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DISPLACEMENT CLOUD POINT EXTRACTION PROCEDURE FOR PRECONCENTRATION OF IRON(III) IN WATER AND FRUIT SAMPLES PRIOR TO SPECTROPHOTOMETRIC DETERMINATION

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ABSTRACT. For the enrichment of iron(III) prior to spectrophotometric determination, displacement cloud point extraction (D-CPE) technique was applied depending on the difference in stability constant of metal complexes. Zinc(II) as gallic acid complex was first separated into a Triton X-100 surfactant. Then, once the aqueous phase has been removed, the sample containing Fe(III) is added, and another CPE process is performed. Because Fe-GA has a higher stability than Zn-GA, Fe(III) can displace Zn(II) from the pre-extracted Zn-GA, allowing for Fe(III) separation from the complex sample matrix and its spontaneous spectrophotometric determination at 560 nm. The effects of pH, ligand, and surfactant quantities, temperature and heating time, centrifuge processes, and interferences were all studied. At the optimal conditions, the calibration graph was linear from 0.5 to 500 µg L^{-1} with enrichment factor of 75.0. The LOD was 0.15 µg L^{-1} and the RSD was 1.3% for 60 µg L^{-1} of Fe(III), n = 10. Accuracy was also evaluated using the standard reference substance (SRS) and flame atomic absorption spectroscopy (FAAS) approaches. This procedure was used for separation and micro-determination of Fe(III) in water and fruit tests (banana, strawberry, lemon, orange, and peaches) with recoveries ranged from 96.5 to 105%.

KEY WORDS: Displacement cloud point extraction, Iron, Spectrophotometry, Fruit samples

INTRODUCTION

Iron is one of the most studied elements due to its biological significance. The level of iron in humans is vital for their health. Iron is stored in the body as iron-ferritin, and its deficiency leads to anemia [1]. High iron levels have been linked to an increased risk of cancer, heart disease, and liver damage [2]. Therefore, many methods have been improved for accurate determination of Fe(III) such as inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), electrochemistry, and ion chromatography. Even though all of these methods are extremely sensitive, the need for expensive and complex apparatus is a major drawback [3].

Spectrophotometric methods still retain their identity among the other methods of metal ions determination due to their cheapness and simplicity. However, they lack sufficient selectivity and sensitivity for the measurement of metal ions in the complicated matrices. This problem could be solved by utilizing a preconcentration step to separate the analyte prior to the determination [4, 5]. The most common preconcentration processes are solid-phase extraction (SPE) [6] and liquid-liquid extraction (LLE) [7]. Both have various limitations such as lengthened extraction times, the use of relatively large amounts of hazardous solvent, and the appearance of a potential cause

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*Corresponding author. E-mail: almullaemad@atu.edu.iq of error caused by multiple stages [8, 9]. Cloud point extraction (CPE) is a modified LLE approach that uses micelles as an extraction solvent instead of hazardous organic solvents [10-13]. Among the preconcentration procedures, CPE represents a simple and environmentally friendly procedure for separation of inorganic and organic species in different samples [14-17].

To reduce background interferences and improve the selectivity of the procedure, a modified CPE procedure, termed as D-CPE, has been developed by Wu *et al.* [18]. According to the D-CPE principle, a metal ion M_1 (the analyte) with better metal-ligand (L) stability can replace a different metal M_2 from M_2-L with lower stability, but the converse process is not allowed. As a result, the analyte can be preconcentrated by utilizing a displacement reaction between analyte M_1 and M_2-L if M_2-L is used as the isolating reagent in place of L. This kind of displacement reaction only seldom happens between M_2-L and those coexisting ions (M_3) whose stability is less than M_2-L. This removes the interference from co-existing ions M_3 brought on by agent competition [19].

In this study, we combined D-CPE with spectrophotometry to determine Fe(III) in water and fruit samples. As a complexing agent, gallic acid (GA), was used. To produce D-CPE, Zn(II), the pre-extraction ion, was complexed with GA before being subjected to CPE. After the aqueous layer was removed, the solution containing Fe(III) was added, and CPE was performed for the second time. Because Fe-GA is more stable than Zn-GA, Fe(III) can replace Zn(II) in the pre-extracted Zn-GA complex. The obtained micellar layer was then subjected to spectrophotometric determination for Fe(III). Additionally, the procedures of standard reference substance (SRS) and flame atomic absorption spectroscopy (FAAS) were utilized to attain accuracy.

EXPERIMENTAL

Instrumentation

A Shimadzu model 1700 double beam UV/VIS spectrophotometer (Shimadzu, Japan) was used for spectrophotometric measurements. For comparison, a flame atomic absorption spectrometry (Shimadzu AA-6800 model, Tokyo, Japan) with a hollow cathode light operating at 12 mA) were employed. A 5986-60 Chemcadet digital pH-meter (Cole-Parmer, Chicago, IL) was used to check pH. Thermo Fisher Scientific's CL30 centrifuge (Bremen, Germany) was utilized to accelerate phase separation.

Solutions preparation

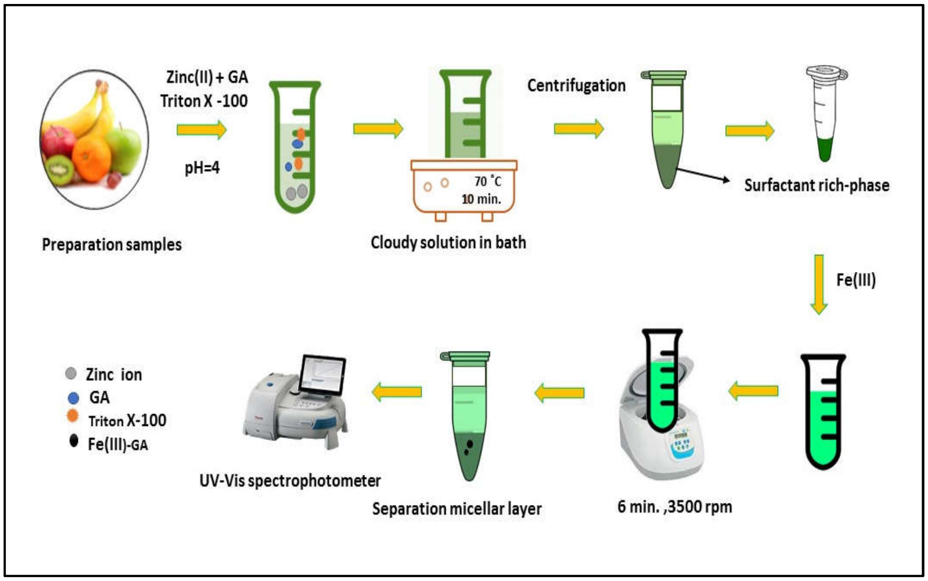
The stock solution for Zn(II) was obtained by dissolving 0.1 g of ultrapure zinc metal in 5.0 mL of HCl and dilute the obtained solution to 100 mL. To prepare 1000 mg L^{-1} standard solution of Fe(III), an adequate quantity of $FeNH_4(SO_4)_2.12H_2O$ were dissolved in 100 mL of 0.5 mol L^{-1} HCl. A gallic acid solution (10.0 mmol L^{-1}) was prepared by dissolving 0.1701 g in 100 mL of ethanol. Before usage, the plastics and glassware were soaked overnight in a 10% (v/v) nitric acid solution and rinsed multiple times with distilled water.

Fruit samples preparation

Fruit samples (banana, strawberry, lemon, orange, and peaches) were gathered from the supermarkets in Al-Najaf, Iraq in Nov. 2021. These samples were dried in electrical oven and then mashed to attain homogenous fine powder. Accurately weighed samples (0.5-0.7 g) were digested with 10 mL of concentrated nitric acid. Near dryness, 5 mL of 30% hydrogen peroxide were added. After that, they were heated again to complete the digestion. The mixture was diluted to 50 mL with distilled water in a volumetric flask.

General D-CPE Methodology

Scheme 1 depicted the general D-CPE approach. At pH 4.0 (0.5 mL acetate buffer), Aliquots of 50 mL solution containing Zn(II), GA (0.1 mmol L^{-1}), and 0.6 mL Triton X-100 (1% v/v) were kept for 10 min in a water bath at 70 °C to obtain a cloud solution. After centrifuging for 6 min at 3500 rpm, phase separation was achieved. To promote the production of viscid micelle layer, the tubes were immersed in ice-bath for 8 min. Then, the upper aqueous layer was removed, and the micelle layer was transferred into an Eppendorf tube. In the second cycle of D-CPE, solution containing Fe(III) was shaken with the surfactant-rich phase and incubated at 70 °C for 10 min to displace Zn(II) from Zn-GA complex. The solution was separated into two phases by centrifugation (6 min at 3500 rpm). The upper phase containing Zn(II) was removed and the surfactant-rich phase was diluted up to 1.0 mL with ethanol for spectrophotometric determination of Fe(III) at 560 nm, against a reagent blank.



Scheme 1. General procedure for D-CPE of Fe(III) from fruit samples.

RESULTS AND DISCUSSION

The solution pH, ligand concentration, amount of Triton X-100, temperature and heating time and conditions of centrifugation, were all optimized to obtain the maximum separation efficiency. According to equation (1), recovery of extraction (R%) was used to measure the procedure efficiency [20].

$$R\ (\%) = \frac{C_m V_m}{C_i V_i} \times 100 \qquad (1)$$

where C_m is the Fe(III) quantity in the micelle phase of volume V_m, C_i and V_i are the initial concentration and volume. The initial studies suggest that the order of addition is critical for achieving quantitative Fe(III) recovery. The sequential addition of Fe(III), gallic acid, buffer, and Triton X-100 resulted in greater recovery.

The pH impact

The pH of the solution has a significant influence on the D-CPE. The pH of the media has a significant effect on the development of stable complexes [21]. The effects of pH were investigated from 1.0 to 8.0 in this study, and the results are shown in Figure 1. The extraction was ineffective at low pH levels due to the protonation of the ligand's active sites, rendering them unusable for metallic ions [22]. The separation was improved by increasing the pH, until reached a maximum level at pH 6.0 for Zn(II) and pH 4.0 for Fe(III). Due to the development of metal hydroxides, the recovery was decreased again at higher value of pH. For this reason, pH 6.0 and 4.0 was chosen as the best value for first and second cycles, respectively.

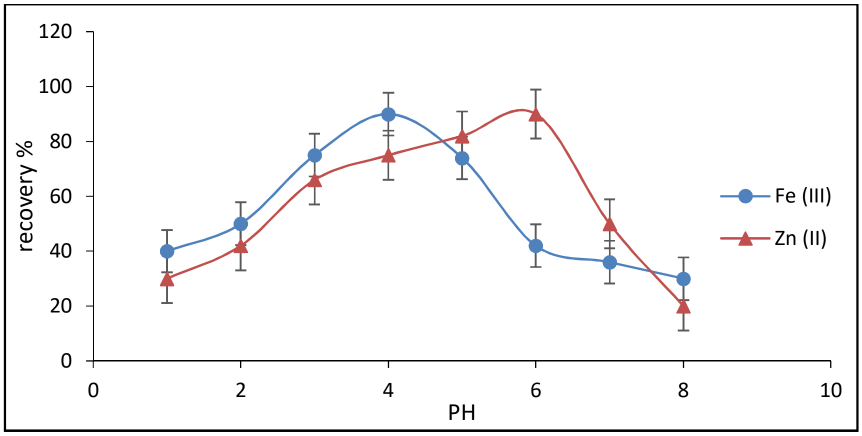


Figure 1.The impact of pH on D-CPE procedure. Sample solution (50 mL), Zn(II) 80 µg L^{-1}, Fe(III) 50 µg L^{-1}, GA 1×10^{-4} mol L^{-1}, Triton X-100, 0.6 mL; other parameters are listed in the experimental section.

The impact of ligand quantity

In CPE, the metal ion should create a stable complex that can be isolated into the surfactant layer [23]. GA was selected as a complexing agent in this work. It can form complexes with both Zn(II) and Fe(III), however, the Fe(III) complex is more stable [24]. Therefore, Fe(III) can replace Zn(II) from its gallate complex. The effect of changing GA concentration from 1×10^{-6} to 1×10^{-3} mol L^{-1} was investigated. As indicated, the maximum recovery of Fe(III) was achieved at 1×10^{-4} mol L^{-1} (Figure 2).

The impact of surfactant volume

Triton X-100 is applied as a surfactant in many of CPE procedures due to its unique properties. It is available in a pure form with a high density, making it easier to create rich-phase samples. The temperature range of clouds is relatively broad [9, 12]. The effect of Triton X-100 (1.0% v/v) volume on Fe(III) preconcentration by the current approach was investigated from 0.1 to 1.5 mL. Figure 3 demonstrates that when the amount of Triton X-100 is 0.6 mL (1% v/v), the recovery of Fe(III) reaches its maximum limit. At volumes less than 0.6 mL, the separation was insufficient, owing to incomplete assemblies that did not completely collect the hydrophobic complexes [25]. Therefore, the volume of 0.6 mL of Triton X-100 (1% v/v) was selected as optimum.

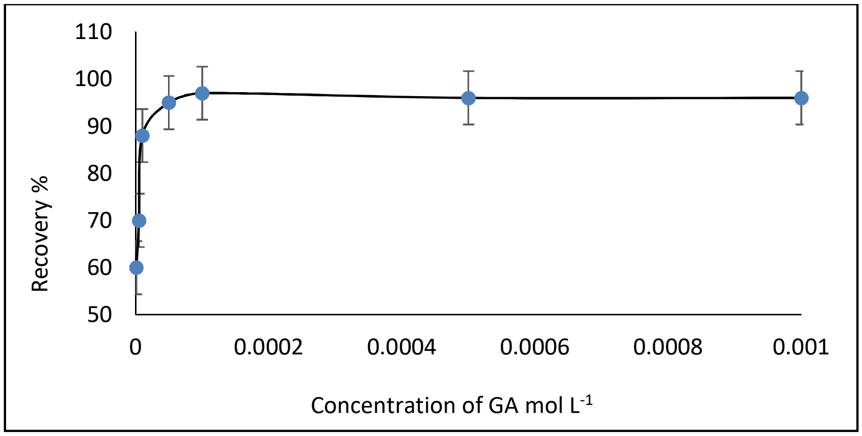


Figure 2.The effect of GA concentration on Fe(III) and Zn(II) determination by the proposed method. sample solution (50 mL), Zn(II) 80 µg L^{-1}, 50 µg L^{-1} Fe(III), GA 1×10^{-4} mol L^{-1}, Triton X-100 0.6 mL; other parameters are listed in the experimental section.

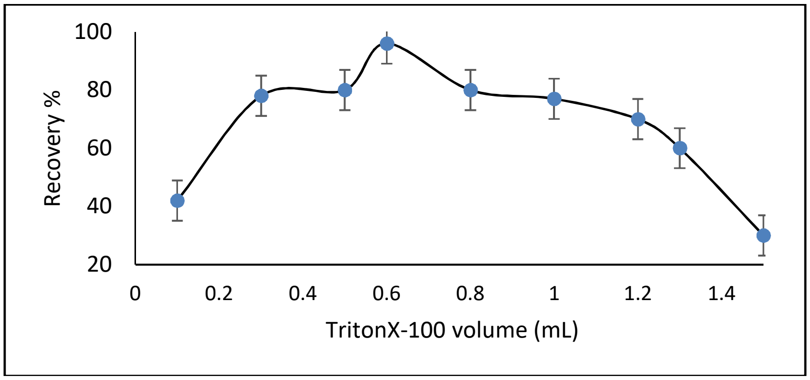


Figure 3. The impact of Triton X-100 volume on Fe(III) determination by the proposed method. 50 mL solution, pH = 4 or 6, 80 µg L^{-1} Zn(II), 50 µg L^{-1} Fe(III), GA 1×10^{-4} mol L^{-1}, Triton X-100 0.6 mL; other parameters are listed in the experimental section.

The impact of temperature and heating time

To enhance micelle-mediated extraction, it is required to warming the solution over the surfactant's cloud temperature. The mixture is split into two isotropic layers as a result. The organic layer, which comprises primarily surfactant aggregation with hydrophobic groups, and the aqueous layer, which includes free ions and other hydrophilic compound [26]. The influence of temperature on extraction of Fe(III) was studied from 30 to 90 °C. The results showed that increasing the temperature above 50 ºC improved the separation, with an ideal value at 70 ºC (R = 99.0 %). Higher temperatures, such as 75–90 ºC, resulted in a considerable decline in extraction efficiency (R ≤ 70 %), attributable to metal complex breakdown. In addition, the effect of an incubation period at 70 °C was examined (Figure 4). The results suggested that 10.0 min of heating was required for optimal extraction (R = 99.0–100.0%). Non-quantitative recovery (R ≤ 90 %) was achieved with shorter times. For the first and second CPE steps, an equilibration temperature of 70 °C for 10 min was chosen (Figure 5) [21].

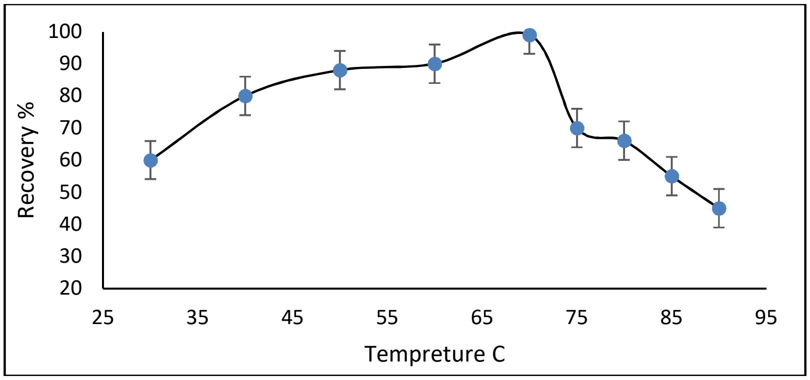


Figure 4.The effect of temperature on D-CPE, 50 mL solution, pH = 4 or 6, Zn(II) 80 µg L^{-1}, 50 µg L^{-1} Fe(III), GA 1×10^{-4} mol L^{-1}, Triton X-100 0.6 mL; other parameters are listed in the experimental section.

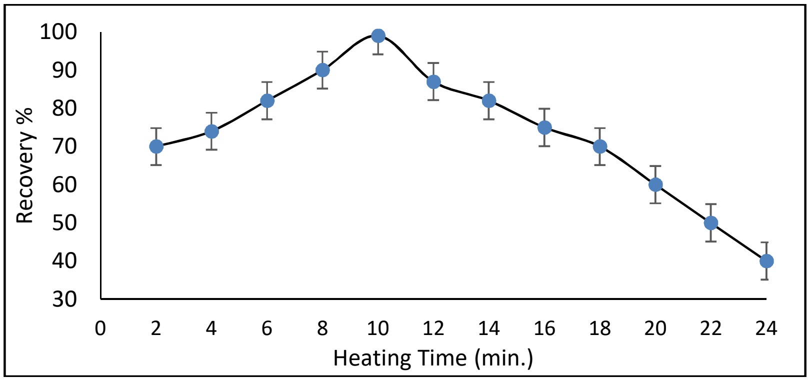


Figure 5.The effect time required for heating, 50 mL solution, pH = 4 or 6, Zn(II) 80 µg L^{-1}, 50µg L^{-1} Fe(III), GA 1×10^{-4} mol L^{-1}, Triton X-100 0.6 mL; other parameters are listed in the experimental section.

The impact of centrifugal time

In CPE, centrifugation is used to hasten the phase separation process. The effect of centrifugation time ranging from 2 to 15 min at 3500 rpm was investigated for this purpose. The results demonstrated that centrifugation at 3500 rpm for 6 min is sufficient for obtaining excellent extraction (R = 99.5%). Longer centrifugation times did not result in any substantial improvement. As a result, these conditions were chosen as optimum [30].

Ionic strength and interference impacts

By using varied amounts of NaCl (0.1-1.5 mol L^{-1}), the influence of ionic strength on the D-CPE was investigated. The findings suggested that ionic strength has no effect on the procedure's efficiency, even when the NaCl concentration is as high as 1.5 mol L^{-1}. To explore the effect of concomitant ions, aliquots containing 80 µg L^{-1} of Fe(III) with varying concentrations of the possible interfering ion were processed by the optimized D-CPE procedure. As shown in Table 1, the tolerance levels of the associated ions were ≤5%. The tolerance level (w/w) is defined as the highest level that causes a change in extraction efficiency of less than 5%. The ions of first and second groups elements (Li^+, Na^+, K^+, Ba^{2+}, and Ca^{2+}) as well as ammonium NH_4^+, and the main anions (Cl^-, Br^-, I^-, SO_4^{2-}, and CO_3^{2-}) did not interact up to 1000-folds. Cd^{2+}, Fe^{2+}, Pb^{2+}, and Ni^{2+} had a tolerance ratio of 600; Cr^{3+}, Cu^{2+}, and Mn^{2+} had a tolerance level of 300; and La^{3+}, In^{3+}, and Zr^{4+} had a tolerance ratio of 200.

Table 1. The impact of several ions on the D-CPE.

Various ions	Ratio of tolerance (w/w)
Li^+, Na^+, K^+, Ba^{2+}, Ca^{2+}, NH_4^+, Cl^-, Br^-, I^-, SO_4^{2-}, and CO_3^{2-}	1000
Cd^{2+}, Fe^{2+}, Pb^{2+}, and Ni^{2+}	600
Cr^{3+}, Cu^{2+}, and Mn^{2+}	300
La^{3+}, In^{3+}, and Zr^{4+}	200

Table 2. Comparison of Fe(III) extraction methods using various CPE methodologies.

Surfactant	Organic reagents	Sample volume (mL)	LOD (µg L^{-1})	Recovery (%)	Linearity	Applied procedure	EF	Ref.
Triton X-114	DOPHHBA	50	1.2	97-98.5	4-500	CPE/ UV-Vis	95.2	[9]
C16MeImCl / Triton X-114	APDC	10	10.0	95-102	50 - 750	CPE/ UV-Vis	20.4	[13]
Triton X-100	HPBN	10	12.0	-	100-10000	CPE/ UV-Vis	29	[27]
CTAB/ Triton X-114	Zincon	50	3.1	96.97.5	0-1000	CPE/ UV-Vis	50	[28]
Triton X-100	BAEE	10	23.0	-	100-100000	CPE/ UV-Vis	46	[29]
Triton X-100	MPTAN	10	41.0	-	50-1000	CPE/ UV-Vis	71	[30]
Triton X-100	GA	50	0.15	96.5–105.1	0.5-500	D-CPE / UV-Vis	75	This work

BAEE; Na-benzoyl-L-arginine ethyl ester hydrochloride, CTAB cethyltrimethyl ammonium bromide, DOPHHBA; 4-(2-(2,4-dioxopentan-3-ylidene) hydrazinyl)-2-hydroxybenzoic acid, ETB; 2-(3-ethylthioureido) benzoic acid, FAAS; flame atomic absorption spectrometry, HPBN; 2-(4-hydroxy phenyl azo)-4-benzene naphthol. MPTAN; methyl phenyl thiazolyl azo]-3-methyl-4-methoxy-2-naphthol.

The applications and accuracy

The calibration curve generated by the optimum D-CPE methodology was linear from 0.5 to 500 µg L^{-1} and had a coefficient of correlation ($R^2 = 0.9994$). The limit of detection (LOD = 3 s/b) was 0.15 µg L^{-1}. The LOD was defined as three times the standard deviation (s) of ten blank measured observations over the calibration graph's slope (b). The quantification limit (LOQ = 10 s/b) was 0.5 µg L^{-1}. The fraction of the slopes of calibrated curves with and without preconcentration was used to obtain the enrichment factor (EF), which was set at 75.0. For ten multiple samplings of aliquots comprising 60 µg L^{-1}, the relative standard deviation (RSD) was 1.3%. Table 2 shows a comparison of Fe(III) D-CPE approaches with other CPE approaches. In comparison to the other techniques, this methodology has a lower LOD, and a better EF [29-32].

The amounts of iron in river water for environmental monitoring were calculated in this article to confirm the validity of the D-CPE technique. The standard reference substance (SRS) (GSB 07–1188–2000, IERM, Beijing, China) is also used to test the method's applicability. Table 3 shows that the recoveries were 102.1 % for two SRSs with a low RSD of 2.11 %, confirming the technique's precision. Also, an independent one-sample t-test was calculated between the two analytical approaches depending on equation 2 [31, 32].

Table 3. Determination of Fe(III) in SRS by D-CPE.

Standard reference substance (SRS) water samples	References values (µg L^{-1})	Measured values (µg L^{-1})	Recovery %	RSD%	t-test *
GSB 202,433	700	715± 18	102.1	2.11	0.40
GSB 202,431	401	410± 11	102.1	2.11	0.44

*paired t-test, 96% confidence level, n = 10, t critical = 4.25.

$$\text{t-test} = \frac{|\text{Mean}_{\text{found}} - \text{Mean}_{\text{certified}}|\ \sqrt{n}}{S\,D} \qquad (2)$$

The approach was successfully used to measure Fe(III) in certain fruit samples, with the results summarized in Table 4. For the analysis of spiked samples, recoveries (96.5–105%) were reported. Table 4 shows the comparison with direct analysis by NO/acetylene FAAS. The two methods are statistically equivalent, but our methodology had a reduced RSD [33].

Table 4. Determination Fe(III) using D-CPE and FAAS in fruit samples (n = 10).

Samples	Added	D-CPE			FAAS
		Found	RSD%	RR %	Found
Banana µg g^{-1}	0	35.51	0.3	-	37.0±0.81
	10	46.51		102.1	-
	25	60.81		100.4	-
Lemon µg g^{-1}	0	20.25	0.3	-	21.3±0.71
	10	30.15		99.6	-
	25	45.31		101.1	-
Orange µg g^{-1}	0	25.70	0.1	-	26.0±0.61
	10	36.65		102.5	-
	25	51.33		101.3	-
Peaches µg g^{-1}	0	22.60	0.2	-	21.5±0.71
	10	32.81		101.8	-
	25	47.35		99.4	-
Strawberry µg g^{-1}	0	40.50	0.2	-	40.3±0.92
	10	50.41		99.8	-
	25	64.80		98.9	-

CONCLUSION

The basis of the D-CPE is the replacement or substituting of one metal for another to create a more stable hydrophobic metal-complex. The current study provides an environmentally friendly, simple, efficient, and inexpensive D-CPE method for Fe(III) preconcentration and quantification using UV-Vis spectrophotometry. By using D-CPE, the process removes the negative effects of organic solvents and surfactants. To the authors' knowledge, this is the first work using D-CPE for isolation Fe(III) complex with gallic acid in presence Zn(II). The proposed technique has a low LOD, a large linear range, and a high enhanced factor. It can be used to analyze Fe(III) in water and fruit samples that are important to the environment because of its flexibility.

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