

# Orthogonal Array Design for the Optimization of Solvent-Assisted Dispersive Solid Phase Extraction for the Determination of Copper Ions in Water and Vegetable Food Samples using Flame Atomic Absorption Spectrometry

Davood Kaviani, Majid Saghi, Maryam Sabonian, Mohammad Hosein Bigtan, Behzad Padidarani

**Abstract**--A simple and efficient dispersive solid -phase micro extraction technique was developed and combined with flame atomic absorption spectrometry, for the extraction and determination of trace amounts of copper in real samples. 2-(5-Bromo-2-pyridylazol)-5-(diethyl amino)-phenol (5-Br-PADAP) was used as chelating agent and benzophenone was selected as extraction solvent. Several possible influential factors such as the type and amount of extraction solvent, amount of chelating agent, sample pH, were optimized using orthogonal array design (OAD) with  $OA_{16}(4)^5$  matrix. Under optimum conditions, an enrichment factor of 13 was obtained. The analytical curves were linear between 5-2000  $\mu\text{g L}^{-1}$ . Based on three SD of the blank, the detection limits was 1.2  $\mu\text{g L}^{-1}$ . The relative SDs for eight replicate measurements of 100  $\mu\text{g L}^{-1}$  of metal ions was 3.2%. The proposed method was successfully applied for determination of copper in environmental waters and some vegetable samples including Pepper, Tomato, Thyme, Aloe vera gel, Morus.

**Keywords:** Solvent-assisted dispersive solid phase extraction, Copper (II), Flame atomic absorption spectrometry, Food and environmental water samples

## I. INTRODUCTION

Sample preparation is one of the most important and crucial steps in the whole analytical process, especially when trace determination is the purpose [1,2]. The main aims of this step are: (a) to transfer the analytes to a physical state that enables their analysis and final detection; (b) to enrich the analytes of interest; and (c) to purify the extracts prior to instrumental determination. Several procedures have been developed for these purposes such as liquid-liquid extraction (LLE) [3–5], co-precipitation [6–8], and solid phase extraction (SPE) [9–17]. However, some of these pretreatment methods need large amounts of sample and/or organic solvents, are time consuming and expensive, and have unsatisfactory enrichment factors. These problems could be addressed by the development of modular and compact processes that provide adequate separation and preconcentration without complex processes.

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SPE is more flexible and efficient than LLE in terms of Simplicity, low consumption of the organic solvents, flexibility in choosing the adsorbent and high enrichment factor. Recently, SPE has been increasingly used for the preconcentration and separation of ultra-trace amounts of inorganic and organic species from complex matrices [18, 19]. Solid phase extraction techniques are surface-dependent processes since their efficiency directly depends on the particle size and the surface area of the sorbent [20]. Dispersive solid phase extraction (DSPE) has risen as an alternative to conventional solid phase extraction. It was, for the first time, proposed by Anastassiades et al. [21] and it can be considered as a quick, easy, cheap, effective, rugged, and safe (Qu ECh ERS) sample treatment method. It is based on the SPE methodology, but the sorbent is added to the extract without conditioning, in small amounts (viz. 50 mg) and the dispersion is carried out assisted by an external energy (usually a vortex stirring). The phases are easily separated just by centrifugation. The phases are easily separated just by centrifugation therefore to an improvement in the extraction kinetics. Moreover, this enhancement allows the use of a smaller amount of sorbent compared to the conventional extraction approaches resulting in the saving of material. In order to obtain analytical information the sorbent is then recovered after the extraction. The analytes can be directly monitored on the sorbent surface by using a spectroscopic technique [22,23] or can be conveniently eluted/desorbed. for the subsequent analysis of the eluted fraction [24–27]. In the present report, for the first time, a novel extraction technique as a high performance and powerful preconcentration method named solvent-assisted dispersive solid phase extraction (SADSPE) is demonstrated. In this method, the appropriate mixture of sorbent and disperser solvent was rapidly injected into the mixture of sorbent and disperser solvent was rapidly injected into the aqueous sample by syringe. Thereby, a cloudy solution formed. The cloudy state resulted from the dispersion of the fine particles of the sorbent in the bulk aqueous sample. Then, this cloudy solution was centrifuged, following which the fine particles sediment at the bottom of the conical test tube. The determination of analytes in the sedimented phase was performed by instrumental analysis. In this extraction method, any component in the solution, directly or indirectly after previous derivatization reactions, interacts with the fine particles of the sorbent, and consequently, gets extracted from the initial solution.

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Simplicity of the operation, rapidity, low sample volume, low cost, high recovery, and high enrichment factor are some advantages of SADSPE. For evaluating the performance of SADSPE, copper was selected as a test analyte and determined in food and water samples using flame atomic absorption spectrometry detection. The effects of various experimental parameters on the extraction were investigated.

## II. MATERIAL AND METHODS

### a. Apparatuses

Following the pre-extraction and pre-concentration, measuring the Cu ions was performed by flame atomic absorption spectrophotometer (Shimadso 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 (Hamwatsu, photonic Co. Ltd L233-series) at a wavelength of 228.8 nm. pH of the Solutions was measured by pH meter (Lab-827) equipped to combined glass electrode. Also Ultrasonic bath (VGT-1730QTD model) was used for homogenizing the extraction and diluting solvent after extraction and centrifuge machine was utilized for centrifuging the samples which highest speed of this machine is 4000 rpm.

### b. Reagents and solutions

Stock solution with concentration of 1000 ppm was prepared through solving 0.3804 gr  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  (purchased from Merck company) in the distilled water. In order to prepare the Cu solutions with lower concentration, serial dilution was utilized. such that in each step of dilution, the solution was diluted ten times. In order to establish stability, 0.5 and 1 ml chloridric acid 1M were added to the stock solution and the diluted solutions respectively. All the used solutions were prepared from ethanol for calibrating flame atomic absorption spectrophotometer. Benzophenon, dibenzyl sulfoxide and naphthalene solvents were utilized for selecting the extraction solvent and methanol, ethanol, acetone and acetonitrile solvents were utilized for dilution solvent all of which are of analytic pure grade and purchased from Merck and Fluka companies.

The Complexing agent 2-(5Bromo-2-pyridylazol)-5-(diethylamino)-phenol (hereafter, 5-Br-PADAP) was used for forming Cu-complex. In the paper Chloridric acid, Phosphoric acid and acetic acid (for buffer preparation), nitric acid and ammonia and Sodium hydroxide bases were used in different stages. The buffer solutions of 5 to 15 were made for pH adjustment. All glass devices were soaked in 10% nitric acid solution for 12 hours before experiment and then washed by the pure distilled water. The metal nitrate salts were utilized to study the effect of disturbances and one solution having various concentrations was made from them and have been used.

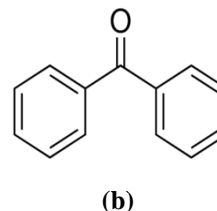
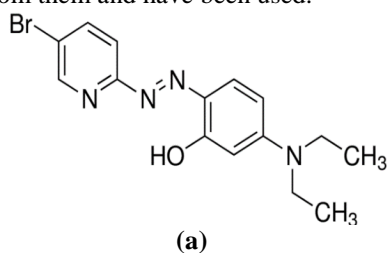


Fig.1.2-(5Bromo-2-pyridylazol)-5-(diethylamino)-phenol (a) and Benzophenon (b)

## III. EXPERIMENTAL SETUP

### a. Actual sample preparation

The plant samples were purchased from local shop and after washing by the pure distilled water and slicing them, were separately kept in an oven at  $100 \text{ }^\circ\text{C}$  for 24 hours and finally were grinded by kitchen mixer. Then 500mg sample was picked up and 100ml pure nitric acid was added to it. The mixture was kept under  $100 \text{ }^\circ\text{C}$  condition for 4 hours until complete drying. Having completely dried, temperature raised to  $25 \text{ }^\circ\text{C}$  and then 3 ml Hydrogen peroxide was added to it. After adding hydrogen peroxide, the mixture was kept under  $130 \text{ }^\circ\text{C}$  condition for one hour. This method was used in order to study all used plant samples. Water samples were filtered through filter paper with mesh size of  $0.45 \text{ }\mu\text{m}$  before using and after acidifying them as 1% by nitric acid, were kept in dark polyethylene bottles.

### b. The solid phase dispersion method of extraction through solvent

2 ml Cu solution (1 ppm), 1 ml optimal buffer solution (PH=8), 0.4 ml ligand 5-BR-PADAP (0.001 M) and 1 ml Tirtion X-100 (0.2 M) were poured into the conical test tube with 12 ml volume and then have been reached to 10 ml volume by distilled water. Then 1 ml Benzophenone 1.5% (methanol solution) were intensively injected into the mixture until it become cloud-shaped. This cloud-shape state is sustainable for long term. Then the test tube was placed into the centrifuge machine for 3 hours at a speed of 4000 rpm to be accumulated as solid sediments at the bottom of the container. The upper solution was discarded through inverting the test tube until no liquid remains inside the tube. Then 45 ml methanol was added to the sediment such that the sediment is fully solved. The resulting solution was injected into the flame atomic absorption spectrometry (FAAS).

Figure 1 shows the procedure.



Fig2. Photography of different steps in SADSPE

IV. RESULT AND DISCUSSION

Various factors contribute to extraction of the diffusion solid phase using solvent. In order to obtain low detection limit and high concentration factor, these vital factors were optimized by experimental design software.

a. Selection of sorbent

Careful attention should be paid in the selection of the sorbent. It should have extraction capability of the concerning compounds, high solubility in disperser solvent and low solubility in water benzophenone, dibenzyl sulfoxide and naphthalene were compared in the extraction of Copper (II). A series of sample solutions were studied using 1 mL methanol containing 1.5% of the sorbent. As Fig 3.

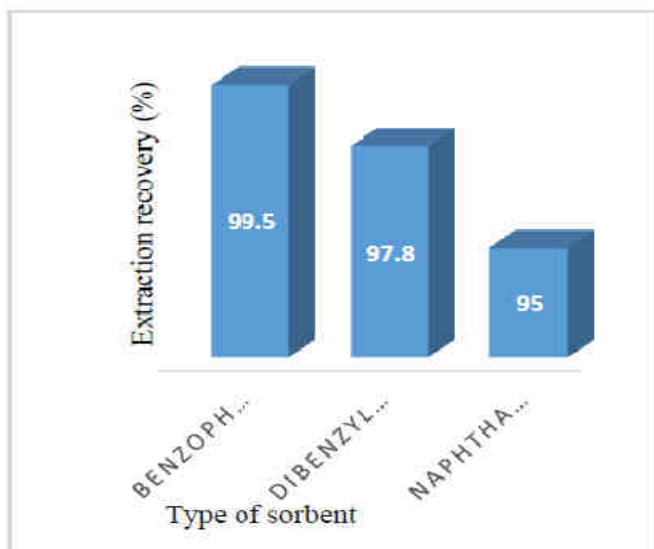


Fig 3. Effect of type of sorbent on the extraction recovery of copper

b. performing experimental design

Orthogonal array design is according to  $L_N(X^Y)$  so in this relation, L devotes to design OAD, N the number of experiments, X the number of factor area and Y is maximum number of factors. In this experimental design, the factors include pH, ligand concentration, surfactant quantity, benzophenone quantity and centrifuging time. The number of experiments is 16 in this procedure. The above factors were entered to the software along with low and high levels and the experimental design was carried out. Table 1 shows level of each parameter and Table 2 shows the average results of three time run obtained of 16 experiments designed by OAD.

Table 1 : level of each parameter

Factors	Level1	Level2	Level3	Level4
pH	4	6	7	8
Ligand	0.000008	0.00001	0.00002	0.00004
Surfactant	0.025%	0.05%	0.1%	0.2%
Benzophenon	0.5%	1%	1.5%	2%
Time	1	3	7	10

Table 2: The results of 16 tests of OAD

M-16	PH	Legend (mol.L <sup>-1</sup> )	Surfactant (%)	Benzophenon (%)	Time (Min)	Absorbance (Average)
1	4	$8 \times 10^{-6}$	0.025%	0.5%	1	0.006
2	4	$10^{-5}$	0.05%	1%	3	0.006
3	4	$2 \times 10^{-5}$	0.1%	1.5%	7	0.023
4	4	$4 \times 10^{-5}$	0.2%	2%	10	0.033
5	6	$8 \times 10^{-6}$	0.05%	1.5%	10	0.070
6	6	$10^{-5}$	0.025%	2%	7	0.060
7	6	$2 \times 10^{-5}$	0.2%	0.5%	3	0.039
8	6	$4 \times 10^{-5}$	0.1%	1%	1	0.103
9	7	$8 \times 10^{-6}$	0.1%	2%	3	0.10
10	7	$10^{-5}$	0.2%	1.5%	1	0.10
11	7	$2 \times 10^{-5}$	0.025%	1%	10	0.090
12	7	$4 \times 10^{-5}$	0.05%	0.5%	7	0.182
13	8	$8 \times 10^{-6}$	0.2%	1%	7	0.104
14	8	$10^{-5}$	0.1%	0.5%	10	0.099
15	8	$2 \times 10^{-5}$	0.05%	2%	1	0.190
16	8	$4 \times 10^{-5}$	0.025%	1.5%	3	0.240

c. The analysis of variance

Table 3 shows the ANOVA related to results obtained in this experiment. In this table, P is the contribution of each factor to perform experiment. The effect of factors has the following order:

pH > Ligand concentration > Surfactant quantity > Benzophenone > Centrifuging time

$SS_i$  is the error square sum of each factor and  $SS_t$  is the total error square sum. F or Fisher coefficient is calculated as dividing variance of each factor ( $V_A$ ) on the error variance ( $V_e$ ) and is used for being significant of factors effect. Since F of ANOVA table (2.20) is greater than F obtained from Fisher table at a 95% confidential level, it is concluded that factors effect is significant at a corresponding confidential level and factors are significant in this confidential level. The optimized condition resulted in this procedure is as following:

pH and ligand concentration in level 4, surfactant quantity in level 2, benzophenone quantity in level 3, centrifuging time in level 1 and confidential interval in level 95% ,  $0.239 \pm 0.013$ . Because of confidential interval obtained, real value should be inside 0.226 to 0.252 range. The obtained result of this experiment in optimal condition equal to 0.240 which shows validity of this procedure.

d. The effect of disturbing ions

The effect of common ions exist in aqueous samples was studied on the Cu extraction (using dispersion solid phase method and at obtained optimal condition) by Taguchi method. Because of competition of disturbing ions in interaction with ligand 5-Br-PADAP, these ions could be effective on the  $Cu^{2+}$  ion extraction. In order to study the effect of disturbing agents,  $Ag^+$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cd^{2+}$ ,  $Fe^{3+}$ ,  $Zn^{2+}$  ionic solutions were made with the specified concentrations

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and then added to the standard Cu solution (100 µg/l). Under the above optimal condition, the extraction was carried out. In studying the disturbing agents, an ion is considered as disturbing which cause to change more than 5 % in cu absorption from sample. The results indicated that greatest disturbance is found at Ni<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup> ions because these ions tend to form complex with ligand 5-Br-PADAP. Table 3 shows effect of the disturbing ions.

**Table 3: effect of the disturbing ions**

Ion	Ions/Cu <sup>2+</sup>	Recovery
Na <sup>+</sup>	10000	98.9
Ca <sup>2+</sup>	10000	98.3
Ag <sup>+</sup>	200	97.6
Cd <sup>2+</sup>	150	96.9
Pd <sup>2+</sup>	200	97.4
Ni <sup>2+</sup>	50	96.2
Zn <sup>2+</sup>	50	96.0
Fe <sup>2+</sup>	100	97.5
Co <sup>2+</sup>	50	96.8
Cl <sup>-</sup>	10000	98.3
SO <sub>4</sub> <sup>2-</sup>	1000	98.6
NO <sub>3</sub> <sup>-</sup>	10000	99.1

## e. Natural water and foods analysis

The actual samples of water including sea water, well water, river water, drinking water and food samples including Bell peppers, strawberries, thyme, Aloe Vera gel and tomatoes were studied for measurement copper using proposed method. The results of the tests are shown in Tables 4 and 5.

**Tables 4: Determination of Copper (II) in Vegetable Food Samples**

Sample	Found (µg L <sup>-1</sup> )	Recovery (%)
Tomato	55	95
Pepper	50	94
Aloe Vera gel	122	102
Thyme	129	103
Morus	80	100

**Tables 5: Determination of Copper (II) in Water**

Sample	Found (µg L <sup>-1</sup> )	Recovery (%)
Well Water (Arak well water, markazi, Iran)	30	99
River Water (Dorud river, Lorestan, Iran)	54	102.0
Sea Water		100.0
Persian Gulf Water, (Iran)	40	
Municipal piping water (Nahavand City water, Hamedan, Iran)	40	99.5
Mineral water gahar mineral water, (Iran)	18	97.9

## f. Figures of merit

Detection limit of one method is concentration of sample machine response of which is significantly different from blank sample response and is defined as the following:

$$DL=3S_b/m$$

Detection limits (DL), Blank standard deviation (S<sub>b</sub>) and slope of calibration line (m) after extraction have been reported in table 6 .

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 6.

**Table 6: Analytical characteristics of the proposed method**

Parameter	Analytical feature
Linear range, µg L <sup>-1</sup>	5-2000
RSD, %(C¼ 50.0 µg L <sup>-1</sup> , n¼10)	3.2
Limit of detection, µg L <sup>-1</sup> (n¼10)	1.2
enrichment factor	13

## g. Comparison with other methods

A comparison of the presented method with other reported preconcentration methods for copper determination is given in Table 7. The presented method has low LOD, high-enrichment factor, and good RSD and these characteristics are comparable or even better than most of the other methods named in

**Table 7: Comparison of different separating and analytical techniques with present methods for the determination of copper.**

Separating /analytical techniques	Linear range (ng/mL/g)	LOD (ng/m L/g)	RSD (%)	EF	Samples	References
SDME/spectrophotometry	5-100	0.15	3.4	33	Food-Water	Wen, Deng, and Guo (2011)
LLE/spectrophotometry	10-400	2.0-4.0	2.0	5	Water-soil	Shrivastava (2010)
DLLME/FAAS	1-600	0.5	1.4	-	-	Mohammadi, Afzali, and Baghelani (2009)
DLLME/FAAS	50-200	3.0	5.1	42	Water	Farajzadeh, Bahram, Mehr, and Jossion (2008)
Coprecipitation/FAAS	-	1.32	2.5	20	Water	Tokahoglu and Yildiz (2009)
SPE/FAAS	10-340	1.9	2.1	33	Food	Ghaedi, Niknam, Taheri, Hossainian, and Soyak (2010)
DLLME/FAAS	0.2-20	0.05	1.5-3.5	55	Cereals Vegetables	Present method

## V. CONCLUSIONS

The analytical potential of solvent-assisted dispersive solid phase extraction is presented and critically evaluated in this article. The SADSPE allows the rapid extraction of copper ions, which was selected as model analyte, using a low amount of sorbent material. This method is environment friendly and robust against very high contents of salt. The other benefits of the method are its simplicity, ease of operation, good accuracy and precision, short extraction time, low cost, and a good enrichment factor. Although the obtained results in this work are related to copper determination, the system could be readily applied for the determination of other metals using various ligands. Future work will be directed toward extending the method for the separation and preconcentration of different inorganic and organic species.

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