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Contributed Paper

# Solid Phase-assisted Determination of Trace Copper(II) and Iron(III) in Food and Water Samples by Cloud Point Extraction: Response Surface Methodology Optimization

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## ABSTRACT

In this study, a simultaneous cloud point (CP) and solid phase extraction (SPE) procedure for the phase separation and pre-concentration of Cu(II) and Fe(III) ions using a synthesized chelating agent, 7'-amino-2,4'-dioxo-2'-thioxo-1',2',3',4'-tetrahydrospiro [indoline-3,5'-pyrano[2,3-d]pyrimidine]-6'-carbonitrile (**L1**), is reported. **L1** is used with *tert*-octylphenoxypoly(ethoxyethanol) (Triton X-114), in a CPE process to concentrate the metal ions followed by adsorption on activated carbon (AC) and determination of the concentration of metal ions by flame atomic absorption spectrometry (FAAS). The main factors affecting the process, such as the concentrations of Triton X-114, and **L1**, AC dose as well as the pH of the sample solutions were optimized by a response surface methodology (RSM). The detection limits ( $3 \text{ sdb} \cdot \text{m}^{-1}$ ) of Cu(II) and Fe(III) ions under optimum conditions ( X1 (pH) = 6, X2 (AC dose) = 15 mg, X3 (Triton X-114 concentration) = 0.22 % (v/v), and X4 (L1 concentration) = 0.40% (w/v) ) were obtained 0.45 and 0.65  $\mu\text{g} \cdot \text{L}^{-1}$ , respectively. The developed method, validated with certified reference materials, was successfully used in determining the concentrations of metal ions in food and water samples.

**Keywords:** solid phase assisted cloud point extraction, copper, iron, flame atomic absorption spectrometry, response surface methodology

## 1. INTRODUCTION

The essential trace elements are necessary for growth, normal physiological functioning, and the general maintenance of life. However, the ingestion or inhalation of these elements in large doses may lead to toxic effects. Trace metals are ubiquitous environmental contaminants and they can be easily taken up by humans, animals, plants, and water

sources in the environment. Cu is an essential trace element in biological systems and living organisms, where it serves as a cofactor in at least 30 important enzymes. The main route of intake for Cu is via food and the metal exists mainly as Cu(II) ions, occurring in food naturally or as a result of pollution. A concentration higher than

1.0  $\mu\text{g}\cdot\text{mL}^{-1}$  of Cu(II) imparts a bitter taste to water, with large oral doses causing symptomatic vomiting and in serious cases, liver damage [1]. Cu in potable water mainly exists as Cu(II) ions, and its concentration is usually very low ( $< 20 \mu\text{g}\cdot\text{L}^{-1}$ ) [1]. The European Commission has fixed the safety limit at  $2 \mu\text{g}\cdot\text{mL}^{-1}$  for Cu in drinking water, while the same is set at 1.3 and  $1.0 \mu\text{g}\cdot\text{mL}^{-1}$  in the USA and Canada, respectively [2]. Therefore, sensitive, reproducible, and accurate analytical methods are required for the determination of trace Cu in biological and water samples. On the other hand, Fe is important in the biosphere, serving as the active center in a wide range of proteins, such as oxidases, reductases, and dehydrases. It plays an essential role in photosynthesis and is a limiting growth nutrient for phytoplanktons in some parts of the open oceans. It is very important to monitor the iron content in vegetables and water samples to prevent an excessive build-up of iron content in our food source.

Currently, the most widely used extraction and preconcentration methods are liquid phase microextraction [3], liquid-liquid extraction [4], ion-exchange [5], resin chelation [6], fiber chelation [7], solid-phase extraction (SPE) [8-11], electrochemical deposition [12], and cloud point extraction (CPE) [13-17]. As one of the more advanced and miniaturized extraction techniques, CPE or alternatively known as micelle-mediated extraction, is well-established and widely applied in many scientific areas, especially the field of analytical chemistry. Compared with other extraction methods such as liquid-liquid extraction, CPE exhibits more environment-friendly properties, and it is safer because small volumes of surfactants are used instead of toxic organic solvents. The CPE technique has also been applied as a procedure for the determination

and removal of dyes, pigments, and food additives, as well as metal analyses [13, 14, 18-21]. Furthermore, combined CPE and SPE has been indicated as an effective method for preconcentration and determination of metal ions and pigments [22, 23].

Traditional optimization or one-factor optimization is the increase in the number of experiments necessary to conduct the research, which leads to an increase of time and expenses as well as an increase in the consumption of reagents and materials. In order to overcome this problem, the optimization of analytical procedures has been carried out by using multivariate statistical techniques that RSM is one of the well-known techniques in analytical optimization. RSM is a collection of statistical and mathematical methods that are useful for the modeling and analyzing engineering problems.

To the best of our knowledge, there are only a couple of published articles regarding combined CPE and SPE. In this work, for the first time we synthesized and used a green and well-known ligand as chelating agent along with AC (as a green sorbent) in the CP/SPE system. In this study, the new method is based on the complexation of Cu(II) and Fe(III) with a derivative, 7'-amino-2,4'-dioxo-2'-thioxo-1',2',3',4'-tetrahydrospiro[indoline-3,5'-pyrano [2, 3-d]pyrimidine]-6'-carbonitrile (**L1**), which works as a chelating agent that forms stable complexes capable of entering the surfactant-rich phase of Triton X-114 to entrap metal complexes. Afterwards, the complexes were adsorbed onto AC as an adsorbent followed by desorbing by a mixture of methanol and nitric acid and determination by FAAS.

To achieve highly efficient phase separation and preconcentration of the Cu(II) and Fe(III) ions by CPE, several

analytical parameters including surfactant and extracting agent concentrations, Ac dose as well as pH of the solution were optimized in this study using RSM methodology. Subsequently, the optimized method was applied to the determination of metal content in food samples. In addition, the method was validated by the determination of Cu(II) and Fe(III) ions in several different certified reference materials.

## 2. MATERIALS AND METHODS

### 2.1 Instrumentation

A Chemtech Analytical Instrument model CTA-3000 atomic absorption spectrometer (Bedford, England) equipped with a flame burner (FAAS) was used for the analysis of Cu(II) and Fe(III) ions, with the lamp current and wavelength set according to the manufacturer's recommendation. A centrifuge machine was used to accelerate the separation process (Universal-320, Hettich centrifuges, England). An inductively coupled plasma (ICP) atomic emission spectrometer (model Varian Liberty 150AX Turbo) was used for the determination of Cu(II) and Fe(III) concentrations. The IR spectrum was recorded using a Shimadzu IR-470 spectrometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were obtained using a Bruker AVANCE-DRX-400 instrument (in DMSO- $d_6$  at 400 MHz and 100 MHz, respectively;  $\delta$  in ppm and  $J$  in Hz). Elemental analyses (C, H, and N) were performed with a Heraeus CHN-O-Rapid analyzer.

### 2.2 Standard Solutions and Reagents

All chemicals used were of analytical reagent grade. All solutions were prepared with deionized water. Standard stock solutions of Cu(II) and Fe(III) ( $1000 \mu\text{g}\cdot\text{mL}^{-1}$ ), and anhydrous  $\text{Na}_2\text{SO}_4$  (1.0 M) were prepared by dissolving appropriate amounts of their salts (Merck, Germany) in deionized water,

containing a few drops of concentrated  $\text{HNO}_3$ . Reference solutions of Cu(II) and Fe(III) were prepared by performing serial dilutions of their stock solutions with deionized water. The non-ionic surfactant Triton X-114 and Triton X-100 (Sigma-Aldrich, Steinheim, Germany) were used without any further purification. 2% (v/v) non-ionic surfactant solutions were prepared by dissolving 2 mL of a non-ionic surfactant in 100 mL of distilled water. Britton-Robinson buffer solutions were used to x the pH in the range of 2.0-10. Buffers with specific pH values were prepared using boric acid, *o*-phosphoric acid, acetic acid, and sodium hydroxide. All of the reagents used in the synthesis of the ligand were purchased from Merck, Germany, and used without further purification. A 2% (w/v) chelating agent was prepared by dissolving 0.2 g of the synthesized ligand in 10 mL of tetrahydrofuran (THF). AC was purchased from Merck Co. Germany.

### 2.3 Synthesis of 7'-amino-2,4'-dioxo-2'-thioxo-1',2',3',4'-tetrahydrospiro[indoline-3,5'-pyrano[2,3-d]pyrimidine]-6'-carbonitrile

Here, we introduce a different and efficient method for the synthesis of the well-known chelating agent using readily available starting materials catalyzed by the ionic liquid, [BMIm]BF<sub>4</sub>. The ionic liquid acts as a solvent for the catalyst and it can be recycled. The chelating agent, 7'-amino-2,4'-dioxo-2'-thioxo-1',2',3',4'-tetrahydrospiro[indoline-3,5'-pyrano[2,3-d]pyrimidine]-6'-carbonitrile (**L1**), was synthesized by a simple and fast method with high yield (>93%) as follows:

A mixture of 0.144 g of 2-thioxo-dihydropyrimidine-4,6-(1*H*,5*H*)-dione, 0.147 g of isatin, 0.066 g of malonitrile, and 5 drops of ([BMIm]BF<sub>4</sub>) as an ionic liquid

catalyst was added to a flask containing a magnetic stirring bar, and 10 mL of a water and ethanol mixture (1:1). The mixture was heated under reflux for 50 min. After the reaction was complete, as monitored by thin layer chromatography (TLC) using silica gel SILG/UV 254 plates, the completed reaction was filtered and washed with warm ethanol ( $3 \times 30$  mL). In order to extract the ionic liquid, the residue was washed with  $2 \times 10$  mL of water. Then, the precipitate was kept in a refrigerator for 1h. To further purify the synthesized ligand, the product was recrystallized in hot ethanol. The yield of the product was determined 91% with the melting point of around 237 °C. Scheme **S1** shows the preparation of **L1**.

The characterization of **L1** was performed to give the following information:

**IR** (KBr):  $\nu_{\text{Max}}$  ( $\text{cm}^{-1}$ ) = [3509, 3205 and 3161 (N-H)]; [3425 and 3313 ( $\text{NH}_2$ )]; [2201 ( $\text{C}\equiv\text{N}$ )]; [1694 and 1656 (C=O)]; [1620, 1615 and 1469 (C=C arom)]; [1343 (C-O and C-N)], [1132 (C=S)].

**$^1\text{H-NMR}$**  (400 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  (ppm) = 6.79 (d, 1H,  $J = 7.8$  Hz, Ar), 6.91 (t, 1H,  $J = 7.6$  Hz, Ar), 7.15-7.20 (m, 2H, Ar), 7.42 (s, 2H,  $\text{NH}_2$ ), 10.54 (s, 1H, NH), 12.50 (s, 1H, NH), 13.90 (br s, 1H, NH).

**$^{13}\text{C-NMR}$**  (100 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  (ppm) = [46.58 (spiro carbon)]; [57.45, 91.52, 109.25, 121.78, 123.97, 128.56, 132.93, 142.10, 152.84, 158.08 and 159.12 (aromatic carbon and C=C)]; [173.90 and 177.13 (C=O)].

The IR,  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra are shown in **Figure S1, S2** and **S3**, respectively, in the Supporting Information.

#### 2.4 CP and SPE Procedures

A 15 mg portion of AC was added to

2.75 mL of 2% (v/v) Triton X-114 and stirred for 2 min. Aliquots of solutions containing Cu(II) and Fe(III) ions, 5.0 mL of buffer solution, 5.0 mL of **L1** (2% w/v), and 3.5 mL of 1.0 M  $\text{Na}_2\text{SO}_4$  were transferred into 25 mL centrifuge tubes, and made up to the mark with bi-distilled water. Each mixture was shaken for 2 min and left to stand in a thermostated bath at 40 °C for 15 min. The mixture was then cooled in an ice bath for 5 min to increase the viscosity of the surfactant-rich phase and to facilitate the removal of the aqueous phase. Subsequent centrifugation at 4000 rpm for 10 min allowed the AC to be separated from the surfactant rich-phase medium. In order to wash the AC and desorb the metal ions adsorbed onto AC, the AC was washed by 0.5 mL of 1 mol·L<sup>-1</sup>  $\text{HNO}_3$  in methanol prior to the determination of Cu(II) and Fe(III) ions by FAAS.

#### 2.5 Experimental Design

RSM based on CCD was used for the modeling and analysis of the effects of experimental variables to optimize them. For the CP/SPE process, important variables such as pH, AC dose, Triton X-114 and **L1** concentrations were chosen as independent variables and denoted as X1-X4, respectively. As shown in Table 1, the pH (X1) ranged from 4 to 8, AC dose (X2) ranged from 5 to 25 mg, Triton X-114 volume (X3) ranged from 0.04 to 0.4% (v/v), and 0.2-0.6% (w/v). According to Eq. (1), the total number of experimental points needed (N) is determined:

$$N = 2^k + 2k + C_0 \quad (1)$$

Therefore, a total of 30 experiments ( $k = 4$  (number of variables),  $C_0 = 6$ ) were performed. Analysis of variance (ANOVA) as the most powerful numerical method

for model validation was performed to estimate the different causes of variation. The significance of each process variable was checked by p and F values.

**Table 1.** Factors and levels used in the CCD study.

Variables	Level of variables		
	-1	0	+1
X1: pH	4	6	8
X2: AC dose (mg)	5.0	15.0	25.0
X3: Triton X-114 conc. (v/v) %	0.04	0.22	0.40
X3: L1 conc. (w/v) %	0.2	0.4	0.6

### 2.5 Analysis of Food Samples

The procedure reported by Gürkan et al. was used for the digestion of spinach, tomato, and tea samples [24]. For the determination of Cu(II) and Fe(III) concentrations, *ca.* 1.0 g of the dried sample was ashed for 6 h at 500 °C in a platinum crucible. After cooling, the ash was carefully moistened with 2 mL of 1:1 (v/v) HNO<sub>3</sub> and the mixture was evaporated to dryness on a hot plate. The residue was dissolved in 5 mL of 1.0 mol·L<sup>-1</sup> HNO<sub>3</sub>. The solution was filtered using filter paper, and the filtrate made up to 25.0 mL with water. Next, 1.0 mL of the resulting solution was transferred into a 50 mL volumetric flask, and made up to volume with the aqueous solution used in the optimization step. The Cu(II) and Fe(III) ions were extracted into the surfactant-rich phase by the CPE process and their concentrations were finally determined by FAAS.

A 20 g chocolate sample was ashed in a silica crucible for 4 h on a hot plate and the charred material was transferred to a furnace for overnight heating at 450 °C. The residue

was cooled and treated with 10 mL of concentrated HNO<sub>3</sub> and 3.0 mL of 30% H<sub>2</sub>O<sub>2</sub>, and it was then kept in a furnace for 2 h at 450 °C. The residual residue was treated with 0.5 mL of concentrated HCl and 1.0-2.0 mL of 70% HClO<sub>4</sub> and evaporated to fumes [1]. The final sample solution was prepared according to the steps described previously, and treated according to the CPE procedure. The Cu(II) and Fe(III) ions were extracted into the surfactant-rich phase by the CPE process and their concentrations were finally determined by FAAS.

A 50 g sample was taken and dried for 48 h in an oven at 120 °C to remove its water content (*ca.* 68% water) and to obtain a constant weight. The dried liver sample was transferred into a glass flask. A mixture of 3 mL H<sub>2</sub>SO<sub>4</sub>, 15 mL HClO<sub>4</sub>, and 15 mL HNO<sub>3</sub> was added into the flask and left to stand overnight. The solution was heated in an oil bath at 50 °C until the foaming stopped. The temperature was then increased to 150 °C and the solution was heated until the evolution of brown nitrogen oxide fumes ceased. When the mixture was dark brown, the flask was cooled for ~2 min before adding a further 5 mL of HNO<sub>3</sub>. The solution was once again heated until nitrogen oxide fumes were no longer given off. The appearance of white perchloric acid fumes in the 1 mL solution is an indication of a complete digestion. The solid residue was then dissolved in water and filtered. pH of the sample solution was adjusted accordingly with the addition of the buffer solution and the procedure as mentioned above was performed.

The method was also used to analyze different water samples, including tap and lake water samples, as well as selected reference materials. All the water samples were filtered through a 0.45 µm membrane to remove any suspended particles. The water

samples were adjusted to the desired pH using buffer solution. The certified reference materials were obtained from CRM TMDA- 51.3 (Environment Canada), SRM 1577B Bovine Liver (NIST), and IAEA 336 Lichen.

## 2.6 Binding Mechanism of Metal-L1 Complex

The binding mechanisms of analyte metals (Cu(II) and Fe(III)) to **L1** can be explained based on the HSAB (Hard-Soft Acid-Base) theory [25, 26]. According to this theory, soft acids react faster and form stronger bonds with soft bases, whereas hard acids react faster and form stronger bonds with hard bases, all other factors being equal. In other words, electron donors and acceptors tend to react in ways that favor hard-hard and soft-soft interactions because thermodynamically, a hard-hard/soft-soft pairing forms a stronger binding interaction in comparison to a hard-soft/soft-hard pairing, which forms a weaker binding interaction. As expected from the HSAB principle [25, 26], a harder Lewis acid such as Fe(III) interacts better with oxygen as a Lewis base than with nitrogen and sulfur, with the former element being harder than the latter two. The interaction of softer Lewis acid such as Cu(II) with softer Lewis base (nitrogen and sulfur) is, to a certain extent, driven by the larger availability of the lone pair in nitrogen and sulfur compared to oxygen.

Furthermore, since the synthesized ligand possesses nitrogen, sulfur, and oxygen donor atoms, in addition to a conjugated  $\pi$  system, it has a high probability to form stable complexes with Cu(II) and Fe(III) ions. Hence, Cu(II) and Fe(III) ions were used as target ions to examine the potential of the ligand **L1** as a reagent for the CPE procedure.

## 3. RESULTS AND DISCUSSION

### 3.1 Model Fitting and Statistical Analysis

As mentioned above, four independent variables were chosen to assess the impact of analytical parameters on the preconcentration/determination of Cu(II) and Fe(III) ions. The variables were assigned one of three levels with coded value (-1, 0, +1) and their star points of +2 and -2 for +  $\alpha$  and - $\alpha$  respectively were selected for each set of experiments. Tables 2a&b show ANOVA results for metal ions determination by combined solid phase-cloud point extraction using the dispersion of AC. The model F values are 215.59 and 425.73 for Cu and Fe respectively indicating that the models are significant. The lack-of-fit for F values of 0.939 and 0.0959 for Cu and Fe respectively pointing out that this term is not significant to the pure error. The Pred-R<sup>2</sup> of 0.9877 and 0.9834 are in reasonable agreement with the Adj-R<sup>2</sup> of 0.9904 and 0.9951 for Cu and Fe respectively. Based on results obtained from Tables 2a&b, an empirical second-order polynomial equations for Cu and Fe were obtained, which in terms of actual factors are as follows:

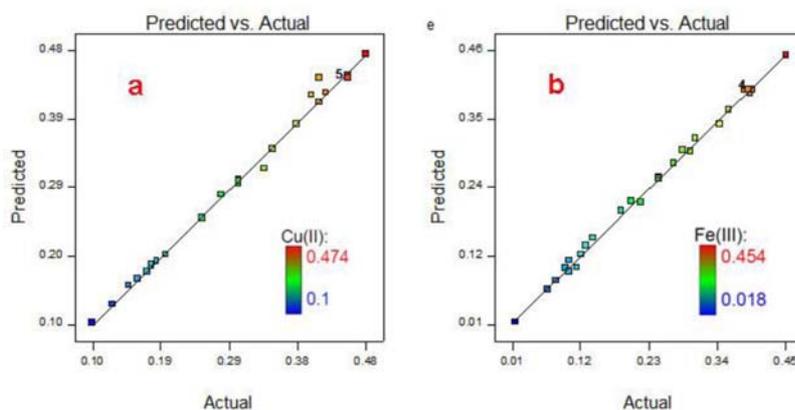
$$Y_{abs,Cu} = -1.22 + 0.45X_1 + 0.012X_2 + 1.48X_3 - 0.085X_4 - 1.64X_1X_2 - 0.07X_1X_3 + 0.046X_1X_4 + 0.013X_2X_3 + 0.18X_3X_4 - 0.036X_1^2 - 2.35X_3^2 \quad (2)$$

$$Y_{abs,Fe} = -1.34 + 0.44X_1 + 0.034X_2 + 0.83X_3 - 0.11X_4 - 1.26X_1X_2 - 0.05X_1X_3 + 0.017X_1X_4 + 7.5E - 003X_2X_3 - 5.75E - 003X_2X_4 + 0.43X_3X_4 - 0.034X_1^2 - 8.75E - 004X_2^2 - 1.36X_3^2 \quad (3)$$

The significance of each model term was checked using P values. P values smaller than 0.05 indicate that the model is statistically significant, while values greater than 0.05 indicate the model terms are not significant.

In this study, all the linear model terms (X1, X2, X3, and X4), the two quadratic model terms ( $X1^2$  and  $X3^2$ ), and the one interaction term (X1X2, X1X3, X1X4, X2X3, X3X4) are significant for Cu and (X1, X2, X3, and X4), the three quadratic model terms ( $X1^2$ ,  $X2^2$ , and  $X3^2$ ), and the one interaction

term (X1X2, X1X3, X1X4, X2X3, X2X4, and X3X4) are significant for Fe. Figures 1a&b show the comparison of predicted and experimental absorption of metal ions that the nearness of the points to the diagonal line indicates the good fit of the model.



**Figure 1.** The comparison of predicted and experimental results for (a) Cu (b) Fe.

### 3.2 Response Surface Methodology Analysis

To further understand the impact of analytical variables on preconcentration and determination of the two metal ions, three dimensional (3D) plots were drawn. Figures 2a&b describe the effect of the combination of pH and AC dose at constant values of chelating agent and Triton X-114 concentrations. The formation of the metal-L1 complex and its chemical stability are two important factors for CPE. The pH, which plays a unique role in the formation of the complex and its subsequent extraction, proved to be a major factor for CPE efficiency [27]. Figures 2a&b show that the maximum absorbance of the metal-L1 complex extracted into the surfactant-rich phase was obtained at pH 6. The complexation process at pH values lower than 6 was incomplete. The incomplete reaction was attributed to the competition

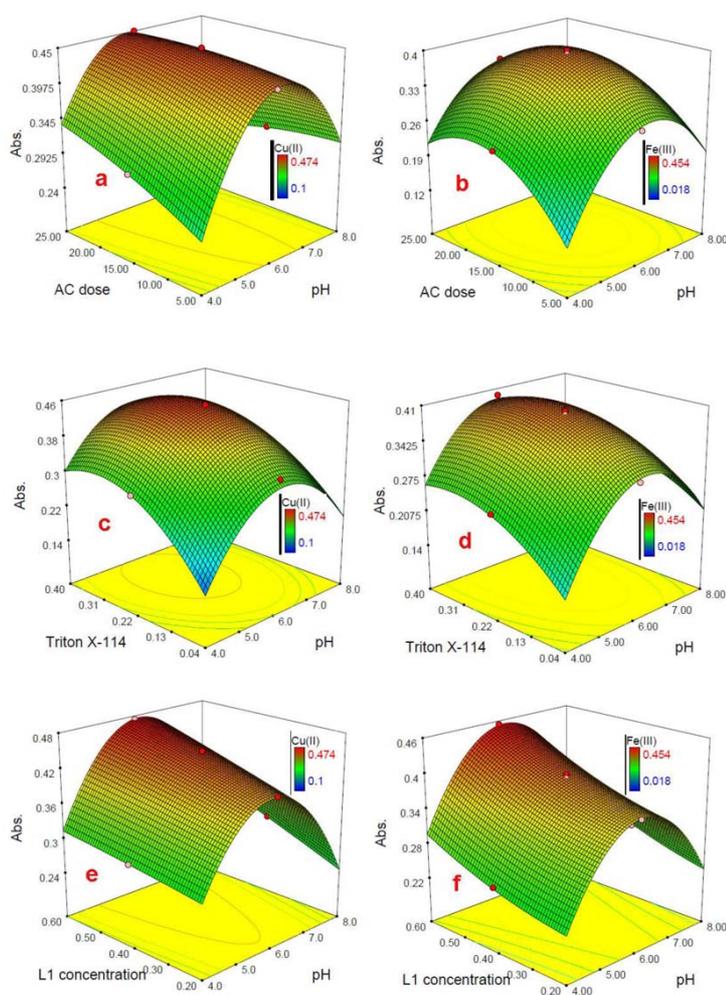
between the metal ions and hydrogen ions for L1 binding, based on the protonation of the ligand. On the other hand, by increasing the pH, the number of active sites on L1 for metal ion binding was increased. However, the decrease in absorbance at pH values higher than 6 was attributed to the formation of metal hydroxide species such as the soluble  $M(OH)^+$  and/or the insoluble precipitate of  $M(OH)_n$ . Based on Figures 2a&b, the adsorption efficiency remarkably increases with increasing the amount of adsorbent at constant pH value as a result of increasing the available surface area for adsorption. After that, it does not affect notably the absorption efficiency.

The surfactant concentration used in CPE systems is a critical factor. At constant pH value (pH 6), the analytes absorption efficiency increases with increasing the Triton X-114 concentration as can be seen if Figures 2c&d. At lower Triton X-114

concentrations, the extraction yield of the metal-complex was low presumably due to an insufficient quantity of the active component in entrapping the hydrophobic complex [28]. However, at higher Triton X-114 concentrations, the extraction yields decreased. This might be related to an increase in the volume of the surfactant-rich phase given the large amount of surfactant present. In addition, an increase in the viscosity of the surfactant-rich phase could also lead to poor sensitivity [29].

Figures 2e&f show the combined influence of pH and **L1** concentration under

constant adsorbent dose and Triton X-114 concentration conditions. At constant pH value, the Cu(II) and Fe(III) ions absorption efficiency increases by increasing in **L1** concentration that it depended on the kinetics of the complex formation and the transference between the phases. However, by using an excess amount of **L1**, a decrease in the absorbance was observed. Adding a larger amount of **L1** to the CPE process might prevent the micelle formation and reduce the extraction efficiency of the target metal ion. For other 3D plots (Figures S4), the same reasons discussed above can be considered.



**Figure 2.** The 3D plots showing the effect of single and combined variables on extraction efficiency.

**3.3 Conformation of Optimal Conditions**

The extraction efficiency and the operation conditions for maximum absorbance of the Cu(II) and Fe(III) ions from aqueous solution were calculated from the second-order equation obtained from the experimental data. The first-order partial differential equations for Cu obtained from Eq. (2) for  $X_i$  are:

$$\frac{\partial Y}{\partial X_1} = 0.45 - 1.64X_2 - 0.07X_3 + 0.046X_4 - 0.036X_1 \quad (4)$$

$$\frac{\partial Y}{\partial X_2} = 0.012 - 1.64X_1 + 0.013X_3 \quad (5)$$

$$\frac{\partial Y}{\partial X_3} = 1.48 - 0.07X_1 + 0.013X_2 - 2.35X_3 = \quad (6)$$

$$\frac{\partial Y}{\partial X_4} = -0.085 + 0.046X_1 + 0.18X_3 \quad (7)$$

The first-order partial differential equations for Fe obtained from Eq. (3) for  $X_i$  are:

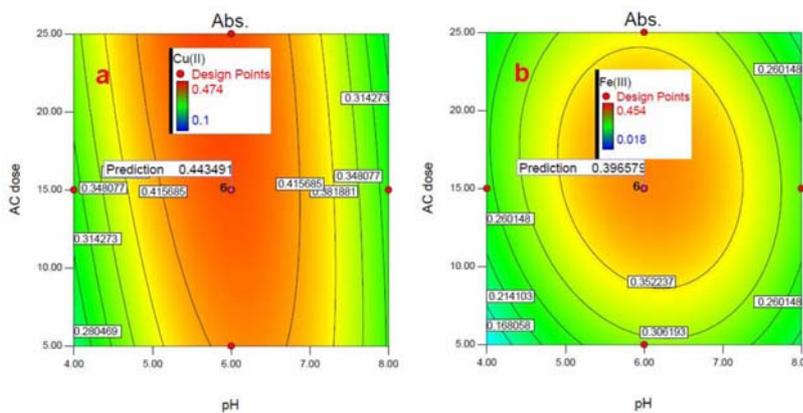
$$\frac{\partial Y}{\partial X_1} = 0.44 - 1.26X_2 - 0.05X_3 + 0.017X_4 - 0.034X_1 \quad (8)$$

$$\frac{\partial Y}{\partial X_2} = 0.034 - 1.26X_1 + 7.5E - 003X_3 - 5.75E - 003X_4 - 8.75E - 004X_2 \quad (9)$$

$$\frac{\partial Y}{\partial X_3} = 0.83 - 0.05X_1 + 7.5E - 003X_2 + 0.43X_4 - 1.36X_3 \quad (10)$$

$$\frac{\partial Y}{\partial X_4} = -0.11 + 0.017X_1 - 5.75E - 003X_2 + 0.43X_3 \quad (11)$$

By considering  $\frac{\partial Y}{\partial X_i} = 0$  resulting in maximum value of Y (absorbance) for Eqs. (4)-(11), the value of  $X_i$  ( $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$ ) could be obtained. The solutions of Eqs. (4)-(7) for Cu were found to be  $X_1 = 0.011$ ,  $X_2 = -28.93$ ,  $X_3 = 0.469$ , and  $X_4 = -1040.60$ . Also, the solutions of Eqs. (8)-(11) for Fe were found to be  $X_1 = 0.033$ ,  $X_2 = 0.32$ ,  $X_3 = 0.26$ , and  $X_4 = -1.11$ . These values were then converted to actual values of  $X_1$  (pH) = 6,  $X_2$  (AC dose) = 15 mg,  $X_3$  (Triton X-114 concentration) = 0.22 % (v/v), and  $X_4$  (L1 concentration) = 0.40% (w/v). In fact, this optimum condition is the best condition for simultaneous extraction of Cu and Fe ions in solution. Under the optimum conditions, the maximums predicted absorbance were 0.443 and 0.396 with satisfactory desirability of 0.963 for Cu and Fe respectively as shown in Figures 3a&b.



**Figure 3a&b.** The contour plot obtained from RSM optimization for optimum extraction efficiency.

### 3.4 Analytical Performances

The precision of the procedure was determined by establishing the relative standard deviation of ten independent measurements carried out in solutions containing the Cu(II) and Fe(III) ions. The limits of detection (LOD) ( $n = 10$ ,  $LOD = X_b + 3s$ , where  $X_b$  is the blank value and  $s$  is the standard deviation (s.d.) of the blank) were found to be  $3 \text{ sdb} \cdot \text{m}^{-1}$  of  $0.45$  and  $0.65 \text{ ng mL}^{-1}$  for Cu(II) and Fe(III) ions, along with preconcentration factor of 50. The enrichment factors (the ratio of the concentration of the analyte after preconcentration to that before preconcentration which give the same absorbance) were 78.1 and 66.6 for Cu(II) and Fe(III) ions, respectively. The limit of quantification (LOQ) is the lowest level of analyte that can be accurately and precisely

measured. The limits of quantification, dened as 10 times the standard deviation of the blank ( $n = 10$ ), were found to be  $3.0$  and  $4.5 \text{ ng mL}^{-1}$  for Cu(II) and Fe(III) ions, respectively. Meanwhile, the relative standard deviations (RSD) at  $100$  and  $150 \text{ } \mu\text{g} \cdot \text{L}^{-1}$  were  $3.1$  and  $2.0\%$  for Cu(II) and Fe(III) ions, respectively. The data presented in Table 3 shows that the method has a high sensitivity and a good precision. Comparative data of some recent studies on the CPE process of Cu(II) and Fe(III) ions are summarized in Table S1. Our method provided better or comparable LOD and preconcentration factor (PF) values compared to the data in in Table S1. The synthesized complexing agent showed a good affinity towards Cu(II) and Fe(III) ions due to the their low LODs

**Table 2a.** ANOVA results of the quadratic model Cu extraction.

Source of variation	Sum of squares	Df <sup>a</sup>	Mean square	F-Value	P-Value	
Model	0.44	14	0.032	215.59	<0.0001	#
X1	1.42 E-005	1	1.42 E-005	308.55	<0.0001	#
X2	2.89 E-003	1	2.89 E-003	19.72	0.0005	#
X3	0.048	1	0.048	327.41	<0.0001	#
X4	0.020	1	0.020	135.67	<0.0001	#
X1X2	0.017	1	0.017	117.19	<0.0001	#
X1X3	0.011	1	0.011	71.74	<0.0001	#
X1X4	5.33 E-003	1	5.33 E-003	36.39	<0.0001	#
X2X3	9.03 E-003	1	9.03 E-003	61.63	<0.0001	#
X2X4	2.40 E-004	1	2.40 E-004	1.64	0.2197	*
X3X4	6.76 E-004	1	6.76 E-004	4.62	0.0484	#
X1 <sup>2</sup>	0.053	1	0.053	365.09	<0.0001	#
X2 <sup>2</sup>	1.94 E-004	1	1.94 E-004	1.32	0.2680	*
X3 <sup>2</sup>	0.015	1	0.015	102.59	<0.0001	#
X4 <sup>2</sup>	7.05 E-006	1	7.05 E-006	0.048	0.8293	*
Residual	2.20 E-003	15	1.46 E-004	-	-	
Lack-of-fit	8.63 E-004	10	8.63 E-004	0.32	0.9392	*
Pure error	1.33 E-003	5	2.67 E-004	-	-	
Cor Total	0.44	29	-	-	-	

<sup>a</sup>Degree of freedom. # Significant. \* Not significant ( $R^2 = 0.9951$ ;  $Adj-R^2 = 0.9904$ ;  $Pred-R^2 = 0.9877$ )

**Table 2b.** ANOVA results of the quadratic model for Fe extraction.

Source of variation	Sum of squares	Df <sup>a</sup>	Mean square	F-Value	P-Value	
Model	0.47	14	0.033	425.73	<0.0001	#
X1	3.56 E-003	1	3.56 E-003	45.49	<0.0001	#
X2	8.54 E-003	1	8.54 E-003	109.21	<0.0001	#
X3	0.026	1	0.026	327.66	<0.0001	#
X4	0.036	1	0.036	457.12	<0.0001	#
X1X2	0.010	1	0.010	130.50	<0.0001	#
X1X3	5.26 E-003	1	5.26 E-003	67.24	<0.0001	#
X1X4	7.02 E-004	1	7.02 E-004	8.98	0.009	#
X2X3	2.92 E-003	1	2.92 E-003	37.30	<0.0001	#
X2X4	2.12 E-003	1	2.12 E-003	27.07	<0.0001	#
X3X4	3.78 E-003	1	3.78 E-003	48.38	<0.0001	#
X1 <sup>2</sup>	0.047	1	0.047	603.98	<0.0001	#
X2 <sup>2</sup>	0.016	1	0.016	204.20	<0.0001	#
X3 <sup>2</sup>	5.01 E-003	1	5.01 E-003	64.14	<0.0001	#
X4 <sup>2</sup>	3.43 E-004	1	3.43 E-004	4.39	0.0535	*
Residual	1.17 E-003	15	7.82 E-005	-	-	
Lack-of-fit	1.02 E-003	10	1.02 E-003	3.37	0.0959	*
Pure error	1.51 E-004	5	3.03 E-005	-	-	
Cor Total	0.47	29	-	-	-	

<sup>a</sup>Degree of freedom. # Significant. \* Not significant ( $R^2= 0.9975$ ;  $Adj-R^2=0.9951$ ;  $Pred-R^2=0.9834$ )

**Table 3.** Analytical performance of the CP/SPE procedure for the determination of Cu(II) and Fe(III) ions.

Parameter	Cu(II)	Fe(III)
Slope	0.019	0.023
Intercept	0.0083	0.0098
Correlation coefficient (r)	0.9997	0.9998
Preconcentration factor	50.0	50.0
Enrichment factor	78.1	66.6
Limit of detection ( $\mu\text{g L}^{-1}$ )	0.45	0.65
Limit of quantitative ( $\mu\text{g L}^{-1}$ )	3.00	4.50

### 3.5 Influence of Co-existing Ions

In order to test the selectivity of the CPE procedure for metal ion analysis, we examined the influence of the co-existing ions, including  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and some other ions commonly found in the various water samples. The effect of the interfering species on the extraction of Cu(II) and

Fe(III) was independently tested. The tolerance limit of the co-existing ions, dened as the largest amount making change the extraction of Cu(II) and Fe(III) ions, is less than 5%, as summarized in Table 4. The results indicate that most of the cations and anions are tolerable at weight ratios of 1000, suggesting the high selectivity of the method.

**Table 4.** Tolerable level of co-existing ions as the quantitative recoveries of Cu(II) and Fe(III) ions (n= 3).

Coexisting ion	Added as	Tolerance ratio
Na <sup>+</sup> , K <sup>+</sup> , Cl <sup>-</sup> , I <sup>-</sup>	NaCl, KI	1000
Ag <sup>+</sup> , SO <sub>4</sub> <sup>2-</sup> , CH <sub>3</sub> COO <sup>-</sup> ,	CH <sub>3</sub> COONa, Na <sub>2</sub> SO <sub>4</sub> , AgNO <sub>3</sub>	750
Mg <sup>2+</sup> , Ca <sup>2+</sup> , Ba <sup>2+</sup> , HPO <sub>4</sub> <sup>2-</sup>	Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O, Nitrate salts	550
F <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , CO <sub>3</sub> <sup>2-</sup>	NaF, KNO <sub>3</sub> , Na <sub>2</sub> CO <sub>3</sub>	400
Mn <sup>2+</sup> , Co <sup>2+</sup> , Zn <sup>2+</sup>	MnSO <sub>4</sub> ·12H <sub>2</sub> O, Nitrate salts	250
Ni <sup>2+</sup> , Pb <sup>2+</sup> , Fe <sup>2+</sup>	Nitrate salts	100

### 3.6 Application to Food and Water Samples

To assess the validity of the developed method, the CPE procedure was used to determine the concentrations of Cu(II) and Fe(III) ions in several food and water samples. The reliability of the procedure was verified using a standard addition method, where a standard solution was spiked to include the target ions of the sample. The results are summarized in Table 5. ICP-AES was used to establish the concentrations of the Cu(II) and Fe(III) ions obtained via the CPE procedure. The recoveries of the metal ions in these food and water samples, estimated from the added versus the measured

concentrations, are reasonable, indicating the suitability of the CPE procedure in the determination of metal ions. The recovery values ranged from 96.4% to 103%. The accuracy of the developed method was also investigated by the analysis of certified reference materials, including TMDA 51.3 fortified lake water, SRM 1577B Bovine liver, and IAEA 336 Lichen. As summarized in Table 6, the results reported using our method were in good agreement with the certified values of the analyte ions. In addition, our CPE procedure could accurately determine target ions without any influences of the co-existing matrixes in various food and water samples.

**Table 5.** Concentrations of Cu(II) and Fe(III) ions in various food and water samples (n = 6).

Sample	Added	Found			Fe(III)		
		Cu(II)			Proposed <sup>a</sup>		
		Proposed <sup>a</sup> (RSD, %)	Recovery, %	ICP-AES	(RSD, %)	Recovery, %	ICP-AES
Spinach <sup>b</sup>	-	25.5 (1.1)	-	25.3	50.2 (1.4)	-	49.2
	50	75.5 (1.5)	100	75.5	100.2 (1.0)	100	99.3
	100	123.6 (1.0)	98.5	123.2	152.2 (1.5)	101	151.3
Tomato <sup>b</sup>	-	95.7 (2.0)	-	95.0	31.1 (0.9)	-	31.9
	100	192.8 (1.8)	98.5	192.0	129.5 (1.4)	98.8	132.0
	200	300.7 (1.7)	102	301.5	226.8 (1.5)	97.8	229.8
Tea <sup>b</sup>	-	11.1 (1.3)	-	11.0	6.6 (0.3)	-	6.3
	10	20.4 (1.0)	96.7	20.2	17.0 (0.5)	102	17.0
	20	30.1 (1.1)	96.8	30.0	26.0 (0.3)	97.7	26.3

**Table 5.** Continued.

Sample	Added	Found			Fe(III)		
		Cu(II)			Proposed <sup>a</sup>		
		Proposed <sup>a</sup> (RSD, %)	Recovery, % ICP-AES	ICP-AES (RSD, %)	Proposed <sup>a</sup> (RSD, %)	Recovery, % ICP-AES	ICP-AES (RSD, %)
Chocolate <sup>b</sup>	-	2.4 (1.8)	-	3.1	4.9 (0.2)	-	5.0
	5.0	7.7 (1.3)	104	7.7	10.2 (0.4)	103	10.2
	10	12.1 (1.0)	97.6	12.3	14.5 (0.5)	97.3	14.1
Liver <sup>b</sup>	-	35.4 (1.5)	-	35.1	41.2 (1.1)	-	43.8
	50	84.4 (1.4)	98.8	84.1	93.5 (1.2)	103	94.0
	100	134.1 (1.9)	99.0	134.0	147.1 (1.0)	104	149.4
Lake water <sup>c</sup>	-	10.7 (1.2)	-	10.4	10.6 (0.9)	-	10.3
	10	21.6 (1.5)	103	21.9	20.9 (0.4)	102	21.0
	20	29.9 (1.0)	97.4	29.5	29.5 (0.8)	96.4	29.7
Tap water <sup>c</sup>	-	6.3 (1.0)	-	6.2	5.7 (0.6)	-	5.6
	5.0	11.0 (1.0)	97.3	11.0	10.4 (0.3)	97.2	10.5
	10	15.8 (1.7)	96.9	16.0	16.0 (0.6)	102	16.4

<sup>a</sup>  $\bar{x} \pm ts \sqrt{n}$  at 95% condence (n = 5).

<sup>b</sup> All values are in  $\mu\text{g g}^{-1}$  after suitable dilution.

<sup>c</sup> All values are in  $\text{ng mL}^{-1}$ .

**Table 6.** The levels of analytes in TMDA-51.3 fortified water, IAEA 336 Lichen. and SRM 1577B Bovine liver certified reference materials established using our method (n = 5).

SRM 1577B Bovine liver certified reference material			
Analyte	Certified value ( $\mu\text{g g}^{-1}$ )	Found value ( $\mu\text{g g}^{-1}$ )	Recovery (%)
Cu	160	157.1 $\pm$ 3.0*	98.2
Fe	184	179.6 $\pm$ 3.5	97.6
IAEA 336 Lichen certified reference material			
Cu	3.60	3.56 $\pm$ 0.2	98.9
Fe	430	414.3 $\pm$ 5.5	96.3
TMDA 51.3 fortified water certified reference material			
Cu	89.2	93.5 $\pm$ 1.5	104.8
Fe	109	106.0 $\pm$ 2.2	97.2

\* mean  $\pm$  standard deviation

### 3.7 Role of AC

AC has two crucial properties that influence the extraction efficiency: (1) they have a relatively high surface area and large number of active sites leading to high adsorption capacity and efficiency (2) they cause very rapid adsorption to the vessel,

only by using a very low amount of them (15 mg).

### 4. CONCLUSIONS

This article presents a new efficient method for preconcentration/extraction of Cu(II) and Fe(III) ions by FAAS in water

samples. The ligand **L1**, synthesized from simple starting materials, was employed as a chelating agent to extract Cu(II) and Fe(III) ions efficiently from food and water samples. The combined and optimized CP/SPE procedure could detect trace levels of Cu(II) and Fe(III) ions by FAAS, and it provided good analytical results, which are comparable to previously reported methods. This procedure is highly sensitive, gives good recovery values, precise, and inexpensive. In line with the green chemistry concept, the small volume of the eluent (500  $\mu$ L) provides a better extraction strategy compared to methods using organic solvents due to its relatively lower toxicity. Moreover, both chelating agent (L1) synthesized and adsorbent (AC) used are categorized as green materials.

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