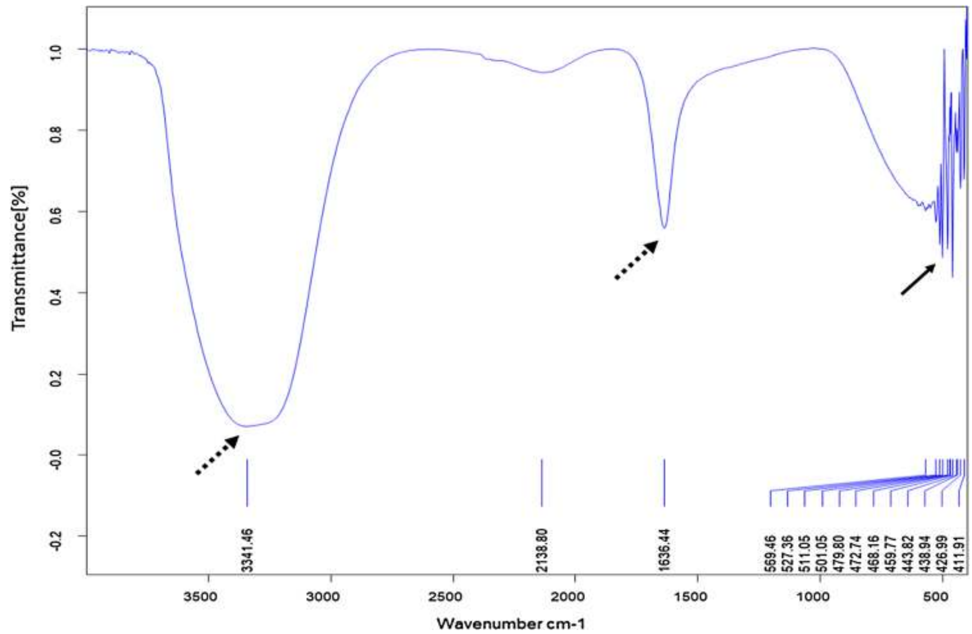


Fig. 1 UV–visible spectroscopy analysis of SPIONs

Fig. 2 Fourier transform infrared spectroscopy analysis of SPIONs. Dotted arrow represents OH stretching; bold arrow represents Fe–O bond



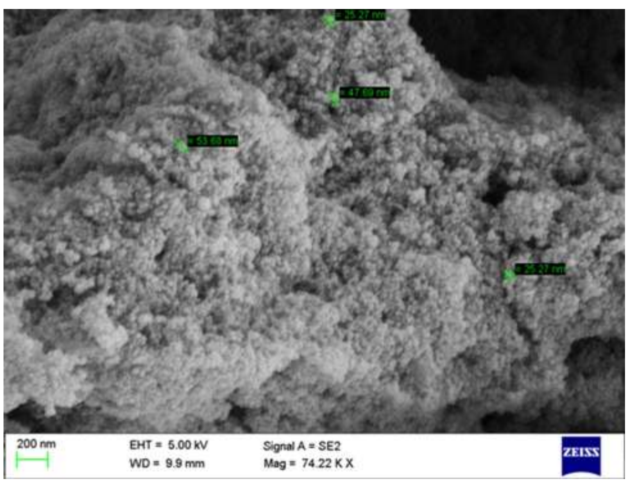


Fig. 3 Scanning electron micrograph of SPIONs

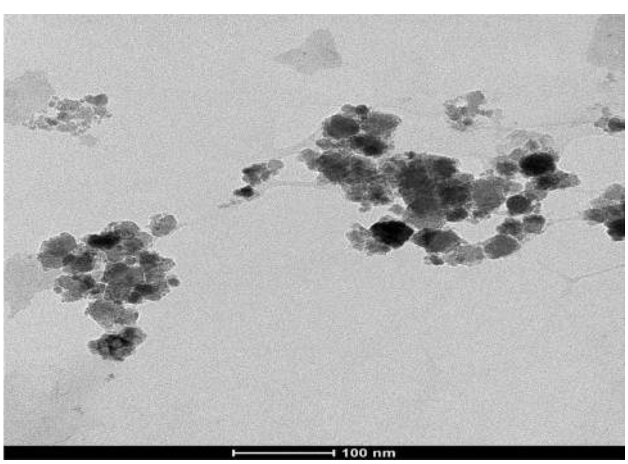


Fig. 4 Transmission electron micrograph of SPIONs

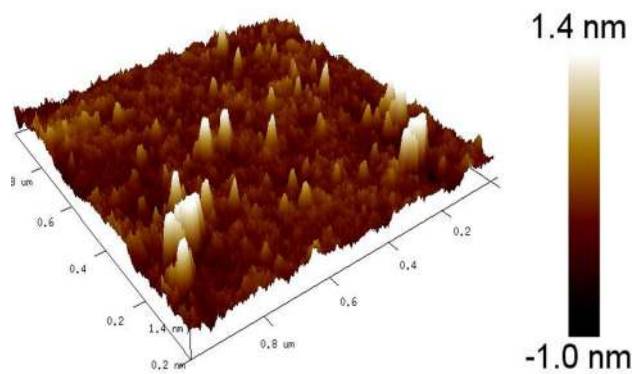


Fig. 5 Atomic force microscopy of SPIONs

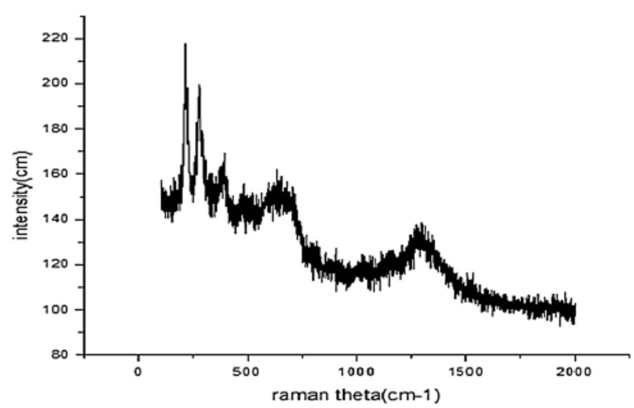


Fig. 6 Raman spectroscopy of SPIONs

be interpreted that the SPIONs were mildly unstable and there might be chances of slight agglomeration [33, 34].

Superconducting quantum interference device (SQUID)

The magnetic moment was steadily increasing up to 200 K temperature and decreased at 300 K; again, it was increasing at 300–310 K, and, finally, fell after 310 K (Fig. 10a). The reduction in coercivity was observed as the mean particle size was decreased [35]. At ZFC mode, the magnetic moment was very low, indicating that the magnetic moments were frozen at this temperature (5 K) (Fig. 10).

Inductive coupled plasma-optical emission spectroscopy (ICP-OES)

The three major metals lead (Pb), copper (Cu), and cadmium (Cd) were found in the waste water, and their concentration was observed to be reduced from 0.0020 to < 0.0000 ppm for lead, 0.0134 to < 0.0000 ppm for cadmium, and 0.1468 to 0.0022 ppm for copper after treating the water with SPIONs, as shown in Table 1. The metabolites of plants would have acted as the functionalizing agent and might have also influenced the absorption. Lanas [36] found SPIONs functionalized with 3-mercapto propionic acid to absorb heavy metals efficiently. Hence, iron-oxide nanoparticles can be used in wastewater treatment for the removal of the heavy metals.

Table 1 ICP-OES analysis of tannery wastewater and treated with SPIONs

S. no.	Line	Concentration (ppm)		element
		Untreated	Treated	
1	Pb 220.353	0.0020	< 0.0000	Lead
2	Cu 324.754	0.1468	0.0022	Copper
3	Cd 214.441	0.0134	< 0.0000	Cadmium

X-ray imaging using chick eggs

From Fig. 11, it can be concluded that the SPIONs acted as contrasting agents and have enhanced the X-ray imaging effect, thereby visualizing yolk of the chick egg clearly. Hence, SPIONs can be used as contrasting agent since iron

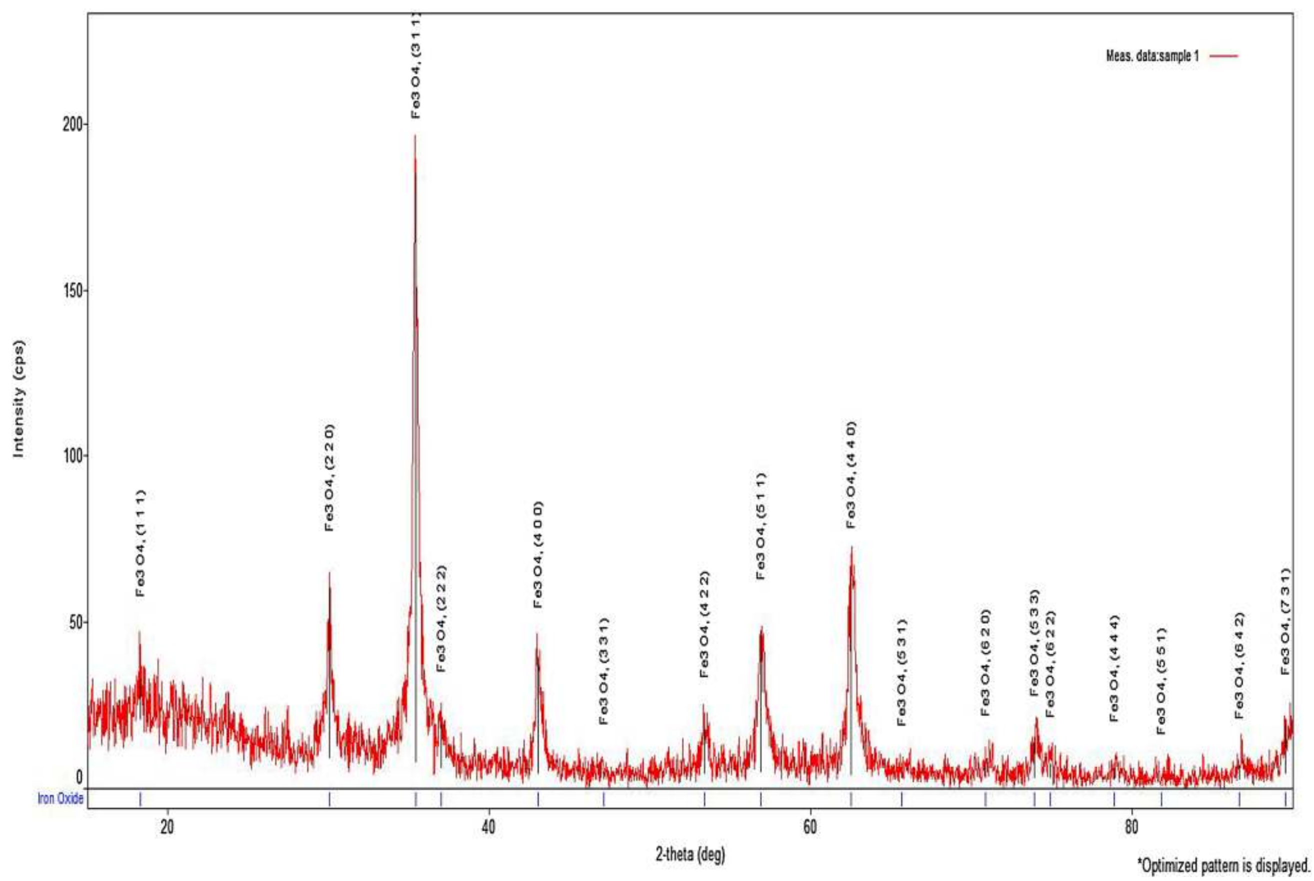


Fig. 7 X-ray diffraction analysis of SPIONs

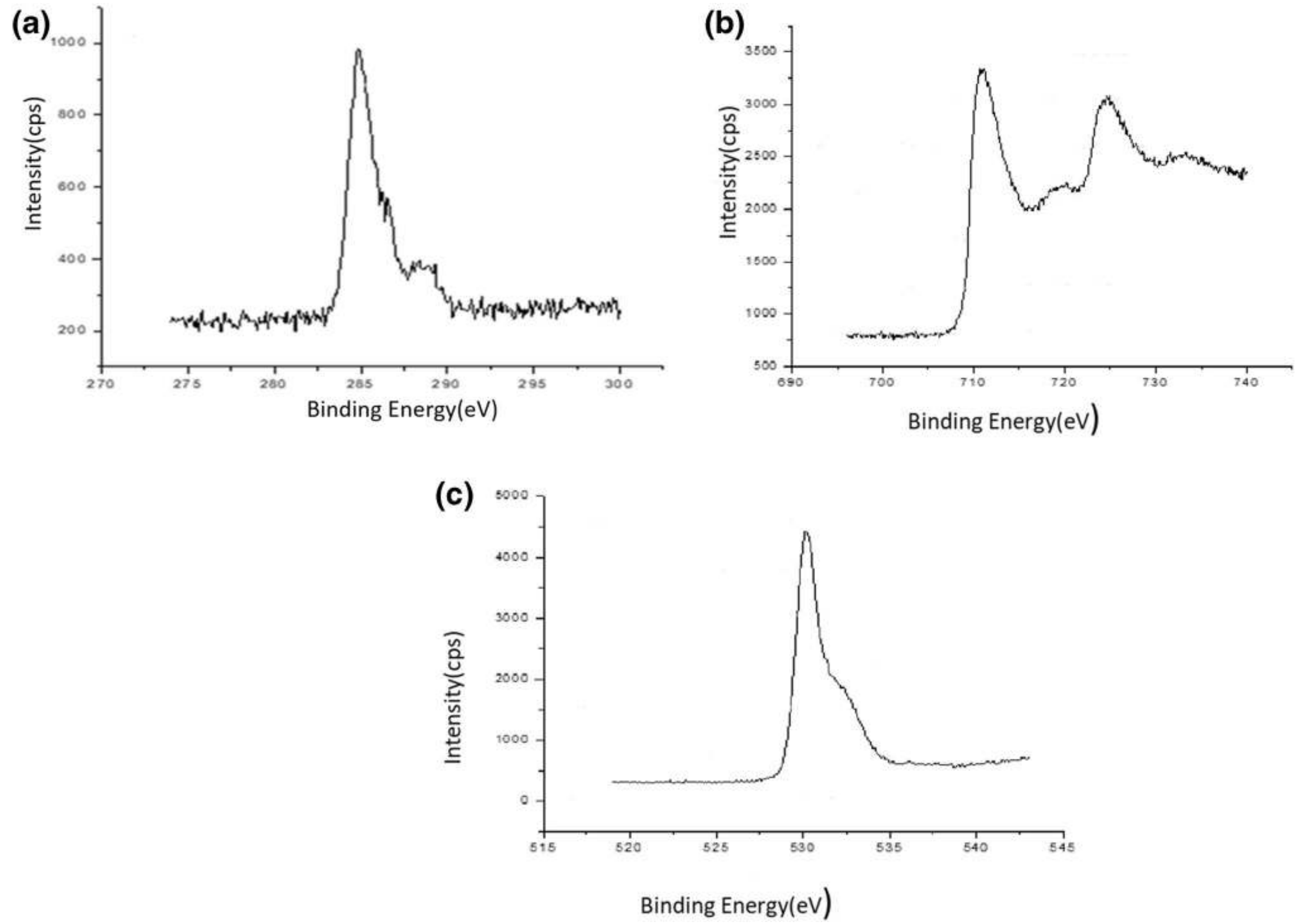
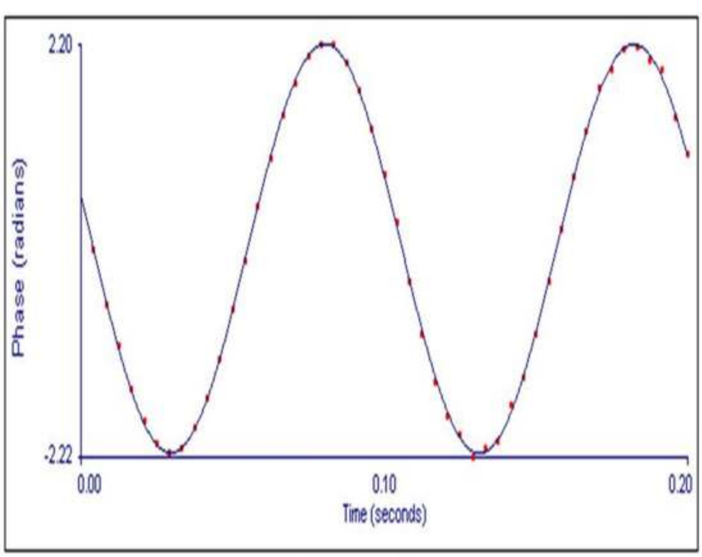


Fig. 8 X-ray photon spectroscopy of SPIONs: **a** for adventitious carbon, **b** $Fe_2p_{3/2}$, and **c** for metal oxide



Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-3.03	-38.74	0.0066
2	-3.01	-38.51	0.0068
Mean	-3.02	-38.62	0.0067
Std. Error	0.01	0.12	0.0001
Combined	-3.02	-38.62	0.0050

Fig. 9 Zeta potential of SPIONs

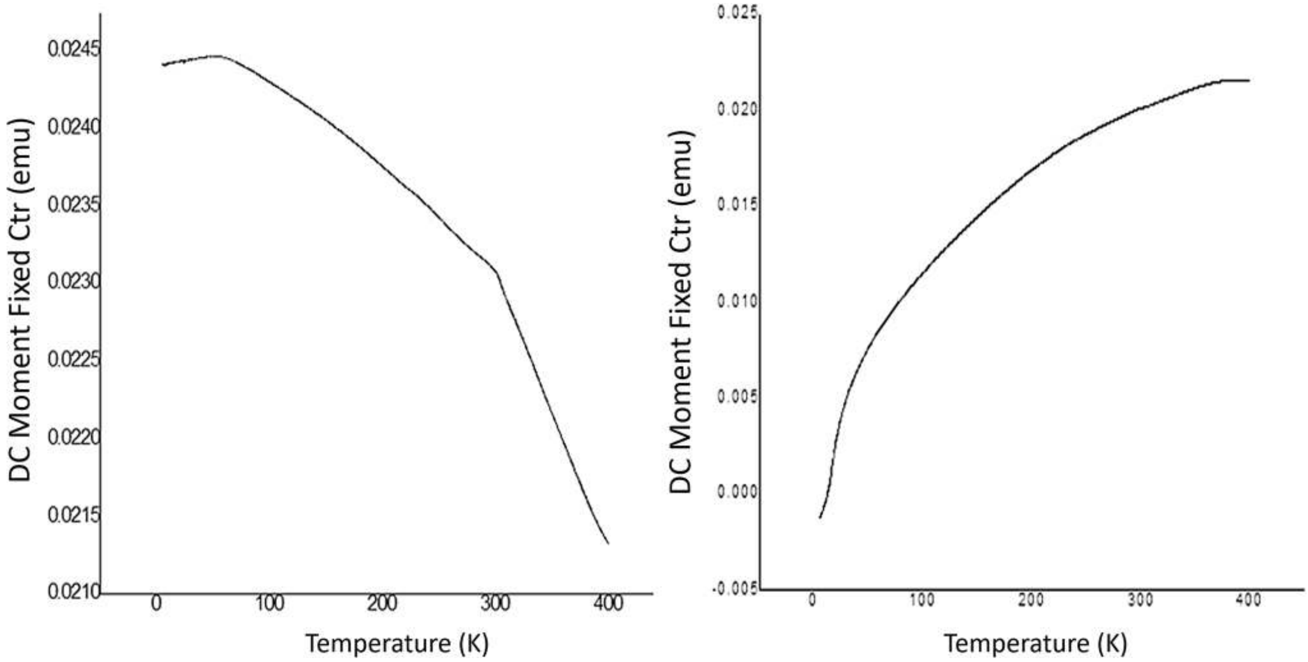


Fig. 10 SQUID analysis of SPIONs. **a** Field-cooled (FC) magnetization measurement, **b** zero-field-cooled (ZFC) magnetization measurement

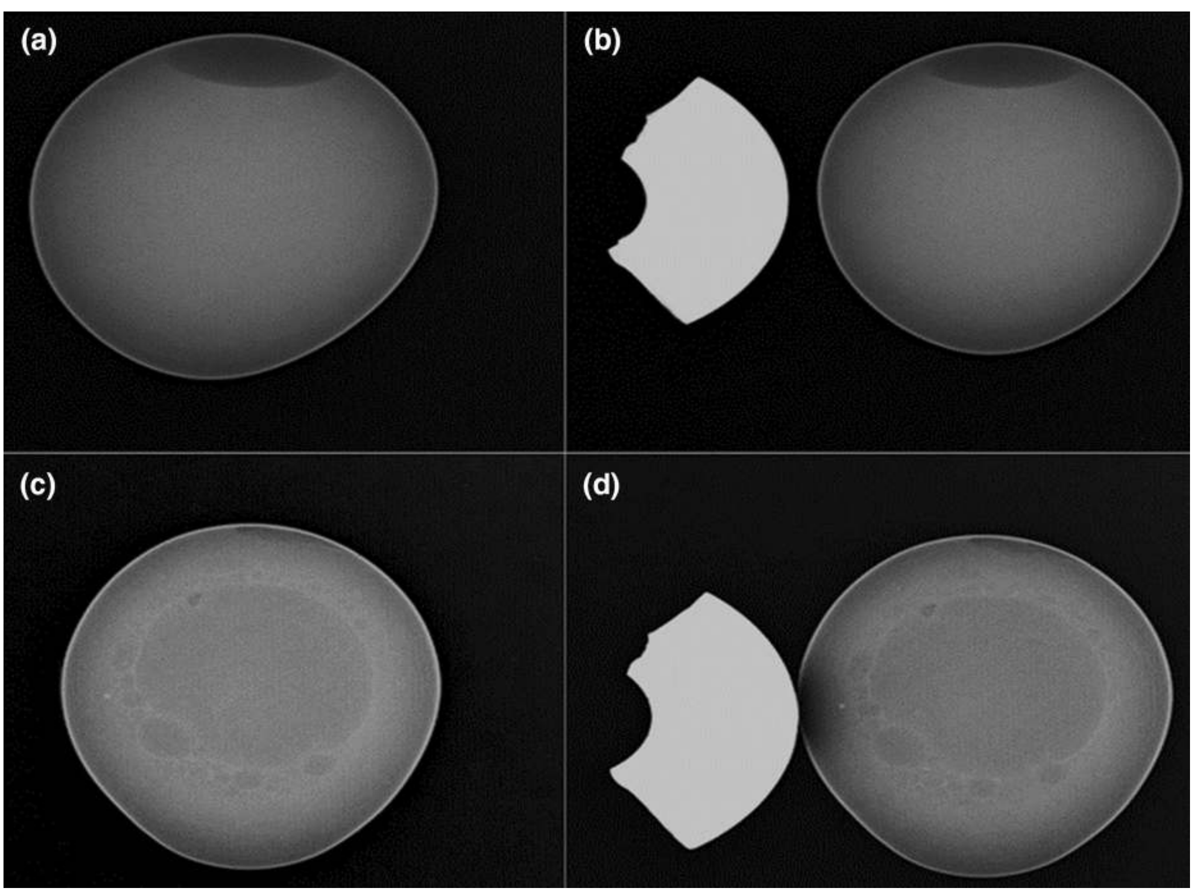


Fig. 11 X-ray imaging using chick eggs. **a** Control egg in the absence of magnetic field, **b** control egg in the presence of magnetic field, **c** egg injected with SPIONs in the absence of magnetic field, and **d** egg injected with SPIONs in the presence of magnetic field

oxides are used in living system; it is less toxic and believed to be metabolized into haemoglobin [37–39].

Conclusion

Super-paramagnetic iron-oxide nanoparticles have been synthesized using *Azadirachta indica* leaf extract as one of the reducing agents. The synthesized particles were confirmed to be in the size range of 25 nm crystalline magnetite (Fe_3O_4). SPIONs were effectively attracting the heavy metals and can be used in the treatment of wastewater for the removal of heavy metals and also as potent contrasting agents in X-ray imaging for diagnostic purposes.

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Compliance with ethical standards

Conflict of interest The authors confirm that there is no conflict of interest.

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