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green synthesis and characterizat on of iron-oxide nanoparticles by guava aqueous leaves extract for doxorubicin drug loading

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Abstract

A green biosynthesis of iron oxide nanoparticles (Fe_3O_4 Nps) was carried out in one step. An aqueous extract of orange peels, green tea, and guava leaves were utilized as precipitating agent for metal precursors. The guava leaves extract was the most powerful one. The shape and size of (Fe_2O_3 Nps) were monitored by transmission electron microscopy. The existence of iron in the yield was studied by UV-visible spectroscopy. The stability of the particles was estimated by hydrogen peroxide reaction. The (Fe_3O_4 Nps) were incubated with human red blood cells (RBCs). The osmotic fragility test for (RBCs) showed no significant shifting from the control. The loading of doxorubicin cytotoxic drug was primitively monitored by scanning electron microscopy for further study plan.

Keywords: iron-oxide nanopatcles, green biosynthesis, doxocirobcin

1 Introduction

Iron-oxide nanoparticles (Fe_3O_4 NPs) have become the strong candidates for many biomedical application due to, their small sizes beside the magnetic properties (Monalisa P et al., 2013)^{1,2}. It is important to choose the raw material for (Fe NPs) preparation otherwise the methods for the adjustable physical and chemical properties of interest. Among the methods of preparation for these (Fe NPs) coprecipitation, thermal decomposition sonochemical methods are the most. In addition electrochemical and green syntheses are introduced by many researches (Akl M et al., 2012)³.

The chemical synthetic procedures generate hazardous by products that could affect the environment directly. Thus there is a great requirement for green chemistry that includes methods which are environment friendly¹. Hence nowadays many researchers are diverting themselves towards biological systems mostly plants for nanoparticle synthesis as it is cost effective and can be easily scaled up to be used for large scale production (Iy-er A et al., 2009)^{1,4}.

The cellular extracts from these biological organisms (such as plant and micro-organism) can be used to synthesize nanoparticles of different size and chemical compositions as they contain certain oxidizing compounds like polyphenol which can reduce metal ion precursor whereas water soluble heterocyclic components stabilize the metal nanoparticles formed. This Biosynthesis of metal nanoparticles extracted from different parts (mostly leaf) of the plant is the most effective process of synthesis at a very affordable cost¹⁻⁵

Entrapping nanoparticles with drugs is a great challenge nowadays. FDA approved doxorubicin hydrochloride drug as liposome based has been used for treatment of cancer (XU et al., 2013)⁶. In the current work, we utilize the aqueous Guava leaves extract to produce iron-oxide oxide nanoparticles. The shape and size of these Fe NPs are observed by Transmission electron microscopy. The Doxocirobcine drug was loaded on the prepared MNPs and the rate of drug loading efficiency was evaluated.

2 Materials and Methods

Preparation of plant extract

Fresh Leaves of guava, green tea, and orange plants were collected from the local markets in Alexandria city, Egypt. The leaves were washed twice with distilled water after that they were left to dry. 200 mg of dried leaves were squashed and incubated in 100

ml double distilled water in 250 ml beaker overnight. The aqueous leaf extract taken and the leaves debris were discarded. The leaves extract was centrifuged then filtered by Whatman filter paper twice to exclude any remnant debris. The clean aqueous extract was preserved in -20 °C for further use.

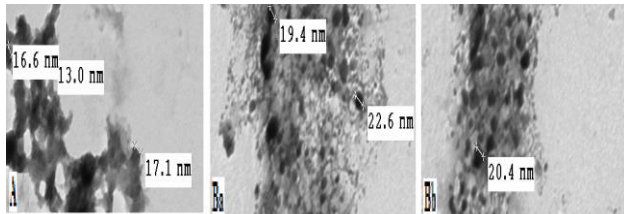


Figure 1: Scanning electron photographic images and corresponding approximate particle sizes, (A) for free Fe₃O₄, (Ba,Bb) Fe₃O₄-Doxo. Loaded particles.

Characterization of iron-oxide nanoparticles

Ultraviolet-Visible absorption (UV-Vis)

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy in the UV-Visible region. This means it uses light in the visible and adjacent (near-UV and near-infrared) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions.

Fourier transformer Infrared analysis (FT-IR)

FTIR was used to identify the peak value of the functional groups of the active components by infrared irradiation. The sulfate group is responsible of the reduction of metal ions via oxidation of aldehyde groups in the molecules to carboxylic acids^{7,8}. The UV Visible spectrum of Fe₃O₄-NPs in the aqueous guava extract is shown in. The two absorption peaks at wavelengths of 402 nm and 415 nm indicate the formation of iron nanoparticles

3 Results and discussion

Determination of total antioxidants

The total antioxidant activity (TAA) of plant leaves aqueous extract were determined by β-carotene bleaching method adopted from Kaur C et al⁹. Oxidation of the β-carotene emulsion was assessed by spectrophotometer at 10-min interval at 470 nm. TAA was expressed as per cent β-carotene inhibition relative to control according to Equation 1

$$TAA = \frac{\text{Absorbance (control - sample)}}{\text{Absorbance of control}} \quad (\text{The total antioxidant activity (TAA) of plant leaves aqueous extract})$$

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Synthesis of Fe NPs

The (Fe₂O₃) were synthesized using a constant volume of the plant extract with ferrous sulfate solution (2 mM), with concomitant stirring at room temperature. The mixture was sonicate to apart the plant debris. The appearance of dark color indicates the formation of (Fe₃O₄) nanoparticles Figure 1.

Table 1: The percentage of TAA in different green leaves extract included in the current work. The values expressed as mean ±SD for duplicates p.>0.05.

Leaves extract	(TAA) (mg/100ml)
Guava extract	50±6
Green tea	38±10
Orange leaves	15 ±8

The preparation procedure was adopted from the previous work of (Akl M. Awwad et al., 2012¹⁰ and Harajyoti M¹¹ et al). Ferrous sulphate solution of 2 m

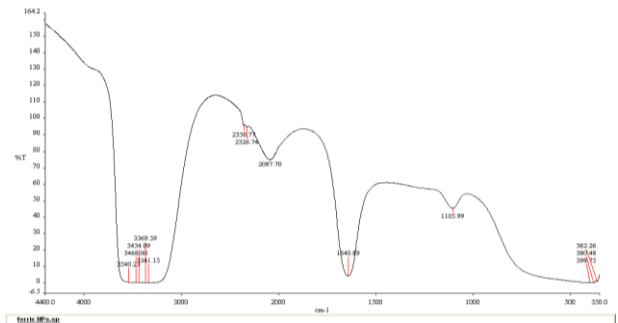


Figure 2: Outprint of FTIR representing the main peaks of functional groups of Fe₃O₄

M concentration was pH was about 5.5. a 100ml FeSO₄ solutions was taken in a 250ml conical flask to which 10 ml of plant leaves extract was added with continuous shaking . The (Fe₃O₄ NPs) were formed by the appearance black color of the solution. The nanoparticles were then precipitated by centrifuging at 13,000 rpm for 15 mins and stored at -4°C for further use.

Osmotic fragility test

Fragility of red blood cells (RBCs) was estimated after incubation of the cells with iron-oxide

nanoparticles. The osmotic fragility was adopted from H. A. Massaldi, et al incubated with BSR20 ul of .¹²⁾ different concentration of Fe₃O₄ nanoparticles for about 6 hours at 37 °C was added to a serial dilution of normal physiological saline solution with different osmolality % from the following Equation 2:

$$\text{Hemolysis\%} = \frac{\text{absorbance of the test}}{\text{absorbance of complete hemolysis}} \times 100$$

Fragility of red blood cells (RBCs) was estimated after incubation of the cells with iron-oxide nanoparticles. The osmotic fragility was adopted from H. A. Massaldi, et al). 20 ul of RBS incubated with different concentration of Fe₃O₄ nanoparticles for about 6 hours at 37 °C was added to a serial dilution of normal physiological saline solution with different osmolality % from the following Equation 2:

DOXO drug loading to MNPs

Loading procedure was adopted from (Davaran et al., 2012)¹³. 2 ml of DOXO. was added to 1 gm of dried MNPS. The mixture was stirring magnetically for 24 hours at room temperature .the Doxo-loaded MNPs was separated with centrifugation for further analysis.The percent of Doxo.loading were deduced by the relation Equation 3

$$\text{Doxo.loading efficiency} = \frac{\text{weight of Doxo. after incubation}}{\text{weight initial Doxo.}} \times 100$$

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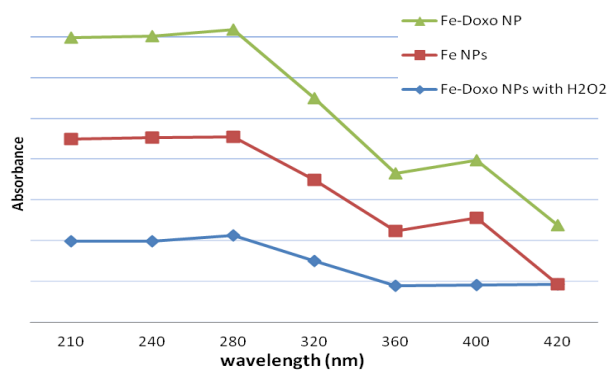


Figure 3: Graphic distribution of absorbance versus the corresponding wavelength showing the maximal absorbance peak.

Fe₃O₄ NPs -H₂O₂ oxidation

Nanoparticle resistance to oxidation was measured by UV-vis before and after the addition of 100 µl of 30% H₂O₂ to 5ml nanoparticle suspension for five minute. as shown **Figure 3**, there is no shift in absorbance peak neither plain Fenps or Fe-Doxo Nps^{3,14}.

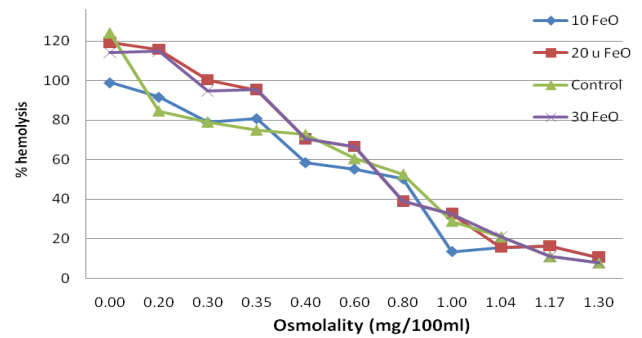


Figure 4:graphical representation of hemolysis percentage of RBCs versus concentration rang of normal saline for control (plain RBCs) and RBCs incubated with Fe₃O₄ of 0.1, 0.2 , and 0.3 % (v/v).the tests were replicated twice represented as mean±SD and pvalue>0.05.

The peaks at 1540 and 1105 cm⁻¹ are attributed to the asymmetric and symmetric stretching vibration of COO⁻¹⁰.The band at 1105.99 cm⁻¹ can be assigned to the symmetric C–O vibration associated with aC–O–SO₃ group . In addition, signals at 3698 cm⁻¹ (OH stretching) and 2358 ad 23269 cm⁻¹ (CH stretching) were also observed^{10,15}.

The presence of magnetite nanoparticles can be seen by two strong absorption bands at around 398 , 380, and 362 cm-1 which **Figure 2**, corresponding to the Fe-O stretching band of bulk magnetite (Fe₃O₄)¹⁶. These results revealed that the C=O groups were bonded on the magnetite particle surface. Overall the observation confirms the presence of organic compounds in guava leaf extract, which acts as a reducing agent and stabilizer for magnetite nanoparticles. These results are in co-ordinance with the results of Mahnaz M et al.^{10,7}

The osmotic fragility results are represented in **Figure 4**. there is no significant skewness of the graph and control graph without Fe₃O₄ NPs .this finding may support that Fe₃O₄ NPs have no osmotic stress on the RBCs integrity under these conditions.

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