Asian Journal of Chemistry; Vol. 29, No. 12 (2017), 2725-2733

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2017.20848

Cloud Point Extraction and Determination of Trace Iron(III) in Urine Samples by Spectrophotometry and Flame Atomic Absorption Spectrometry

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Received: 8 June 2017; *Accepted*: 17 September 2017; *Published online*: 30 October 2017; AJC-18623

Cloud point technique used effectively for extraction and pre-concentration of iron(III) in the urine samples of occupational workers prior measured by using flame atomic absorption spectrometry and UV-visible spectrophotometer. The metal responds with benzidine as reagent in a non-ionic surfactant Triton X-114 medium. The main factors affecting cloud point extraction efficiencies, such as pH of sample solution, concentration of benzidine reagent, type of surfactant, concentration of Triton X-114, effect of salt out, influence of interferences and impact of equilibration temperature and time were studied. The calibration curve was linear in the range of 0.25-3.0 μg mL^{-1} with r^2 = 0.9655 for UV-visible spectrophotometer at λ_{max} 425 nm. The limit of detection was 0.25 μg mL^{-1}. The relative standard deviation for six replicates was 3.071 %.

Keywords: Cloud point extraction, Triton X-114, UV-visible spectrophotometer, Iron(III), Benzidine, Urine samples.

INTRODUCTION

The increase in the concentration of trace components relative to the total component concentration can be achieved with pre-concentration techniques [1]. There are multiple methods of pre-concentration for metals, like solid phase extraction (SPE), cloud point extraction (CPE), electrochemical deposition, co-precipitation and precipitation, liquid-liquid extraction (LLE) and ion-exchange [2-5]. In this study, emphasis was placed on the use of cloud point extraction technique for several reasons *viz*., high recovery efficiency, high concentration coefficient [6], green chemistry, less consuming reagent, less production of chemical residues, surfactant used is safe, non-volatile and non-toxic simplicity [7,8]. A pre-concentration method including the process of cloud point extraction is depended on the use of non-ionic varieties of surfactants in aqueous solutions and thus the formation of micelles and by heating these solutions to a certain temperature will become cloudy or turbid this temperature is known as the cloud point temperature [9]. Cloud point extraction is used with coupling with different techniques for estimating various elements in different samples such as, cloud point extraction to pre-concentration and analysis of strontium (II) ion by turbidimetric method using Schiff base derivative [10].

The increases in the concentration of iron in the body have negative effects including endocrine problems and diseases of the liver, lung and other diseases [11-14]. The previous literature review conducted to determine the iron element in various samples by using cloud point extraction method that is coupled with different techniques such as: flame atomic absorption spectrometry (FAAS) [12,15,16], UV-visible spectrophotometer [17,18], inductively coupled plasma mass spectrometry [19], graphite furnace atomic absorption spectrometry [20] and capillary electrophoresis [21]. The urine has a pH of 5.5- 7.0 and a range of 6.2 [22], while the concentration of iron element in the urine is estimated to be about the median and range concentration of iron in urine was 4.9 ng mL^{-1} and < 2.1-16.4 ng mL^{-1} [23-26]. The aim of this study was to develop simple spectrometric method for the determination of Fe(III), using benzidine as a complexing agent and cloud point extraction. The proposed method is successfully applied for the determination iron(III) in urine of occupational worker samples.

EXPERIMENTAL

A pH meter WTW (model 720) with a combined glass electrode was used for pH measurements. A Hettich centrifuge (model EBA-20, Germany) with 10 mL calibrated centrifuge tubes was used for phase separation at 3600 rpm for 5 min. A Lab Line Super mixer (model 129) Hitech Trader, USA was used to mix the solutions. Shimadzu double beam UV-VIS Spectrophotometer, UV-1800, Japan is used to measure all

absorption spectra. The metals ions have been determined by using flame atomic absorption spectrophotometer (FAAS) (Buck scientific model 210 VGP, USA) with deuterium background correction equipped with 10 cm of air/acetylene flame burner head and hollow-cathode lamp that can be changed with metal ion.

Most of the chemicals have been prepared by using analytical grade chemicals and deionized water. Benzidine reagent (1% w/v) was prepared by dissolving 1 g of benzidine in 1-2 mL dimethyl sulfoxide and then add absolute ethanol to 100 mL in a volumetric flask. Solutions of non-ionic surfactant Triton X-114 have prepared at 20 % (v/v) concentration by diluting 20 mL of Triton X-114 to 100 mL (hot deionized water) in a volumetric flask. A stock solution of 1000 mg/L of Fe(III) was prepared by dissolving 0.2920 g of $FeCl_3$ in 100 mL deionized water.

Sample collection: A 44 urine samples were collected from occupational workers workers in oil refineries and terminals engaged in dyeing, welding, plumbing, *etc*. The urine samples were collected in a new polyethylene bottles (120 mL) and stored at 0-5 ºC [27].

Urine sample digestion: In a 100 mL beaker, 25 mL of urine sample accurately measured, was treated with a mixture of 5 mL H_2O_2 and 2.5 mL of conc. HNO_3, and was placed on a hot plate. Then the samples are heated until the sample become complete dry. Thereafter, a dry residuum with a dark colour is added to 2.5 mL of conc. HNO_3 and heated again to the point of drought. This step is repeated several times until a white ashes were obtained. The white ashes were dissolved in the final step by using 2.5 mL of 3M of HCl [28,29].

Cloud point extraction: The precocentration and extraction procedure for iron(III) by using cloud point extraction method aliquots of 10 mL of Fe(III) solution containing 2 μg mL^{-1} and 0.5 mL of 1 % (w/v) of benzidine reagent was mixed. The green colour solution product formed has λ_{max} 425 nm depending on the absorption spectra of UV-visible spectrometry for Fe(III)-benzidine complex. After adjusted the pH at 4.2 by using 0.1 M of HCl and 0.1 M of NaOH, added 0.1 mL of 20 % (v/v) Triton X-114. Then the mixture placed on the super mixer to mix and heated on a water bath at 70 ºC for 10 min. This step follows the separation of the solution into two layers *via* the centrifuge at 3600 rpm for 5 min. After that the phases are cooled using an ice bath at 0-5 ºC to increase the viscosity so that surfactant-rich phase separated easily by converting tube. The remaining micellar phase containing Fe (III)-benzidine complex was dissolved in 0.75 mL of absolute ethanol and transferred into 1 cm quartz cell (1 mL). Then measured the absorbance of solution against a blank solution prepared in the same way. The proposed reaction mechanism for Fe(III)-benzidine complex formation is shown in Fig. 1.

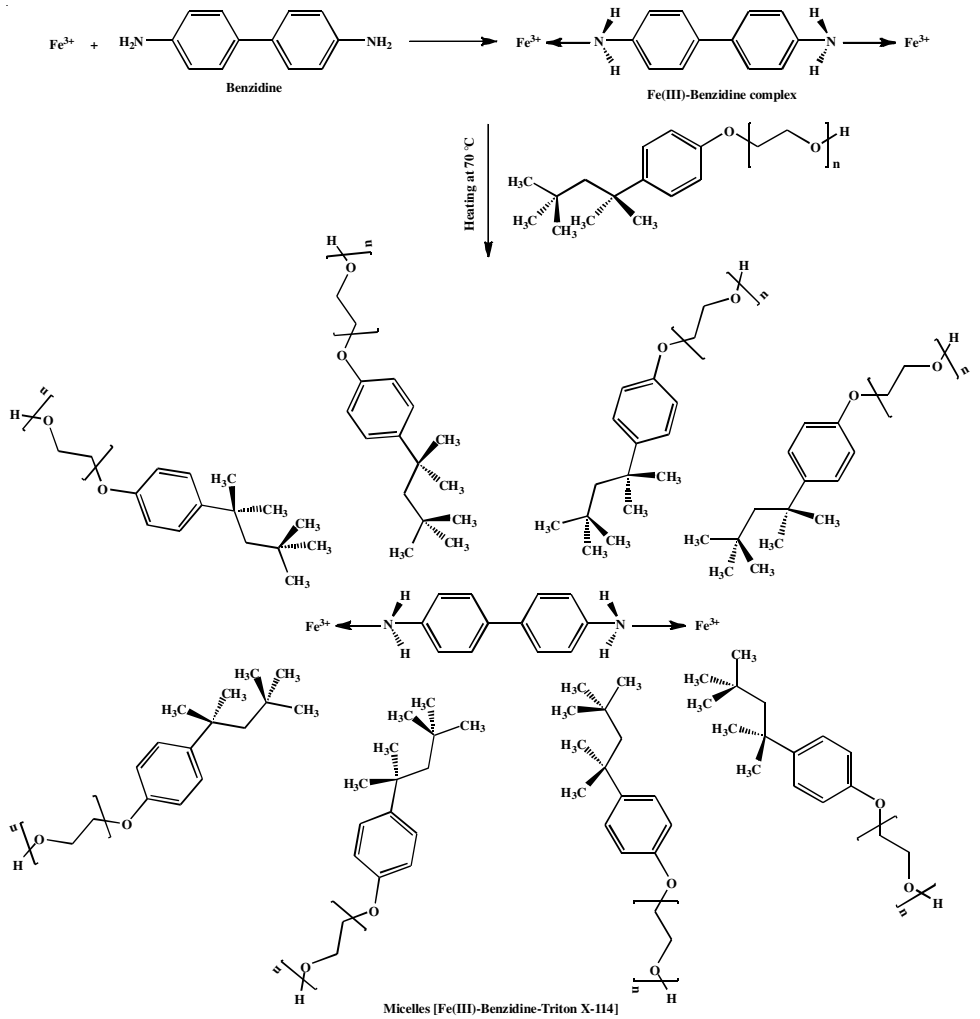

Fig. 1. Proposed reaction mechanism for Fe(III)-benzidine complex formation

Absorption spectra for Fe(III)-benzidine complex: The absorbance spectra for benzidine, iron(III) solution and Fe(III)-benzidine complex in surfactant rich phase are domenstrated Fig. 2. The absorbance spectra of benzidine reagent shows λ_{max} 288 nm, iron(III) solution has λ_{max} 320 nm, while Fe(III)-benzidine complex has 425 nm.

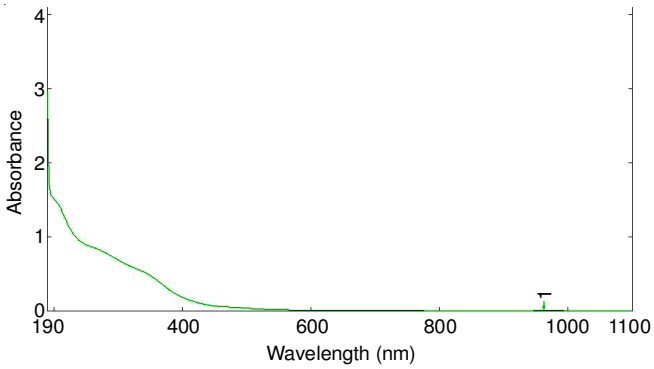

Fig. 2A. Absorption spectra of iron(III) solution

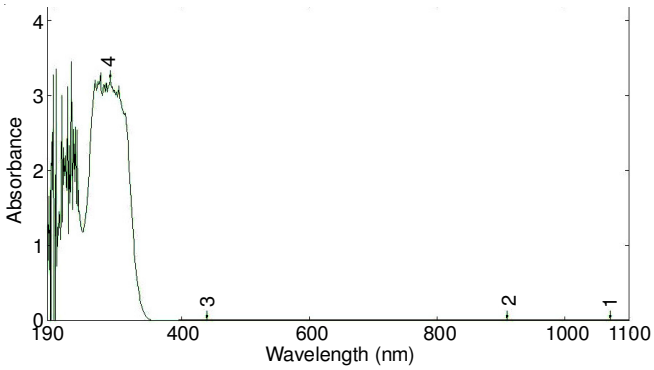

Fig. 2B. Absorption spectra of benzidine reagent solution

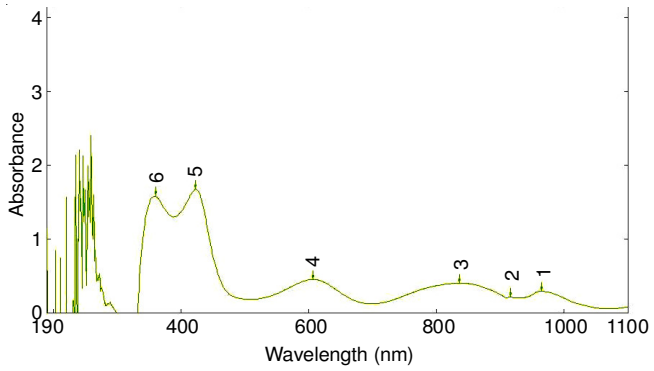

Fig. 2C. Absorption spectra of iron(III)-benzidine complex

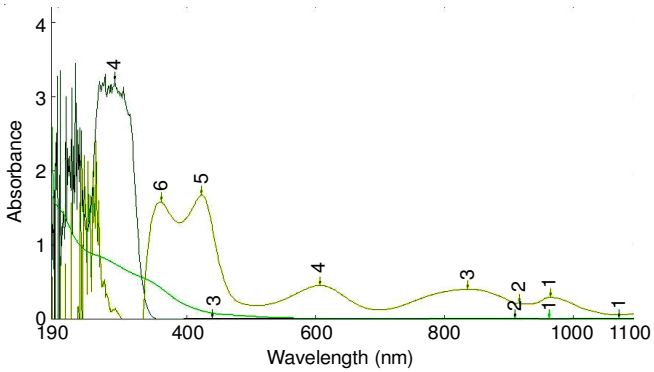

Fig. 2D. Over lapping for Fe(III)-benzidine complex

RESULTS AND DISCUSSION

The optimal conditions for high efficiency of cloud extraction were studied and stabilized. The most important conditions are order addition, pH , concentration of reagent, types of surfactant, concentration of surfactant, equilibrium temperature, time of incubation, salt out effect and interfering ions.

Order addition effect: The most important factors that have been studied are order additions where it changed the order of (2 μg mL^{-1},10 mL) of Fe(III) and (1% w/v, 0.5 mL) of benzidine reagent (Table-1). The greatest absorbance value was chosen accordingly.

TABLE-1
EFFECT OF ORDER ADDITION

Type of complex	Order addition	Absorbance of an aqueous layer (Aq)	Absorbance of a rich phase surfactant (As)
Fe(III) complex	M + R + T	0.000	0.295
	R + M +T	0.000	0.301

Effect of pH: pH is one of the most important factors that directly affect the formation of the iron complex by using cloud point extraction. The test was applied using the range of pH 2.7-10.4. These values were adjusted using by 0.1 M NaOH and 0.1 M HCl solution. The absorbance increased, when the pH value equal 4.2, the complexation reaction at pH values lower than 4.2 is incomplete probably due to the protonation of benzidine (Fig. 3). On the other hand at pH value higher than 4.2 is due to hydrolysis of Fe(III). The effects of different types of acids (0.1 M) are shown (Fig. 4). The effect of hydrochloric acid on Fe(III)-benzidine complex formation is stronger as compared to other acids, where it gave the highest absorbance. It is also found that volume 0.75 mL of 0.1 M of HCl gave best absorbance (Table-2).

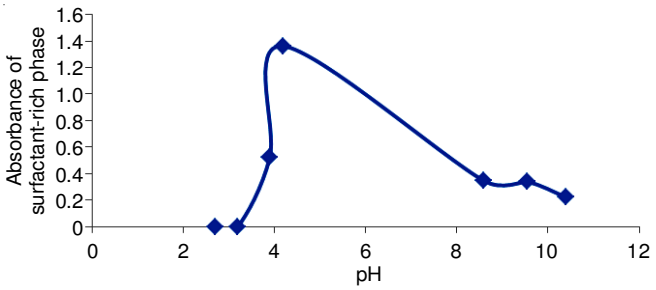

Fig. 3. Effect of pH

TABLE-2
EFFECT CONCENTRATION OF HCl

Volume of HCl (mL)	Absorbance		
	Original solution (A_o)	Aqueous layer (Aq)	Rich phase surfactant (As)
1.00	0.000	0.000	0.899
0.75	0.000	0.000	1.060
0.50	0.000	0.000	0.454
0.25	0.000	0.000	0.084
0.10	0.000	0.000	0.007
0.05	0.000	0.000	0.010

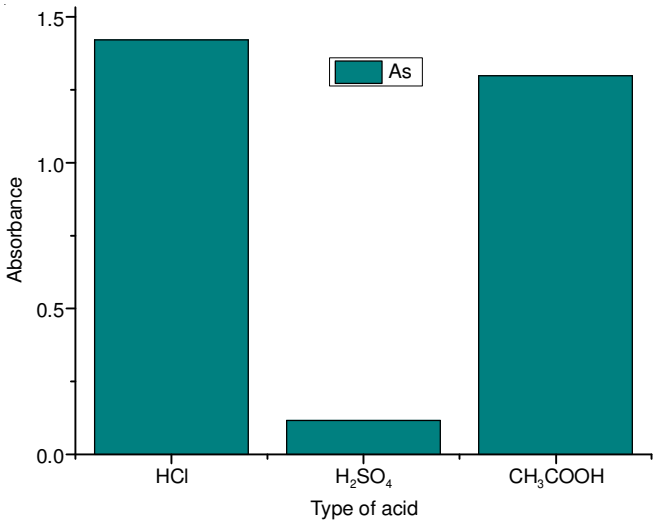

Fig. 4. Effect of different acids

Effect of reagent concentration: The concentration effect of chelating agent *i.e.*, benzidine on absorbance value was studied by using various volumes from 0.05 to 1.00 mL of 1 % w/v of benzidine while keeping other factors constant. The results (Fig. 5) show that the absorbance value increased with increasing amount of added reagent benzidine. The best result was relied upon when the amount of reagent is 0.5 mL.

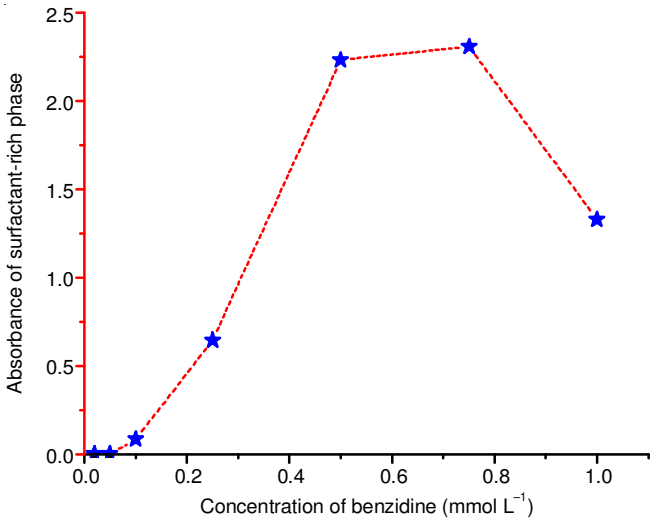

Fig. 5. Effect of concentration of reagent

Influence of type and concentration of surfactant: The efficiency of cloud point extraction dependent on the type of surfactant used. Fig. 6 shows the effect type of surfactants (Triton X-100, sodium dodecyl sulfate (SDS) and Triton X-114) and its concentration on absorbance of Fe(III)-benzidine complex. Fig. 7 shows the absorbance of the complex increased by increasing the volume of 20 % (v/v) Triton X-114.

Effect of equilibrium temperature and time: Fig. 8 shows the highest absorbance signal at 70 ºC. In order to achieve easy phase separation and efficient pre-concentration in cloud point extraction processes, it was desirable to employ the shortest incubation time. The effect of incubation time was investigated in the ranges 1-25 min. The results demonstrate that in the incubation time of 5 min was chosen for further

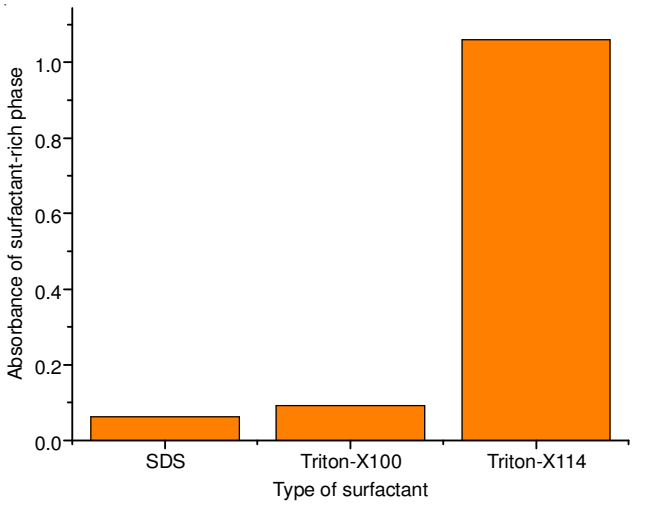

Fig. 6. Effect of type of surfactant

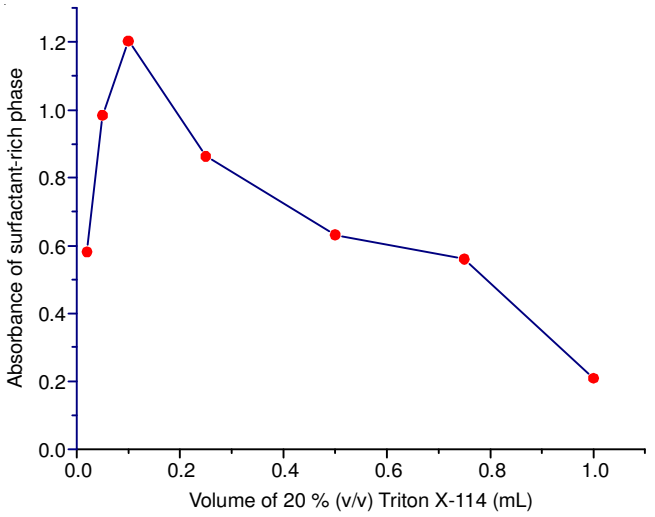

Fig. 7. Effect of concentration of Triton X-114

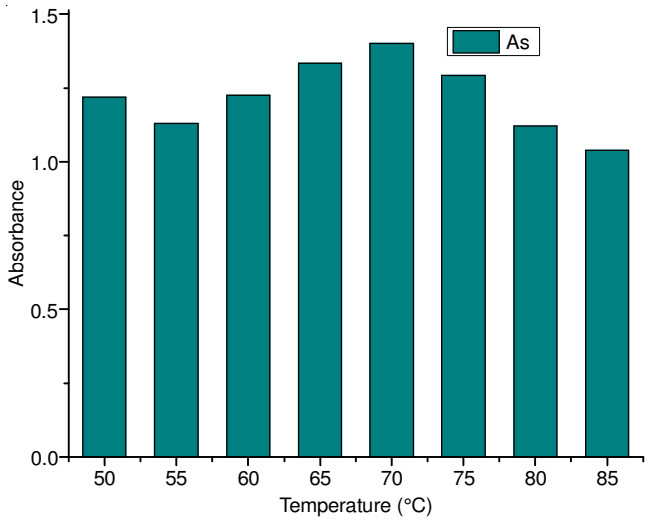

Fig. 8. Effect of temperature

experiments. Fig. 9 showed the effect of incubation time on the complex formation.

Effect of salts: The effect of salts on cloud point extraction were studied by added 0.5 mL of 15 % w/v of NaCl, KCl, Na_2CO_3 and Na_2SO_4 to solution of Fe(III). The results demons-

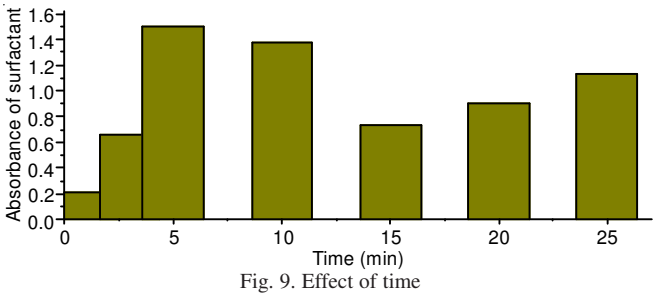

Fig. 9. Effect of time

trate that the presence of some salts lead to increase the absorbance value which is attributed due to the increase in cloud point extraction efficiency since these salts will pull water molecules and destroy the hydration shell of iron ion which leads to increase the bonding between the reagent benzidine and iron. Table-3 shows the effect of salt out on complex.

TABLE-3
EFFECT OF SALT

Salts 0.5 mL of 15 % (w/v)	Absorbance of a rich phase	Recovery (%)	E_{re} (%)
NaCl	0.442	39.21917	60.780
KCl	0.401	35.58119	64.418
Na_2CO_3	0.404	35.84738	64.152
Na_2SO_4	2.103	186.6016	86.601

Effect of interference ions: The effects of foreign ions on the extraction of 2 μg mL^{-1} of Fe(III) were studied, cations that may react with benzidine or species may react with analytes and decrease the extraction efficiency to perform this study, 100 μg mL^{-1} of interfering ions were added to a solution of 2 μg mL^{-1} of Fe (III) and were subjected to the complete procedure. The results demonstrate that the presence of large amounts of species commonly present in water samples have significant effect on the cloud point extraction efficiency. Table-4 shows the effect of interferences on the complex.

TABLE-4
EFFECT OF INTERFERENCES IONS

Interfering ion	Added as	Concentration of Interfering ion ppm/Conc. of Cu(II) ppm ratio	Absorbance
Co(II)	$Co(NO_3)_2$	50	1.921
Ni(II)	$Ni(NO_3)_2$	50	1.642
Cu(II)	$Cu(NO_3)_2{\cdot}3H_2O$	50	1.803
Cr(III)	$Cr(NO_3)_3$	50	0.972
Al(III)	$Al(NO_3)_3{\cdot}9H_2O$	50	0.721
Pb(II)	$Pb(NO_3)_2$	50	1.721
Zn(II)	$ZnCl_2$	50	1.812

Stoichiometry of the complex: The stoichiometry of the complex determined by using two methods:

Job's method: In Job method, solution of reagent benzidine and iron(III) ion 1×10^{-3} M were prepared and mixed in continuous variation then diluted with 10 mL of deionized water after that measured the absorbance of solution by UV-visible spectrophotometer at 425 nm. The stoichiometry of the complex determined Job's method was found to be 0.7-0.3. Fig. 10 showed the Job's plot of absorbance at 425 nm *versus* mole fraction of Fe(III) ion.

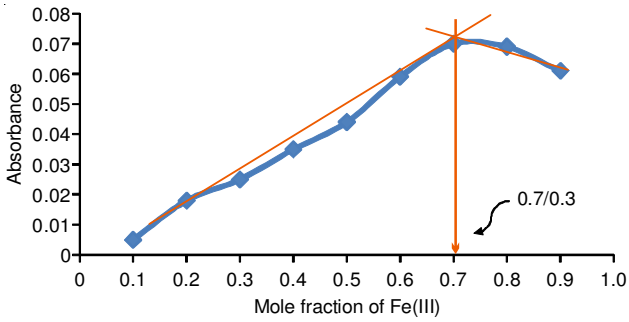

Fig. 10. Job method

Mole ratio method: In the mole ratio method, fixed the volume of Fe^{3+} ion (1×10^{-3} M) and change volume of reagent benzidine (1×10^{-3} M) or *vice-versa*, then diluted with 10 mL of deionized water and finally measured the absorbance of the solution by UV-visible spectrophotometer at 425 nm. The stoichiometry of the complex determined by mole ratio method was found to be 2.33:1. Fig. 11 showed the mole ratio plot of benzidine-Fe(III) complex.

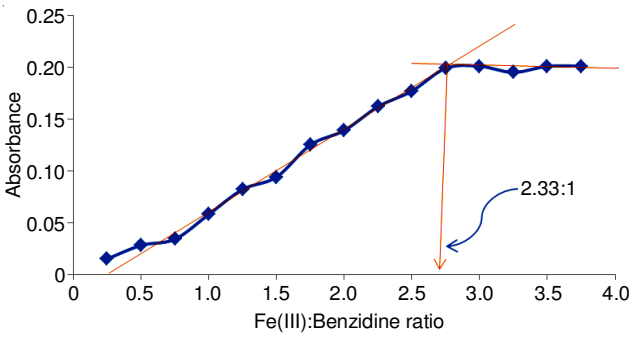

Fig. 11. Mole ratio

Calibration curve of Fe(III) by UV-visible with cloud point extraction: Calibration graphs were established by plotting absorbance *versus* concentration of Fe^{3+} ion. A series 0.1-7 μg mL^{-1} Fe^{3+} ion were prepared. Table-5 and Fig. 12 showed the range of concentration of Fe(III), results of absorbance for linear regression analysis using cloud point extraction and calibration plot for Fe(III).

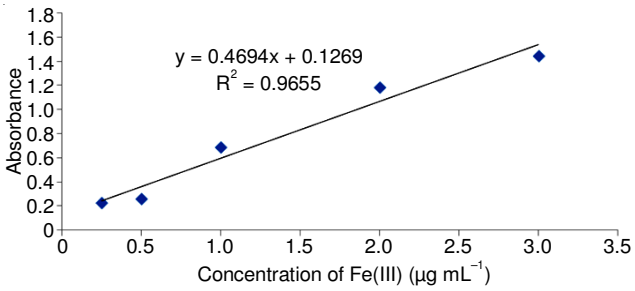

Fig. 12. Calibration graph for Fe(III) by UV-visible spectrophotometry

Precision and accuracy

Repeatability: This test is applied to the concentrations (0.5 and 2.0) μg mL^{-1} of Fe(III). The values of relative standard deviation (RSD %) for Fe^{3+} ion is tabulated in Table-6. The percentage relative standard deviation less than 5 % can be achieved using this method.

TABLE-5
RESULTS OF CALIBRATION GRAPH OF Fe(III) BY UV-VIS-CPE METHOD

Concentration of Fe(III) (μg mL^{-1})	Absorbance (y_i)	Mean $\bar{y}$	Standard deviation σ_{n-1}	RSD (%)	Confidence interval of mean $\bar{y} \pm t_{0.05/2} \frac{\sigma_{n-1}}{\sqrt{n}}$
3.00	1.294, 1.595, 1.566	1.485	0.1660	11.1814	1.485 ± 0.412
2.00	1.299, 1.068, 1.265	1.210	0.1247	10.3015	1.210 ± 0.309
1.00	0.700, 0.675, 0.694	0.689	0.0130	1.8923	0.689 ± 0.0324
0.50	0.204, 0.317, 0.321	0.280	0.0664	23.6670	0.280 ± 0.164
0.25	0.242, 0.212, 0.223	0.223	0.0151	6.8057	0.223 ± 0.037

TABLE-6
REPEATABILITY OF Fe(III) COMPLEX AT OPTIMUM PARAMETERS

Concentration of metal (μg mL^{-1})		Absorbance $\bar{y}_i$	Mean $\bar{y}$	Standard deviation σ_{n-1}	Repeatability RSD (%)	Confidence interval of mean $\bar{y} \pm t_{0.05/2} \frac{\sigma_{n-1}}{\sqrt{n}}$
Fe(III)	0.5	0.252, 0.355, 0.295, 0.332, 0.300, 0.313	0.307833	0.035153	3.515347	0.307 ± 0.0368
	2.0	0.396, 0.401, 0.411, 0.429, 0.399, 0.399	0.405833	0.012465	3.071363	0.405 ± 0.0130

Calibration curve of Fe(III) by FAAS: A series of standard iron(III) solutions ranging from 0-5 μg mL^{-1} were used at λ_{max} 248.3 in order to determine the calibration curve for Fe(III) in FAAS technique. Fig. 13 shows the range of concentration of Fe (III), results of absorbance for linear regression analysis using FAAS.

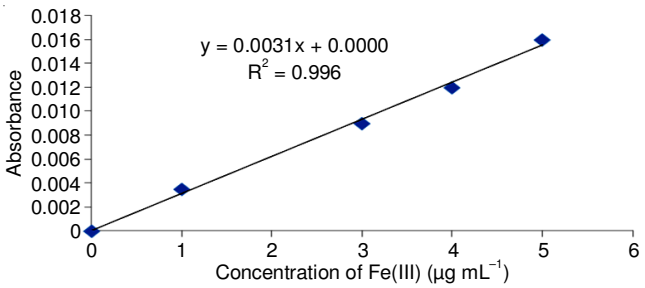

Fig. 13. Calibration graph for Fe(III) by FAAS

Analytical applications: After the digestion of urine sample pre-concentrated by cloud point extraction and determine the Fe(III) concentration in urine of 44 samples of occupational workers by two methods UV-visible-CPE and FAAS-CPE at the optimum conditions. The results are presented in Tables 7 and 8.

Statistical treatment of results: By applying a Grubbs-test to outlier of the sample concentrations after estimating by UV-visible spectrophotometry, FAAS techniques, the results were excluded as shown in Table-9. This test was applied to 44 samples as shown in Table-10. The significance tests described so far are used for comparing means, and hence for detecting systematic errors. The F-test was used to compare the variances between the concentration of iron by two methods UV-Vis-CPE and FAAS-CPE to appear have significant or not between them [30]. Table-11 shows the results of applied F-test. And the comparison of a sample mean with true mean

TABLE-7
RESULT OF Fe(III) CONCENTRATION IN URINE SAMPLE BY USING UV-VISIBLE SPECTROPHOTOMETER

ID	Gender	Age	Type of occupational worker	Duration of work (year)	Absorbance of Fe(III) by UV-visible spectrophotometer	Concentration of Fe(III) μg mL^{-1} in urine samples by UV-Vis-CPE method
H001	Male	55	Oil refineries	35	0.980	0.065427
H002	Male	37	Oil refineries	12	0.110	-0.001296
H003	Male	28	Oil refineries	7	1.019	0.068418
H004	Male	26	Welding	2	1.179	0.080689
H005	Male	27	Welding	6	1.233	0.084830
H006	Male	50	Welding	25	1.261	0.086978
H007	Male	23	Plumbing	8		
H008	Male	22	Plumbing	7	1.439	0.100629
H009	Male	60	Plumbing	20	0.715	0.045103
H010	Male	49	Plumbing	35		
H011	Male	28	Plumbing	2	0.981	0.065504
H012	Male	29	Welding	15	1.012	0.067881
H013	Male	53	Dyeing	10	1.109	0.075320
H014	Male	45	Welding	20	0.813	0.052619
H015	Male	32	Dyeing	9		
H016	Male	25	Dyeing	11	2.101	0.151400
H017	Male	27	Oil refineries	10	1.009	0.067651
H018	Male	26	Dyeing	2	1.200	0.082299
H019	Male	35	Dyeing	20	1.393	0.097101
H020	Male	45	Oil refineries	4	1.112	0.075550

H021	Male	24	Welding	3	0.224	0.007446
H022	Male	22	Welding	8	0.579	0.034673
H023	Male	35	Welding	10	0.656	0.040578
H024	Male	42	Oil refineries	20		
H025	Male	35	Welding	25	1.109	0.075320
H026	Male	25	Welding	10	1.270	0.087668
H027	Male	38	Welding	2	0.820	0.053156
H028	Male	55	Oil refineries	12	0.954	0.063433
H029	Male	47	Terminals to provide fuel	11	0.813	0.052619
H030	Male	45	Oil refineries	10	0.791	0.050932
H031	Male	38	Oil refineries	12	1.102	0.074783
H032	Male	48	Oil refineries	3	0.611	0.037127
H033	Male	41	Oil refineries	11	0.511	0.029458
H034	Male	34	Terminals to provide fuel	2	0.814	0.052696
H035	Male	55	Terminals to provide fuel	35		
H036	Male	53	Oil refineries	11	1.113	0.075627
H037	Male	22	Oil refineries	2	0.987	0.065964
H038	Male	28	Oil refineries	8	1.130	0.076931
H039	Male	43	Oil refineries	15	0.813	0.052619
H040	Male	33	Oil refineries	12	0.934	0.061899
H041	Male	52	Terminals to provide fuel	15	1.554	0.109449
H042	Male	41	Terminals to provide fuel	9	0.313	0.014272
H043	Male	40	Welding	15	1.009	0.067651
H044	Male	48	Terminals to provide fuel	24	0.813	0.052619

Samples were lost due to damage during work

Sample was neglected because applied G-test

Sample was deleted because it is illogical

TABLE-8
RESULT OF Fe(III) CONCENTRATION IN URINE SAMPLE BY USING FAAS

ID	Gender	Age	Type of occupational worker	Duration of work (year)	Absorbance of Fe(III) by FAAS	Concentration of Fe(III) μg mL^{-1} in urine samples by FAAS-CPE
H001	Male	55	Oil refineries	35	0.004	0.052083
H002	Male	37	Oil refineries	12	0.002	0.104166
H003	Male	28	Oil refineries	7	0.009	0.023148
H004	Male	26	Welding	2	0.007	0.029761
H005	Male	27	Welding	6	0.003	0.069444
H006	Male	50	Welding	25	0.003	0.069444
H007	Male	23	Plumbing	8		
H008	Male	22	Plumbing	7	0.008	0.026041
H009	Male	60	Plumbing	20	0.005	0.041666
H010	Male	49	Plumbing	35		
H011	Male	28	Plumbing	2	0.005	0.041666
H012	Male	29	Welding	15	0.004	0.052083
H013	Male	53	Dyeing	10	0.001	0.208333
H014	Male	45	Welding	20	0.006	0.034722
H015	Male	32	Dyeing	9		
H016	Male	25	Dyeing	11	0.004	0.052083
H017	Male	27	Oil refineries	10	0.004	0.052083
H018	Male	26	Dyeing	2	0.005	0.041666
H019	Male	35	Dyeing	20	0.002	0.104166
H020	Male	45	Oil refineries	4	0.003	0.069444
H021	Male	24	Welding	3	0.004	0.052083
H022	Male	22	Welding	8	0.005	0.041666
H023	Male	35	Welding	10	0.002	0.104166
H024	Male	42	Oil refineries	20		
H025	Male	35	Welding	25	0.010	0.020833
H026	Male	25	Welding	10	0.005	0.041666
H027	Male	38	Welding	2	0.005	0.041666
H028	Male	55	Oil refineries	12	0.005	0.041666
H029	Male	47	Terminals to provide fuel	11	0.004	0.052083
H030	Male	45	Oil refineries	10	0.006	0.034722
H031	Male	38	Oil refineries	12	0.009	0.023148
H032	Male	48	Oil refineries	3	0.004	0.052083
H033	Male	41	Oil refineries	11	0.007	0.029761
H034	Male	34	Terminals to provide fuel	2	0.007	0.029761
H035	Male	55	Terminals to provide fuel	35		

H036	Male	53	Oil refineries	11	0.002	0.104166
H037	Male	22	Oil refineries	2	0.001	0.208333
H038	Male	28	Oil refineries	8	0.007	0.029761
H039	Male	43	Oil refineries	15	0.006	0.034722
H040	Male	33	Oil refineries	12	0.002	0.104166
H041	Male	52	Terminals to provide fuel	15	0.012	0.017361
H042	Male	41	Terminals to provide fuel	9	0.003	0.069444
H043	Male	40	Welding	15	0.01	0.020833
H044	Male	48	Terminals to provide fuel	24	0.006	0.034722

Samples were lost due to damage during work Samples were neglected because applied G-test

TABLE-9
RESULTS OF GRUBBS-TEST APPLIED ON Fe(III)

No. of sample		Readings excluded		Factor of concentration cf	Mean of concentration	
		UV-Visible	FAAS		UV-Visible	FAAS
Fe(III)	44	7	7	22.5	0.007-0.151	0.017-0.208

TABLE-10
CLASSIFICATION OF TYPES OF SAMPLES AND STATISTICAL RESULTS OF Fe(III) TO THEM ACCORDING TO DIFFERENT TECHNIQUES

Type of occupational worker	Type of measurement	No. of sample	Mean of conc. $\bar{y}$ (μg mL^{-1})	Standard deviation σ_{n-1}	Confidence interval of the mean $\bar{y} \pm t_{0.05/2}\frac{\sigma_{n-1}}{\sqrt{n}}$
Oil refineries	UV-Vis-CPE	14	0.0636	0.0192	0.0636 ± 0.0111
	AAS-CPE	14	0.0539	0.0300	0.0539 ± 0.0173
Welding	UV-Vis-CPE	12	0.0612	0.0246	0.0612 ± 0.0156
	AAS-CPE	12	0.0481	0.0237	0.0481 ± 0.0150
Plumbing	UV-Vis-CPE	3	0.0704	0.0281	0.0704 ± 0.0695
	AAS-CPE	3	0.0364	0.0090	0.0364 ± 0.022
Terminals to provide fuel	UV-Vis-CPE	5	0.0522	0.0236	0.0522 ± 0.0292
	AAS-CPE	5	0.0406	0.0203	0.0406 ± 0.0252
Dyeing	UV-Vis-CPE	3	0.0849	0.0111	0.0849 ± 0.0276
	AAS-CPE	3	0.0659	0.0334	0.0659 ± 0.0829
Total	UV-Vis-CPE	37	0.06359	0.0219	0.0635 ± 0.0073
	AAS-CPE	37	0.05127	0.0261	0.0512 ± 0.0087

TABLE-11
APPLICATION F-TEST ON THE Fe(III) SAMPLES

Type of occupational worker	Type of measurement	d.f =n-1	Standard deviation σ_{n-1}	Variance σ^2	F-calculate $F = \frac{\sigma^2}{\sigma^2}$	F-critical value	P-value	Type of significant
Oil refineries	UV-Vis-CPE	13	0.0192	0.000386	1.9736	2.69	0.13007	Non-significant
	AAS-CPE	13	0.0274	0.000754				
Welding	UV-Vis-CPE	11	0.0246	0.000605	1.0714	2.80	0.45633	Non-significant
	AAS-CPE	11	0.0237	0.000562				
Plumbing	UV-Vis-CPE	2	0.0281	0.00079	9.6296	19.00	0.0941	Non-significant
	AAS-CPE	2	0.0090	0.000081				
Terminals to provide fuel	UV-Vis-CPE	4	0.0236	0.000557	1.3414	6.39	0.3917	Non-significant
	AAS-CPE	4	0.0203	0.000412				
Dyeing	UV-Vis-CPE	1	0.0104	0.000109	17.889	161.45	0.14815	Non-significant
	AAS-CPE	1	0.0441	0.00195				
Total	UV-Vis-CPE	34	0.0223	0.00049	44.76	1.79	< 0.00001	Significant
	AAS-CPE	34	0.0244	0.000681				

must be based on Student's t test to compare the mean of iron concentration by two methods UV-visible-CPE and FAAS-CPE [31]. Table-12 shows the result of t-Test.

Conclusion

A sensitive, selective and environmentally friendly spectrometric method of determination of low concentrations of Fe(III), using benzidine as a complexing agent and cloud point extraction is developed. The proposed method was succcessfully applied for the determination of urine samples of occupational workers.

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TABLE-12
STUDENT t-TEST OF Fe(III) RESULTS OF UV-Vis-CPE AND AAS-CPE

Type of occupational worker	Type of measurement	d.f =n-1	Mean of conc. $\bar{y}$ ($\mu g\ mL^{-1}$)	Standard deviation σ_{n-1}	Confidence interval of the mean $\bar{y} \pm t_{0.05/2}\frac{\sigma_{n-1}}{\sqrt{n}}$	t-calculated $t = \bar{y} - \mu\frac{\sqrt{n}}{\sigma_{n-1}}$	P-value	Type of significant
Oil refineries	UV-Vis-CPE	13	0.0636	0.0192	0.0636 ± 0.0111	11.437	< 0.00001	Non-significant
	AAS-CPE	13	0.05007	0.02747	0.0500 ± 0.0173	5.9283	0.00069	Non-significant
Welding	UV-Vis-CPE	11	0.0612	0.0246	0.0612 ± 0.0156	7.9277	< 0.00001	Non-significant
	AAS-CPE	11	0.0481	0.0237	0.0481 ± 0.0150	6.3141	0.000058	Non-significant
Plumbing	UV-Vis-CPE	2	0.0704	0.0281	0.0704 ± 0.0695	4.0400	0.05615	Significant
	AAS-CPE	2	0.0364	0.0090	0.0364 ± 0.022	6.0485	0.026266	Significant
Terminals to provide fuel	UV-Vis-CPE	4	0.0522	0.0236	0.0522 ± 0.0292	4.4814	0.010978	Non-significant
	AAS-CPE	4	0.0406	0.0203	0.0406 ± 0.0252	3.9322	0.017074	Non-significant
Dyeing	UV-Vis-CPE	2	0.0849	0.0111	0.0849 ± 0.0276	12.4828	0.006357	Non-significant
	AAS-CPE	2	0.0659	0.0334	0.0659 ± 0.0829	3.1632	0.087094	Significant
Total	UV-Vis-CPE	35	0.06359	0.0219	0.0635 ± 0.0073	16.2742	< 0.00001	Non-significant
	AAS-CPE	35	0.05127	0.0261	0.0512 ± 0.0087	10.764	< 0.00001	Non-significant

True value $\mu = 4.9\ ng\ mL^{-1}$ [Ref. 25], $t_{0.05/2\ (n-1)}$, n = 3(4.303), n = 4(3.182), n = 5(2.776), n = 12(2.201), n = 14(2.160), n = 15(2.145), n = 37(2.028), n = 39(2.024).

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