



Karaj branch

## **Pre-concentration and Determination of Low-traces of Copper Ions in Different Mediums Using Dispersive Liquid-liquid Micro-extraction (DLLME)**

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### **Abstract**

In the paper, in order to pre-concentrate and measure the low values of Cu ions, the new, simple, quick and very sensitive method of Dispersed Liquid-Liquid Micro Extraction (DLLME) coupled with flame Atomic Absorption spectrometry (AA) has been utilized. The DLLME technique coupled with AA (AA-DLLME) was run inside one narrow tube which constitute aqueous samples. In this method, a mixture of toluene and methanol 90:10 (v/v) was injected into the aqueous sample as extractor and disperser agent and then Cu and diethyldithiocarbamate (DDTC) complex was concentrated inside the toluene and was collected on the sample solution. In order to optimize the factors affecting on the process, different parameters affected on the Cu extraction such as kind and volume of extractor, volume of dispersing solvent, pH of sample solution, ligand concentration, time for bubble formation and salt effect were studied. Under optimal condition, limit of detection (LOD), relative standard deviation (RSD) and limit of linearity (LOL) for calibration curve were measured and were obtained as: 1.2  $\mu\text{g}\cdot\text{l}^{-1}$ , 3.64% and 0.01-1  $\text{mg}\cdot\text{l}^{-1}$ , respectively. This method was successfully applied to determine Cu in environmental waters, milk powder, liquid milk and orange juice.

**Keywords:** *Microextraction, Pre-concentration, Copper, DLLME.*

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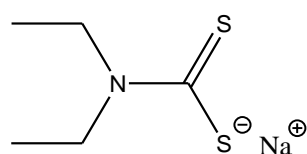
## **Introduction**

Analytical techniques including several steps such as: sampling, sample preparing, analysis, calculation and finally statistical evaluation of results [1]. Each of these steps directly effect on the validity, precision and sensitivity. Meanwhile, the step of sample preparation is specifically important [1-5]. Preparing the sample includes transforming one real sample into the state that is suitable for analyzing through separation technique or other technique. Preparing the sample is important to degree which involves about 60% volume of analytical process [3, 5-7]. Generally, preparing the sample is for achieving purposes such as eliminating potential interferences (for separating and measuring stages) from the sample to increase the technique selectivity, species pre-concentration and increase the technique sensitivity, to transform the species into the state which is suitable for separating/detecting or preparing one reproducible and powerful technique which is independent of changes in the sample texture [7-9]. The ideal extraction is a simple, cheap and reproducible technique and makes quantitative recovery of the species without losing and eliminating them. Also it should be performed with low volume of sample and should have high selectivity and low losses, solvent consumption of it should be at minimum level, it should be automation-featured and continuously used with analytical systems and finally it needs no concentration and lowering the volume of extraction phase [10-11]. These classic extraction techniques require very much consumption of solvent with high purity which is more costly and high-toxic while it has low selectivity [12]. Liquid-liquid extraction is one separating technique which has been based on the unequal distribution of one object among two non-miscible solvents. This technique traditionally had been applied to the industry of preparing and refining aromatic oils, drugs and dyes etc. In 1867, liquid-liquid extraction has been applied for separation of cobalt from nickel, gold from platinum and iron from other metals. Application of liquid-liquid extraction become extensively common for separating along with synthesizing dithizone in 1925 and after that the extraction technique has obtained very good status because of simplicity, execution speed, use of not complex devices and its use in micro and macro scale among various techniques. In the paper, in order to pre-concentrate and measure the Cu quantities in aqueous environments, new, quick and very high sensitive technique of dispersed liquid-liquid microextraction (DLLME) coupled with the flame atomic absorption spectrometry (AA-DLLME) has been utilized. The proposed technique in the paper is economically very effective because of the amount of used solvent and low cost of the solvent [13-15].

## **Experimental**

### *Material and apparatuses*

The 1000 ppm Cu solution (stock solution) was prepared through dissolving 0.3804 g of  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  in distilled water. In order to prepare copper solutions with lower concentrations than stock solution, the successive dilution method was used. Undecanol, 1-dodecanol, n-octanol and toluene was used as extracting solvents and methanol, ethanol, acetone and acetonitrile were used for selecting dispersing solvent. Also in order to form Cu complex, complexing agent of sodium diethyldithiocarbamate (DDTC) (Figure 1) had been used and salts of metal nitrate such as Nickel, Mercury, Cobalt, Bismuth, Lead, Cadmium, Iron, Zinc and Palladium had been utilized for studying the interference effect. All the chemicals used in the paper were purchased from Merck Germany ltd with high analytical purity grade.



**Figure 1.** Sodium diethyldithiocarbamate.

The glass tubes with diameter of 4, 8 and 12 mm and various lengths were used which is connected to air pump with adjustable output air pressure equipped to terminal. In order to inject the solvent and the distilled water to the column, insulin syringe was used to remove organic phase extracted from 100  $\mu\text{l}$  Hamilton syringe (due to need to very high precision) and rubber septum GC was utilized to close the glass column end. Following the pre-extraction and pre-concentration, measuring the Cu ions was performed by flame atomic absorption spectrophotometer (Shimadzu AA680, model) equipped to double-beam background correction system, air-acetylene flame with flow rate of  $1.8 \text{ min}^{-1}$  and Copper (Cu) Lumina Hollow Cathode Lamp (Hamamatsu, photonic Co. ltd L233-series) at a wavelength of 228.8 nm. pH of the solutions was measured by pH meter (Lab-827, model) equipped to combined glass electrode. Also ultrasonic bath (VGT-1730QTD, model) was used for homogenizing the extraction and diluting solvent after extraction.

#### *Milk powder preparation*

0.5 g milk powder and 2 ml acetic acid solution and concentrated nitric acid with ratio of 4:10 were mixed together and then yellow solution was yielded. Then it was placed under the mild temperature and each time the green solution was seen, 2 to 3 drops of acid was added to it. It has done until approximately solution was dried; finally the dried solution and yellow powder remained in the bottom of the container. Then obtained solid was placed at room temperature to cool. Then, 2

ml concentrated nitric acid and 40 ml distilled water was added to it and pH was adjusted to 2. The resulting solution is passed through the filter and then was kept in the polyethylene containers.

#### *The liquid milk preparation*

5 ml milk was moderately heated and 20 ml nitric acid was added drop by drop to it. After stopping heating, milk temperature was reached to the room temperature and finally 5 ml hydrogen peroxide (30%) was added to it. The solution was heated until its volume was approximately reached to 0.5 ml. Having it cooled, 40 ml distilled water were added to it and after adjusting pH to 2, its volume was brought to 100 ml by distilled water.

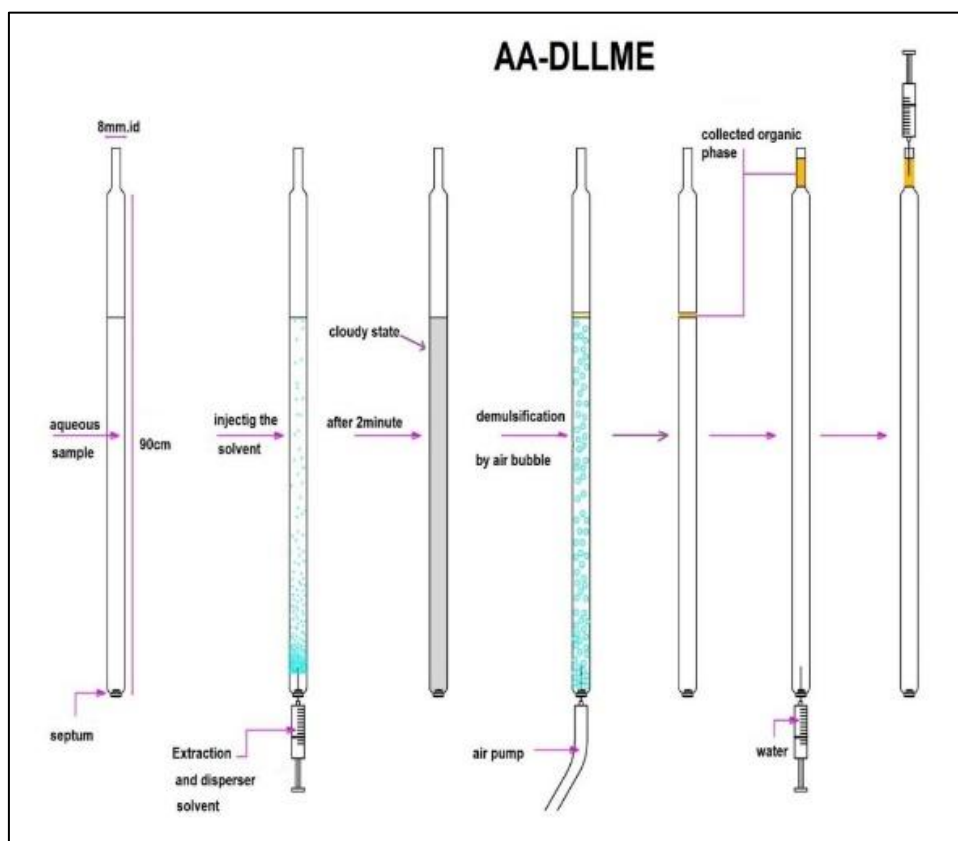
#### *Orange juice preparation*

100 ml orange juice was heated to dry and having it dried, was placed on the fire to fully burn the sample. Then, it was placed inside the furnace for 4 hours at 450 °C. Finally, 30 ml distilled water was added to the obtained while ash and after adjusting pH to 2, its volume was brought to 100 ml by distilled water.

#### *Cu extraction using AA-DLLME technique*

20 ml Cu standard solution and 1 ml DDTC ligand 0.05 M were added to the sample solutions. Then, 3 ml buffer with pH=2 was added to the yellow solution and finally its volume was brought to 25 ml using pure distilled water. The glass extraction column end of which is was blocked with a rubber septum, become joint on the base by a clamp. Because of special physic of extraction column, the solution should pour from top into it by 10 ml syringe. When the solution was positioned in the column, 1000 µl solvent (including 900 µl methanol and 100 µl toluene) was progressively injected from bottom by insulin syringe through septum. As soon as the beginning of the injection of solvent, the cloudy form was formed from the bottom of the solution and was slowly risen and whole solution gradually become cloudy. This lead to increase contact surface of toluene with aqueous solution and finally it leads to extract the Cu complex within itself. In order to separate organic phase from aqueous phase and to accumulate it upon the column, blowing small air bubbles was utilized into the solution. Blowing bubble was carried out by pump and through very narrow needle connected to the septum. Time for bubble formation was considered as 18 min. Bubbles cause to clear the aqueous solution and collect the yellow organic phase which contains species of Cu extracted complex. Then the distilled water was injected into the tube using 2 ml syringe through septum which cause to rise the organic phase from glass column and to gather it one narrow section of the tube. The organic phase was carefully removed by Hamilton syringe. It is

necessary to note that the organic phase amount was removed, was 80  $\mu\text{l}$  in all experiments. It is noteworthy that when separating the organic phase, the aqueous phase should never enter into the syringe. The organic phase was poured into the vial and in order to have capability of injection to atomic absorption machine; it was diluted by 100  $\mu\text{l}$  ethanol. Then vial contents were injected to the atomic absorption machine for measuring. The general scheme of liquid-liquid micro-extraction using air bubbles has been illustrated in Figure 2.



**Figure 2.** Schematic of used technique in the paper.

## Results and Discussion

### *Pareto chart*

A chart which could be helpful for evaluating the factors and interactions is Pareto chart where the t-test was used to determine whether the effects are significant or not? According to Figure 3, in this chart, those elements which are positioned at right side of vertical line (the critical value t), at confidential interval of 95% are significant.

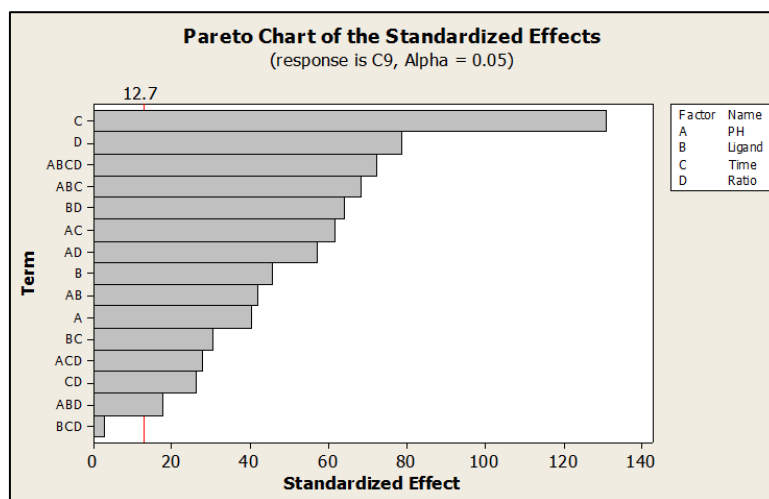


Figure 3. Pareto chart of the standardized effects.

### Box-Behnken experimental design

The relative significance of various variables and the possible interaction among them were studied using Box-Behnken experimental design method. In experimental design, through this method, total number of experiments (N) is equal to:

$$N = 2k(k-1) + C \quad (1)$$

Where k is the number of main factors and C is the number of central point's repetitions. In the method, four parameters were selected for optimization and two levels were considered for each which are carried out in one block with three repetitions of central point. For this, considering to results of previous experiments, these levels has been considered for each parameter. In design method of response level, kind of used scheme for optimization was Box-Behnken which is performed by Minitab 16 software. These parameters included the pH effect, ligand concentration, percent of extracting solvent, extraction time which had greatest effect on the extraction. Table 1 shows parameters and change levels considered for each of them. It should be noted that the number of experiments for Cu is 27. Order of experiments run was randomly selected. The presence of systematic error can lead to misinterpret the data. Two effective ways to avoid systematic error are to block and to randomly run experiments.

**Table 1.** Box-Behnken design for Cu determination.

Factor	High level (+)	Low level (-)
pH	6	1
Ligand concentration (M)	0.05	0.0005
Extraction time (min)	20	4
Percent of extracting solvent	20	5

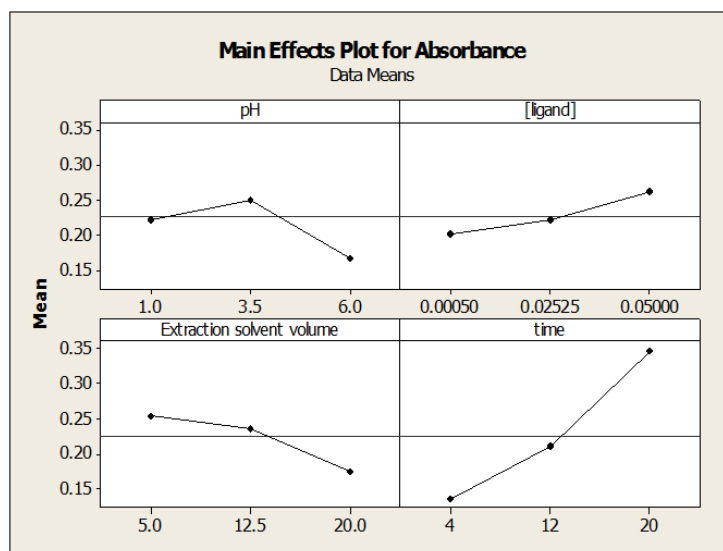
Therefore Box-Behnken design was carried out through Minitab software. The results have been reported in the Table 2.

**Table 2.** The results related to the Box-Behnken design for measuring Cu.

Number	pH	Ligand concentration (M)	Extraction time (min)	Solvent extraction (ml)	Absorbance
1	6	0.0005	12	12.5	0.181
2	3.5	0.02525	4	20	0.050
3	6	0.02525	12	5	0.115
4	1	0.02525	12	20	0.117
5	6	0.02525	4	12.5	0.095
6	3.5	0.00050	4	12.5	0.127
7	3.5	0.02525	20	20	0.236
8	1	0.05000	12	12.5	0.226
9	3.5	0.02525	12	12.5	0.268
10	3.5	0.02525	12	12.5	0.257
11	1	0.02525	4	12.5	0.202
12	3.5	0.05000	12	20	0.270
13	1	0.00050	12	12.5	0.194
14	3.5	0.02525	12	12.5	0.274
15	1	0.02525	20	12.5	0.358
16	6	0.02525	20	12.5	0.286
17	3.5	0.00050	12	5	0.312
18	3.5	0.05000	20	12.5	0.410
19	1	0.02525	12	5	0.227
20	3.5	0.02525	4	5	0.181
21	3.5	0.00050	20	12.5	0.193
22	3.5	0.05000	12	5	0.215
23	6	0.02525	20	5	0.472
24	6	0.02525	12	20	0.173
25	3.5	0.05000	4	12.5	0.166
26	3.5	0.00050	12	20	0.198
27	6	0.05000	12	12.5	0.153

*Diagram of the main effects*

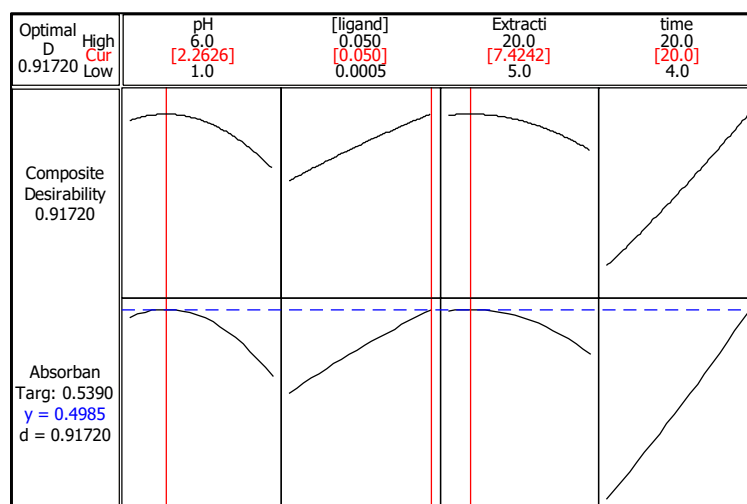
According to the Figure 4, firstly pH has an increasing effect to a certain point and then has a decreasing effect. Responses of ligand concentration increase with a mild slope as the concentration increases. As the time of extraction increases, the response increase but as expected, when percentage of the extracting solvent rather dispersing solvent increases, response decrease.



**Figure 4.** Diagram of the main effect for measuring Cu.

*The optimal points chart*

Optimal point for the four studied factors through Minitab program has been shown in Figure 5. It is seen that pH and percentage of optimal extracting solvent from multivariate calibration is approximately similar to one-variable optimization. So high levels of extraction time and ligand concentration were considered, were obtained as optimal points from multi-variable calibration.



**Figure 5.** The chart of optimal points for measuring Cu using Box-Behnken design.



Mathematical equation of which these charts are deduced is as Table 3:

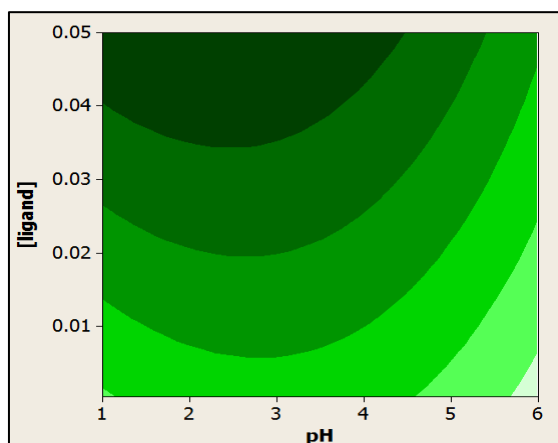
**Table 3.**Mathematical equation and its term and coefficients.

Term	Coef
Constant	0.201799
pH	0.0322645
Ligand	-4.89161
Ve	0.000888788
Time	0.00361285
pH×pH	-0.0100133
Ligand×Ligand	-10.7472
Ve×Ve	-5.81481E-04
Time×Time	0.000152995
pH×Ligand	-0.242424
pH×Ve	0.00224000
pH×Time	0.000437500
Ligand×Ve	0.227609
Ligand×Time	0.388889
Ve×Time	-4.37500E-04

$$A = 0.201799 + 0.0322645 \text{ pH} - 4.89161 [\text{L}] + 0.000888788 [\text{V}_e] + 0.00361285 t - 0.0100133 \text{ pH}^2 - 10.7472 [\text{L}]^2 - 5.81481 (10^{-4}) [\text{V}_e]^2 + 0.00015299 t^2 - 0.242424 \text{ pH} [\text{L}] + 0.00224000 \text{ pH} \text{ V}_e + 0.000437500 \text{ pH} t + 0.227609 [\text{L}] \text{ V}_e + 0.388889 [\text{L}] t - 4.37500(10^{-4})\text{V}_e t + \dots$$

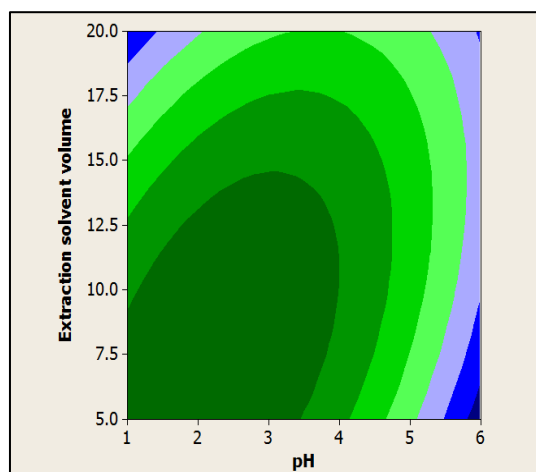
### Boundary charts

In Figure 6 through 11 which are the boundary charts, changes of two parameters on the absorption has been shown while third and fourth parameter were kept constant. In order to extract Cu, according to the following charts, the effect of factors could be found together and the effect of factors on the absorption and finally on the extraction of Cu could clearly be seen.



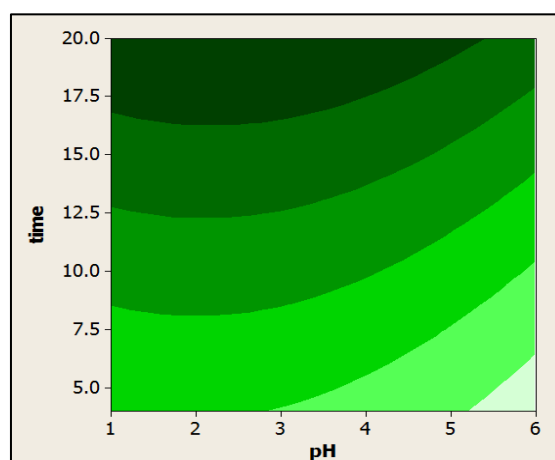
**Figure 6.** The boundary chart of  $\text{Cu}^{2+}$  absorption by pH and ligand concentration parameters.

The parameters of volume extracting percent and extraction time were kept optimal and constant.



**Figure 7.** The boundary chart of  $\text{Cu}^{2+}$  absorption by pH and volume extracting percent parameters.

The parameters of ligand concentration and extraction time were kept optimal and constant.



**Figure 8.** The boundary chart of  $\text{Cu}^{2+}$  absorption by pH and extraction time parameters.

The parameters of volume extracting percent and ligand concentration were kept optimal and constant.

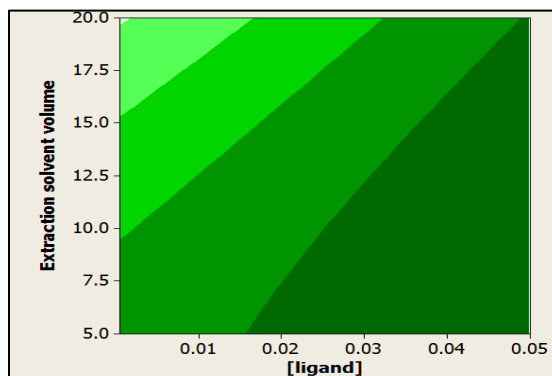


Figure 9. The boundary chart of  $\text{Cu}^{2+}$  absorption ligand concentration and volume extracting percent parameters.

The parameters of pH and extraction time were kept optimal and constant.

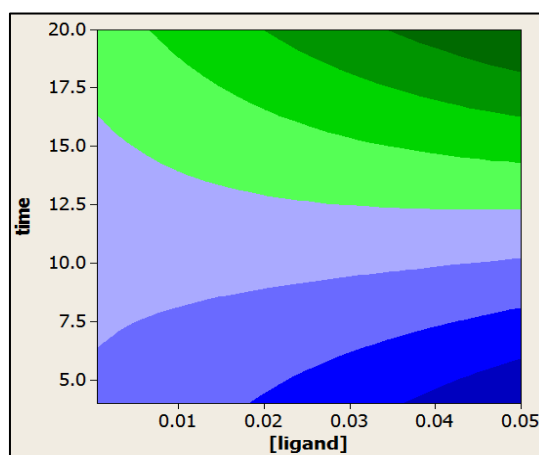


Figure 10. The boundary chart of  $\text{Cu}^{2+}$  absorption by ligand concentration and extraction time parameters.

The parameters of volume extracting percent and pH were kept optimal and constant.

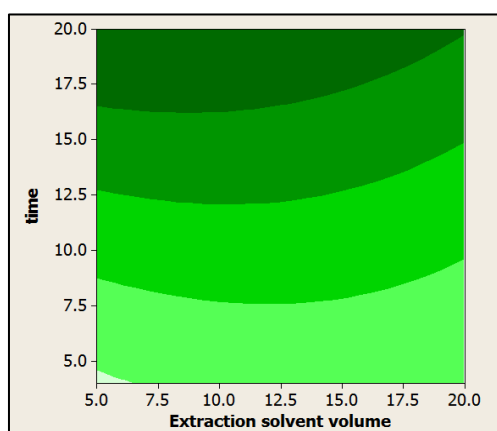


Figure 11. The boundary chart of  $\text{Cu}^{2+}$  absorption by volume extracting percent and extraction time parameters. The parameters of pH and ligand concentration were kept optimal and constant.

*Studying the interference effect*

The effect of common ions existed in the aqueous samples on the Cu extraction were studied using AA-DLLME at optimal condition which has obtained from Box-Behnken method. The interfering ions could compete with Cu ions from view of reaction with ligand DDTC and as result could be effective on the extraction of Cu<sup>2+</sup> ion. In order to study the interference effect, Hg<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Bi<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, Fe<sup>3+</sup>, Zn<sup>2+</sup> and Pd<sup>2+</sup> ions with certain concentration were prepared and were added to the Cu standard solution (100 µg/l) and then extraction was carried out under the optimal condition. The results of this section are found in Table 4.

**Table 4.** Study the interfering ions in Cu measuring.

Ions	[Ions]/[Cu <sup>2+</sup> ]	Extraction recovery (%)
Ni <sup>2+</sup>	400	98.37
Co <sup>2+</sup>	400	108.11
Bi <sup>3+</sup>	400	99.76
Pb <sup>2+</sup>	400	100.1
Cd <sup>2+</sup>	400	96.98
Fe <sup>3+</sup>	400	99.04
Pd <sup>2+</sup>	400	98.75
Zn <sup>2+</sup>	400	79.87
Zn <sup>2+</sup>	200	96.42
Hg <sup>2+</sup>	400	72.07
Hg <sup>2+</sup>	200	82.39
Hg <sup>2+</sup>	20	97.13

*Figures of merit*

Limit of detection (LOD) of one method is concentration of sample machine response of which is significantly different from blank sample response and is defined as the following:

$$DL=3S_b/m \quad (2)$$

Detection limits (DL), Blank standard deviation (S<sub>b</sub>) and slope of calibration line (m) after extraction has been reported in Table 5. In order to determine detection limit of the procedure, 10 blank solutions were prepared and have been extracted according to the proposed method. Then absorption signal of extracted solution was determined by flame atomic absorption spectrometry so their results have been shown in Table 5.

**Table 5.** Analytical parameters of Cu measurement by AA-DLLME.

Parameter	value
RDS %	3.64
Increasing factor	220.8
Concentration factor	140
LOL ( $\mu\text{g.l}^{-1}$ )	20-1000
R <sup>2</sup>	0.9931
LOD ( $\mu\text{g.l}^{-1}$ )	1.2

### *Cu measurement in actual samples*

The actual water sample including spring water, well water, river water, well water, milk powder, low-fat liquid milk and orange juice (this sample 30 min before experiment and the sample was prepared from three 200 g oranges) were tested to measure Cu using the proposed method, so their results have been illustrated in Tables 6 and 7.

**Table 6.** Cu measurement in aqueous samples.

Sample	Cu <sup>2+</sup> added ( $\mu\text{g.l}^{-1}$ )	Cu <sup>2+</sup> found ( $\mu\text{g.l}^{-1}$ )	Extraction (%)
	0	4	-
Urban water	40	42.6	96.8
	80	84.5	100.5
	0	0	-
River water	40	39.3	98.2
	80	78.1	97.6
	0	23	-
Spring water	40	58.6	93.1
	80	107	103.8
	0	6	-
Well water	40	44.5	96.7
	80	82.1	95.4

**Table 7.** Cu measurement in food samples.

Sample	Cu <sup>2+</sup> added (µg.l <sup>-1</sup> )	Absorption
	0	0.411
Milk powder	40	0.457
	80	0.514
	0	0.303
The liquid milk	40	0.337
	80	0.393
	0	0.444
Orange juice	40	0.536
	80	0.603

## Conclusion

In the paper, DLLME technique using aeration was proposed as new method for extracting and concentrating and measuring Cu<sup>2+</sup> in aqueous environments and environments with complex textures such as liquid milk, milk powder and orange juice. Simplicity, high speed, low cost and being environmentally friendly could be pointed out as typical advantages of this method. The results indicated that enrichment factor; extraction efficiency, standard deviation and detection limit of this technique is higher or at an acceptable level than other measurement methods. This technique could measure the low amounts of Cu in the aqueous samples, milk powder, liquid milk and orange juice with very good accuracy.

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