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DETERMINATION OF MICRO AMOUNTS OF Fe (II) AND Fe (III) IN TEA AND RICE SAMPLES BY CLOUD POINT EXTRACTION-SPECTROPHOTOMETRY USING A NEW CHELATING AGENT

ZIANAB T. IBRAHIM, ZUHAIR A-A KHAMMAS[*] and KHALID J. KHADHIM[a]

Department of Chemistry, College of Science for Women, University of Baghdad, Jadiyriah, BAGHDAD, IRAQ
[a]Department of Chemistry, College of Education, University of Al-Qadisiya, Diwanya, AL-QADISIYA, IRAQ

ABSTRACT

A new chelating agent namely, 2-[2-(5-nitro thiazolyl) azo]-8 hydroxyquinoline (5-NTA8HQ), was synthesized, characterized and used for the determination of iron species in rice and black tea samples by combination of cloud point extraction method and spectrophotmetric techniques. The method involved a selective hydrophobic complex formation between iron (II) and 5-NTA8HQ at pH 5, which can efficiently be extracted in surfactant-rich phase of Triton X-114 and determined sepectrophotometrically at λ_{max} 650 nm. Also, Iron (III) can selectively form a chelate with 5-NTA8HQ at pH 6 and extracted with Triton X-114 but with less sensitivity than with Fe(II) counterpart. Thus, this limitation is treated via the reduction of Fe(III) to Fe(II) with ascorbic acid and the amount of Fe(III) in samples is found by subtraction. All significant factors for both Fe(II) and Fe(III) species that influence the separation and determination steps were investigated in detail by one factor-at-a-time (OFAT) optimization. At optimum conditions, the enrichment factors of 126 and 52 fold were obtained for Fe(II) and Fe(III) ions respectively, leading to detection limits of 0.35 ng Fe(II) mL^{-1} and 3.96 ng Fe(III) mL^{-1} in aqueous solution. The linear dynamic range, relative standard deviation (n = 7 at 10 ng Fe(II) mL^{-1} and 30 ng Fe(III) mL^{-1}), and the recoveries by the standard additions method were of 0.5-50 ng $mL^{-1}$and 10-150 ng mL^{-1}, 0.54% and 1.58%, 98.3 ± 2.9 and 98.5 ± 1.26 for Fe(II) and Fe(III) ions, respectively. The described method is sensitive, easy to apply and has a slight interference; thereby, the determination of iron species in rice and black tea samples were performed.

Key words: New chelating agent, Fe(II) and Fe(III), Cloud point extraction (CPE), UV-Vis spectrophotometry, Flame atomic absorption spectrometry (FAAS).

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[*]Author for correspondence; E-mail: dr_zuhair52@yahoo.com

INTRODUCTION

At present, the analytical chemists face a great challenge to so-called the speciation analysis in order to provide a high-precise data for workers and specialists, who are interesting in food, environment, medicine and occupational health. This challenge may be ascribed to the existence, distribution and binding state of metallic ion species in the origin of the sample beside the difficulty of choosing the appropriate method for the separation and detection, especially when the metal species are with varying and at very low concentration in the sample. However, there is a determined effort and great attention by researchers in recent years to develop more precise and appropriate analytical procedures in this domain. These are reflected in the emergence of several published papers and reports, particularly for metallic species that have an impact directly to humans and environment such as mercury, chromium, arsenic, antimony, selenium, tin, iron, etc. Of these, iron, which is like many elements can exist in more than oxidation state (-2 to +6), but the two most important forms are Fe(II), in which the iron ion shares two of its electrons, and Fe(III), in which it shares three electrons[1]. Principally, these two chemical forms make iron suitable for numerous biochemical reactions in all life forms, from bacteria to man [2]. But, an excess concentration of iron (i.e. iron overload) is potentially toxic to human due to its pro-oxidant activity and cause diseases like hemochromatosis, while iron deficiency is the most common type of anaemia and can be related to severe pathologies[3,4]. Therefore, the U.S. Recommended Daily Allowance (USRDA) for iron is 18 mg for male and 11 mg for female between the ages of 19-50 years[5]. Since, iron has two readily inter converted oxidation states that occur especially in biotransformation processes, the determination of iron species is very important from the point of view of biochemical and nutritional studies because of the influence of the two forms on the bioavailability of iron and physicochemical and toxicological properties of other trace elements and organic substrates[6-8]. In the light of this information, it seems that the speciation analysis of iron is of prime importance from the standpoint of health effect to get enough information on its biotransformation and toxicity in its different oxidation forms.

The most widely applied techniques for the speciation analysis of iron include, solvent extraction-flame atomic absorption spectrometry[9,10], molecular absorption spectrophotometry[11], micro-column packed-electrothermal vaporization inductively coupled plasma-optical emission spectrometry[12], sequential injection analysis[13,14], flow injection analysis[15], flow injection-assisted optical sensor[16], flow injection-electrochemical/atomic absorption detectors[17], capillary electrophoresis[18] and HPLC [19].

Recently, a cloud point extraction (CPE) in conjunction with molecular spectroscopy and/or atomic absorption spectrometry has received wide acceptance in the scientific

community for the separation, extraction and detection of iron species in different matrices, mainly because it complies with the "green chemistry" principles[20-22]. However, few reports were published including; speciation of iron in certified reference material using the modified ferrozine method, FIA-spectrophotometry and flame atomic absorption spectrometry after CPE[23], in water and milk samples using cloud point extraction-flow injection flame atomic absorption spectrometry[24] and in beer sample by CPE-Spectrophometry[25]. In all the above methods, the classical ligands such as ammonium pyrrolidinecarbodithioate (APDC), Ferron and 2-(bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) were used as complexing agents for cloud point extraction of both iron species, in an attempt to improve the selectivity and elaboration of the analytical characteristics of CPE methodology in combination with instrumental analytical techniques.

In the present work, a new thiazolylazo dye reagent, namely 2-[2^{-}-(5-nitro thiazolyl) azo]-8 hydroxyquinoline (5-NTA8HQ) was synthesized, characterized, and exploited as a laboratory-made complexing agent to investigate the cloud-point extraction methodology for preconcentration of ultra trace amounts of iron species using Triton X-114 as an extracting medium and their determination by UV-Vis spectrophotometry and flame atomic absorption spectrometry (FAAS). The developed method was applied for the determination of ultra trace amounts of iron (II) and (III) in rice and black tea samples.

EXPERIMEMTAL

Apparatus

A PG Instrument T80 + UV/Vis spectrometer (England) equipped with 10-mm optical path cell was used for the scanning study of absorption spectra of the complexes formed, while absorbance measurements were carried out with spectrophotometer UV-7804C (China). A PG model Spectra A 500 (England) flame atomic absorption spectrometer equipped with a deuterium lamp background correction and an iron hollow cathode lamp (operated at 15 mA) as the radiation source at the wavelength of 248.3 nm with 1.0 nm spectral band pass was used. All of the absorbance measurements were performed using an air/acetylene flame at flow rates of 3.5 and 1.5 L min^{-1}, respectively. The pH measurements were conducted with a pH meter Philip PW model 9421 (Holland) with an accuracy of ± 0.01 pH unit. The effect of temperature was investigated by using a water bath WB 710 model (OPTIMA, Japan).

Reagents and solutions

All the chemicals used were of analytical reagent grade, and used without further purification. Distilled and deionized water was used for diluting the samples and reagents.

2-Amino-5-nitro thiazole, 8-hydroxyquinoline (RIEDEL-DE HAEN AG SEELZE, Germany), sodium nitrite (BDH), hydrochloric acid (BDH), and ethanol were purchased from (GCC, England). Non-ionic surfactant Triton X-114, whose chemical formula is t-Oct-C_6H_4-(OCH_2CH_2)nOH, with (n) equal to 7-8 and an average molecular weight of 537 g/mol, was obtained from ACROS ORGANICS (New Jersey, USA) and was used without further purification. The aqueous solution (10% v/v) of Triton X-114 was prepared by diluting 10 mL of concentrated solution to 100 mL with water. Stock solutions of Fe(II) and Fe(III) ions (1000 mg L^{-1}) were prepared by dissolving 7.027 g and 4.84 g of $Fe(NH_4)_2$ $(SO_4)_2.6H_2O$ (BDH) and $FeCl_3.6H_2O$ (Merck) in deionized water, respectively. Working standard solutions of each metal ion were freshly prepared by appropriate dilution of the stock standard solutions. An acetate buffer solution (0.1 mol L^{-1}) was prepared from acetic acid and sodium acetate at different pH.

Synthesis procedure of (5-NTA8HQ) reagent

The synthesis of 2-[2-(5-nitro thiazolyl) azo]-8 hydroxyquinoline (5-NTA8HQ) was carried out according to general procedure described elsewhere[26,27] with some modifications of starting materials as shown in Figure 1. 2-Amino-5-nitro thiazole (1.45 g, 0.01 mol) was dissolved in 25 mL of distilled water and 5 mL of concentrated hydrochloric acid and diazotized below 5°C with (0.69 g, 0.01 mol) sodium nitrite. The resulting diazonium chloride solution was added dropwise with cooling to the solution of (1.450 g, 0.01 mol) 8-hydroxyquinoline dissolved in 50 mL of ethanol and the mixture left in the refrigerator overnight. The mixture was neutralized with dilute hydrochloric acid to (pH = 6.0).

The solid product was filtered off, washed with cold distilled water, crystallized twice from hot ethanol and dried over $CaCl_2$ to give purple crystals. Yield 78%; mp. 272-274°C; mass spectrum analysis shows that the chemical structure of the reagent is $C_{12}H_7N_5S$ (301.285 g mol^{-1}); IR(KBr) ν_{max}/cm^{-1}, 3379, (w, Ar-OH), 3109 (w, Ar C-H), 2900 (w, C-H aliphatic), 1604 (m, C-O) 1585 (m, C=N), 1512 (s, N=N), 1234 w, 1164 m (C-S), 1110 (m, C-N), 887, 825, 779 (s, δ C-H); ^{1}H NMR (DMSO-*d*6, 298 K) d/ppm) (1) Single peak at δ = 2.5 ppm for OH group (2) Mutiple peaks at δ = 7.08-7.28 ppm for H3 proton of 8-HQ ring (3) Doublet peaks at δ = 8.25-8.36 ppm for H4 proton of 8-HQ ring (4) Multiple peaks at δ = 7.8-7.94 ppm for the H6 proton of 8-HQ ring 5-Multiple peaks at δ = 8.8-8.98 ppm for the H7 proton of 8-HQ ring (6) Doublet peaks at δ = 9.0-9.06 ppm for H4 proton of thiazole ring (7) Doublet peaks at δ = 8.62-8.68 ppm for H5 proton of 8-HQ ring. The chemical structure of 2-[2-(5-nitro thiazolyl) azo]-8 hydroxyquinoline abbreviated as (5-NTA8HQ) is shown in Fig. 1. The reagent is insoluble in water but very soluble in some organic solvent like methanol, ethanol, acetone, dimethyl sulfoxide (DMSO) and dimethylformamide (DMF).

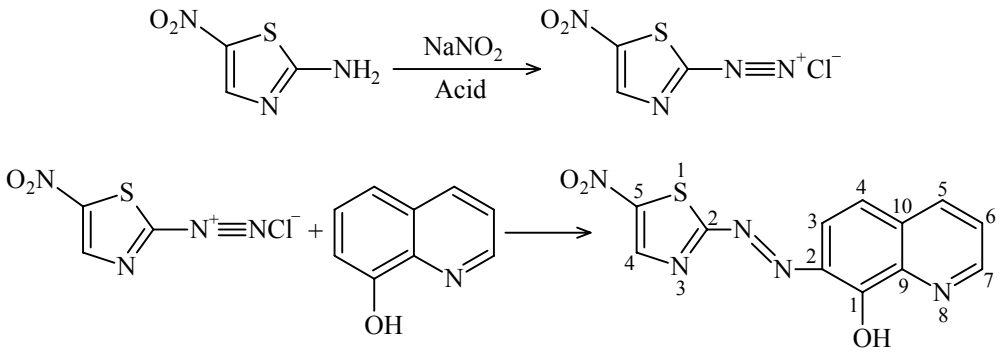

Fig. 1: Synthetic path of reagent (5-NTA8HQ)

General procedure for CPE

To an aliquot of 2 mL of a solution containing known amount Fe(III) and Fe(II) standard or sample solution, 0.3 mL of 1.0 x 10^{-3} mol L^{-1} (5-NTA8HQ) reagent solution for both Fe(III) and Fe(II), 1.0 mL of acetate buffer solution at pH = 6.0 for Fe(III) and 5.0 for Fe(II), 0.2 mL of Triton X-114 (10%) for Fe(III) and Fe(II) were mixed in a 5-mL volumetric flask and diluted to mark with deionized water. The contents of the flask were transferred into a 10 mL centrifuging tube and the phase separation was induced by heating the contents in a water bath at 60ºC for 15 min.

For Fe(II), the separation of the phases was accelerated by centrifuging at 4000 rpm for 10 min. The viscosity of the surfactant-rich phase was increased by cooling the system in an ice-bath for 5 min. After decantation, the surfactant-rich phase that remained adhered to the tube was dissolved with a 3 mL of 0.1 M nitric acid in ethanol and the concentration of the Fe(II) ions was determined spectrophotometrically at λ_{max} 650 nm. For Fe(III), its content was reduced to Fe(II) by adding 1 mL of 0.1 M ascorbic acid followed the above-mentioned CPE procedure, from which the concentration of total iron was determined by FAAS and the amount of Fe(III) in standards or samples was calculated by difference.

Preparation of sample solution

Preparation of rice sample[28]

The rice samples were first grinded into fine powder using a glass mortar, then an accurately amount of 1.00 g of a powdered rice sample was transferred into a glass beaker and digested with 10 mL of concentrated nitric acid (65% w/v) and 10 mL of hydrogen peroxide (30% v/v) on a hot plate at a fairly low temperature. The content was cooled to room temperature and the residue was dissolved in 1.0 mL of 0.1 M HNO_3. After dilution

with deionized water, the pH was adjusted to nearly 5 by the addition of dilute NaOH solution. Then, the solutions were transferred into a 50.0 mL volumetric flask and diluted to mark with the deionized water. The concentration of Fe(II) and Fe(III) were determined according to the general CPE procedure.

Preparation of black tea sample[29]

1.0 g of black tea sample was transferred quantitatively into a 250 mL round bottom digestion flask. Exactly 6 mL of freshly prepared 5:1 mixture of concentrated HNO_3 and concentrated $HClO_4$ was added to the sample. The sample was swirled gently to homogenize, fitted to a reflux condenser and digested continuously for three hours at a maximum temperature of 300^{o}C. The digest was quantitatively transferred to a 50 mL volumetric flask and diluted to mark with deionized water. The concentration of Fe (II) and Fe(III) were determined according to the general CPE procedure.

Statistical analysis

All mathematical and statistical computations were made using Excel 2007 (Microsoft Office) and Minitab version 14 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of [(5-NTA8HQ)$_2$Fe] and [(5-NTA8HQ)$_2$Fe] Cl complexes were recorded in the presence of surfactants against a reagent blank prepared under the identical conditions. The spectra of Fe(II) and Fe(III) complexes show the absorption maxima of 650 and 590 nm with molar absorpitivities of 1.64×10^7 and 1.71×10^6 L mol^{-1} cm^{-1}, which allowed the speciation of Fe. Whilst, the ligand (5-NTA8HQ) gave the absorption maxima of 520 nm (Fig. 2).

The reagent (5-NTA8HQ) reacts with Fe(II) and Fe(III) ions at pH 5 and 6 forming a deep green and pale green complexes, respectively, and the absorbance reached its maximum within 5 min and remained stable, for at least 24 hr. The stoichiometry of [(5-NTA8HQ)$_2$Fe] and [(5-NTA8HQ)$_2$Fe]Cl complexes were studied, under the established experimental conditions, by Job's and mole ratio methods. The obtained results indicated that the composition of both complexes was 1: 2 with stability constant of 1.9×10^{11} and $1,2 \times 10^{10}$ L^{2}M^{-1} for [(5-NTA8HQ)$_2$Fe] and [(5-NTA8HQ)$_2$Fe] Cl complexes, respectively. In addition, these complexes were characterized on the basis of spectroscopic techniques and the suggested related chemical structures are shown in Fig. 3.

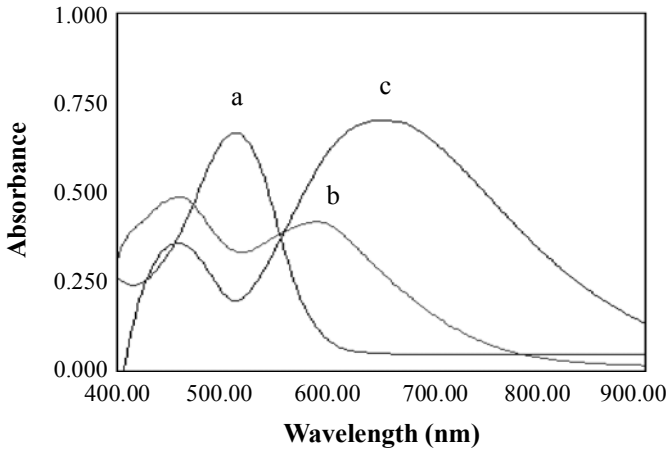

Fig. 2: Absorption spectra (a) Reagent (5-NTA8HQ) = 1 x 10^{-3} M (b) Fe (III) -(5-NTA8HQ) complex, Fe (III) = 150 ng mL^{-1}, 0.3 mL of (5-NTA8HQ) = 1 x 10^{-3} M, Buffer pH = 6 (1 mL), 0.2 mL of 10% (v/v) Triton X-114 (c) Fe (II))- (5-NTA8HQ) complex, Fe (II) = 30 ng mL^{-1}, 0.3 mL of (5-NTA 8 HQ) = 1 x 10^{-3} M, Buffer pH = 5 (1 mL), 0.2 mL of 10% (v/v) Triton X-114

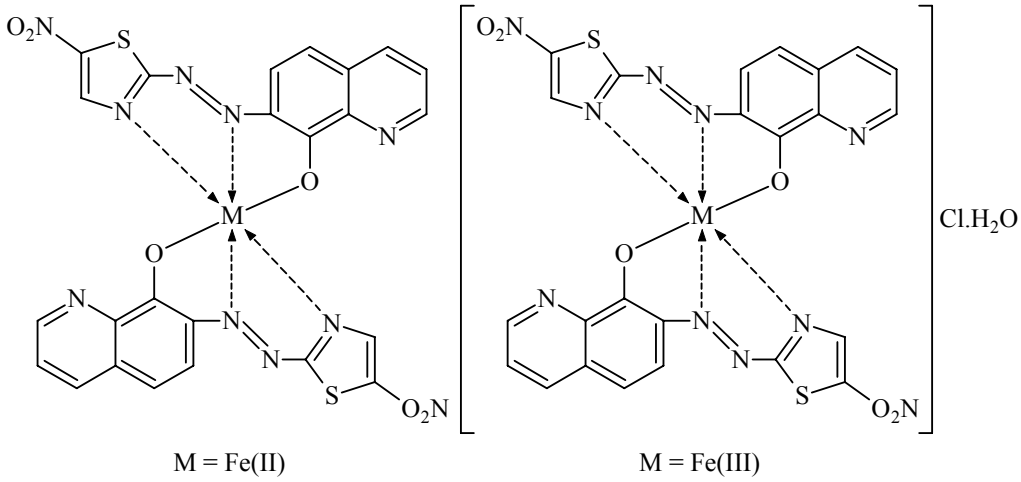

Fig. 3: The probable chemical structures of the complexes

Optimization of CPE procedure

The effect of different experimental variables, which impact the CPE procedure for the two species such as, pH, concentration of reagents, amount of non-ionic surfactant, temperature and incubation time were investigated using one variable-at-a-time (OVAT) strategy in searching the optimum conditions, to maximize recovery percentage and other

analytical figures of merit such as sensitivity and detection limit of both species in the selected matrices. Each experiment of the following variable was conducted following the general CPE procedure

Effect of pH

The solution pH plays a significant role in the formation of metal complex with the chelating agent and their subsequent extraction by CPE methodology. Thus, the effect of pH was studied in the range of 2 to 8 using different pH acetate buffer solutions. The results are depicted in Fig. 4. As can be seen from Fig. 4 that the absorbance first increased with increasing pH and reached a maximum at pH 6.0 and 5.0 for Fe (III) and Fe (II) complexes, respectively. Thereafter, the absorbance gradually decreased because of partial dissociation of the complexes at higher pH, which may result in incomplete extraction of both complexes. Therefore, pH 5.0 and 6.0 were selected as the optimum pH for complete formation of for Fe (II) and Fe (III) complexes, respectively.

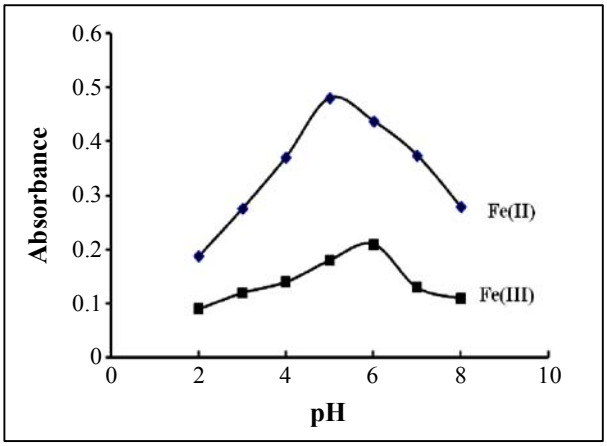

Fig. 4: Effect of pH on the formation of (5-NTA8HQ) complex formed with Fe(II) and Fe(III) ions by CPE [Conditions: 20 ng mL^{-1} Fe (II) or 80 ng mL^{-1} Fe(III), 0.3 mL of 1 x 10^{-3} M (5-NTA8HQ) 0.2 mL of 10% (v/v) Triton X-114, Temp. 60^{o}C, Time 15 min]

Effect of (5-NTA8HQ) concentration

The effect of the 5-NTA8HQ concentration was investigated by measuring the absorbance signal according to the general CPE procedure of solution containing 20 ng mL^{-1} Fe(II) or 80 ng mL^{-1} Fe(III) and varying volume from 0.1 to 0.5 mL of 1 x 10^{-3} mol L^{-1} (5-NTA8HQ). In both cases, Fe(III) or Fe(II), the analytical responses increase rapidly as the volume of 5-NTA8HQ increases and reach maximum up to 0.3 mL and decrease thereafter

with further increase in the chelating agent indicating that any excessive amount of chelating reagent was not necessary (Fig. 5). Consequently, 0.3 mL of 1 x 10^{-3} mol L^{-1} of 5-NTA8HQ was chosen as optimum for both species.

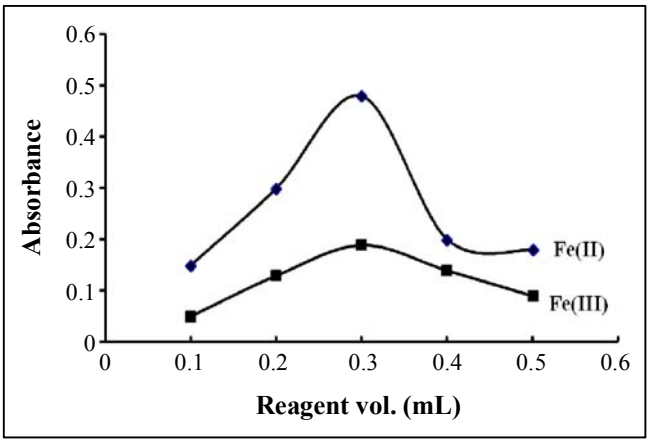

Fig. 5: Effect of concentration of 5-NTA8HQ on the CPE of Fe(II) and Fe(III) [Conditions: 20 ng mL^{-1} Fe (II) or 80 ng mL^{-1} Fe(III), X mL of 1 x 10^{-3} M (5-NTA8HQ), pH 5 or 6 for Fe(II) or Fe(III) 0.2 mL of 10% (v/v) Triton X-114, Temp. 60^{o}C, Time 15 min]

Effect of Triton X-114 amount

Triton X-114 was chosen as nonionic surfactant in the present study because of its low cost, commercial availability with high purity, low toxicological properties, and its high viscosity provides the opportunity for easy phase separation by centrifugation[24]. The effect of variation of triton X-114 amount on the absorbance magnitude for the determination of Fe(III) or Fe(II) ion was evaluated. Different volumes of triton X-114 (10% v/v) ranging from 0.1 to 0.5 mL were used in this study at previously established optimum conditions. As shown in Fig. 6, the absorbance for both ions increased by increasing the triton X-114 concentration up to 0.2 of 10% (v/v) for Fe(III) and Fe(II) and then suddenly decreased at higher amounts. Therefore, 0.2 mL of 10% (v/v) triton X-114 was used as the optimum amount for Fe(III) and Fe(II) for subsequent experiments.

Effect of the equilibrium temperature and incubation time

For reaction completion of the metal ion with chelating agent and to attain efficient phases separation by CPE method, the influence of the equilibrium temperature and the incubation time were considered. Consequently, a study was carried out to choose the range of temperature that enhances higher absorbance signals for Fe(III) and Fe(II) ions. The

temperature was varied from 30°C to 80°C at incubation time of 15 min keeping other variables constant in search of optimum value. It was shown (Fig. 7) that a maximum absorbance signal was achieved, when the temperature was 60°C. Thus, an equilibration temperature of 60ºC for maximum extraction of Fe(II) and Fe(III) was chosen as optimal. On the other hand, it was observed that the incubation time of 15 min was sufficient for the maximum absorbance for the two complexes (Fig. 8).

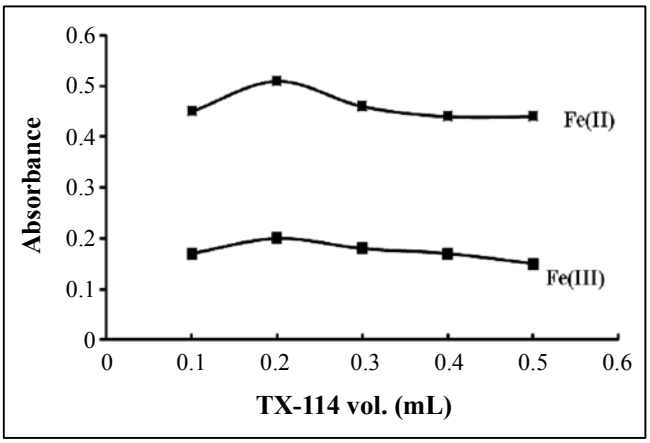

Fig. 6: Effect of Triton X-114 amount on the CPE of Fe(II) and Fe(III) complexes [Conditions: 20 ng mL^{-1} Fe(II) or 80 ng mL^{-1} Fe(III), 0.3 mL of 1 x 10^{-3} M (5-NTA8HQ), pH 5 or 6 for Fe(II) or Fe(III) X mL of 10% (v/v) Triton X-114, Temp. 60^{o}C, Time 15 min]

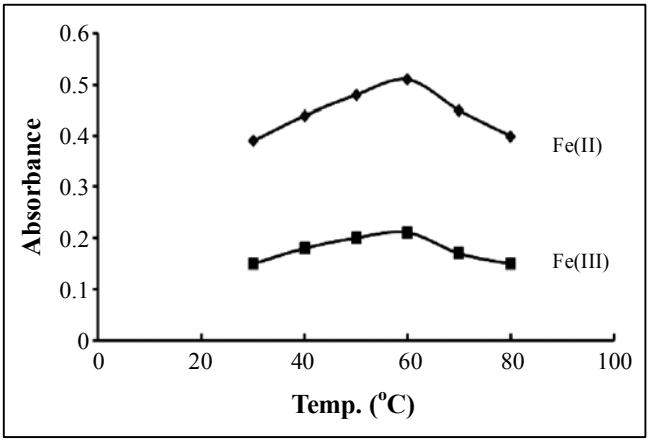

Fig. 7: Effect of equilibration temperature on the CPE of Fe(II)/Fe(III) [Conditions: 20 ng mL^{-1} Fe (II) or 80 ng mL^{-1} Fe(III), 0.3 mL of 1 x 10^{-3} M (5-NTA8HQ), pH 5 or 6 for Fe(II) or Fe(III) 0.3 mL of 10% (v/v) Triton X-114, Time 15 min]

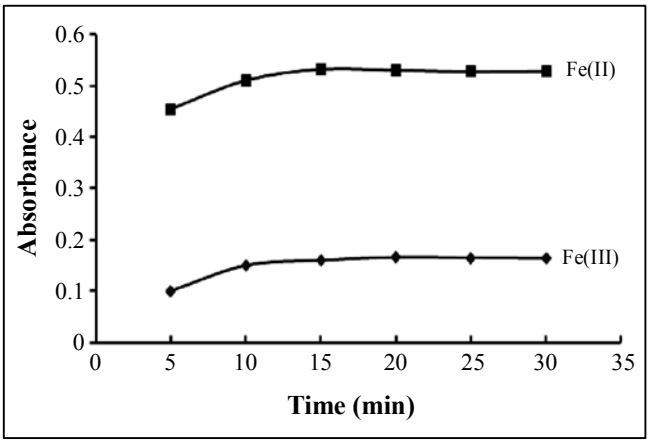

Fig. 8: Effect of incubation time on the CPE of Fe(II)/Fe(III) [Conditions: 20 ng mL^{-1} Fe(II) or 80 ng mL^{-1} Fe(III), 0.3 mL of 1 x 10^{-3} M (5-NTA8HQ), pH 5 or 6 for Fe(II) or Fe(III) 0.3 mL of 10% (v/v) Triton X-114, Temp. 60^{o}C]

The effect of centrifugation rate and time was also taken into account on extraction efficiency. A centrifuging time of 10 min at 4000 rpm was selected for the entire CPE procedure as being optimum and beyond this time, no confirmation was observed for improving extraction efficiency. It is worth mentioning that the sensitivity and extraction efficiency of Fe(II)-(5-NTA8HQ) complex was higher than those of Fe(III)-(5-NTA8HQ) complex in all the aforesaid CPE optimization procedures. This was obvious from the value of the stability constant of the former, which was 16 fold higher than the latter. So it is required to convert Fe(III) by its reduction into Fe(II), when conducting the general CPE procedure for the determination of Fe(III) in the real samples.

Calibration graphs

Under the optimized conditions established by CPE procedure, a series of standard Fe(II) and Fe(III) solutions ranging from 0.5-50 and 10-150 ng mL^{-1}, respectively, were taken and subjected to the general CPE in order to test the linearity of the method. The Fe(II) ion was determined spectrophotometrically at λ_{max} 650 nm, while total iron was determined by FAAS and Fe(III) ion calculated by difference. The statistical evaluation for the calibration graphs has shown that a strong correlation between signal and Fe(II) or Fe(III) concentration may exist (r = 0.9999 or 0.9997). The analysis of variance (ANOVA), On the other hand, also proved that the linear regression equations [y = 0.0195 ± 0.003851) + (0.02507 ± 0.000145) x] and [(0.00900 ± 0.004596) + (0.002617 ± 0.000058) x] for Fe(II) and Fe(IIII), respectively, were statistically valid. This because of the ratio (MSreg/MSerror) for 1 and 6 dof and 1 and 8 dof, larger than critical value ($F_{1,6}$ = 5.99 and $F_{1,8}$ = 5.32 at 95%

CI), indicating that the predication based on the regression line is satisfactory as listed in Table 1.

Table 1: Analysis of variance table of regression line for Fe(II) and Fe(III)

Source	dof	SS	MS	F	P	Ion
Regression	1	1.54919	1.54919	179235.47	0.000	
Residual error	6	0.00005	0.00001			Fe(II)
Total	7	1.54924				
Regression	1	0.128996	0.128996	10787.16	0.000	
Residual error	8	0.000096	0.000012			Fe(III)
Total	9	0.129092				

Dof = Degrees of freedom, SS: sum of squares, MS: mean of squares, F (Fisher F-test)

The statistical analytical results for the calibration data for both species are summarized in Table 2.

Table 2: Method validation of the determination of Fe(III)-(5-NTA8HQ) and Fe(II)-(5-NTA8HQ) using the proposed method

Fe(III)	Fe (II)	Parameter
y = 0.009000 + 0.002617 x	y = 0.01955 + 0.02507 x	Regression equation
0.9995	0.9999	Correlation coefficient ®
0.00345808	0.00293995	Std. dev. of regression line ($s_{y/x}$)
0.002617 ± 0.000058	0.02507 ± 0.000145	C.L. for the slope (b ± tsb*)* at 95%
0.009 ± 0.0045969	0.01955 ± 0.003851	C.L. for the intercept (a ± tsb*)* at 95%
10-150	0.5-50	Concentration range (ng mL^{-1})
3.96	0.35	Limit of detection (ng mL^{-1})
13.21	1.17	Limit of quantitation (ng mL^{-1})
3.83×10^{-4}	3.99×10^{-5}	Sandell's sensitivity (µg cm^{-2})
1.71×10^{6}	1.64×10^{7}	Molar absorptivity ($L.mol^{-1}.cm^{-1}$)

Cont…

Fe(III)	Fe (II)	Parameter
1:2	1:2	Composition of complex (M:L)*
1.58% at 30 ng Fe(III) mL^{-1}	0.54% at 10 ng Fe(II) mL^{-1}	RSD% (n = 7)
98.5 ± 1.26	98.3 ± 2.9	Recovery (%)
55	71	Preconcentration factor (PF)*
52	126	Enrichment factor (EF)**

*PF is calculated as the ratio of volumes of aqueous phase to that of surface-rich phase
**EF is calculated as the ratio of slope of calibration curves obtained with and without CPE

The proposed method has achieved enriching factor of 126 and 52 fold and this is what was allowed to get on the detection limit of 0.35 and 3.96 ng mL^{-1} for Fe(II) and Fe(III) in aqueous solution, respectively. It can be concluded that the prepared ligand in this work beside CPE-Spectrophotometry/FAAS gave satisfactory analytical figures of merit for Fe(II) and Fe(III), which were much better than with those obtained by some previous studies (Table 3). But, they were in harmony with most studies that used analytical methods in combination with CPE.

Table 3: Comparison of the proposed of CPE method with reported methods in chemical literatures for the determination of Fe(II)/Fe(III)

Ref.	EF	Detection limit (ng mL^{-1})		Linear range (ng mL^{-1})	Technique	Sample
		Fe(II)	Fe(III)			
11	-	0.22	0.22	-	UV-Vis Spec./FAAS	Wine
12	-	0.053	0.053	-	SPE-ETV-ICP-OES	Water
13	-	0.1a	0.15a	Fe(II) : 0.15-100a Fe(III): 0.3-80a	SIA	-
14	-	0.05a	0.1a	-	SIA	Waters
15	-	0.25a	0.17a	Fe(II) 0-30a Fe(III) 0-50a	FIA	Drugs
16	-	80	80	80-500	FIA-Optical sensor	Synthetic
18	-	0.06[a]	0.1[a]	-	EC	
23			7.0	-	CPE-Spec./FAAS	CRM

Cont…

Ref.	EF	Detection limit (ng mL^{-1})		Linear range (ng mL^{-1})	Technique	Sample
		Fe(II)	Fe(III)			
24	-	1.7	1.7	10-250	CPE-FI-AAS	Water and milk
25	-	0.8	1.0	5.0-112	CPE- UV-Vis Spec.	Beer
29	Fe(II) 167 Fe(III) 158	0.2	0.5	0.01-0.4[a]	FO-LADS	Different samples
30	Fe(III) 19.6	-	0.4[a]	Fe(III) 40-1000	CPE-FAAS	Environmental and biological
This work	Fe(II) 126	0.35	3.96	Fe(II) 0.5-50 Fe(III) 10-150	CPE-Spec./FAAS	Tea and rice

[a] In (μg mL^{-1}); CRM: Certificate reference materials; UV-Vis Spec.: UV.Vis Spectrophotometry; SPE-ETV-ICP-OES: Solid Phase extraction-Electrothermal Vaporization–Inductively Coupled–Optical Emission Spectrometry; FAAS: Flame Atomic Absorption Spectrometry; FIA: Flow Injection Analysis; FO-LADS; Optic-Linear Array Detection Spectrophotometry SIA : Sequential Injection Analysis; CE: Capillary Electrophoresis

By considering a limit of detection (LOD) of 0..35 μg $L^{-1}$for Fe(II) and 3.96 μg L^{-1} for Fe(III) in aqueous solution and 1 g of solid sample in 50 mL solution , the LOD of the method would be 0.0175 and 0.198 μg g^{-1} for Fe(III) and Fe(III) respectively. On that basis, the proposed method has been successfully used for the determination of both species in various samples of tea and rice that were selected randomly from Iraqi markets in order to test the applicability and reliability of the method.

Recovery test

Since the certificate reference materials (CRM's) for the determination of the iron species in samples are not available, accuracy has been tested through the recovery percent evaluation. In this test, the effect of Fe(II)/Fe(III) ratio was studied by taking up the binary mixtures prepared at Fe(II)/Fe(III) concentration ratios ranging from 0.1 to 3 after reducing of Fe(III) with ascorbic acid (pH 5 acetate buffer) and the analyzed by the suggested method. The results obtained are summarized in Table 4.

As could be seen from the results, Fe(II) and Fe(III) are completely separated for further studies, and quantitatively recovered ranging from 96- 99.5% for Fe(II) and in range of 98.0-99.0% for Fe(III).

Table 4: The results of speciation analysis of Fe in binary mixture obtained by the proposed method

Fe(II)				
E_{rel} (%)	Recovery (%)	Found (µg L^{-1})	Added (µg L^{-1})	Fe(II)/ Fe(III) Ratio
-3.0	97.0	9.7	10	0.1
-1.0	99.0	14.9	15	
-1.0	99.0	19.8	20	
-1.5	98.5	19.7	20	0.5
-4.0	96.0	28.8	30	
-1.4	98.6	29.6	30	2.0
-0.5	99.5	39.8	40	
-1.25	98.75	39.5	40	3.0

Fe(III)					
E_{rel} (%)	Recovery (%)	Found (µg L^{-1})	Total Fe (µg L^{-1})	Added (µg L^{-1})	Fe(ll)/Fe(lll) Ratio
-2.0	98.0	19.6	29.6	20	0.1
-1.5	98.5	19.7	34.7		
-1.0	99.0	19.8	39.8		
-1.0	99.0	29.8	49.8	30	0.5
-2.0	98.0	29.6	59.6		
-1.0	99.0	39.7	69.7	40	2.0
-2.0	98.0	39.5	79.5		
-1.0	99.0	49.6	89.6	50	3.0

Interference study

The effect of most diverse ions expected in the rice and tea matrices on the determination of 50 ng/mL−Fe(II) solution was studied following the general CPE procedure. It is agreed that an extraneous ion deemed to interfere seriously, when it gives a relative error percent of more than ± 5%. The results indicated that some of metal ions like, Ca(II), Na(I), K(I), Mg(II), Co(II) and Zn(II) have no appreciable effect on the iron species responses, while the other metal ions such as Cu(II), Cd(II) and Ni(II) have exceeded the

allowable limits of interferences for Fe(II) as shown in Table 5. Therefore, a mixture of 0.01 M oxalic acid, tartaric acid or sodium fluoride was used to control the interferences of Cd(II), Cu(II) and Ni(II) without any appreciable masking of Fe species.

Table 5: Effect of divers ions on the absorption signal of Fe(II) (50 ngmL^{-1}, Abs = 1.270) by CPE-spectrophotometry

E_{rel} (%)	ΔA	A	Interferent/Fe (II)	Interfering ion
0.15	0.002	1.272	1000	Na^+
-0.47	-0.006	1.264	1000	K^+
0.94	0.012	1.282	1000	Ca
1.90	0.025	1.295	1000	Mg
0.55	0.007	1.277	1000	Co(II)
6.20	0.080	1.35	500	Ni(II)
9.40	0.120	1.39	500	Cu(II)
8.60	0.110	1.38	500	Cd(II)
-0.62	-0.008	1.262	1000	Zn(II)

Determination of iron species in real samples

To test the applicability of the proposed CPE method, three imported black tea samples beside four local and two imported rice samples available in the Iraqi markets were analyzed for both Fe species. The results are tabulated in Tables 6 and 7, respectively.

Table 6: Determination of iron species in black tea samples

**Fe(III) (µg g^{-1})	*Fe(II) (µg g^{-1})	Tea sample
30.35 ± 0.15	29.11 ± 0.091	1
55.3 ± 0.2	27.1 ± 0.126	2
82.81 ± 0.2	22.21 ± 0.189	3

*The mean value and its standard deviation of three replicate measurements at 95% confidence level. ($x \pm t.\ s/\sqrt{n}$); **The results determined by calculating the difference between the total Fe and Fe(II) amounts before and after reduction of Fe(III) with 1.0 mL of 0.01 M ascorbic acid

Table 7: Determination of iron species in rice samples

**Fe(III) (μg g^{-1})	*Fe(II) (μg g^{-1})	Rice sample
34.6 ± 0.58	11.36 ± 0.159	Iraqi 1
34.12 ± 0.55	6.21 ± 0.151	Iraqi 2
23.77 ± 0.22	12.23 ± 0.227	Indian
20.2 ± 0.7	19.11 ± 0.04	Vietnamese

*The mean value and its standard deviation of three replicate measurements at 95% confidence level ($x \pm t.\ s/\sqrt{n}$)

**The results determined by calculating the difference between the total Fe and Fe(II) amounts before and after reduction of Fe(III) with 1.0 mL of 0.01 M ascorbic acid

The results in Table 6 revealed that the value for Fe(II) obtained in all black tea samples were in the range 22.21-29.11 μg g^{-1}, while the Fe(III) was in the range of 30.35-82.81 μg g^{-1}. Likewise, the results in Table 7 have shown that the value for Fe(II) obtained in all rice samples were in the range 11.36-19.11 μg g^{-1}, while the Fe(III) was in the range of 20.2-34.6 μg g^{-1}.

CONCLUSION

To the best of our knowledge, the separation and preconcentration of iron species into surfactant rich phase (TX-114) in a single-step extraction is scanty and just few articles appeared in the chemical literatures since establishment of CPE. In this work, we have exploited use of laboratory-made azo dyes ligands instead of the commercial thiazolylazo dyes such as 1-(2-thiazolylazo)-2-naphthol (TAN), 4-(2-thiazolylazo) resorcinol (TAR) and 2-(2-thiazolylazo)-p-cresol (TAC), for the first time, for cloud point extraction in the selective separation and enrichment of iron species as a prior step for the determination of ultra-trace quantities in real samples by means of spectrophotometric techniques. The analytical figures of merit and enrichment for Fe(II) and Fe(III) species were satisfactory compared to those reported in chemical literatures especially with those using sophisticated and expensive instrumentation such as SPE-ETV-ICP-OES, FIA-Optical sensor, FO-LADS etc.

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