ORIGINAL ARTICLE

The application of Flame Atomic Absorption Spectrometry to evaluate Lead (II) in Vegetable Samples after Its Preconcentration by SA-DLLME

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ABSTRACT: In this paper, a new and environmentally friendly approach has been developed to determine the levels of lead in plant samples. To preconcentrate the lead ion, SA-DLLME (surfactant-assisted dispersive liquid-liquid micro-extraction) was used in the study of flame atomic absorption spectrometry (FAAS). The influence of the following analytical parameters on the quantitative recoveries of lead was studied: pH, type and amount of the extraction solvent, type and amount of the dispersing solvent, type and amount of the surfactant, centrifugation time and rate, amount of (APDC), sample volume and amount of matrix. The approach was validated using vegetable samples. The approach offered a (LOD) of 0.28 μg l^{-1}, (LOQ) of 0.933 μg l^{-1}, (RSD) of 1.2-4.3%, (EF) of 85, PF of 30, and ER% of (100.6-102.1)%.

INTRODUCTION

Contamination of agricultural soils with heavy metals (HMs) in some areas causes the accumulation of these elements in products and causes risks for humans. The entry and accumulation of various metals, especially HMs, in the environment is one of the problems that modern mankind has faced due to activities incompatible with nature. Among the HMs, lead and zinc have a high degree of importance and degree of pollution, and the contamination of agricultural products grown in soils contaminated with these elements can threaten and endanger human health. HMs are a special category of metals that have a specific weight of more than 6 grams per cubic meter or an atomic mass of more than 50. HMs have very harmful impacts on human health and enter the human body through various methods. HMs are found in abundance in the earth's crust and are needed in varying amounts for the proper functioning of the body. Some of

these HMs such as zinc, iron, and cobalt are essential for health and others are harmless such as silver, ruthenium, and indium. However, HMs such as arsenic, aluminum, cadmium, mercury, and lead are very harmful to health. Arsenic is one of the most toxic elements known to the human body. This element is abundant in the earth's crust, but it is also found in smaller amounts in air, water, soil, and rocks. This element is widely used in the production of munitions, batteries, electrical tools, semiconductor industries, wood industries, and pesticides. Most arsenic poisoning is often seen in mines and agricultural activities. Poisoning with this element causes nausea, headache, dizziness, lethargy, and flu-like symptoms. Rupture of blood cells, anemia, fainting, and lowering of blood pressure are other things that arsenic poisoning causes in the human body. Long-term exposure to arsenic can cause chronic diseases such as

neurological diseases, diabetes, heart diseases, and cancer. Aluminum poisoning can also have many risks for the body. Generally, a high level of aluminum mainly endangers brain and bone health. Not only does this cause brain diseases, but evidence has shown that bone pain, bone diseases, and reduced bone regeneration capabilities are other chronic diseases caused by aluminum poisoning. In addition to serious damage to the lungs and kidneys, cadmium poisoning also disrupts the functioning of body enzymes

Lead is considered to be one of human health's most dangerous metals because it affects the central nervous system, and is the main cause of anemia and gastrointestinal damage. It is also linked to gene expression alterations [1-3]. Lead could get into our bodies through the ambient air, water, and food [4]. Inorganic lead (Pb^{2+}) attaches to the –SH enzyme or proteins and acts as an enzyme inhibitor. This interferes with calcium synthesis and is stored in the body. Organic compounds such as tetramethyl lead contain lead in their structure and are highly poisonous. It is because the body absorbs easily by them quickly through the skin and the mucus membrane. Acute human lead exposure induces serious harm to the lungs, liver, brain, and reproductive system that often ends in death. In addition to that, moderate lead exposure may cause anemia, fatigue, and weak muscles, and the patient to feel exhausted and irritable. Both findings pose significant public safety issues and these call for the correct detection of this metal ion at trace and sub-trace levels in biological samples [5]. Numerous approaches have been developed to detect low levels of metals, such as Pb, in samples using various analytical tools, such as atomic absorption spectroscopy [6]. However, measuring HMs in many real samples is a complex analytical task due to the complex matrix and traces concentrations and requires sensitive detection approaches and pre-concentration procedures [7]. Separation and purification approaches were developed, such as liquid-liquid extraction [8], solid-phase extraction [9], Cloud point extraction (CPE) [10, 11] and dispersive liquid-liquid microextraction (DLLME). Nowadays, the sample preconcentration technique is given noticeable attention, it is quick, low cost, and greener due to the reduced use of toxic organic solvents [12]. DLLME

is an efficient, simple, and inexpensive way to provide high concentration and recovery by extraction [13]. FAAS is currently the most widely used analytical approach for the determination of HMs such as lead. However, due to the requirements of the liquid phase sampling technology and the low concentration of metal ions, it is essential to perform efficient separation and pre-concentration steps before the analytical measurements [14, 15]. In the current study, it was tried to develop a new, sensitive, simple, reliable, selective, and accurate analytical approach for the determination of pb (II) trace levels in plant samples using FAAS based on the SA-DLLME approach. The selective chelating agent Ammonium pyrrolidine dithiocarbamate (APDC) was used for the separation and preconcentration of pb(II). There may be a strong link between pb (II) surgery and the ligand containing the sulfur atom as a soft base [16, 17]. Among the sulfur-containing ligands, dithiocarbamate is a useful ligand that stabilizes different metals in different oxidation states [18]. To fully characterize the proposed approach, numerous parameters were tested and optimized such as pH, volume and types of extraction solvent and dispersing solvent, types of surfactant concentration, surfactant, centrifugation time and rate, and amount of (APDC), sample volume and amount of matrix.

MATERIALS AND METHODS

The analytical grade reagents were used when obtained from various companies without further purification. It should be mentioned that deionized water was used for the dilution of the standard reagents and samples. APDC (Ammonium pyrrolidine dithiocarbamate) was bought at Sigma as the chelating agent. Triton X-100 and Triton X-114 were purchased from Sigma (Sigma Ultra> 99.6%).

Instrumentation

WTW Laboratory Equipment, E163694 (Germany) was used for measuring and controlling the pH of the aqueous media. A Z200A centrifugation device (HERMLE / Germany) was used to accelerate and facilitate the phase separation of turbid solution. Electrical furnace (Oven Philip Harris Limited, Shenstone), Ser. No. T 7981S1 (England) was used to digest solid samples.

General procedure

Lead extraction and measurement by using SA-DLLME / FAAS

A schematic diagram of lead measurements using SA-DLLME in a sample solution is shown (see Figure 1). Aliquots of normal lead/water sample solutions were placed in 15 ml centrifuge tubes. For this, 1ml of each buffer solution, 0.1% APDC, 0.1% TX-100, and 0.1% TX-114 solution were added and made up to a total volume of 10 ml using Deionized water. After that, 0.3 ml of dispersion solvent (ethanol) and 0.2 ml of extraction solvent (chloroform) were added. The tube consists of a cloudy solution of small drops. The tubes were then transferred to a centrifuge and centrifuged at 3000 rpm for 5 minutes [19].
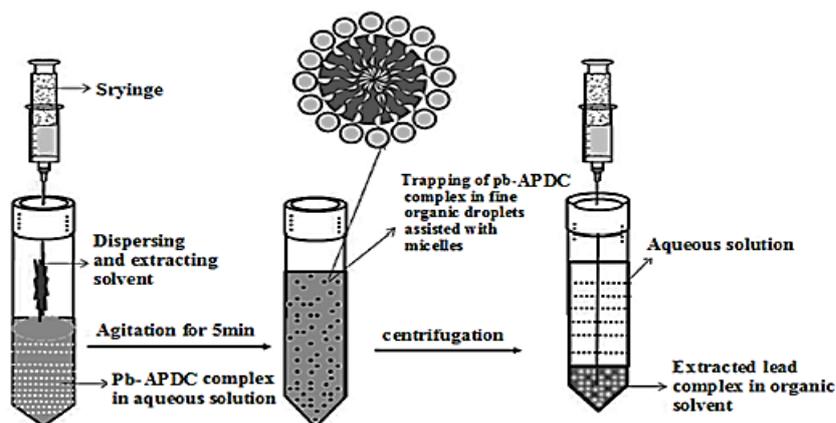

Figure 1. SA-DLLME process schematic for determining lead in the sample solution.

Application of real samples

Vegetable samples (turnip, onion, carrot, radish, potato, spinach, and beta Vulgaris) were collected from the local market in Nasiriya City. The samples were chopped into small pieces and dried in an oven at 80°C for 48 h. The samples were finely ground to powder using an electrical grinder after drying was finished. 0.10gm of the vegetable sample was placed in a 100ml beaker and 10ml of concentrated HNO_3 (65%, w/w) was added. The mixture was steamed almost to dryness on a hot plate at 150 ° C. It should be noted that 3 ml of concentrated hydrogen peroxide (30% w / w) was added, after cooling to room temperature [20].

RESULTS AND DISCUSSION

Optimization of SA-DLLME parameters impact of pH

The sample's pH value plays a vital role in the pre-concentration procedure. This is because the formation of soluble metal complexes and their aqueous solution stability is strongly linked to the medium's pH. Complex formation interaction between metal and ligand, and thus the efficiency of extraction, is pH dependent [21, 22]. The impact of pH on the extraction and formation of the Pb-APDC complex was examined by the addition of appropriate buffer solutions at a pH range of (1–12) while maintaining all other parameters constant (see Figure 2). It can see from the figure that the recovery of lead with APDC was maximized and remained almost constant (98%) in the pH range of 6–8. It should be noted that the recoveries of lead in the highly acidic pH were not quantitative due to the lack of complex formation between lead and APDC. Therefore, pH 6 was selected as the optimal pH for further studies.
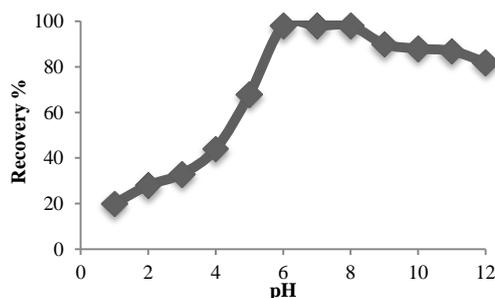
Figure 2. Impact of pH on the recovery of pb(II).

Impact of extracting solvent and its volume

The type of solvent extracted is also an important factor in the extraction process. During the SA-DLLME procedure, it plays an important role in cloudy solutions, including drops. This cloudy solution provides a favorable environment for the analyte to drop. The low aqueous solvent of the extractor ensures a high recovery. Therefore, the choice of extraction solvent has a significant impact on recovery [23, 24]. Herein, the extraction solvents such as hexane, toluene, chloroform, carbon tetrachloride, and 1,2-dichloromethane were evaluated in this study, resulting in recoveries of 25, 15.8, 97.5, 78.2 and 84%, respectively. We selected chloroform for further study. The results presented in (Figures 3 and 4) indicate that 200µl of chloroform was sufficient to provide quantitative recovery (98%).
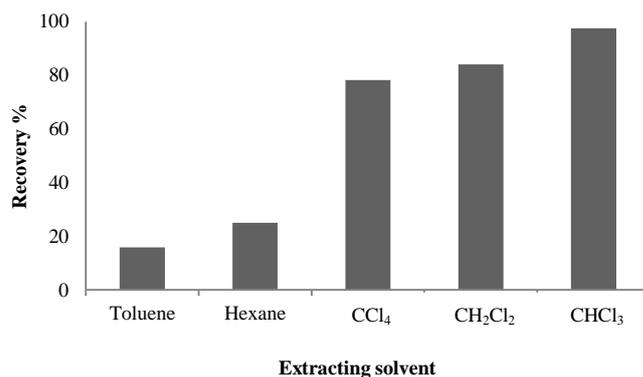

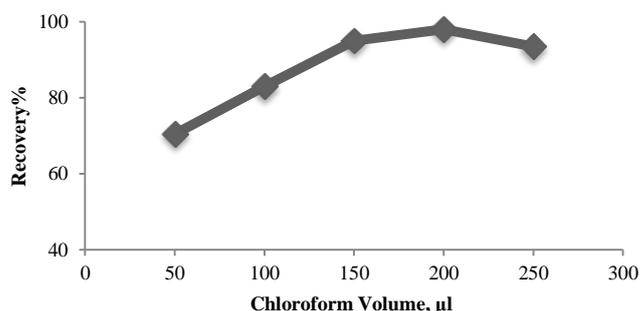**Figure 3.** Impact of extracting solvent type on the recovery of Pb(II).

Figure 4. Impact of the volume of chloroform on the recovery of Pb(II).

Impact of dispersing solvent and its volume

The impact of the dispersing solvent was investigated to maximize the recovery. Tests solvents of acetonitrile, acetone, ethanol, and methanol were tested. The restoration is shown (see Figures 5 and 6). Quantitative recovery (99%) was performed with ethanol. The lowest yields were methanol, acetone, and acetonitrile: 60, 87, and 72.5%, respectively. When ethanol was used as a dispersant, high extraction was possible due to its excellent compatibility with the extraction solvent (chloroform). Also, different volumes of ethanol were

tested (100–500μl), while maintaining all other parameters constant. The highest recovery (99%) was obtained with 300μl. Hence, 300μl was selected as the optimum volume of the dispersive solvent for further experiments.
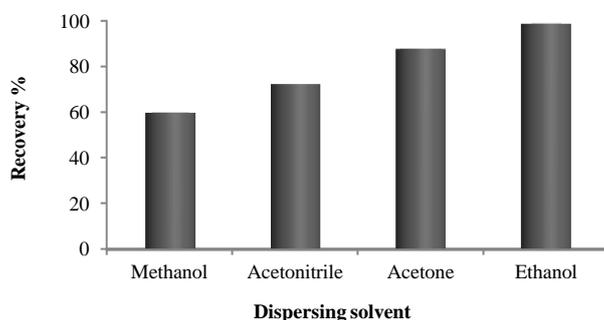

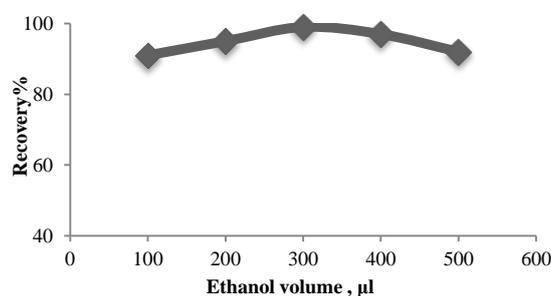**Figure 5.** Impact of dispersing solvent type on the recovery of Pb(II).

Figure 6. Impact of the volume ethanol on the recovery of Pb(II).

Impact of a non-ionic surfactant on the extraction and amount

The usage of nonionic TX-100 and TX-114 was studied for the extraction of the pb(II)–APDC complex into the chloroform. TX-114 was used as the surfactant for the extraction and determination of lead (see Figures 7 and 8). The volume of TX-114 was also examined between (100-600) µl of 0.1% TX-114 solution at optimal conditions. In the results presented in Figure 8, quantitative pb ion extraction increased in volume but it remains constant, therefore 400µl of TX-114 was sufficient to provide quantitative recovery (99%).
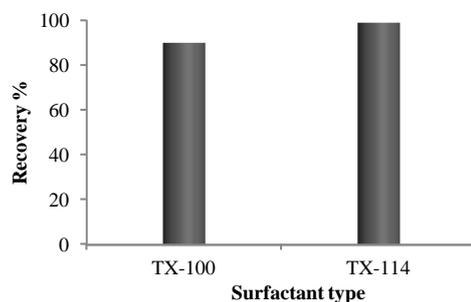

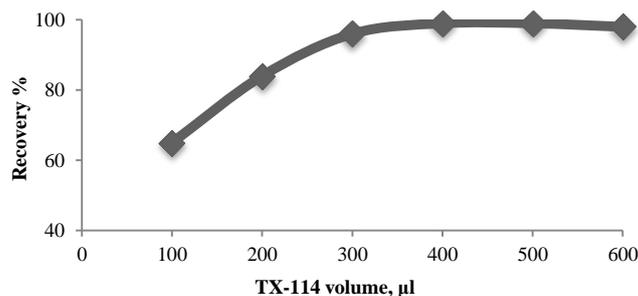**Figure 7.** Impact of surfactant type on the recovery of Pb(II).

Figure 8. Impact of TX-114 volume on the recovery of Pb(II).

Impact of centrifugation time and rate

Rate and time of centrifugation were simultaneously examined at 1000 – 4000 rpm from 2 to 10 min. It was observed that efficient quantitative extraction and phase separation were adequate for 5min at 3000 rpm.

Impact of the amount of (APDC)

Different amounts of a 0.1% solution of APDC in methanol were tested, using 300µl ethanol and 200µl of chloroform. The obtained results which showed in Figure 9 reveal that 200µl was sufficient to provide quantitative lead recovery.
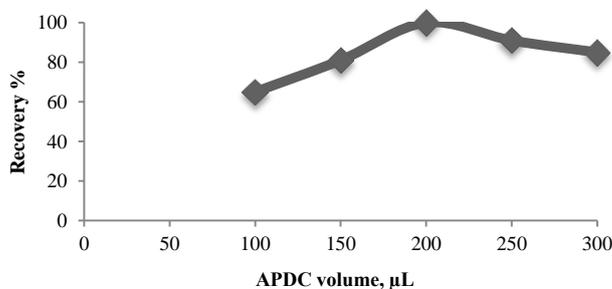

Figure 9. Impact of APDC volume on the recovery of Pb(II).

Impact of sample volume

In the current study, various quantities of the sample were tested at optimal conditions: 10,15,20,30, and 40ml. The obtained results shown in Figure 10 reveal that quantitative recovery was obtained using volumes of up to 15ml. Hence, a pre-concentration factor of 30 can be achieved when using 15ml of the sample and 500µl of 0.1M HNO_3

Figure 10. Impact of sample volume on the recovery of Pb(II).

Impact of matrix

The existence of matrix or coexisting ions can interfere with the SA-DLLME procedure because of competition for the chelating agent between ions [25-27]. Under optimum conditions the impact of Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Cl^-, was tested. The obtained results which showed in Table 1, emphasize that the recovery was 100%, 99% with Na^+, K^+, Cl^-, NO_3^-, Zn^{2+}, Ni^{2+} and Cu^{2+}, and not less than 95% with the other ions tested.

Table 1. The impact of matrix on the recovery of Pb(II)

Ion	Salt added	Concentration mg l^{-1}	Recovery%
Na^+	NaCl	1000	100
K^+	KCl	1000	100
Ca^{2+}	$CaCl_2$	2000	95
Mg^{2+}	$Mg(NO_3)_2 \cdot 6H_2O$	5000	98
Fe^{3+}	$Fe(NO_3)_3 \cdot 9H_2O$	1000	98
Zn^{2+}	$Mg(NO_3)_2 \cdot 6H_2O$	1000	99
Co^{2+}	$Co(NO_3)_2 \cdot 6H_2O$	1000	97
Cu^{2+}	$Cu(NO_3)_2 \cdot 6H_2O$	1000	99
Ni^{2+}	$Co(NO_3)_2 \cdot 6H_2O$	1000	99
Cd^{2+}	$Cd(NO_3)_2 \cdot 4H_2O$	1000	95
Cl^-	NaCl	100	99
NO_3^-	KNO_3	500	100
SO_4^{2-}	Na_2SO_4	2000	98

Determination of lead in vegetable samples

The present procedure has been used to determine the amounts of lead in vegetable samples. Lead concentration in the extracted samples with an acceptable correlation coefficient of 0.9986 was calculated from the installed calibration curve (y = 0.0057x + 0.0213). Table 2 shows the amount of lead present in the vegetable samples. The concentration of lead in vegetable samples ranged (from 0.056-0.642 µg g^{-1}).

Applications to real samples

The initial SA-DLLME approach was applied in vegetable s joint, the concentration of Pb in onions, potatoes, spinach, and beta Vulgaris exceeded safe limits, maximum permitted level in vegetables (µg g^{-1}) were defined as 0.2 [28]. The most possible source of road and/or highway emissions could be a runoff of irrigation water, and agricultural soil [29, 30].

Table 2. The addition of recovery tests to real samples for preconcentration of lead using SA-DLLME/FAAS

Sample	Added (µg g^{-1})	Found (µg g^{-1})	%RSD	% Recovery
Turnip	0	0.056	3.7	-
	0.2	0.258	3.2	100.8
Carrot	0	0.152	3.1	-
	0.2	0.356	2.6	101.1
Onion	0	0.344	2.5	-
	0.2	0.547	2.1	100.6
Potato	0	0.332	3.4	-
	0.2	0.538	3.0	101.1
Radish	0	0.088	3.8	-
	0.2	0.290	3.3	100.7
Spinach	0	0.429	2.4	-
	0.2	0.642	1.2	102.1
Beta vulgaris	0	0.211	4.3	-
	0.2	0.415	3.9	101.0

Analytical performance of the procedure

The analytical efficiency of SA-DLLME with FAAS for the pre-concentration and lead determination under optimized experimental conditions has been routinely examined (Table 3).

Table 3. The analytical characteristics of the approach

Parameter	Analytical feature
Linear range µg l^{-1}	5-400
Limit of detection (LOD) µg l^{-1}(n=5)	0.28
Limit of quantification (LOQ) µg l^{-1}(n=5)	0.933
RSD %	1.2 - 4.3
Enrichment factor (EF)	85
Preconcentration factor (PF)	30
Percent extraction recovery (PR%)	100.6-102.1

A comparison between the proposed approach and some other reported DLLME techniques for the determination of Pb(II) ion was performed, and the results obtained were provided in Table 4. Comparing the results, it can be seen that the LOD and the enrichment factor of this approach are superior to those of the other approaches.

RSD was relatively low. The results indicate that SA-DLLME-FAAS is a reproducible, fast and sensitive approach to pre-concentration and measurement of lead in vegetable samples by injecting microprobes after SA-DLLME.

Table 4. Comparison between the proposed approach and other reported DLLME techniques for determination of lead (II) ion.

Extraction approachs	Chelating agent	Dynamic range (µg l^{-1})	LOD	EF	%RSD	Ref.
TDLLME/µs-FAAS	5-Br-PADAR[a]	3-300	0.31	45	6.9	32
DLLLME/GFAAS	PMBP[b]	0.1-20	0.04	78	3.2	33
DLLME-based home - made tablet disperser/FAAS	Na-SDDTC[c]	1-20	0.430	130	1.3	14
UA-DLLME-SFO-BE	Congo red[d]	10-500	1.9	81	3.4	34
IL-DLLME/FAAS	[C$_8$MIM (PF$_6$)][e]	5-190	0.8	135	2.8	35
DLLME/AAS	rac-CBCD[f]	-	2.6	75	5.2	36
SA-DLLME/FAAS	APDC[g]	5-400	0.28	85	1.2	this work

a: 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol; b: 1-phenyl-3-methyl-4-benzoylpyrazol-5-one; c: Sodium diethyldithiocarbamate; d: 3,3′-[[1,1′ biphenyl]-4,4′-diylbis-(azo)] bis[4-amino-1-naphthalene-sulfonic acid; e: 1-octyl-3-methylimidazolium hexafluorophosphate; f: rac-(E,E)-N,N-bis(2-chlorobenzylidene)cyclohex-ane-1,2-diamine; g: Ammonium pyrrolidinedithiocarbamate.

CONCLUSIONS

A liquid-to-liquid dispersion microextraction system was created using surfactants to determine traces of lead in plant samples prior to atomic absorption spectroscopy measurements. This approach showed a wide linear range, a high amplification coefficient, a low detection limit, and a high reserve concentration ratio for lead ions. It eliminates the need for highly qualified professionals and high operating costs because the equipment needed today is cheap and easy to practice. In addition, it is environmentally friendly because it mainly uses low toxicity surfactants and organic solvents.

ACKNOWLEDGMENTS

Conflict of interests

No conflict.

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