



Preconcentration and spectrophotometric determination of Fe (III) by cloud point extraction using Zincon as complexing agent

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Abstract

A cloud point extraction (CPE) process was described for the separation and spectrophotometric analysis of Fe(III) in different water samples and blood as chelating agent. The complexation reaction between Fe(III) and Zincon was elaborated at pH 5.0 using acetate buffer and the complex was quantitatively recovered in a mixed micelle system composed of cetyltrimethyl ammonium bromide (CTAB) and Triton X-114. This reaction was carried out at room temperature in the presence of 0.05 mol L⁻¹ Na₂SO₄ as salting-out electrolyte. The linearity was up to 1000 µg L⁻¹. The preconcentration factor was 50. The precision (as relative standard deviation) and the limit of detection were 2.5% and 3.1 µg L⁻¹, respectively. The proposed procedure was used for the spectrophotometric detection of Fe(III) in water and blood samples and the data were statistically comparable to those achieved using ICP-OES technique.

Keywords: Cloud point extraction; Iron; Zincon; Mixed micelle; spectrophotometry.

Introduction

Iron (Fe) is a critical element for life. It is used by human in many industries and activities. It is required for many biological activities such as heme and myoglobin synthesis. Its deficiency leads to anemia and other diseases [1]. The precise detection of trace ions in environmental samples is an inspiring mission for analytical chemists [2, 3]. Generally, a preconcentration process is needed prior to analysis of metal ions to increase sensitivity and selectivity. Among these procedures, liquid-liquid microextraction [4], coprecipitation [5], solid phase extraction [6] and cloud point extraction (CPE) do well [7]. The use of CPE has involved significant consideration because it is in agreement with the "green chemistry" principle. The procedure reduce the generation of toxic substances therefore termed as eco-friendly procedure [8, 9]. The surfactants used in CPE are inflammable, not volatile, not toxic which are considered different from organic solvents exhausted in the traditional liquid-liquid extraction [10]. The CPE depend on extraction of the analyte into a micelle-rich phase. When the surfactant solution is heating over a critical temperature, it will separate into two different phases; an aqueous phase

contain no or very little amount of the surfactant and the other is the surfactant-rich phase that contain the analyte [11]. Zincon, 2-carboxy-2'-hydroxy-5'-sulfoformazylbenzene is an analytical reagent used for detection of many metal ions including Zn, Cu and Co ions [12]. It is also used as metallochromic indicator in complexometric titrations [13]. However, the use of Zincon as a complexing agent in CPE approach is limited.

In the present work, Zincon is applied for the CPE and spectrophotometric analysis of Fe(III) in aqueous media. Mixed surfactant system of nonionic surfactant (Triton X-114) and cetyl trimethylammonium bromide (CTAB) was used in the separation of analyte. The parameters affecting the extraction efficiency of CPE were systematically considered and optimized. The process was used for extraction of Fe(III) from blood and water samples and the results were compared with those achieved using ICP-OES analysis technique.

2. Experimental

2.1. Apparatus

The absorbance was measured by monochromatic UV-Vis Spectrometer UV2 (Unicam Ltd., Cambridge, UK), in the range of 400-700 nm. Measurements of pH was justified by (Hanna Instruments, Woonsocket,

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RI, USA). The phase separation was enhanced by Sorvall centrifuge TC-6 (Thermo Fisher Scientific™, Waltham, Massachusetts, USA). For comparison, Fe(III) was determined by a Thermo Scientific iCAP™ 7400 ICP-OES (Thermo Fisher Scientific, Bremen, Germany) according to the manufacturer illustrations. Plasma samples were digested according to a previously optimized procedure using microwave system (Speedwave®four, Berghof Products, Germany) [12].

2.2. Chemical reagents

All chemicals were of analytical reagents grade and were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Double-distilled water was used through this work. A stock of standard solution (1000 mg L^{-1}) of Fe(III) was prepared by dissolving the proper amount of ammonium ferric sulfate dodecahydrate ($\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) in 100 mL double-distilled water acidified with H_2SO_4 . The mixed surfactant of Triton X-114 (1.0% v/v) and CTAB ($1.0 \times 10^{-2} \text{ mol L}^{-1}$) were prepared by dissolving the suitable amounts of each in double-distilled water. A stock solution of Zincon ($1.0 \times 10^{-2} \text{ mol L}^{-1}$) was obtained by dissolving the appropriate quantity of the reagent in 2 mL of saturated NaOH (1M) solution and complete the volume to 100 mL by double-distilled water. Solution to control pH 2 was prepared by using 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} KCl. The pH 3.0-6.0 buffer solutions were prepared using 0.1 mol L^{-1} acetic acid and 0.1 mol L^{-1} sodium acetate, pH 7.0-8.0 were prepared by 0.5 mol L^{-1} hexamine and 0.1 mol L^{-1} HNO_3 or 0.1 mol L^{-1} NaOH, Finally, pH 9.0 was prepared by 0.5 mol L^{-1} sodium tetraborate and 0.1 mol L^{-1} HCl.

2.3. CPE procedure

In a 50 mL polyethylene test tube, a sample or standard solution containing Fe(III) was mixed with 500 μL of $1.0 \times 10^{-2} \text{ mol L}^{-1}$ Zincon, 2 mL acetate buffer (pH 5.0), 500 μL of 1.0×10^{-2} CTAB and 5.0 mL of Triton X-114 1.0% (v/v). The cloud solution was formed immediately by the addition of 1.0 mL of 2.5 mol L^{-1} of Na_2SO_4 solution. The phase separation was achieved by centrifugation (4000 rpm for 5 min). When cooling (5 min) in an ice-bath, a viscous surfactant-rich phase was obtained and the aqueous phase can be removed easily. The micellar phase was transferred to an Eppendorf tube and its volume was made up to 1 mL by ethanol. The absorbance was measured at 603 nm against blank. For the ICP-OES analysis, the surfactant rich-phase was diluted by 1-propanol to prevent the deterioration effect of ethanol on the plasma performance [14].

2.4. Collection of samples

Tap and river water samples were collected from Mansoura city and stored in polyethylene containers

after adjustment of pH to 2.0. Blood samples were centrifuged at 3000 rpm for 10 min to separate plasma. One mL of plasma was placed in Pyrex beaker together with 5.0 mL of HNO_3 and 2.0 mL HClO_4 . The mixture was gradually heated on an electrical heater. Near dryness 5.0 mL of deionized water and boiled again. Then the content was transferred carefully to 10.0 mL volumetric flask and the final volume was completed to the mark by double-distilled water.

3. Results and discussion

3.1. The stoichiometry of the complex

The stoichiometry of zincon complex with Fe(III) was estimated by continuous variation and mole ratio methods was found to be 1:3 metal to ligand complex (Fig 1). In molar ratio method, the absorbance at 603 nm is plotted against the molar ratio of two reactants, by keeping the concentration of Zincon constant at $1 \times 10^{-3} \text{ mol L}^{-1}$ and varying Fe(III) concentration in the range of 1×10^{-4} - $1.8 \times 10^{-3} \text{ mol L}^{-1}$. The stoichiometry was also confirmed by using continuous variation method and it was found to be 1:3 Fe(III)-Zincon. The reaction can be summarized as

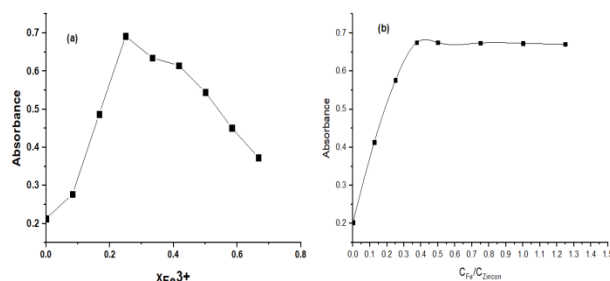


Fig. 1. (a) The continuous variation and (b) the mole-ratio plots for zincon and Fe(III).

3.2. Optimization of cloud point extraction procedure

3.2.1. Effect of pH

The formation of the metal complex and its stability depends mainly on the solution of pH, in CPE method [15]. Accordingly, the effect of pH was evaluated 2.0 to 9.0. In (Fig. 2), and the optimum recovery was achieved at pH 5.0. The extraction efficiency decreased at lower pH because of the competition for the active site of Zincon between H^+ and Fe(III), while at higher pH than 5.0, the extraction decreases due to Fe(III) hydroxides were formed [16]. Therefore, pH 5.0 was applied for the subsequent steps.

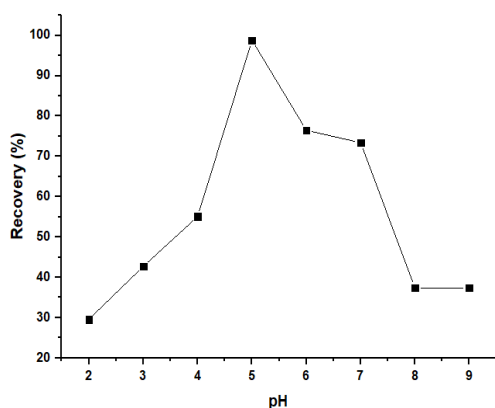


Fig. 2 Effect of pH on the separation recovery of Fe(III). CPE procedures: 50 mL of Fe(III) $250 \mu\text{g L}^{-1}$, Zincon 0.1 mmol L^{-1} , CTAB 0.1 mmol L^{-1} , Triton X-114 0.05% (v/v), Na_2SO_4 0.05 mol L^{-1} , at 4000 rpm centrifugation rate for 5 min.

3.2.2. Effect of Zincon concentration

The effect of amount of Zincon on the efficiency of the CPE procedure was studied from 0.25 to 2.0 mmol L^{-1} . As presented in Fig. 3, the recovery increases by rising Zincon amount until get constant value at 0.1 mmol L^{-1} . Therefore, 0.1 mmol L^{-1} of Zincon was used as optimal amount for the determination of Fe(III).

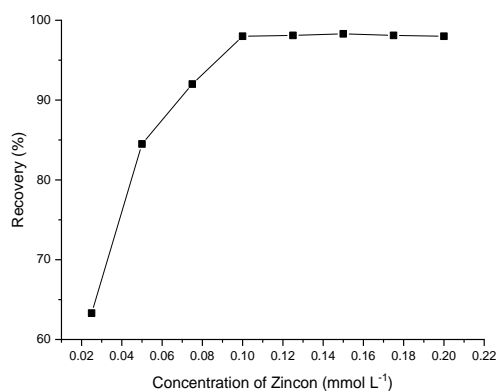


Fig. 3 Effect of Zincon concentration on the separation recovery of Fe(III) complex. CPE procedures: 50 mL of Fe(III) $250 \mu\text{g L}^{-1}$, pH 5.0, CTAB 0.1 mmol L^{-1} , Triton X-114 0.05% (v/v), Na_2SO_4 0.05 mol L^{-1} , at 4000 rpm centrifugation for 5 min.

3.2.3. Effect of Triton X-114 and CTAB

The use of Triton X-114 alone as a surfactant in our study didn't make the solution turbid. So, Fe(III) can't be extracted in the solution even in presence of various concentration of Na_2SO_4 or by heating. On the other hand, the use of CTAB (cationic surfactant) with Triton X-114 produced a turbidity in the solution, probably due to formation of ternary

complex (Fe(III)-Zincon-CTAB) that can aggregates and incorporates into the micellar system [17]. Therefore, the concentration of CTAB and Triton X-114 should be controlled to get the better extraction efficiency.

The effect of nonionic surfactant (Triton X-114) on the separation of Fe(III) by the advised procedure was applied in the range of 0.05 - 0.5% (v/v) in the presence of fixed amount of CTAB (Fig. 4). It was noticed that upon increasing the amount of Triton X-114, the recovery was increased until 0.1% (v/v). Therefore, this amount was applied in this work.

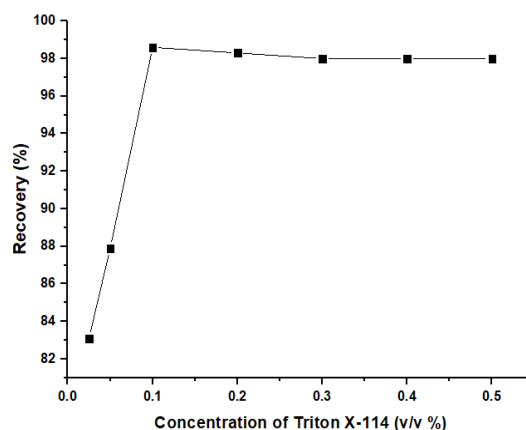


Fig. 4 Effect of non-ionic surfactant (Triton X-114) concentration on the separation recovery of Fe(III). CPE procedures: 50 mL of Fe(III) $250 \mu\text{g L}^{-1}$, pH 5.0, Zincon 0.1 mmol L^{-1} , CTAB 0.1 mmol L^{-1} , Na_2SO_4 0.05 mol L^{-1} , at 4000 rpm centrifugation rate for 5 min.

Also, the effect of cationic surfactant (CTAB) on the recovery of Fe(III) by the presented procedure was evaluated from 0.25 to 2.0 mmol L^{-1} . The results (Fig. 5) confirmed that recovery rises with increasing concentration of CTAB and reaches a stable value at 0.1 mmol L^{-1} . So, this concentration of CTAB was applied in this study.

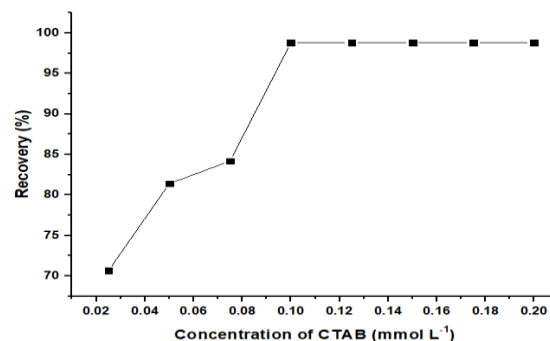


Fig. 5 Effect of cationic surfactant (CTAB) concentration on the separation recovery of Fe(III). CPE procedures: 50 mL of Fe (III) $250 \mu\text{g L}^{-1}$, pH 5.0,

Zincon 0.1 mmol L⁻¹, Na₂SO₄ 0.05 mol L⁻¹, at 4000 rpm centrifugation rate for 5 min.

3.2.4. Effect of strong electrolyte

One of the main purposes of CPE is to minimize the total analysis time and simplicity of procedure by getting a cloud point at room temperature. This has been occurred by addition of strong electrolytes to the micellar solution, such as Na₂SO₄ and KCl (salting-out effect)[16]. The effect of addition of different concentrations of Na₂SO₄ or KCl were studied at a extent from 0.025 to 0.1 mol L⁻¹ to get a good separation of cloudy solution at room temperature. Data presented in (Fig. 6), shows that Na₂SO₄ gives better extraction efficiency than KCl. The Fe(III) complex recovery by the existent method was increased by rising the concentration of Na₂SO₄ until attain a maximum at 0.05 mol L⁻¹. Hence 0.05 mol L⁻¹ of Na₂SO₄ was applied during the work.

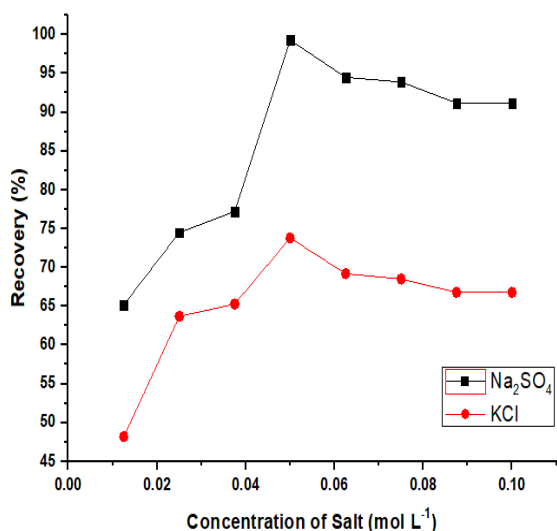


Fig. 6 Effect of adding strong electrolyte of Na₂SO₄ and KCl on the separation recovery of Fe(III). CPE procedures: 50 mL of Fe(III) 250 µg L⁻¹, pH 5.0, Zincon 0.1 mmol L⁻¹, at 4000 rpm centrifugation rate for 5 min.

3.2.5. Effect of the rate and the time of centrifugation

The centrifugation rate was investigated at a range of 1500-4000 rpm from 3 to 10 min. The results signify that 4000 rpm centrifugation for 5 min led to the best recovery of Fe(III) (Table 1).

Table 1. Effect of rate and time of centrifugation on the recovery of Fe(III).

Centrifugation rate (rpm)	Centrifugation time (min)	Recovery (%)
1500	3	61.5 ± 2.9
	5	68.6 ± 3.8
	10	76.3 ± 4.2
2000	3	67.0 ± 5.0
	5	75.1 ± 4.1
	10	81.9 ± 3.3
2500	3	79.4 ± 4.2
	5	86.4 ± 3.7
	10	90.9 ± 2.1
3000	3	83.3 ± 3.6
	5	92.5 ± 4.0
	10	98.4 ± 2.9
4000	3	93.5 ± 2.2
	5	99.1 ± 2.4
	10	99.0 ± 1.9

3.2.6. Effect of interfering ions

The interfering anions and cations effecting on the recovery of Fe(III) extraction and determination were estimated below the optimized requirements, in order to confirm the selectivity of the study. The molar ratio between the interference cation or anion and analyte was described as the tolerance ratio that creating an error ±5% in the recovery of Fe(III). As shown in (Table 2), most concomitant cations or anions did not affect even at high concentration. This indicates the possibility of the procedure in extraction of Fe(III) in real samples with different environments. However, Cu²⁺, Co²⁺ and Ni²⁺ interfered at 100-fold excess. These interfering cations can be reduced by addition 0.05% (w/v) CN⁻ as masking agent. So, during real sample analysis 0.05% (w/v) CN⁻ was used to reduce the interfering ions.

3.3. Analytical features

At the optimized the condition, the dynamic linear range was from 10 to 1000 µg L⁻¹ (R² = 0.9997). The precision, calculated as relative standard deviation (RSD), was 2.5% for determination of 200 µg L⁻¹ of Fe(III). The preconcentration factor was 50 for 50 mL sample.

Comparison with other CPE indicated that the presented procedure is comparable or better than the other methods in term of linearity, LOD, and preconcentration factor (Table 3).

3.4. Application

The procedure was finally utilized for the extraction of Fe(III) in water and plasma samples (Table 4). The effects of spiked samples indicate the accuracy of the procedure (recovery = 95.0 – 100%). Good

agreement between the procedure and direct analysis by ICP-OES support the applicability of our method (Table 5).

Table 2 Effect of interfering ions on the recovery of 100 µg L⁻¹ of Fe(III) by the offered method

Interfering ion	Added as	Tolerance limit (mg L ⁻¹)	Recovery (%)
Na ⁺	NaCl	2000	99.2±2.4
K ⁺	KCl	2000	98.1±1.8
Mg ²⁺	MgSO ₄ ·7H ₂ O	500	98.4±2.9
Ba ²⁺	BaCl ₂ ·2H ₂ O	200	99.1±1.5
Ca ²⁺	CaCl ₂ ·2H ₂ O	500	99.2±2.0
Cd ²⁺	CdCl ₂ ·H ₂ O	200	96.4±1.9
Hg ²⁺	HgCl ₂	200	98.0±2.8
Pb ²⁺	Pb(NO ₃) ₂	200	95.8±3.1
Co ²⁺ *	CoCl ₂	100	96.4±2.7
Ni ²⁺ *	NiSO ₄	200	96.5±2.5
Cu ²⁺ *	Cu(NO ₃) ₂	100	95.6±2.8
Zn ²⁺	ZnSO ₄	200	97.0±1.4
Fe ²⁺	FeCl ₂	100	97.6±2.2
Al ³⁺	AlK(SO ₄) ₂ ·12H ₂ O	250	96.9±3.1
Cr ³⁺	CrK(SO ₄) ₂	200	98.0±2.4
Cr ⁶⁺	K ₂ Cr ₂ O ₇	500	98.8±3.0
Cl ⁻	NaCl	2000	96.9±1.8
NO ₂ ⁻	NaNO ₂	2000	96.9±2.1
NO ₃ ⁻	NaNO ₃	1000	98.4±2.3
HCO ₃ ⁻	NaHCO ₃	1000	98.3±1.9
SO ₄ ⁻²	Na ₂ SO ₄	1000	99.0±2.9
PO ₄ ⁻³	NaH ₂ PO ₄ ·2H ₂ O	100	98.7±2.5

Table 3 Comparison of the proposed method with other mentioned CPE methods for analysis of Fe(III) metal ion.

CPE system	Detection technique	Sample volume (mL)	EF	LOD ($\mu\text{g L}^{-1}$)	Linearity ($\mu\text{g L}^{-1}$)	RSD (%)	Ref
2-(5-bromo-2-pyridylazo)-5-diethylaminophenol/Triton X-114	Spectrophotometry	10	20	0.8-1.0	0.5-112	2.0 - 2.6	[15]
2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol/Triton X-114	Spectrophotometry	10	NP	4.0	50-300	1.5-3.9	[17]
1-(2-pyridylazo)2-naphthol/Triton X-114	FAAS	50	9.6	3.0	20-400	2.5	[18]
3-amino-7-dimethylamino-2-methylphenazine (Neutral Red)/ Triton X-114	FAAS	25	98	0.7	2.5-200	2.1	[19]
8-hydroxy-7-iodoquinoline-5-sulfonic acid/ Triton X-114	FAAS	15	19.6	0.4	10-400	2.4	[20]
Zincon/Triton X-114/CTAB	Spectrophotometry	50	50	3.1	10-1000	2.5	Our work

Table 4. Determination of Fe(III) in water samples by the suggested procedure

Sample	Added ($\mu\text{g L}^{-1}$)	Found ($\mu\text{g L}^{-1}$)	Recovery (%)
Tap water	-	40.5±5.3	-
	10.0	50.1±6.1	96.0
	20.0	60.0	97.5
River water	-	123.3±9.6	-
	20.0	142.8±11.0	97.5
	40.0	162.0±10.5	96.8

Table 5. Determination of Fe(III) in plasma samples by the suggested method and comparison with ICP-OES

Sample	The present method (n = 5)		ICP-OES (n = 5)		<i>t</i> - value
	Results ($\mu\text{g L}^{-1}$)	RSD (%)	Results ($\mu\text{g L}^{-1}$)	RSD (%)	
Plasma (blood)	1410±65.4	4.6	1395±59.9	4.3	0.38

Tabulated *t*-value is 2.31 (95% confidence interval)

Conflicts of interest

"There are no conflicts to declare".

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4. Conclusion

The CPE method was successfully applied for extraction and spectrophotometric analysis of trace amount of Fe(III). The procedure is safe, rapid, accurate and economic, beside; it is the first time to use of Zincon as chelating ligand for CPE of Fe(III). The method exhibits good analytical characteristics

such as low LOD, reasonable preconcentration factor and wide dynamic analytical range. The process can be employed for routine analysis of Fe(III) in water and plasma blood samples.

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