

## Determination of Zinc Ions in Environmental Samples by Dispersive Liquid-Liquid Micro Extraction and Atomic Absorption Spectroscopy

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**Abstract:** In this work preconcentration of the Zn ions was investigated in water sample by Dispersive liquid-liquid micro extraction (DLLME) using chloroform as an extraction solvent, methanol as a disperser solvent and 8-Hydroxyquinoline as a chelating agent. The determination of extracted ions was done by graphite furnace atomic absorption spectrometry. The influence of various analytical parameters including pH, extraction and disperser solvent type and volume and concentration of the chelating agent on the extraction efficiency of analyses was investigated. After extraction, the enrichment factor was 26 and the detection limit of the method was  $0.0033 \mu\text{g l}^{-1}$  and the relative standard deviations (R.S.D) for five determinations of 1 ng/ml Zn were 7.41%.

**Keywords:** Dispersive liquid-liquid micro extraction (DLLME), Graphite furnace atomic absorption spectrometry, Zinc, water samples.

### INTRODUCTION

Trace heavy metals are essential micro-nutrients and have a variety of biochemical functions in all living organisms [1–3]. Zinc is a component of hundreds of proteins involved in intermediary metabolism, hormone secretion pathway, and immune defense, and Zn enzymes participate in the synthesis and decomposition of proteins, fats, carbohydrates, and nucleic acids [4, 5]. It is also mobile and easily taken up by plants in the environment [6]. Deficiency of zinc leads to several disorders such as growth retardation, diarrhea, the decrease of the immunological defense, eye and skin lesions, malfunctioning of wound healing and other skin diseases [7]. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity [8]. Zinc deficiency in the human diet has been found to retard growth and maturity and to produce anemia. On the other hand, zinc is a human-made environmental pollutant. The concentration of zinc in unpolluted natural water is low, and the sensitivity of analytical techniques is often insufficient for its determination [9]. Thus, the development of simple and inexpensive test methods that do not require considerable sample preparation, sophisticated laboratory equipment, and highly skilled personnel for the rapid determination of these metals in water is of interest [10]. Traditional solvent extraction has been used as a basic and powerful method of concentrating for a long time. However, it requires extensive amounts of organic solvents. A special attention is nowadays focused on techniques, which are characterized by a considerable reduction or complete elimination of organic solvents. The solvent-free techniques such as gas phase extraction, membrane extraction and solid phase extraction are limited to volatile and relatively volatile organic compounds, volatile non polar compounds and relatively low volatile compounds [11].

Despite good developments in the modern analytical instruments, which allow great enhancement in aspects of analysis, in many cases the available analytical instrumentation does not have enough sensitivity for the analysis of natural samples. Sample preparation is still a bottleneck for overall throughput because the involved steps often employ large volumes of hazardous organic solvents [12]. Although, the determination of trace metal ions in natural waters is difficult due to various factors, particularly their low concentrations and matrices effects. Pre-concentration and separation can solve these problems and can lead to a higher confidence level and easy determination of the trace elements. Several methods have been reported for the separation and preconcentration of metal ions, such as coprecipitation [13], liquid-liquid extraction (LLE) [14], solid-phase extraction (SPE) [15] and cloud point extraction (CPE) [16].

DLLME is a modified solvent extraction method and its acceptor-to-donor phase ratio is greatly reduced compared with other methods. In DLLME, the appropriate mixture of the extraction and disperser solvents is rapidly injected by syringe into aqueous samples containing analytes. Thereby, a cloudy solution forms. In fact, the cloudy state results from the formation of fine droplets of the extraction solvent which disperse in the sample solution. Then, this cloudy solution is centrifuged and the fine droplets become sediment at the bottom of the conical test tube. The determination of analytes in the sediment phase can be performed by instrumental analysis. In this extraction method any component in the solution, directly or indirectly after previous (or simultaneous) derivatization reaction, interacts with the fine droplets of the extraction solvent and consequently gets extracted from the initial solution and concentrates in the small volume of the sediment phase. Simplicity of the operation, rapidity, low sample volume, low cost, high recovery and high enrichment factor are some advantages of DLLME [17].

DLLME is a miniaturized sample pre-treatment technique. On the other hand, graphite furnace atomic absorption spectrometry (GF AAS) is a micro amount sample analysis technique. Therefore, it makes it perfect when a combination of both DLLME and GF AAS is used. The applicability of the approach has been demonstrated for the determination of zinc in water samples.

## EXPERIMENT

### Reagents and solutions

All solutions were prepared using ultra pure water. The stock solution of zinc ( $1000 \text{ mg L}^{-1}$  for atomic spectroscopy standard) was purchased from Merck (Darmstadt, Germany). Working standard solutions were prepared by serial dilutions of the stock solution with ultra pure water prior to analysis. Working solutions of Zn were prepared daily by appropriate dilution of stock solutions. A range of solutions at different pH values was prepared by adjusting with  $\text{HNO}_3$  (65%, suprapure) or  $\text{NaOH}$  (suprapure).

Other chemicals used were: carbon tetrachloride (analytical grade for determination with dithizone), chloroform (analytical grade for determination with dithizone), carbon disulfide (for spectroscopy) as extraction solvent, methanol (suprasolv), ethanol (for spectroscopy), acetone (suprasolv) and acetonitrile (HPLC grade) as disperser solvent, 8-Hydroxyquinoline (analytical grade) as chelating agent,  $\text{HNO}_3$  (65%, suprapure) and  $\text{NaOH}$  (suprapure). The mentioned chemicals were also obtained from Merck.

### INSTRUMENT

The experiments were performed using a PerkinElmer atomic absorption spectrophotometer AAnalyst800 with a graphite furnace atomizer. A zinc hollow cathode lamp, operated at a current of 10 mA and a wavelength of 213.9 nm was used. Pyrolytically coated graphite tubes were used. The sample injection volume was 20  $\mu\text{L}$  in all experiment. The temperature program for the graphite atomizer is listed in Table 1. The pH values were measured with a Metrohm pH-meter (Model: 827, Herisau, Switzerland) supplied with a glass-combined electrode. The Centurion

Scientific centrifuge (Model ALC 4232-D) was used for centrifuging.

### Dispersive liquid-liquid microextraction procedure

In DLLME method, 5 ml of aqueous sample containing Zn was placed in a 10 ml screw cap glass test tube. Then, 1.5 ml of methanol (as disperser solvent) containing 250  $\mu\text{L}$  of chloroform (as extraction solvent) and 0.05  $\text{mmol.L}^{-1}$  8-hydroxyquinoline (as chelating agent) was rapidly injected into a sample solution by using 2.00 ml syringe. A cloudy solution was formed and separation of the phases was achieved by centrifugation at 3000 rpm for 3 min. After this process, a small droplet of chloroform was sediment in the bottom of conical test tube. 190  $\mu\text{L}$  sediment phase was withdrawn into the microsyringe and then injected into the GFAAS for analysis.

## RESULTS AND DISCUSSION

The influence of various analytical parameters including pH, extraction and disperser solvent type and volume, concentration of the chelating agent and interfering ions on the extraction efficiency of analyses was investigated.

### Effect of disperser solvent type and volume

The disperser solvent must be miscible in extraction solvent (organic phase) and also sample solution (aqueous phase). Therefore, acetone, methanol, ethanol and acetonitrile were tested. The effect of these solvents on the extraction efficiency of DLLME was investigated using 1.5 ml of each solvent containing 250  $\mu\text{L}$  of  $\text{CHCl}_3$  as the extraction solvent. The maximum extraction efficiency of DLLME was obtained by using methanol as a disperser solvent. That is due to the high compatibility of methanol with aqueous solution than other solvents. Therefore, methanol was selected for further experiments.

After finding the disperser solvent type, it was necessary to find the optimum volume of it. Various experiments were performed with different volume of methanol in the range of (0.5 – 3 ml). However, by increasing the volume of methanol, the solubility of complex in water increased also, thereby, analytical signal decreased. So, 1.5 ml methanol was chosen in the following work.

Table 1 Temperature program of GF-AAS in analysis

Step	Temperature ( $^{\circ}\text{C}$ )	Ramp (s)	Hold (s)	Argon flow rate ( $\text{mLmin}^{-1}$ )
1: Dry	130	15	30	250
2: Pyrolysis	1200	10	20	250
3: Atomization	2000	0	5	0
4: Cleaning	2450	1	3	250

### Effect of extraction solvent type and volume

For the selection of extraction solvent it is important to consider some properties such as higher density than water, low solubility in water and capacity of extraction of interested ions.

Dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), Chloroform ( $\text{CHCl}_3$ ), Bromobenzene ( $\text{C}_6\text{H}_5\text{Br}$ ), Carbon disulfide ( $\text{CS}_2$ ) and carbon tetrachloride ( $\text{CCl}_4$ ) were employed and compared in the zinc extraction. The results showed (Fig 1) that extraction efficiency of  $\text{CHCl}_3$  is higher than  $\text{CH}_2\text{Cl}_2$ ,  $\text{C}_6\text{H}_5\text{Br}$ ,  $\text{CS}_2$  and  $\text{CCl}_4$ .

Therefore,  $\text{CHCl}_3$  was selected as extraction solvent for subsequent experiments.

To study the effect of volume the extraction solvent, a series of solutions containing increasing volumes of  $\text{CHCl}_3$  (100–500  $\mu\text{L}$ ) dissolved in a fixed volume of methanol were subjected to DLLME. The results was observed that the extraction efficiency was increased

for increasing volume of  $\text{CHCl}_3$  (100–250  $\mu\text{L}$ ) and then extraction efficiency was decreased for increasing volume of  $\text{CHCl}_3$  (300–500  $\mu\text{L}$ ). The decreased ratio lowers the number of droplets formation available for extraction thereby lowering the extraction efficiency. Based on these observations,

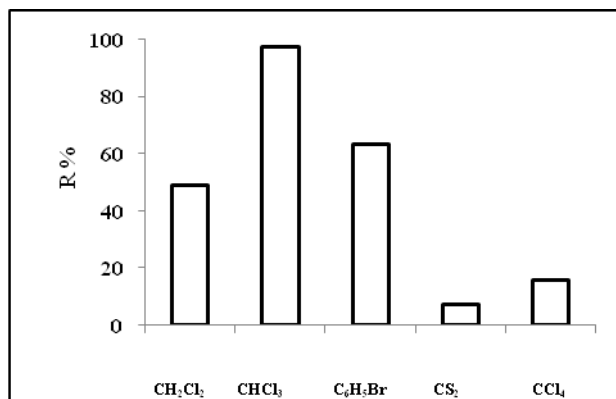


Fig. 1 Effect of different extraction solvent on Zn determination

#### PH Effect

The pH of the sample solution is an important factor affecting the formation of complexes and the subsequent extraction. The effect of pH on the

DLLME extraction of Zn was studied in the pH range of 4 to 12 (Fig 2). The highest recovery intensity of Zn was obtained at pH 6.0. Therefore, pH 6.0 was selected for further study.

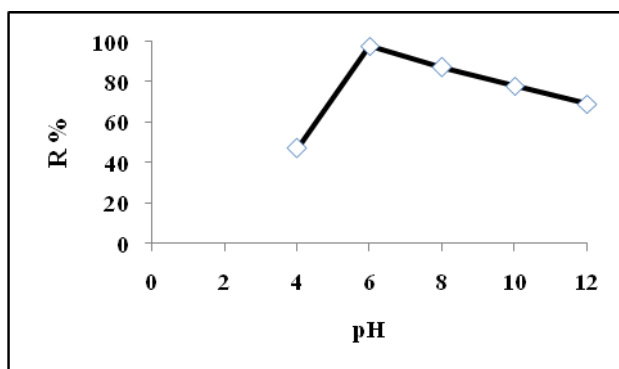


Fig. 2 Effect of pH on Zn determination

#### Effect of 8-HQ concentration

The influence of the amount of 8-HQ as chelating agent was also evaluated and the results showed that the signals of Zn was increased with the increase of 8-

HQ amount from 0.005 to 0.05  $\text{mmol.L}^{-1}$  and then decreased to 0.75  $\text{mmol.L}^{-1}$ . In this study, 8-HQ amount of 0.05  $\text{mmol.L}^{-1}$  was employed.

Table 2 Effect of matrix ions on zinc determination

Interfering ions	Tolerable concentration (analyte: interfering ion)
$\text{Si}^{4+}$ , $\text{As}^{3+}$ , $\text{Co}^{2+}$ , $\text{Ni}^{2+}$ , $\text{Cr}^{3+}$ , $\text{Al}^{3+}$ , $\text{Fe}^{3+}$	1:10
$\text{Mn}^{2+}$ , $\text{Cu}^{2+}$	1:100
$\text{Sr}^{2+}$ , $\text{Ba}^{2+}$ , $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$	1:2500
$\text{SO}_4^{2-}$ , $\text{H}_2\text{PO}_4^-$ , $\text{CH}_3\text{COO}^-$ , $\text{Cl}^-$ , $\text{NO}_3^-$ , $\text{PO}_4^{3-}$	1:5000

### Study of interferences

Interferences were studied in the presence of a constant concentration of analyte ( $1 \mu\text{g.L}^{-1}$ ) and different amounts of foreign ions (analyte: foreign ion ratio 1:10, 1:100, 1:2500 and 1:5000). Tolerable concentration of foreign ions was considered that concentration in which less than 10% deviation in absorbance reading was observed in comparison with the case in which interfering ion was absent. The obtained results are given in Table 2.

### Figures of merit

The calibration graph was linear in the range of  $1\text{--}4 \text{ ng.mL}^{-1}$  for Zn.

The limit of detection based on three times of the blank standard deviations was  $0.0033 \mu\text{g.L}^{-1}$ . The

relative standard deviation (R.S.D.) for seven replicate measurements of  $1 \mu\text{g.L}^{-1}$  was 7.41%.

### Natural water analysis

**Sample collection:** Samples used for the developing of the analytical procedure were collected from agriculture water in the city of Saveh– Iran. All samples were collected in pre-cleaned high density polyethylene bottles. Collected samples acidified at pH lower than 2.0 by adding concentrated nitric acid in order to avoid metal adsorption on to the inner bottles walls then samples were filtered through a  $0.45 \mu\text{m}$  polycarbonate membranes nucleopore filter. Well water, Mineral water and Tap water were analyzed by DLLME combined with GFAAS (Table 3).

Table 3 Determination of Zn in tap, well and mineral water samples

Sample	Zn concentration ( $\text{mg.L}^{-1}$ )	RSD % n=5
Well water	0.250	7.25
Mineral water	0.104	4.37
Tap were	0.259	5.19

### CONCLUSION

The aim of this study was determination of Zn by Dispersive liquid-liquid microextraction (DLLME) using chloroform as an extraction solvent, methanol as a disperser solvent and 8-HQ as a chelating agent. The determination of extracted ions was done by graphite furnace atomic Absorption spectroscopy. Enrichment factor and recovery for the target analyte was obtained about 26 and 97%, respectively. The method can be advantageously applied to the analysis of environmental samples containing  $\text{Zn}^{2+}$  ion at trace levels.

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