ORIGINAL RESEARCH PAPER

Magnetic solid-phase extraction and Uv/vis Spectrophotometric determination of trace amount of Copper in vegetable and fruit samples after preconcentration of its Pentetate complex

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ABSTRACT

Core-shell structure Magnetite@polydopamine (Fe_3O_4@PDA) nanoparticles have been successfully synthesized and were characterized by FT-IR, TEM, FE-SEM, XRD, EDX, Raman Spectroscopy, VSM and Zeta potential. The effects of the different parameter on copperpentetate complex (CuDTPA) sorption on Fe_3O_4@PDA were investigated by using Box-Behnken design for Spectrophotometric determination of trace amount of Copper in vegetable and fruit samples after preconcentration of its Pentetic complex. Under optimal experimental conditions, L.R., LOD, and LOQ of Cu (II) were 2-700 µg.L^{-1}, 0.0146 µg.L^{-1} and 0.0487 µg.L^{-1} respectively. The Preconcentration Factor 100 was obtained. Low relative standard deviations (RSD=0.7 %) for 10 replicates are achieved. The method was applied to the determination of trace amount of Cu (II) in food samples and the results showed that no significant difference between the proposed method and Flame Atomic Absorption Spectroscopy.

Keywords: *Box-Behnken Design; DTPA; Fe_3O_4@PDA; Magnetic Solid Phase Extraction; Vegetable and Fruit Samples*
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INTRODUCTION

Metals are normal ingredients in Earth's crust. The ultra-trace amounts of poisonous metals are usually available in trace concentrations in environmental samples (1). Heavy metals perhaps available in foods naturally, or from contamination during protection, cooking and industrial processes or as an outcome of human processes (such as exhaust gases, industrial emissions, etc.). Among the attendance of heavy metals in foods and long-term effect on health, a relevance exists therefore, it is necessary to keep them at an acceptable low level of toxicity. (2).

Trace elements such as Copper are necessary for trace amount in human health and is straight involved in the Fe metabolism. Consumption of fundamental metal ions from food and beverages

are the main origin for human (3). However, the higher level of fundamental trace metals in our body makes well-being danger, such as hepatic difficulty. But owing to these reasons, the precise and accurate determinations of trace metals for analytical chemists are significant (4).

FAAS and/or GFAAS, ICP-OES and/or ICP-MS and electro analytical techniques are the main instrumental techniques for the measuring trace amounts of heavy metal ions (5-8). One of the main difficulty of these instrumental techniques is the attendance of a trace amount of heavy metals lower than the limit of detection (9). Preconcentration techniques, for example, liquid-liquid extraction (LLE), membrane filtration, coprecipitation, cloud point extraction (CPE), solid-phase extraction (SPE) and electro deposition, are common to eliminate this difficulty are used by researcher

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around the world (10-13).

Different chelators like ethylenediamine tetra acetic acid (EDTA), triethylenetetramine hexaaceticacid (TTHA), diethylenetriamine pentaaceticacid (DTPA), 5, 10, 15, 20- tetrakis(penta fluorophenyl)porphyrin (H$_2$TFPP)(14) and methyl-2-pyridylketoneoxime (MPKO) (15) have been widely studied, however EDTA and DTPA, are more than other common chelators have been used. EDTA is a hexa dentate chelator capable of complexing stoichiometrically with almost any transition metal in the periodic Table (16). The effectiveness of chelator for a specific metal ion by the stability constant (log K) is governed. The log K of EDTA-Cu (II) is 18.8 and the log K for DTPA-Cu (II) is 21.4 at 20 ^{o}C in 0.1M ionic strength.

DTPA (LD$_{50}$=0.59 g kg^{-1} for rat; λ_{max}=237 nm) is one of the synthetic poly amino poly carboxylic acids that can form stable complexes with a large number of metal ions, like Cu $^{2+}$, Ni $^{2+}$, Co $^{2+}$, Cd $^{2+}$, Zn $^{2+}$, Mn $^{2+}$ and Ca $^{2+}$. Free DTPA contains 5 carboxylic acid and 3 amine groups, and 5 pKa values of 1.79, 2.56, 4.42, 8.76 and 10.42 have been reported (17). Like EDTA, DTPA chelates Cu ions at equimolar ratio over pH 2.5 to 11.0.

Magnetic-SPE (MSPE) which has received worthy attention in this decade, is based on magnetic sorbents with great extraction efficiency and comfortable operation, (18, 19). Magnetic nano particles (MNPs) have recently been the focus of continued research as the adsorbent for MSPE thanks to their high extraction capacity, high surface area, diverse functionalized possibilities and easy separation sufficiency (20). Between the Magnetic nano particles sorbents used, magnetite (Fe$_3$O$_4$) is most popular due to its low toxicity, unique magnetic property and low- price (18, 19). Extraction efficiency and selectivity of bare Fe$_3$O$_4$ MNPs could be improved via the surface modification (21, 22). Fe$_3$O$_4$ MNPs with organic modification like dopamine could be used as a sorbents for the extraction of low concentration of heavy metals from the food samples (23, 24).

Dopamine (DA) that includes amine and catechol functional groups is the first catecholamine. Polydopamine (PDA) films as multifunctional coatings can be easily grown and adhered to a large variety of organic and inorganic materials in pH>7 through self-polymerization of DA (22). Owing to the versatility and easy polymerization process, PDA used for surface modification of solid materials. The use of PDA as a coating agent for the construction of multifunctional nanocomposites can provide various advantages like enhancement of water dispersibility and stacking interaction between the analyte and sorbent (24, 25).

The multivariate design approach is a famous collection of statistical and calculation techniques for designing a set of experiments, expanding a mathematical model, measuring the efficacy of factors and obtaining the best condition of factors for appropriate responses. (26).These methods let the concurrent optimization of a number of variables, are quicker and more cost-effective than normal optimization strategy "one variable at a time (OVAT)". (19, 27). The first step in multivariate optimization screening the factors that studied in order to get the significant efficacy of the analytical system. After determining the significant factors, in the second step, the optimum operating conditions are gained by using more complex experimental designs like Box-Behnken design (BBD). BBD as a type of multivariate design approach, is a class of rotatable second-order designs based on imperfect factorial designs and by the equation 2 k (k + 1) + cp; in this equation cp is the number of duplications at the central point and k is the number of factors; the number of experiments is obtained (19, 26, 28, 29).

The aim of this study is that stablishing a new, speed, effective and simple MSPE procedure by uv/vis for determination Cu(II) ions after preconcentration of its Pentetate complex in vegetable and fruit samples on Fe$_3$O$_4$@PDA.The magnetic sorbent is characterized by TEM, FT-IR, FE-SEM, XRD, EDX, Raman Spectroscopy, VSM and Zeta potential. The effects of different parameter on the sorption of complex CuDTPA on Fe$_3$O$_4$@PDA were investigated by using BBD.

MATERIALS AND METHODS
Reagents
Tris (hydroxy methyl) amino methane hydro chloride (H$_2$NC (CH$_2$OH)$_3$. HCl) (PubChem CID: 93573), dopamine hydrochloride(PubChem CID: 65340), Penteticacid (diethylenetriamine pentaaceticacid or DTPA) (PubChem CID: 3053), Calcium chloride(PubChem CID: 24854), triethylamine (TEA) (PubChem CID: 8471), ferricchloridehexahydrate (FeCl$_3$.6H$_2$O) (PubChem CID: 24810), ferrouschloridetetrahydrate (FeCl$_2$.4H$_2$O) (PubChem CID: 16211588), sodium hydroxide, acetic acid, hydrochloric acid, potassium hydrogen phthalate, nitric acid, hydrogen peroxide, ethanol and copper(II) chloride (PubChem CID:

24014) were purchased from Merck.

A 100.0 mg L^{-1} stock solution of Cu^{2+} was prepared by dissolving 0.0212 gr of $CuCl_2$ in a 100.0 mL volumetric flask and was diluted to the mark with doubly distilled water (DDW). DTPA solution was prepared by dissolving 1.967 gr DTPA, 13.1 mL TEA and 0.5 gr of $CaCl_2$ in approximately 1000.0 mL of DDW and pH was adjusted to 7.3 with 1:1 HCl solution (30). Acetate (pH 5.0, 0.1 M) and/ or Tris-HCl (pH 8.5, 0.1 M) buffer solution was prepared by dissolving an appropriate amount of CH_3COOH or $H_2NC (CH_2OH)_3$. HCl in DDW and pH was adjusted by adding appropriate amounts of 0.1 M NaOH or HCl solution.

Apparatus

The pH measurements were performed with a JENWAY model 3510 pHmeter. An ultrasonic water bath (SONICA 2200ETH S3) was used to disperse the nanoparticles in solution. The UV-Vis absorbance spectra were recorded using double beam UV-Visible spectrophotometer (PG Instrument, T80+), equipped with 10 mm quartz cuvettes. Flame atomic absorption spectrometer (Perkin–Elmer Analyst 800) equipped with Zeeman-effect background correction and air/acetylene burner (17 L. min $^{-1}$) was used for the determination of Cu(II) as reference method. FT-IR spectra on a Perkin-Elmer spectrometer (Spectrum 65) were determined over a potassium bromide pellet in the range 4000-400 cm^{-1}. TEM images at the accelerating voltage of 80 KV were taken with a Zeiss -EM10C. FESEM images were obtained by Hitachi S-4160-30KV scanning electron microscope on Au grid. The crystalline structures of MNPs were studied by XRD analysis on a Bruker D8 Advance diffractometer with Cu Kα radiation at 20 mA and 40 kV. Raman spectra were evaluated by WITec Confocal Raman instrument with a 514 nm laser wavelength. Magnetization measurements were carried out on a BHV-55 VSM. Malvern Zeta sizer ZS were applied for recording zeta potential of particles.

Synthesis of Fe_3O_4 and Fe_3O_4@PDA magnetic nano particles

Via the chemical co-precipitation method the Fe_3O_4 MNPs were prepared (19, 24, 31, 32). 10.4 g of $FeCl_3.6H_2O$, mixed with 4.0 g of $FeCl_2.4H_2O$, and 1.7 ml of HCl (concentrated) in 50 mL water and in next step the mixture was added drop by drop to 0.5 L NaOH (1.5 M) solution under N2 protection

and vigorous stirring at 80°C for half hour. The precipitate were separated by external magnet and washed with 4×50.0 mL DDW, then the products at 50°C for 6 h were dried.

In next step, 1.0 g Fe_3O_4 nanoparticles were dispersed in 0.5 L Tris-HCl (pH 8.5, 0.1 M) buffer solution for 5 min by using ultrasonic, and then 1.0 g dopamine hydrochloride was added, then the mixture solution was mechanically stirred at 25 °C for 24 h. After reaction, the precipitate Fe_3O_4@PDA nanoparticles were separated by external magnet and washed with DDW and anhydrous ethanol (1:1) and finally dried at 60 °C for 4 h (32).

Procedure of Magnetic Solid Phase extraction

To prepare working solutions, transfer 2-700 μg L^{-1} of Cu^{2+} stock solution (100 mg. L^{-1}) to a series of 150.0 mL beakers, then add 15.0 mL acetate (pH=5, 0.1M) buffer solution and finally dilute each to the mark with DTPA solution. Then, 28 mg of magnetic sorbent (Fe_3O_4@PDA) was added to the 150.0 mL of solution and the mixture stirred for 25 ~~min~~ minutes and finally, external disk magnet (1.5 T) was used to separate magnetic sorbent (Fe_3O_4@PDA) from the solution. The adsorbed complex onto the magnetic sorbent was desorbed by 1.5 mL of ethanol and collected for the determination Copper pentetate complex by UV/Vis in $λ_{max}$=276 nm.

Preparation of vegetable and fruit samples

The vegetable (12 samples) and fruit (6 samples) samples were purchased from local markets in Kermanshah city (west of Iran). Each kind of product, if possible, is chosen from different manufacturers. Each sample was washed thoroughly with tap water and DDW. The shells of samples were robbed when necessary. Finally, the samples were dried for 24 h at 105 °C and grounded.

One gram of each sample was put in a beaker, and 10.0 mL of HNO_3 (concent.) was added to the beaker. The mixture was evaporated near to dryness for 4h at about 130 °C on a hot plate. After cooling to 25 °C, 3.0 mL of H_2O_2 (30%, w/w) was added. The mixture was again evaporated near to dryness. The resulting solution was diluted to 25.0 mL with distilled water (33). The filtration procedure by whatman filtration paper no.1 was made for some samples which were not completely dissolved. Then 2.5 mL of each sample transfer to 200 mL beaker, add to 20.0 mL acetate buffer solution (pH=5, 0.1M) and finally dilute each to

the mark with DTPA solution (150.0 mL of this solution used for UV/Vis analysis and 50.0 mL used for FAAS analysis as a reference method). The MSPE technique that described above was used for the intended sample solutions.

Box-Behnken Design

A three-factor, three levels Box-Behnken design was employed in order to achieve the highest amount of the Cu (DTPA) adsorbed by the Fe_3O_4@PDA. Sorbent mass, pH and the uptake time were investigated as independent variables and Cu (DTPA) desorption was considered as the dependent variable. The experimental domains of these factors were established as preliminary experiments. The variables and levels of the BBD model are given in Table 1. The multinomial equation generated by this experimental design is as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

Where Y is the estimated response (dependent variable), b_1 to b_{33} are the regression coefficients, X_1 to X_3 are independent variables and b_0 is the intercept (19).

RESULT AND DISCUSSION
Characterization studies

FT-IR spectroscopy was used to verify the functional groups of the synthesized Fe_3O_4 and Fe_3O_4@PDA nanoparticles. In the spectrum of bare Fe_3O_4 (Fig. 1a) the peaks at 443 and 588 cm^{-1} are respectively related to the octahedral bending and tetrahedral stretching vibration of the Fe-O function group and the peaks at 1625 and 3410 cm^{-1} are corresponding to the surface-adsorbed water and hydroxyl groups (31). For the Fe_3O_4@PDA, new broad and strong absorption bands can be seen in the range of 1000-1800 cm-1, which can be attributed to the C-O stretching of phenol compounds and the stretching vibration of the aromatic rings. Finally, result confirms the

formation of the polydopamine shell on the surface of Fe_3O_4 NPs via both chemical and physical adsorption (24).

TEM measurement was used to confirm the nanostructure of Fe_3O_4@PDA. As shown in Fig. 1b TEM image of Fe_3O_4@PDA suggest an obvious PDA coat (about 6-10 nm-in-thickness) is immobilized on the surface of Fe_3O_4 nanoparticles, revealing the core/shell structure of Fe_3O_4@PDA.

The size and morphology of the Fe_3O_4@PDA were studied by field-emission scanning electron microscopy (FE-SEM). The FE-SEM image of Fe_3O_4@PDA was shown that the sorbent was formed in nanometer-sized particles as quasi-spherical with an average diameter of 20±5 nm (Fig. 1c).

In Fig. 1d the existence of PDA shell on the surface of magnetite NPs by the EDX detector coupled to the SEM which showed the presence of Fe, C, N and O were also confirmed, and as well as that figure shown, Au peak refer to grid that by EDX operator was used.

The crystal nano structure of the magnetic sorbent was investigated by powder XRD, as shown in Fig. 1e. The characteristic diffraction peaks in the Fe_3O_4@PDA at 2θ of 18.1 º, 30.2º, 35.6º,36.2 º, 43.4º, 53.8º, 57.2º ,and 62.8º have corresponded to the diffraction of (111), (220), (311), (222), (400), (422), (511), and (440) of the Fe_3O_4. The entire diffraction peaks match with the magnetic face centered cubic (FCC) structure of Fe_3O_4 (JCPDS card number 19-0262). No other characteristic peaks owing to the contamination of hydroxides or hematite were detected. Notably, the XRD pattern of the Fe_3O_4@PDA has similar diffraction peaks with Fe_3O_4, and the result have indicated that the PDA coating does not change the crystalline phase of Fe_3O_4 and polymer shell is formless in nature.

To evidently confirm the formation of PDA shell, Raman spectroscopy was used in investigating the changes of the interface of magnetic sorbent. Four strong and broad bands around 398, 489, 526 and 687 cm^{-1} can be observed in the Raman spectrum of bare magnetic cores, which correspond to the T_{2g} and A_{1g} modes of symmetry, As shown in Fig. 1f (34). However, after coating with the PDA polymer shell,

Table 1. The experimental elements and levels of the Box–Behnken design

Variables	Coded symbols	Levels		
		Low	Middle	High
PH	X_1	4.50[a]	5.75[a]	7[b]
Uptake time (min)	X_2	5	20	35
Sorbent mass (mg)	X_3	20	50	80

[a] Acetate buffer solution (0.1M)
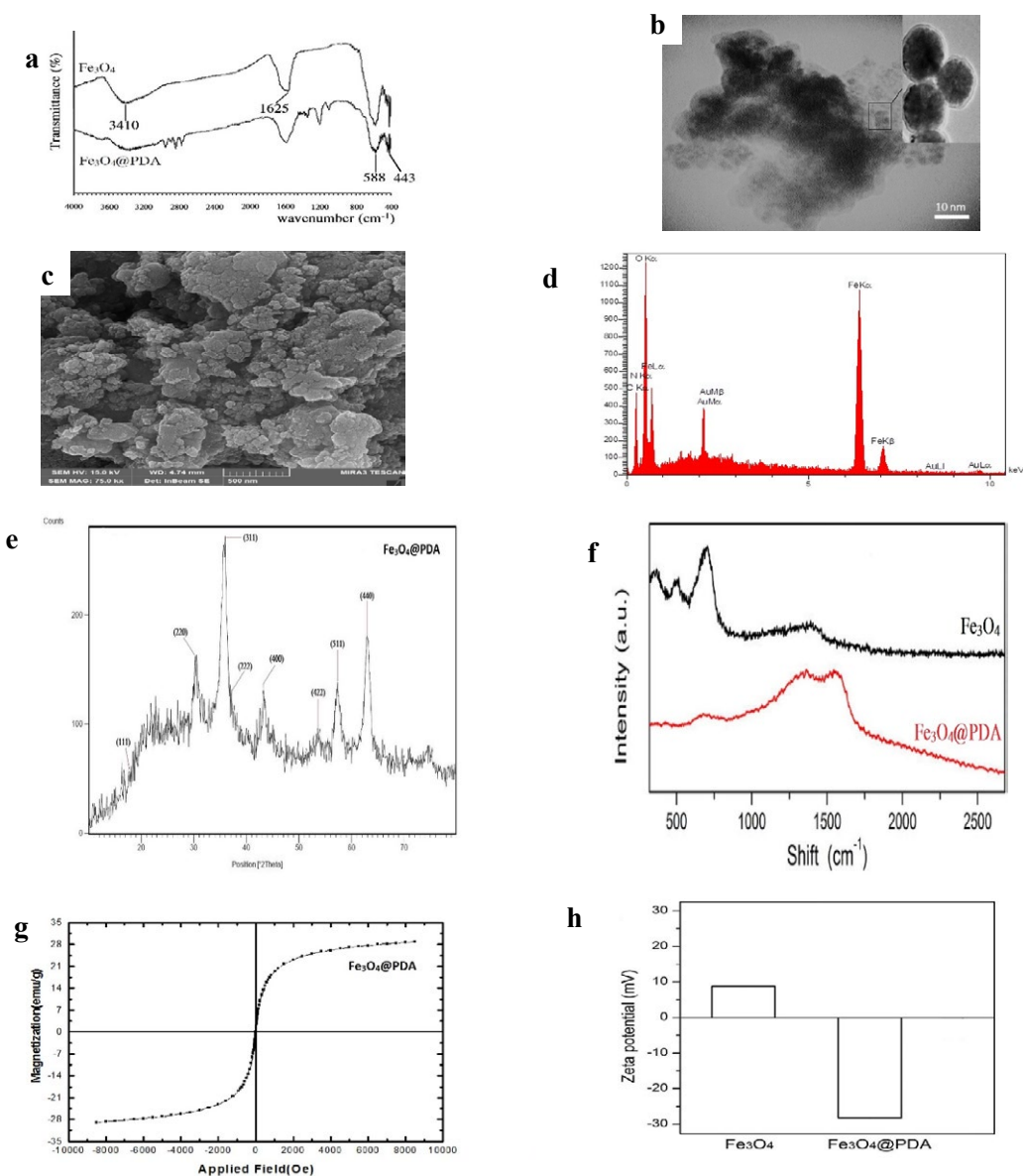[b] Phosphate buffer solution (0.1M)

Fig. 1. (a) FT-IR spectrum, (b) TEM image, (c) FE-SEM image, (d) EDX data, (e) XRD pattern, (f) Raman spectra, (g) VSM and (h) Zeta potential of Fe_3O_4 and Fe_3O_4@PDA.

the intensity of these characteristic bands decreased, whereas two new broad bands around 1577 and 1355 cm^{-1} are present in the Raman spectrum. They are characteristic bands of polydopamine that can be imputed to the deformation of the catechol group.

The magnetic evaluations were accomplished by VSM at 25 °C. In the VSM magnetization curve of Fe_3O_4@PDA nanoparticles, there is no hysteresis, and the residue and coercivity is negligible, result indicating the super-para magnetism of these nanoparticles (Fig. 1g). The saturation

magnetization (M_s) value of Fe_3O_4@PDA (30.4 emu g^{-1}) is lower than Fe_3O_4 (61.2 emu g^{-1}) (data was not shown) that owing to the existence of nonmagnetic PDA coating. However, the magnetic sensitivity of the prepared sorbent is strong enough to provide a quick, cheap, easy, effective and reusable way to separate the sorbent from samples.

Also, the successful grafting of dopamine molecules was confirmed by the zeta-potential analysis. As shown in Fig. 1h, the naked magnetic cores show positive values(8.89 ± 0.94 mV),

while the Fe_3O_4@PDA nano sorbent because of the deprotonation of the phenolic group on the polydopamine shells, shows a negative zeta-potential (-28.42 ± 0.47 mV).

Optimization conditions for the solid phase extraction of Cu (DTPA).

Comparison of the preliminary results on the desorption of 100.0 mL of Cu(DTPA) solution (500.0 µg.L^{-1}) by 0.2 g unmodified and modified Fe_3O_4 by 2.0 mL ethanol showed that the desorption of Cu (DTPA) was 0.382 ± 0.007 and 1.092 ± 0.006, respectively. Thus, bonded PDA on the surface of Fe_3O_4 had an effective role in increasing desorption of Cu (DTPA). In order to achieve the best performance, the conditions of desorption of Complex onto Fe_3O_4@PDA were optimized separately.

Optimization of the sorption step

The optimization step of the Cu (DTPA) sorption onto Fe_3O_4@PDA was performed using a BBD. The design matrix and the relevant results

of BBD experiments to determine the influences of the three variables including pH, uptake time, and sorbent mass are shown in Table 2.

According to ANOVA analysis (Table 3), the model F-value was 98488.47. This shows that the terms in the model have a notable efficacy on the response. The determination coefficient (R^2) and adjusted determination coefficient (R^2_{adj}) values were obtained 0.9999 and 0.9999, respectively. The value of R^2 showed that the value of R^2_{adj} was very close to 1, which shows the high significance of the model. The P value for the lack of fit (0.00) also shows that multinomial model is statistically notable for the response. According to the model, X_1 to X_2X_3 are significant model terms for the sorption of Cu (DTPA). Based on BBD, empirical relationships among the Cu (DTPA) sorption (Y) and significant independent variables were expressed by the following:

$$Y=0.753500-0.096230X_1+0.003297X_2+0.009315X_3 + 0.002800\ X_1^{\ 2} - 0.000241X_2^{\ 2} + 0.000013\ X_3^{\ 2} + 0.000467\ X_1X_2 - 0.000920\ X_1X_3 + 0.000213\ X_2X_3$$

Table 2. Design matrix in the Box–Behnken model.

Run order	PH	Uptake time (min)	Sorbent mass (mg)	desorption of Cu(DTPA)	
				observed	predicted
1	7	20	20	0.399	0.400
2	5.75	5	80	0.806	0.807
3	5.75	20	50	0.763	0.763
4	7	35	50	0.702	0.701
5	4.5	20	20	0.583	0.583
6	7	5	50	0.474	0.473
7	5.75	20	50	0.763	0.763
8	5.75	35	20	0.443	0.442
9	4.5	5	50	0.742	0.743
10	4.5	20	80	1.228	1.227
11	7	20	80	0.906	0.906
12	5.75	35	80	1.209	1.209
13	5.75	5	20	0.424	0.423
14	4.5	35	50	0.935	0.936
15	5.75	20	50	0.763	0.763

Table 3. Analysis of variance (ANOVA) for Box-Behnken design

Source of variation	DF [a]	SS [b]	MS [c]	F-Value	P-Value [d]
Model	9	0.930716	0.103413	98488.47	0.000
X_1	1	0.000598	0.000598	569.77	0.000
X_2	1	0.000304	0.000304	289.92	0.000
X_3	1	0.008805	0.008805	8386.08	0.000
$X_1^{\ 2}$	1	0.000071	0.000071	67.31	0.000
$X_2^{\ 2}$	1	0.010817	0.010817	10301.59	0.000
$X_3^{\ 2}$	1	0.000499	0.000499	475.22	0.000
X_1X_2	1	0.000306	0.000306	291.67	0.000
X_1X_3	1	0.004761	0.004761	4534.29	0.000
X_2X_3	1	0.036864	0.036864	35108.57	0.000
Lack-of-Fit	3	0.000005	0.000002	*	0.000
Pure Error	2	0.000000	0.000000		
Total	14	0.930721			

[a] degree of freedom; [b] sum of square; [c] mean square; [d] P values < 0.05 were considered to be significant

Whereas it is noted in Table 1, X_1 to X_3 denotes coded values of the pH of experiment, uptake time and utilized sorbent mass, respectively.

Fig. 2.demonstrates response surface plots for the relationship between pH, Uptake time and Sorbent mass on the sorption of Cu (DTPA) from the aqueous solution. The curvatures of these plots show the practicable interaction between the variables.

Fig. 2a represents the efficacy of Uptake time and Sorbent mass on the sorption of Cu (DTPA) at constant pH (5.0, 0.1 M). This plot shows that sorbent mass and uptake time have positive mutual effect on sorption of Cu (DTPA) complex. As seen in Fig. 2b by increasing the pH, the absorption of complex; due to hydrolysis of copper ion; is reduced. Thus it can be concluded that with adding time and keep solution in weak acidic pH can be achieved to the greatest absorption of copper complex. And finally, as shown in Fig. 2c, in weak acidic pH with increasing the mass of sorbent, the absorption of complex owing to more absorption capacity of nano-sorbent increased clearly. According to the results of the optimizing absorption step, pH 5.0

(acetate buffer solution, 0.1 M), 28 mg sorbent mass and 25min uptake-time were selected as the optimum conditions.

Optimization of the volume sample.

The efficacy of changes in the volume of sample solution was investigated by using different sample volumes (50.0, 100.0, 150.0, 200.0 and 250.0 mL) with the identical concentration of Cu (DTPA) in optimum pH, sorbent mass and uptake time to get the highest preconcentration factor. Result represented that 150.0 mL of volume sample has the highest sorption (Table 4). Therefore, the suitable volume (150.0 mL) was chosen as volume sample for next studies.

Optimization of type and volume of the eluent

In optimum condition, desorption of Cu (DTPA) complex from the Fe_3O_4@PDA sorbent was studied by using different eluents including chloroform, Ethyl acetate, Acetone, Aceto nitrile, Methanol, Ethanol, Acetic acid, Methanol-acetic acid (1:1) and ethanol-acetic acid (1:1) (eluent volume of 2.0 mL). Consequently, base on result obtained
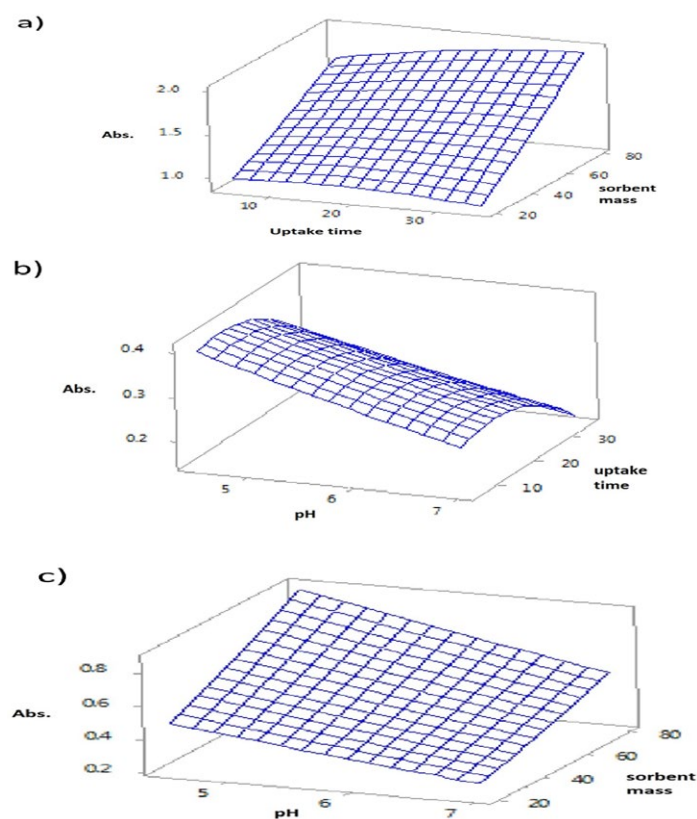

Fig. 2. RSM plot for solid phase extraction of Cu(DTPA)

(Table 5A), Ethanol has been used as an eluent in subsequent studies. To choose the proper volume of eluent, the retained copperpentetate complex on the sorbent were stripped with varying volumes (0.5, 1.0, 1.5, 2.0, 2.5 mL) of Ethanol, therefore, the suitable volume of Ethanol (1.5 mL) was selected as eluent (Table 5B).

Analytical figures of merit.

By employing optimal experimental conditions, the developed sorbent presented good linearity (r = 0.998) in the concentration range of 2-700 µg L^{-1}. The linear regression equation for this range was $A = 8 \times 10^{-3}C - 0.027$, where A is the absorbance and C is the concentration of Cu (DTPA) complex, µg L^{-1}. The limit of detection (LOD) and quantification (LOQ) were 0.0146 and 0.0487 µg L^{-1}, respectively. The relative standard deviation (RSD) resulting from the analysis of 10 replicates of 150 mL solutions

containing 100 µg L^{-1} of Copper pentetate complex was 0.7 %. The preconcentration factor (P.F.) (the ratio of the volume aqueous phase/organic phase) of this method in optimum condition was 100.

Efficacy of diverse ions.

The efficacy of a potential interference caused by common species present in samples was examined. For this purpose diverse ions (up to a maximum tolerance ratio of 200) were added to solutions containing 100 µg L^{-1} of Copper pentetate complex, and the solutions submitted to the recommended procedure. An ion was considered as interfering when it caused a variation in the absorbance of the analyte greater than 3%. The results (Table 6) show that excess amounts of common cations and/or anions not interfere with the determination of trace quantities of Cu (DTPA) complex. The obtained results show that EDTA have seriously

Table 4: effect of volume on desorption of Cu (DTPA) complex from the Fe_3O_4@PDA sorbent (pH: 5 acetate, time: 25 min, sorbent mass: 28 mg, volume of eluent (Ethanol): 2 mL, concentration of Cu(II): 500 µg. L^{-1})

Volume of solution (mL)	Amount of desorption (Abs $_{\lambda=276\,nm}$)
100	0.974
150	1.202
200	0.986
250	0.832
300	0.651
400	0.492
500	0.398

Table 5: desorption of Cu (DTPA) complex from the Fe_3O_4@PDA sorbent (pH: 5 acetate, time: 25 min, sorbent mass: 28 mg, volume of initial solution: 150 mL, concentration of Cu(II): 500 µg. L^{-1}): A) by using different eluents for 2 replicates (volume of eluent = 2 mL) B) by using different volume of ethanol for 2 replicates.

	Type of eluent	Amount of desorption (Abs $_{\lambda=276\,nm}$)
A	chloroform	0.032
	Ethyl acetate	0.087
	Acetone	0.448
	Aceto nitrile	0.732
	Methanol	1.142
	Ethanol	1.204
	Acetic acid	1.152
	Methanol-acetic acid (1:1)	1.096
	ethanol-acetic acid (1:1)	1.175
B	Volume of Ethanol (mL)	Amount of desorption (Abs $_{\lambda=276\,nm}$)
	0.5	0.762
	1	1.127
	1.5	1.511
	2	1.203
	2.5	0.879

Table 6. Effect of diverse ions in the determination of copper pentetate complex by SPE method.

Species	Tolerance ratio
Na^+, K^+, Ca^{2+}, Ba^{2+}, Mn^{2+}, Ag^+, Hg^{2+}, Cl^-, Br^-, I^-, SO_4^{2-}, NO_2^-, NO_3^-, PO_4^{3-}, CrO_4^{2-}, $Cr_2O_7^{2-}$	200:1
Ni^{2+}	50:1
Co 2+	10:1
Fe 3+	7:1
Cr 3+	5:1
EDTA	1:1

interfering effects, whereas it doesn't exist in real sample matrix.

Reusability of sorbent.

To evaluate the reusability of sorbent on Copper complex, sorbent was used for 10 times. Sorbent with 3 ml of DDW after each absorption was washed and then dried again as a sorbent was used. This procedure was repeated up to 10 times. The results showed that sorbent can be used up to 5 times with high recovery up to 90 percent (Fig. 3).

Real sample analysis

To evaluate the applicability of this method Copper in vegetable and fruit samples was measured. Moreover, the accuracy of results FAAS measurements were done by official AOAC methods (35). Then, to verify the results and

accuracy of the proposed approach and ensure the absence of systematic error T-test was used. Results measured 6 times in these samples (3 times for each method) (Table 7) , and a comparison of the mean concentration of Cu in some vegetable and fruit samples from this study with the results obtained from the some other country (Brazil, Turkey and Egypt) studies are summarized in Table 8.

CONCLUSION

The magnetic nano-adsorbent coated with PDA was successfully synthesized and used as a novel sorbent for SPE of trace amount of Copper by UV/Vis spectroscopy. The results showed that the nano-sorbent was successfully absorbed Cu (DTPA) and desorbed with the least amount of eluent. In optimal experimental condition (obtained by BBD), the linear range 2-700 $\mu g\ L^{-1}$, limit of detection (LOD)
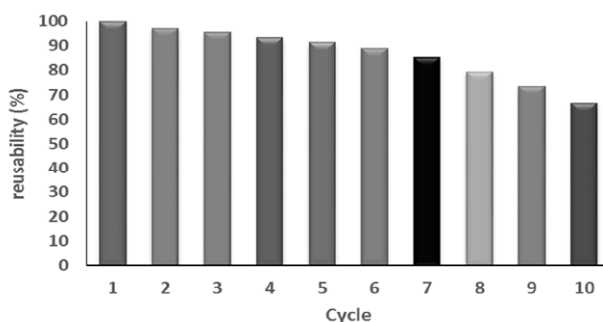

Fig. 3. Reusability of sorbent.

Table 7. Concentrations of Cu ($\mu g\ g^{-1}$) in vegetable and fruit samples (n=3) found in this study

sample	Mean concentration ($\mu g\ g^{-1}$)± SD (n=3)		
	This study		
	UV/Vis spectroscopy (proposed method)	FAAS (comparison method)	T_{cal} (d.f^a=4)^b
Vegetable			
Bell pepper	25.20±0.53	24.91±0.72	0.562
Carrot	2.12±0.09	2.21±0.08	1.295
Dill	7.86±0.34	7.93±0.20	0.307
Lettuce	3.53±0.42	3.66±0.25	0.461
Onion	2.13±0.08	2.18±0.05	0.918
Parsley	5.18±0.18	5.21±0.12	0.240
Potato	N.D ^{c}	0.94±0.08	—
Spinach	4.62±0.37	4.48±0.23	0.557
Tomato	2.23±0.18	2.31±0.12	0.641
Truffle	7.37±0.19	7.42±0.25	0.276
Mushroom	5.34±0.22	5.51±0.16	1.082
Zucchini	6.51±0.09	6.59±0.12	0.924
Fruit			
Apple	11.69±0.24	11.55±0.21	0.760
Banana	2.28±0.21	2.32±0.16	0.262
Cucumber	6.42±0.20	6.45±0.17	0.198
Data	22.87±0.81	22.41±0.42	0.873
Kiwi	2.96±0.60	2.91±0.24	0.134
Peach	2.35±0.08	2.41±0.05	1.102

[a.] degree of freedom
[b.] $T_{critical}$ with degree of freedom 4 at confidence level 95% is equal 2.776
[c.] not detected

Table 8. Comparison of Concentrations of Cu (μg g^{-1}) in vegetable and fruit samples
(n=3) found in this study and those in some literature in different country

samples	Brazil [36]	Egypt [37]	Turkey [33]	This work (Iran)
Vegetable				
Bell pepper	—	—	—	25.20
Carrot	0.7	1.51	2.94	2.12
Dill	—	—	9.67	7.86
Lettuce	0.40	1.97	6.54	3.53
Onion	0.80	1.49	1.71	2.13
Parsley	—	—	6.47	5.18
Potato	0.79	0.83	1.10	Not detected
Spinach	0.6	4.48	5.88	4.62
Tomato	0.60	1.83	3.43	2.23
Truffle	—	—	—	7.37
Mushroom	—	—	6.54	5.34
Zucchini	—	—	—	6.51
Fruit				
Apple	0.40	1.47	1.33	11.69
Banana	0.90	2.51	2.28	2.28
Cucumber	0.40	5.69	2.51	6.42
Data	—	18.30	—	22.87
Kiwi	3.10	—	2.89	2.96
Peach	1.10	1.46	—	2.35

0.0146 μg L^{-1}, limit of quantification (LOQ) 0.0487 μg L^{-1}, low relative standard deviation (RSD) 0.7% (n=10) and preconcentration factor (P.F) (the ratio of the volume aqueous phase/organic phase) 100 was obtained. The method was used for the determination of trace amount of Cu in different real samples and the results showed that no significant difference between the proposed method and FAAS. Compare the results with other studies also showed that the proposed method due to high preconcentration factor, good accuracy, high sensitivity, ease of method, low cost, high selectivity and biocompatibility; and can be used successfully for determination of trace amounts of copper in different real samples.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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