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Speciation, Preconcentration and Determination of Iron in Legumes Sample Based on a Novel Magnetic Mixed Hemimicell Solid Phase Extraction Technique

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ABSTRACT
A novel magnetic mixed hemimicell solid phase extraction (MMHSPE) technique for speciation analysis of soluble, ferrous and ferric iron in legumes sample by flame atomic absorption spectrometry analysis (FAAS) was developed. MMHSPE system consisting of alumina-coated magnetite nanoparticles (Fe_3O_4/Al_2O_3NPs) modified by sodium dodecylsulfate-1-(2-pyridylazo)-2-naphthol (SDS-PAN) have been successfully synthesized as an extracting agent. The procedure is based on complexation of Fe(II) with PAN that immobilized on the SDS-coated Fe_3O_4/Al_2O_3 NPs. Total iron is subjected to a similar extraction procedure after reduction. Then Fe(III) has been calculated by subtracting Fe(II) from the total iron. The new and rapid method of analyses (MMHSPE technique) has been successfully applied for the determination of iron ions in certified reference materials (NCS DC 73349—bush, branches and leaves; and TM-23.2—fortified water) and legumes samples with high efficiency. Under the optimum conditions of parameters, the recoveries of Fe(II) by analyzing the seven spiked some legumes samples were between 96.0% and 103.6% and detection limits of Fe(II) were between 1.7 and 3.1 ng mg^{-1}. The results have been also indicated that Fe_3^{+} concentrations obtained are always higher than the Fe^{2+} contents in legumes sample and Soy bean snack and Peanut have the maximum content of Fe^{2+}.

KEYWORDS: Alumina-Coated Magnetite Nanoparticles; Magnetic Mixed Hemimicell Solid-Phase Extraction; Speciation; Fe(II); Fe(III);. Iron in Legumes Sample.

1. INTRODUCTION

Iron is an essential nutritional element for all life forms, i.e. it is a cofactor in many enzymes and essential for oxygen transport and electron transfer [1]. Although some food products contain iron, but the nutritional value of a food of a given mineral depends not only on the mineral content, but also on its bioavailability for humans. In the case of iron, the effect that its solubility in water, oxidation state and extent of complex formation has on its bioavailability has been evaluated [2]. It is generally accepted that only soluble nonheme iron can be absorbed; thus, only a fraction of the soluble iron is available [3]. So, it is well known that iron (II) is more available than iron (III), because the latter has a low solubility in the gut. However, iron (III) can be reduced to the more soluble iron (II) in the gut by the action of gastric hydrochloric acid and reducing agents, such as ascorbic acid [4]. Therefore, chemical speciation has gained an increasing interest in environmental and toxicological analyses. The most important reason, therefore, lies in the fact that the toxicity of an element, its biological availability and transport mechanisms highly depend on the chemical form in which it appears [5].

Legumes are one of the most important food groups for people especially vegetarians because it is a good source of protein which is nutritionally available at low cost [6]. The iron content of legumes together with their high consumption in different areas of the world means that they are a good source of dietary iron for large population groups. In Iran, three species of legumes stand out for their high consumption: beans (Phaseolus vulgaris L.), chickpeas (Cicer arietinum L.) and lentils (Lens culinaris L.) .

Many methods for speciation of iron (II) and (III) has been studied with different techniques such as accelerator MS [7], ICP-AES and polarography [8], cathodic stripping voltammetry [9], capillary electrophoresis [10], flow injection chemiluminescence [11] ion chromatography [12], FAAS [13] and automated analysis [14]. However, the sample pretreatment processes in these studies, especially in samples with complicated matrixes (such as food samples), were labor intensive, time consuming and organic solvents wasting. To overcome this problem and also solve the problem of the trace concentration of iron in real samples, it is necessary to develop a simple, rapid and reliable sample pretreatment method, without filtration and centrifuges steps with highly sensitive determination for analysis of iron in foods and waters at environmentally relevant concentrations.

Recently, magnetic materials have received increasing attention. Magnetic materials as adsorbents have several advantages in comparison with traditional adsorbents. The separation process can be performed directly in crude samples containing suspended solid material without additional centrifugation or filtration, which makes separation easier and faster [15].

In this research we used, a new and rapid method of analyses by alumina-coated magnetite nanoparticles (Fe_3O_4/Al_2O_3 NPs) were successfully synthesized, then modified by SDS in acidic media to form mixed hemimicelles and using PAN as complexing reagent for Fe_2^+. These new magnetic adsorbents were applied for an accurate, fast and cheap method for the determination of the oxidation state and determination of iron (soluble, ferrous and ferric iron) in legumes sample based on MMHSPE technique assisted by ultrasonic. This study was conducted to determine iron element in seven varieties of legumes. Due to the high surface area and the excellent adsorption capacity of these nano-magnetic adsorbents, satisfactory extraction recoveries of Fe(II) ions could be produced with only 0.05 g Fe_3O_4/Al_2O_3 NPs without any matrix interferences. Moreover, the unique super paramagnetic property made these adsorbents separated from the matrix rapidly with an adscititious magnet. Compared with conventional SPE methods, the proposed method still have advantages of simple operation procedures and short analysis time. This method shows great analytical potential in processing complicated samples.

2. EXPERIMENTAL

2.1. Apparatus

A model Sens AA atomic absorption spectrometer equipped (GBC Scientific Equipment, USA) equipped with deuterium background correction with iron hollow-cathode lamps as the radiation source were used for absorbance measurements. All measurements were carried out in an air/acetylene flame. The instrumental parameters were adjusted according to the manufacturer's recommendations. The magnetic property of nanoparticles was analyzed using a vibrating sample magnetometer (VSM, JDM-13, China). The functional groups on the surface site of sorbents were detected by a Fourier transform infrared spectrometer (model FT/IR 400 type A, JASCO, Tokyo, Japan). A background spectrum was measured on pure KBr. A scanning electron microscopy (SEM) image was obtained using SEM instrument (SEM, Cambridge, S360, England). A Sonics vcx750 USA ultrasonic bath (Ultrasonic Instrument) was employed in this process. The operating frequency of the ultrasonic bath was constant and fixed at 40 kHz. The output power was 100 W. The ultrasonic assisted extraction was performed at room temperature. An Edmund Buhler GmbH model mechanical shaker was used throughout the experiments. A Hot-Air oven Memmert, model 5 ^0C, Germany was employed in this process. A digital pH meter Jenway model 3510 equipped with a combined glass calomel electrode was used for the pH adjustment.

2.2. Chemicals and materials

Iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), Aluminum isopropoxide sodium dodecyl sulfate (SDS) and all chemicals used in this work, were from Merck (Darmstadt, Germany) used for the preparation of all standard and sample solutions. Throughout all analytical work, de-ionized water was used. All glass apparatus have been kept permanently full of 1.0 mol L^{-1} nitric acid when not in use. In the speciation and preconcentration procedures, 1-(2-pyridylazo)-2-naphthol (PAN) (Merck), Pyrex glass and concentrated nitric acid (65%, Merck) were used. The iron (II) stock solutions were prepared by dissolving 0.702 g of Mohr's salt ($(NH_4)_2$ Fe $(SO_4)_2$ 6 H_2O) and 1.0 g of ascorbic acid (to prevent oxidation of Fe(II) to Fe(III)) in a volumetric flask. The Fe(III) stock solutions were prepare by dissolving $NH_4Fe(SO_4)_2.12H_2O$ in 0.1 mol L^{-1} HCl. The working solutions of metals were prepared by series dilution of their stock solutions immediately prior to use. The solutions of sodium thiosulfate, sodium pyrophosphate, KI and $SnCl_2$ were prepared in distilled water to examine the reduction of Fe^{3+}–Fe^{2+}. The PAN (chelating) solution was prepared by dissolving 0.250 g of PAN in 100 mL of 95% ethanol .

The following certified reference materials were used for method validation: the standard bush, branches and leaves sample (NCS DC 73349) purchased from China National Analysis Center for Iron and fortified water sample (TM 23.2) purchase from LGC (Teddington, UK).

2.3. Procedure

2.3.1. Food sampling

Common, general and scientific names of seven varieties of legumes selected are shown in Table 1. Samples were bought from two major wholesale markets in Iran, namely Saman market in Shiraz and Khorsandi market in Tehran. From each market, 2 kg of each sample were randomly collected from five retail outlets. Each category of sample bought from a particular market was pooled together as one composite sample (10 kg each). Each composite sample was divided into 3 sub-groups (A, B and C). Finally, six sets of samples were employed in this study. After purchasing, the samples as such were transported to the

Chemistry Laboratory, the Shiraz Payame Noor University.

Table 1. English and scientific names and the number of food samples collected and their methods of preparation

Legumes samples	Scientific name	n[a]	Preparation methods
White beans	Vigna unguiculata	6	Raw
Chickpeas	Cicer arietinum L.	6	Raw
Peanuts	Arachis hypogeal L.	6	Raw
Long beans	Vigna sinensis	6	Raw
Mung beans	Phaseolus radiatus	6	Raw
Soy beans snaks	Glycine max L. Merrill	6	Raw
Lentils	Lens culinaris L	6	Raw

[a]N, number of samples collected from markets or households.

2.3.2. Analysis of legumes

The seven legumes studied were: white beans, chickpeas, peanuts, long beans, mung beans, soy beans and lentils. These legumes were provided by an Iranian manufacturer (Explain in Food sampling section) as raw legumes cleaned once with tapwater, twice with deionized (DI) water and then dried in a hot-air oven (Memmert, model 5C^0, Germany) at 60 ± 2 ºC for 4 h. After that the samples were first ground manually with a mortar and then mechanically in an electrical mill to obtain small particles. The water content of all the raw legumes was determined so that the results could be expressed in terms of dry weight. 2.0 g of a ground sample of raw legumes were weighed in Erlenmeyer flasks (250 mL). 500 mL of deionized water was then added to the raw products. Nitrogen was bubbled to remove oxygen and the flask was stoppered with parafilm. The whole was shaken for 5 min at room temperature and transferred to conical polyethylene tubes fitted with screwed caps then heated in a boiling water bath for 10 min [4]. For recovery studies, spiked samples were prepared by adding up to 500 mL standard solution of Fe(II) and Fe(III) to of legumes sample prepared in the above. The speciation-preconcentration procedure given above was applied to the sample.

2.3.3. Preparation of alumina-coated magnetite nanoparticle

The Fe_3O_4 nanoparticles (Fe_3O_4 NPs) were prepared by chemical coprecipitation method [16]. The Fe_3O_4/Al_2O_3 nanoparticles (Fe_3O_4/Al_2O_3 NPs) were prepared according to Li et al. [17] with minor modification. The magnetic property of Fe_3O_4 NPs and Fe_3O_4/Al_2O_3 NPs was analyzed using a JDM-13 vibrating sample magnetometer. Both Fe_3O_4 nanoparticles and the Fe_3O_4/Al_2O_3 nanoparticles show super paramagnetic behavior at room temperature due to no hysteresis. The saturation magnetization (Ms) values of the Fe_3O_4 nanoparticles, the Fe_3O_4/Al_2O_3 nanoparticles and PAN-SDS alumina coated Fe_3O_4 nanoparticles were determined 54.00, 10.2 and 9.8 emug^{-1}, respectively. It is noticed that the saturation magnetization of Fe_3O_4 nanoparticles is higher than that of Fe_3O_4/Al_2O_3 nanoparticles, because the surface of Fe_3O_4/Al_2O_3 nanoparticles is coated with a layer of nonmagnetic Al_2O_3. However, the saturation magnetization for Fe_3O_4 NPs and Fe_3O_4/Al_2O_3 NPs is sufficient for magnetic separation with an external magnetic field. A scanning electron microscopy (SEM) image of Fe_3O_4/Al_2O_3 NPs, which illustrates the uniform size distribution of the nanospheres and to confirm that Al_2O_3, SDS and PAN are bonded to the Fe_3O_4 NPs, the characterizations were performed by FT-IR spectroscopy .

2.3.4. Speciation procedure based on Magnetic Mixed hemimicelles solid-phase extraction (MMHSPE) procedures

The MMHSPE procedure was carried out as follows. Firstly, 0.05 g Fe_3O_4/Al_2O_3 was added to 500 mL solution containing 80 mg SDS and 0.9 mg L^{-1} PAN solution in a 500 ml flask. The pH of solution was adjusted to 2.0-2.8 with 3.0 mol L^{-1} HCl solutions and then the flask was shacked mechanically for 8 min to form mixed hemimicelles assemblies. Subsequently, the SDS-PAN coated Fe_3O_4/Al_2O_3 NPs were isolated by placing a strong magnet and the supernatant was poured away and there were washed with high purity deionized water.

500 mL of legumes sample solution containing Fe(II) and Fe(III) were prepared (according to that as mentioned in Food sampling Section) and the pH value was adjusted to 3.0 with 0.1 mol L^{-1} HCl solution was added into the SDS- PAN coated Fe_3O_4/Al_2O_3 NPs and then, the mixture was homogenized and the extraction was performed under ultrasonic action for 8 min. The ultrasonic-assisted extraction was performed at room temperature. Subsequently, the SDS- PAN coated Fe_3O_4/Al_2O_3 NPs were isolated by placing a strong magnet and the supernatant was poured away. The preconcentrated target analyte absorbed on SDS- PAN coated Fe_3O_4/Al_2O_3 NPs was eluted with using 3 mL of 2 mol L^{-1} HNO_3. The analytes in the effluent were determined by FAAS. The procedure is the same for Fe(III), except that 0.161 mol L^{-1} sodium thiosulfate was added to each conical flask, as reduction agent, in order to reduce Fe(III) into Fe(II) ions, before added samples solutions to sorbents were carry out. The eluate was used directly for further flame atomic spectrometry (FAAS) analysis. Illustration of the whole procedure of the preparation of SDS- PAN coated Fe_3O_4/Al_2O_3 NPs and their application as SPE sorbents for enriching the analyte was shown in Fig. 1. The procedure is the same for Fe(III), except that of sodium thiosulfate, as reduction agent, was added to the analyte solution in order to reduce Fe(III) into Fe(II) ions.

3. RESULTS AND DISCUSSION

3.1. Characterization of the adsorbent

A scanning electron microscopy (SEM) image of Fe_3O_4/Al_2O_3 NPs, which illustrates the uniform size distribution of the nanospheres were performed (Fig. 2).

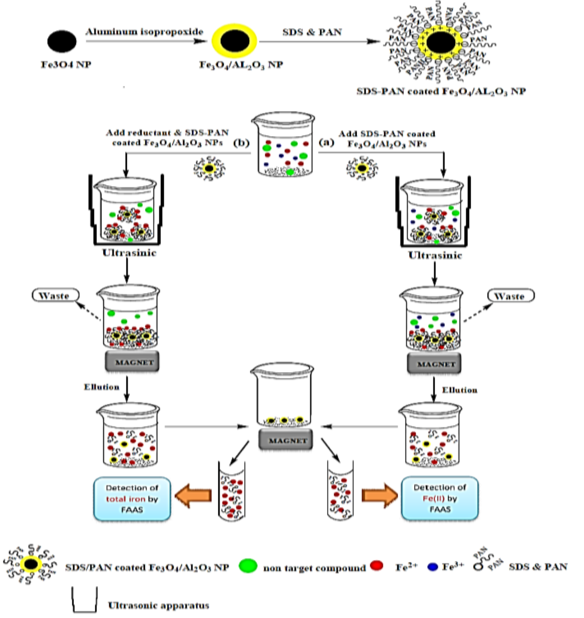

Fig. 1. Schematic illustration of the preparation of SDS-PAN-coated Fe_3O_4/Al_2O_3 NPs and their application for speciation and preconcentration of the analyte based on MHSPE.(a) detection of Fe^{2+}.(b) detection of total iron (The general conditions: modified Fe_3O_4/Al_2O_3 NPs amount, 0.05 g; pH= 2.0, SDS amount, 80 mg, 0.9 mg L^{-1}PAN solution, legumes sample volume, 500 mL; ultrasonic-assisted extraction time, 8 min; Fe^{2+} concentration, 80 ng mg^{-1}).

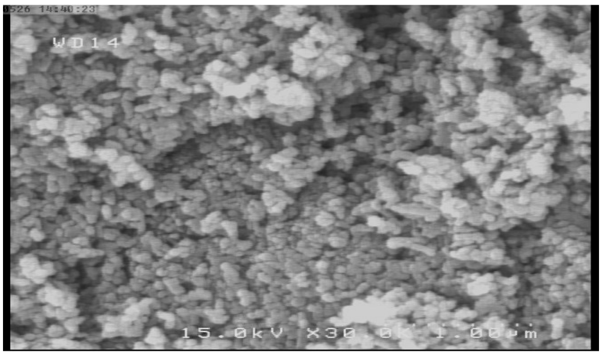

Fig. 2. SEM image of the proposed sorbent Fe_3O_4/Al_2O_3 NPs.

FT-IR spectroscopy was employed to confirm that Al_2O_3, SDS and PAN are bonded to the Fe_3O_4 NPs, the characterizations were performed by FT-IR spectroscopy. The FT-IR spectra for Fe_3O_4 NPs, Fe_3O_4/Al_2O_3 NPs and SDS- PAN Fe_3O_4/Al_2O_3 NPs are shown in Fig. 3.

In Fig. 3A the peaks at 560–660cm^{-1} were assigned to Fe–O bond vibration of Fe_3O_4 [18]. The broad band region at 3100–3700 cm^{-1} results from the stretching vibrations of O–H attached by the hydrogen bonds to the iron oxide surface and also the water molecules chemically adsorbed to the magnetic particle surface [19]. After Fe_3O_4 nanoparticles are functionalized with Al_2O_3, the characteristic peak at 560–660 cm^{-1} splits to two peaks. The transmittance wavebands in this region correspond to the metal–oxygen bonds; as a result, these two peaks can be an indication of two metal–oxygen bonds, Al–O and Fe–O, Fig. 3B. In Fig. 3C, after the immobilization of PAN on the coated Fe_3O_4/Al_2O_3 NPs, the FT-IR spectrum showed a signal at 1625.46 cm^{-1}. The transmittance waveband from 1400 to 1650 cm^{-1} assign to the C=C stretching vibrations of the ring. C–H stretching vibrations also observed at 2912.29 cm^{-1}. Once Fe_3O_4 / Al_2O_3 NPs are coated with PAN and SDS the strong peaks at the range of 750-1000 cm^{-1} (752.22 and 805.28 cm^{-1}) and 1175-1350 cm^{-1} (1195.44 cm^{-1}) are attributed to S–O and S=O bands stretching vibrations respectively. All these indicate that SDS and PAN are successfully attached to the magnetic nanoparticles (Fig. 3D).

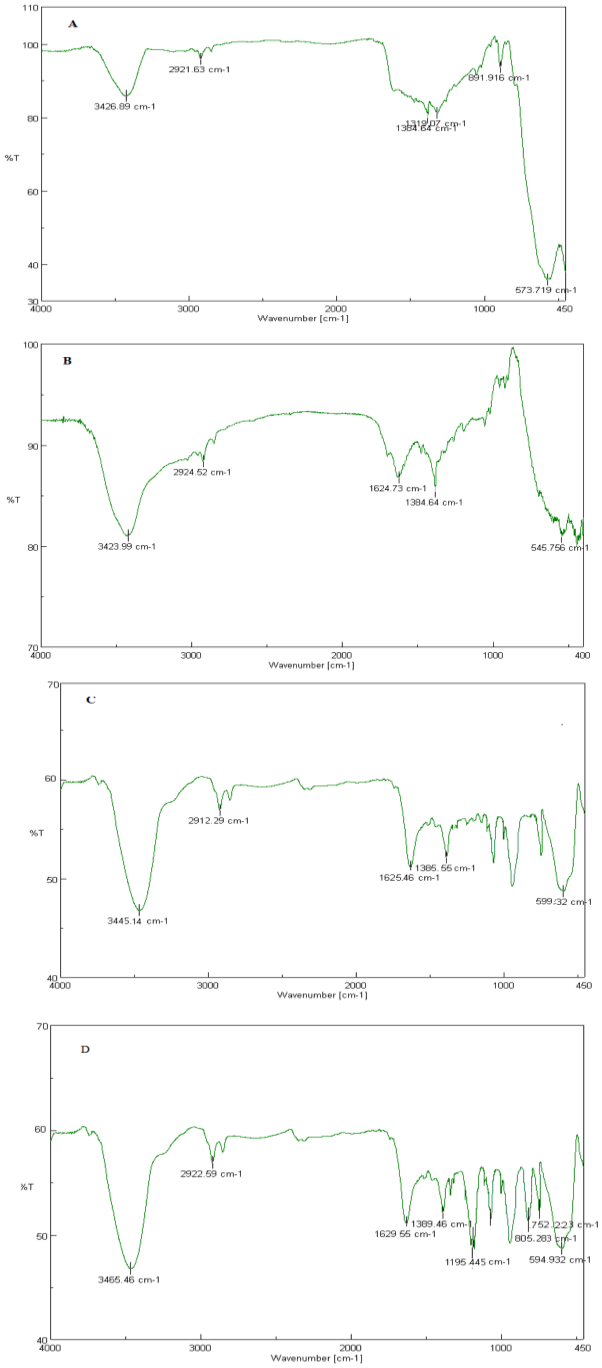

Fig. 3. FT-IR spectra for (A) Fe_3O_4 NPs, (B) Fe_3O_4/Al_2O_3 NPs, (C) Fe_3O_4/Al_2O_3 NPs @ SDS @ PAN.

3.2. The optimization of MMHSPE conditions

In this study, one-variable-at-a-time optimization approach was used for the optimization of MMHSPE conditions. The optimization was carried out by analyzing spiked legumes samples (80 ng mg^{-1} Fe(II) and Fe(III)) according to that as mentioned in Analysis of legumes Section. The parameters affecting the performance of the extraction were investigated. When one parameter changed, the other parameters were fixed at their optimized values.

3.2.1. Effect of the amount of magnetic adsorbents

Different magnetic adsorbents amounts (0.02–0.12 g) in 500 mL acidified of legumes sample solution containing Fe(II) and Fe(III) assisted by ultrasonic. The recovery of Fe^{2+} increased with the increase of magnetic adsorbents amount. Maximum recovery have achieved when the amount of adsorbents was above 0.05 g, then it kept invariant. According to the above results, 0.05 g was selected as the final amount of magnetic adsorbents used in the following studies.

3.2.2. Effect of the amount of SDS on adsorption of PAN and Fe^{2+}

The adsorption of anionic surfactant, SDS, on the positively charged alumina surfaces in acidic solution, is a favourable process and based on the added surfactants, they can form various aggregation on the surface (hemimicelles, mixed hemimicelles and admicelles) [20]. The effect of SDS amounts on the extraction efficiency was considered in the ranges of 0–100 mg for the surfactants. The recovery of extraction increased remarkably with the increasing amount of SDS. Maximum recovery was obtained when SDS amounts were between 70 and 140 mg, for larger amounts, the adsorption of the analyte. After that, the adsorption of the analyte decreased gradually, it may could because the excess of SDS forms micelles in the bulk aqueous solution and therefore, PAN is redistributed into the solution again. So 80 mg of SDS was the chosen optimum amount in order to achieve the highest possible extraction efficiency [21].

3.2.3. Effect of concentration of chelating reagent

In order to achieved maximum recovery of Fe^{2+}, different concentration of chelating reagent was investigated that the recovery of Fe^{2+} was increasing by the addition of PAN concentration. Maximum recovery of Fe^{2+} was obtained at 0.9 mg L^{-1} of chelating reagent. Above of this amount it remained constant. That is because of saturating of the sorbent magnetic surface by the ligand. Also, volume of chelating reagent was optimized for achieved the best recovery of Fe^{2+} absorption. Quantitative recoveries for the analytes were obtained at 0.9 mg L^{-1} of PAN solution.

3.2.4. Effect of pH

Variety of pH amounts result in change of the charge density on the Fe_3O_4/Al_2O_3 NPs surface, therefore, pH is one of the factors influencing the adsorption behavior of mixed hemimicelles system. Maximum adsorption of SDS on positively charged Fe_3O_4/Al_2O_3 NPs surfaces via formation of self-aggregates [22], was achieved at pH 1–2.9 by shaking the solution containing SDS and Fe_3O_4/Al_2O_3 NPs for 15 min. In acidified solution, SDS would form hemimicelles on Al_2O_3 NPs by strong sorption [23]. The critical role of pH solutions in the sorption of target ions on the sorbents was also investigated in the range of 1.0–10.0. The pH of the solution was adjusted at the required value by the addition of 1.0 mol L^{-1} NaOH or 1.0 mol L^{-1} HCl. The quantitative recovery (>95%) was found for Fe^{2+} at the pH range of 0.75- 4.0. In order to preconcentrate ions simultaneously, a pH of 3.0 was selected as the compromise condition. For examine the effects of acidified media on nanoparticles, SDS-PAN coated Fe_3O_4/Al_2O_3 NPs were stirred for 15 min in an acidic environment and the analyte in the effluent was determined by FAAS. The results were shown that there is not any iron signal. Usually, an inert coating on the surface of magnetite nanoparticles prevents their aggregation in solution, improves their chemical stability and provides better protection against toxicity [24].

3.2.5. The amount and type of reduction agent

For the determination of Fe^{3+}, the samples were reduced by using the reducing reagents. For this purpose, different reducing reagents such as: sodium thiosulfate, sodium pyrophosphate, $SnCl_2$ and KI were examined. The results were shown that the recoveries for both of Fe^{2+} and Fe^{3+} decrease when sodium pyrophosphate was used. In addition, Fe^{3+} was not reduced in the presence of $SnCl_2$ and KI. But, using sodium thiosulfate gave the favorite results. Therefore, sodium thiosulfate was chosen as the most effective reducing agent. It must be note that the precipitate was seen in case of using the concentration as high as 0.161 mol L^{-1} sodium thiosulfate. This can be attributed to the reduction of the sulfur in thiosulfate ions to elemental sulfur in the acidify matrix. After using sodium thiosulfate as the reducing reagent during to extraction procedure, total iron concentration was could found. The concentration of Fe^{3+} in the samples was calculated by the subtraction of Fe^{2+} from total iron concentration.

3.2.6. Standing and magnetic separating time

The experimental results indicated that the extraction time had an obvious effect on the legumes sample solution adsorption. In this study shaking to assist the extraction of Fe^{2+} from sample solutions compare with ultrasonic-assisted extraction. When ultrasonic-assisted extraction applied instead of shaking, the extraction efficiency was improved. The results showed that the maximum recovery of Fe^{2+} (82-98%) could obtain, when ultrasonic-assisted extraction time was 8 min. Therefore, cause of ultrasonic-assisted extraction was superior to shaking in the extraction of Fe^{2+} from samples, 8 min of ultrasonic-assisted extraction time was chosen for the extraction of samples. In the experiment, SDS-PAN coated Fe_3O_4/Al_2O_3 NPs possessed super paramagnetism properties and large saturation magnetization, which enabled them to be completely isolated in a short amount of time (less than 1min) by a strong magnet.

3.2.7. Desorption condition

Some experiments were carried out in order to choose a proper eluent for the adsorbed Fe^{2+} ions by modified nano-magnetite. In order to obtain the highest recovery of Fe^{2+}, different concentrations (1–4 mol L^{-1}) of various acids were investigated in this study. From the data given in Fig. 4, it is obvious that 2.0, 3.0 and 4.0 mL of 2.0 mol L^{-1} nitric acid could accomplish the quantitative elution of Fe^{2+} from the modified magnetic NPs. Thus, 3.0 mL of 2.0 mol L^{-1} nitric acid was selected as eluent for stripping of studied metal ions in this study. For eluent effects on nanoparticles, before extraction procedures SDS-PAN coated Fe_3O_4/Al_2O_3 NPs was eluted with using 3 mL of 2 mol L^{-1} HNO_3 and the analyte in the effluent were determined by FAAS. The results were shown that there is not any iron signal.

3.2.8. Sample volume

Sample volume is a main parameter for obtaining high preconcentration factor. The decreased recovery with sample volume maybe due to the low concentration of metal ions in the solution when sample volume was increased. Fortunately, using magnetically assisted separation of the adsorbent (modified magnetic NPs), made collect the adsorbent from larger volumes of spiked legumes sample solutions, possible. Thus, different experiments were performed using different sample solution amounts, in the range of 50–1000 mL legumes sample solution containing Fe(II) and Fe(III). The metal were preconcentrated on the modified magnetite NPs by applying the proposed procedure. Quantitative recovery (>95%) of Fe^{2+} was obtained up to 500 mL of sample solution. The result proved that Fe_3O_4/Al_2O_3 NPs MMHSPE method showed great analytical potential in preconcentration large volume water samples. It should be pointed out that in the case of larger quantity of sample solution; the more time was required for loading of the analytes on solid phase. The sample volume of 500 mL was selected as the ideal volume for trace analysis of Fe(II) ions in sample solutions.

3.2.9. Interference effects

To study the effect of foreign ions on the extraction efficiency of Fe(II) ions, their extraction from 500 mL of spiked real sample solutions containing different concentrations of foreign ions was studied. The tolerance limits of different cations are shown in Table 2. No significant interferences from these commonly encountered matrix components were observed because they could not form complexes with PAN and hence could not be preconcentrated. As could be seen, the tolerance limits of Cu(II), Zn(II) and Hg(II) were50, 50 and 10 times of Fe(II), respectively. Considering this fact that the contents of these coexisting ions in real samples maybe higher than their tolerance limits, EDTA was used as the masking agent to reduce the possible interference because EDTA can form stable ionic complexes with the interfering cations, but does not show affinity to Fe(II).

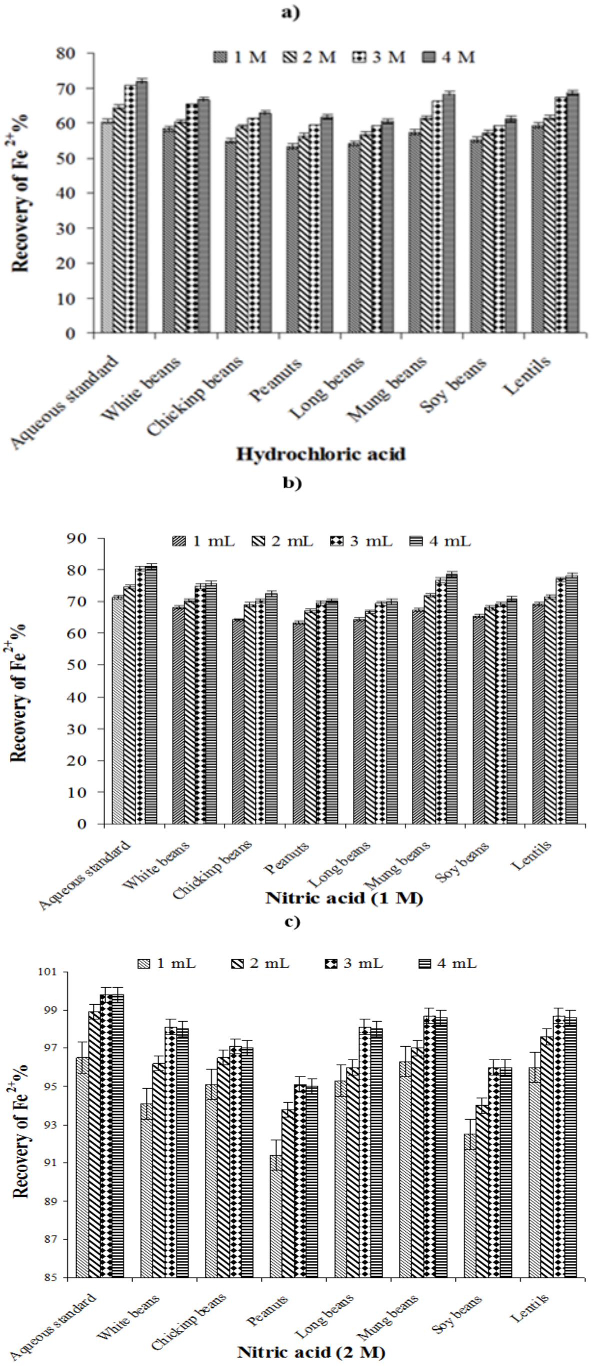

Fig. 4. Effect of different desorption solvents on the recoveries of Fe^{2+} (a) 2 ml hydrochloric acid solution; (b) HNO_3 1 M (c) HNO_3 2.0 $molL^{-1}$. The general conditions: modified Fe_3O_4/Al_2O_3 NPs amount, 0.05 g; pH= 2.0, SDS amount, 80 mg, 0.9 mgL^{-1}PAN solution, legumes sample volume, 500 mL; ultrasonic-assisted extraction time, 8 min; Fe^{2+} concentration, 80 ng mg^{-1}.

For this purpose, EDTA was added in the solution with a concentration of 0.1 mmol L^{-1} as masking reagent to lower these interferences. By this treatment, it was found that the common coexisting ions did not have significant effect on the separation and determination of Fe(II) (Table 2).

Table 2. Tolerance limit of interfering ions

Interfering ion	Ratio of [Interfering ion]/ [Total Fe ion]	Recovery(%) [a]
Na^+	2000	99.5(2.2)
SO_4^{2-}	1500	99.2(3.1)
Cl^-	1500	98.9(2.6)
F^-	1500	99.4(2.7)
Br^-	1500	98.9(2.7)
NO_3^-	1000	99.8(3.1)
I^-	1000	99.5(2.5)
K^+	500	99.0(2.9)
CO_3^{2-}	500	98.5(2.6)
Mg^{2+}	250	102.1(3.0)
Ca^{2+}	250	101.0(2.7)
Ba^{2+}	250	99.8(2.9)
Ni^{2+}	100	100.1(3.1)
Cr^{6+}	100	95.2(2.8)
Cu^{2+}	50	96.7(3.5)
Zn^{2+}	50	99.3(2.7)
Hg^{2+}	10	95.8(2.2)
Cu^{2+}	500 [b]	98.4(3.0)
Zn^{2+}	500 [b]	99.6(2.5)
Hg^{2+}	500 [b]	97.8(2.4)

[a] Values in the parentheses are R.S.D. (N=4)

[b] 0. 1 mmol.L^{-1} EDTA added.

As can be seen, the recovery of total iron was almost quantitative in the presence of these ions which supported the idea of the interference-free determination of Fe(II) and Fe(III) in real samples by the proposed method.

3.2.10. Reusability of the solid phase after desorption

To study the reusability of the solid phase, it was reused after desorptions from 1-30 times and the extraction efficiency of Fe(II) ions was calculated for each one. These extractions were studied from 500 mL of spiked real sample solutions. The results showed that the maximum recovery of Fe^{2+} (96-99%) could be obtained when the sorbent was reused between 1-25 times. Reusing more than 25 times reduced the recovery of Fe^{2+} to 86-95%. Therefore Fe_3O_4/Al_2O_3 NPs can be reused at least 20 times on average without the obvious decrease of recovery after wash/calcine in one step procedures.

3.3. Validity of the method

Once the procedure had been set up its validity was checked. The magnetic mixed hemimicells solid phase extraction (MMHSPE) method was applied to white beans, chickpeas, peanuts, long beans, mung beans, soy beans and lentils to detect matrix interferences. A ''*t*-test'' was applied to compare the slopes of the regression equations corresponding to the added matrix with those of aqueous standards; differences between them indicated matrix interferences. The results are reported in Table 3 which shown no significant differences between the calibration curve slopes obtained in aqueous standards solution and in the complicated matrixes.

Finally, the linearity of the response and the precision were measured by applying the earlier described procedure and the addition's method to assess the validity of the proposed method. Limit of detection (LOD) was estimated as the analyte concentration producing signal/noise ratio of 3:1. The details on the linearity ranges, calibration curves, correlation coefficients and LODs and RSD% of the proposed method were shown in Table 3. Each of the analytes exhibited good linearity with correlation coefficient r^2 > 0.9978 in the studied range. LODs were between 1.7 and 3.1 ng mg^{-1}. As the amount of Fe(II) in the sample solution was measured after a final volume of nearly 3.0 mL, the solution was concentrated by a factor of 166.6.

3.4. Analysis of standard reference materials

In order to study the accuracy of the proposed method, standard reference materials were analyzed. The presented MMHSPE procedure was applied to certified water samples TM-23.2 (fortified water) and NCS DC 73349 (bush, branches and leaves sample). During the experiments the proposed method was applied for 50 mL of TM-23.2 (fortified water). Whereas sample treatment procedure which was explained in Analysis of legumes Section was applied to 300 mg of NCS DC 73349 (bush, branches and leaves sample) before introduction to the proposed method. The results are given in Table 4 and are in good agreement with the certified values and the recoveries are in acceptable range.

3.5. Application of MMHSPE to legumes sample

To validate the feasibility of the method, seven real legumes sample (white beans, chickpeas, peanuts, long beans, mung beans, soy beans and lentils) were analyzed.

Among the seven legumes sample, Fe^{3+} and Fe^{2+} at detectable levels was found. Then the recoveries of $Fe^{2+}$and Fe^{3+} were studied by adding a certain amount of $Fe^{2+}$and Fe^{3+} standard solution with two concentrations (30 and 50 ng mg^{-1}) into legumes sample (Table 5). The recoveries of $Fe^{2+}$and Fe^{3+} were in the range of 96–103.2%, 96.5–103.6%, respectively. The present method is able to detect both Fe(II) and Fe(III) ions, it was found that the Fe(III) concentrations obtained are always higher than the Fe(II) contents and Soy bean snack and Peanut have the maximum content of Fe^{2+} (Shown in Fig. 5.).

Table 3. Matrix interferences study- addition's method.

Set	Regression equation	Correlation coefficient	Linearity range (ng mg^{-1})	RSD[a]%	Detection Limit (ng mg^{-1})	Confidence interval of slope (95%)
Aqueous standard	y=0.0083x+0.004	0.9992	5-110	1.6	1.7	0.0076-0.0090
Added white beans	y=0.0081x+0.001	0.9981	25-108	2.3	2.4	0.0078-0.0084
Added chickpeas	y=0.0082x+0.003	0.9978	26-110	1.7	2.9	0.0077-0.0086
Added peanuts	y=0.0079x+0.002	0.9985	29-102	2.5	3.1	0.0078-0.0080
Added long beans	y=0.0083x+0.002	0.9991	23-105	1.9	2.5	0.0076-0.0089
Added mung beans	y=0.0079x+0.007	0.9988	16-108	2.1	1.9	0.0077-0.0080
Added soy beans	y=0.0082x+0.003	0.9990	21-112	2.2	2.5	0.0077-0.0087
Added lentils	y=0.0087x+0.005	0.9981	27-107	2.5	2.8	0.0085-0.0089

[a] R.S.D$_s$ Values was obtained for five measurements (C=50 ng mg^{-1})

Table 4. Results obtained for Fe(III) ion determination in certified samples.

Samples	Certificate value	Found value	Recovery (%)
NCS DC 73349 (bush, branches and leaves sample)	1070±57 µg g^{-1}	1051±8 µg g^{-1}	98.2
TM-23.2 (fortified water)	12.7 ±1.3 µg mL^{-1}	12.8 ±0.4 µg mL^{-1}	100.8

(±S.D.) Average of ten measurements

Table 5. Total soluble iron (II) and iron (III) soluble in the legumes expressed as ng g^{-1}dry sample

Samples	Spiked (ng mg^{-1})		Found (ng mg^{-1})		Relative recovery (%)	
(Specific name)	Fe(II)	Fe(III)[a]	Fe(II))	Fe(III)[a]	Fe(II)	Fe(III)[a]
White beans	—	—	10 ± 2[b]	39 ± 4	—	—
(Vigna unguiculata)	30	30	41 ± 3	68 ± 2	103.0	96.5
	50	50	59 ± 5	90 ± 3	97.7	101.6
Chickpeas	—	—	ND[c]	10 ± 2	—	—
(Cicer arietinum L.)	30	30	31 ± 2	39 ± 3	102.7	97.3
	50	50	50 ± 4	61 ± 3	99.8	101.8
Lentils	—	—	ND	19 ± 4	—	—
(Lens culinaris L.)	30	30	30 ± 2	50 ± 3	96.0	103.5
	50	50	51 ± 5	68 ± 4	101.5	98.0
Peanut	—	—	15 ± 2	35 ± 3	—	—
(Arachis hypogaea L.)	30	30	44 ± 4	66 ± 1	97.1	102.9
	50	50	66 ± 3	87 ± 4	102.2	103.6
Soy bean snack	—	—	15 ± 2	45 ± 5	—	—
(Glycine max L. Merrill)	30	30	46 ± 3	74 ± 5	102.6	97.0
	50	50	66 ± 2	96 ± 4	102.2	101.8
Long bean	—	—	14 ± 2	40 ± 5	—	—
(Vigna sinensis)	30	30	45 ± 3	69 ± 6	102.5	97.1
	50	50	66 ± 2	91 ± 4	103.2	101.8
Mung bean	—	—	12 ± 2	32 ± 5	—	—
(Phaseolus radiates)	30	30	41 ± 3	62 ± 5	96.6	99.4
	50	50	63 ± 2	81 ± 4	102.2	98.1

[a] Fe (III) content was calculated by difference, subtracting Fe (II) to total soluble iron., [b]Average of six measurements (±SD).
[c] ND cannot be determined using the proposed method.

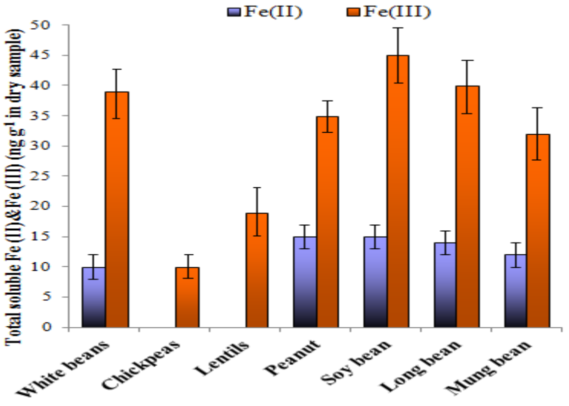

Fig. 5. Comparison total soluble iron (II) and iron (III) soluble in the legumes expressed as ng g-1dry sample.

4. CONCLUSION

The new and rapid method of analyses reported here is useful for measuring soluble iron, iron (II) and (III) in legumes sample. In this research, modified magnetite NPs (SDS- PAN coated Fe$_3$O$_4$/Al$_2$O$_3$ NPs) were successfully synthesized and a new type of magnetic mixed hemimicells solid phase extraction (MMHSPE) method assisted by ultrasonic was proposed for speciation and preconcentration of iron (II) in legumes sample. Easy and low cost synthesis of sorbents compared with the commercial adsorbents and rapid extraction of Fe^{2+}, simple isolation of adsorbents from sample matrix by using external magnetic field without additional centrifugation or filtration after analyte adsorption, are some advantages of this work. On the other hand, these Fe$_3$O$_4$/Al$_2$O$_3$ NPs can be reused at least 20 times on average without the obvious decrease of recovery after wash/calcine procedures. Compared with classical methods, it is obvious that the sample preparation used in the proposed method is simple and time-saving. So, MMHSPE provide a simply and fast speciation method with great potential in pretreatmeant of complicated samples. This methodology also gives good accuracy, low limits of detection and excellent precision on the target analytes, which show its potentiaity in trace analysis in various samples with complicated matrix. The results have been also indicated that Fe^{3+} concentrations obtained are always higher than the Fe^{2+} contents in legumes sample and Soy bean snack and Peanut have the maximum content of Fe^{2+}.

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