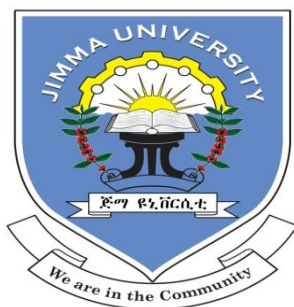


**JIMMA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL SCIENCE
DEPARTMENT OF CHEMISTRY**



**MSc THESIS ON
OPTIMIZATION OF DISPERSIVE LIQUID-LIQUID
MICROEXTRACTION FOR SPECTROPHOTOMETRIC
DETERMINATION OF LEAD IN WATER**

SHETAHUN AWEKE

**FEBRUARY, 2021
JIMMA, ETHIOPIA**

**OPTIMIZATION OF DISPERSIVE LIQUID-LIQUID
MICROEXTRACTION FOR SPECTROPHOTOMETRIC
DETERMINATION OF LEAD IN WATER**

SHETAHUN AWEKE

ADVISORS: ABERA GURE (PhD)

MEAZA DEJENIE (MSc)

**An MSc THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA
UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY**

FEBRUARY, 2021

JIMMA, ETHIOPEA

JIMMA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL SCIENCE
DEPARTMENT OF CHEMISTRY
MSc THESIS APPROVAL SHEET

We, the under signed, member of the Board of Examiners of the final open defense by **Shetahun Aweke** have read and evaluated his thesis entitled “*Optimization of Dispersive Liquid-Liquid Microextraction for Spectrophotometric Determination of Lead in Water*” and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirement for the degree of Master of Science in Chemistry (Analytical Stream).

Dr. Abera Gure

Name of Advisor (1)

Signature

Date

Mrs. Meaza Dejene

Name of Advisor (2)

Signature

Date

Dr. Fekadu Melak

Name of Internal Examiner

Signature

Date

Dr. Bayissa Leta

Name of External Examiner

Signature

Date

TABLE OF CONTENT

LIST OF TABLE	III
LIST OF FIGURE.....	IV
ACKNOWLEDGMENT.....	V
ABBREVIATION.....	VI
ABSTRACT.....	VII
1. INTRODUCTION	1
1.1. Background of the study	1
1.2. Statement of the problem.	3
1.3. Objective of the study.	4
1.3.1. General objective	4
1.3.2. Specific objective	4
1.4. Significance of the study	4
2.1 lead	6
2.2. Use of lead.....	7
2.3 Chemical and physical properties of lead	7
2.3.1. Physical properties.....	7
2.3.2 Chemical properties.....	7
2.4 Sources of lead in our environment.....	8
2.5 Toxicity of lead	8
2.6 Dispersive liquid-liquid micro-extraction	9
2.7 DLLME development and application	9
2.8 Principle of DLLME	10
2.9 Spectrophotometric determination of lead	11
2.10. Sample preparation method.....	11
3. MATERIALS AND METHODES	12
3.1. Chemicals and Reagents.....	12
3.2 Instruments and Apparatus.....	12
3.3. Sample collection	12
3.4 Standard and working solution preparation	13

3.4. DLLME Extraction procedure	13
3.6 Optimization parameters	13
3.7 Method of Validation	14
4. RESULT AND DISCUSSION	15
4.1 UV-Vis absorption spectra of Lead-dithizonate.....	15
4.2 Optimization of DLLME procedure.....	16
4.2.1 Effect of the type of Extraction Solvent	16
4.2.2 Effect of the type of the dispersant solvent	17
4.2.3 Effect of the volume of extraction solvent	17
4.2.4 Effect of the volume of disperser solvent.....	18
4.2.5 Influence of pH.....	19
4.2.6 Effect of salt.....	20
4.2.7 Effect of centrifugal time.....	21
4.3 Method of validation	22
4.3.1 Calibration curve construction.....	22
4.3.2 Limit of detection and Limit of Quantification	22
4.3.3 Precision study.....	23
4.3.4 Analysis of real sample and recovery studies.....	24
4.3.5 Effect of Interfering Ions.	25
4.3.6 Comparison of the proposed method with other methods	26
5. CONCLUSION AND RECOMMENDATION.....	27
5.1 Conclusion.....	27
5.2 Recommendation.....	27
6. REFERENCES	28

LIST OF TABLE

Table 1: Figure of merits of the proposed DLLME method	23
Table 2: Intra- and inter-day precisions (RSD) of the proposed method.....	23
Table 3: Relative recoveries (%RR) of the method	24
Table 4: Tolerance limits of interfering ions for the determination of 0.05 mg/L Pb ²⁺	25
Table 5: Comparison of the proposed DLLME with other methods	26

LIST OF FIGURE

Figure 1: Structure of Lead-dithizonate complex	2
Figure 2: DLLME Procedure for the analyte after addition of a chelating agent	11
Figure 3: UV-Vis absorption spectra of experimental lead-dithizonate complex.....	15
Figure 4: The effect of the type's extraction solvent. Condition: Extraction solvent volume, 300 μ L Concentration of Pb, 1 mg/L; volume of acetone, 800 μ L; Sample pH 9; Centrifugation time, 3 min (3000 rpm); and NaCl, 0.3 g.....	16
Figure 5: Effect of disperser solvent type. Condition: Similar to Figure 4.....	17
Figure 6: Effect of the extraction solvent volume. Condition: Similar to Figure 4, except for the volume extraction solvent.....	18
Figure 7: Effect of the disperser solvent volume. Condition: Extraction solvent volume, 200 μ L; Disperser solvent volume, different and other parameters are similar to Figure 4.....	19
Figure 8: Effect sample pH. Condition: Disperser solvent volume, 800 μ L and other parameters are similar to Figure 7.....	20
Figure 9: Effect of salt. Condition: Sample pH 9 and other parameters are similar to Figure 8.....	20
Figure 10: Effect of centrifugation time. Condition: Salt amount, 3% (w/v) & other parameters are similar to Figure 9.....	21
Figure 11: Calibration curve for lead, Pb (II)	22

ACKNOWLEDGMENT

First and for most, I would like to offer praise and glory to Almighty God for providing me good health, peace and strength in all my life. Next I want to express my sincere gratitude and heartfelt appreciations to my advisors, Dr. Abera Gure and Mrs. Meaza Dejene for their initiation, continued and invaluable guidance as well as substantial encouragement for supporting the present study. My special thanks goes to Dr. Abera Gure in going through all activities always alongside me in guiding with constructive and scientific way of writing, critical feedbacks, valuable comments and suggestions, a person who played a great role in bringing this thesis work to final and also my strength and moral as an advisor and father in every difficulties and challenges.

ABBREVIATION

WHO	World health organization
ACN	Acetonitrile
ICP-MS	Inductive coupled plasma mass spectroscopy
DMSO	Dimethyl sulfoxide
DLLME	Dispersive liquid-liquid micro extraction
LLE	Liquid-liquid extraction
EF	Enrichment factor
LOD	Limit of detection
LOQ	Limit of quantification
PAH	Polycyclic aromatic hydrocarbon
ET-AAS	Electro thermal atomic absorption spectroscopy
ICP-AES	Inductive coupled plasma atomic emission spectroscopy
FAAS	Flame atomic absorption spectroscopy
Ppm	Parts per million
Rpm	Rate per minute
RR	Relative recovery
RSD	Relative standard deviation
UV-Vis	Ultraviolet-Visible Spectrophotometry
EF	Enrichment factor

ABSTRACT

In this study, dispersive liquid-liquid micro extraction (DLLME) was developed for extraction and pre-concentration of lead ion (Pb^{2+}) prior to its quantitative determination by UV-Vis spectrophotometer. The metal ion was extracted after making a complex with 1,5-diphenylthiocarbazone. Different experimental parameters affecting the extraction efficiency of the complex by DLLME including types and volume of solvent effect, mass of salt effecting, pH, and centrifugal time were assayed and the optimum conditions were established. Calibration curve was constructed by spiking and extracting distilled water containing Pb^{2+} at eight concentration levels, ranging from 0.005 - 2 mg/L. The obtained calibration curve has good linearity with a coefficient of determination (r^2) 0.9952. The limits of detection (LOD) and quantification (LOQ) of the method were 0.003 and 0.009 mg/L, respectively. The precision studied in terms of intra-day and inter-day at two concentration levels showed acceptable %RSD values that were less than 3% in both cases. Applicability of the method was investigated by analyzing different water samples and demonstrated satisfactory recoveries ranging from 79.1-108.1%. Generally, the developed DLLME method could be used as attractive alternative for extraction and pre-concentration of the lead as a complex of 5-diphenylthiocarbazone prior to quantitative determination by UV-Vis spectrophotometer.

Keywords: *Dispersive liquid-liquid microextraction; Lead; 1,5-Diphenylthiocarbazone; Water sample; UV-Vis spectrophotometer*

1. INTRODUCTION

1.1. Background of the study

Environmental pollution is defined as the addition of contaminants into the natural environment that cause adverse change to the environment. It is an undesirable change in the chemical, physical, and biological characteristics of air, water and soil, thus causes the health problem to all the living beings any physical, biological or chemical change in water that adversely affects living organism can be considered pollution [1]. The contamination of the environment with heavy metals is a serious problem because of industrial activities and sewage sludge discharge to the environment. Traces of heavy metals are widespread in the environment through soil erosion, industrial and agricultural processes [2].

In Ethiopia, 90% of industries discharge effluent to water bodies, rivers and land without treatment and are the major source of water pollution [3]. Heavy metals can come from a variety of sources such as mining, energy and fuel production, fertilizer and pesticide industries, metallurgy, electroplating, and atomic energy facilities, etc. [4, 5]. Lead (Pb) is a typical toxic heavy metal that tends to accumulate in people's vital organs and as such causes serious health problems [4, 6]. The presence of as low as 100 µg/L of Pb in the blood can have negative effects on the health of children. According to the World Health Organization (WHO) guideline the recommended maximum concentration of Pb in the drinking water is 10 µg/L [7]. For Pb determination dispersive liquid-liquid micro extraction (DLLME) has been coupled to different spectrometry detection techniques, such as atomic absorption (AAS), atomic fluorescence, inductively coupled plasma with optical emission (ICP-OES), and total reflection X-ray [8-9]. However, the above methods are slow, costly, difficult to use, unavailable in our laboratory, and bulky in the experimental process [10]. Therefore, in this work, DLLME method has been proposed for selective extraction and preconcentration as well as quantitative determination of Pb in water samples by UV-Vis spectrophotometry has only been used in this experimental study [11, 12].

On the way to achieving that final objective important advance has been made in miniaturization and simplification of sample preparation procedures This was done to minimize the consumption of samples and reagents (and hence the cost of analysis). Maintain a high degree of selectivity

and recovery using environmentally friendly methods and speed up the sample processing process which is currently considered to be the key stage in the rate of the analytical process. Traditional methods for sample preparation, including liquid-liquid extraction (LLE), soxhlet extraction, chromatography, distillation and absorption, usually suffer from the disadvantage of time consuming, multistage operation, production of toxic organic solvents and large amounts of toxic organic solvent to be used and difficulty in automation to some extent [13,14]. Therefore, analytical chemists have focused on the development of new, sample preparation methods which are less time consuming, more effective, fast, low cost and require smaller amounts of organic solvents, yet provide accurate and precise data with reasonable quantization limits.

One of those methods which has currently taken special attention for extraction and/or pre-concentration of organic and inorganic pollutants from aqueous matrices is DLLME [15]. DLLME provides many benefits such as high recoveries, high improvement factors and speed. Furthermore, it is an environmentally friendly method, since it uses very small volumes (in microliter level) of organic solvents. In addition, it is simple and inexpensive, as it does not require specific reagents or expensive lab equipment [16]. Thus, development of DLLME method for extraction of a toxic heavy Pb after complexing with appropriate reagent such as dithizone has paramount importance for analysis the metal form water and other related matrices. Dithizone (1,5-Diphenylthiocarbazon) is one of the important complexing reagent for analysis of Pb UV-Vis analysis. The chemical structure of lead-dithizonate complex is shown in Figure 1.

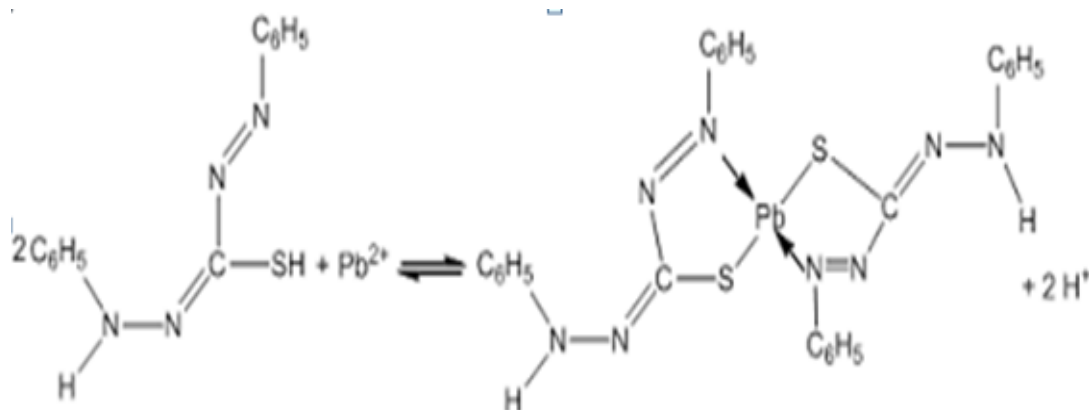


Figure 1: Structure of Lead-dithizonate complex

In this experiment, it is used due to its merits such as simplicity, usability, low cost and high sensitivity. Moreover, it is often difficult to directly determine Pb, since it is available at very low concentrations in the environment water and other samples. Interferences from the matrix in the real samples also cause another complication. Thus, sample preparation that involves extraction and/or and pre-concentration step is often needed [17]. Several sample preparation methods have been used for analysis of Pb in water samples. For example, solid phase extraction, cloud point extraction, capillary microextraction, and solid phase microextraction were some of the reported methods [18, 19]. But, some of these methods are time consuming and need special equipment. Thus, developing other alternative methods such as DLLME that are less laborious and use simple laboratory apparatus for determination of Pb in water and other matrices is important.

These days, DLLME is one of the most widely used sample preparation method because of its special merits such as simplicity, rapidness, low cost, low consumption of organic solvents, and high enrichment factor [18]. The method involves formation of cloudy solution after the fast injection of a suitable mixture of extraction and disperser solvents into the aqueous phase. After extraction, phase separation is achieved by centrifugation [19]. DLLME has been applied for the extraction of various inorganic compounds; however, to the best of the researcher knowledge, DLLME has not been reported for analysis of Pb after complexing with dithizone prior to UV-Vis spectrophotometric analysis. Therefore, in this work, DLLME method has been proposed for selective extraction and pre-concentration of Pb in water samples after complexing with dithizone prior to its quantitative determination by UV-Vis spectrophotometer.

1.2. Statement of the problem.

The environment contains an infinite number of complex pollutants, including heavy metals like Pb that are potentially harmful to the environment and/or the health of humans and other animals. For example, environmental waters, including surface waters (lakes, ponds, and rivers) as well as underground water can certainly contain Pb, originating from industrial waste, municipal discharges and natural processes [20]. It can be transferred from a particular environment to another by natural phenomena or by organisms including human beings. The presence of these and several varieties of their transformation products at different concentration

Pb²⁺ levels in the waters has a direct or indirect impact on the human health. For instance, Pb, a toxic heavy metal, it affects the kidneys, brain cells and liver membrane permeability, reducing some of their functions [21]. As a result, continuous assessment and monitoring of the contamination level of different types of environmental waters is important to identify their suitability for human consumptions. On the other hand, Pb metal exists at trace level in the environment and thus, requiring sensitive and selective sample preparation method which can simultaneously extract and/or pre-concentrate the analyte prior to its instrumental determination. However, no report was reported in the analysis of Pb extraction from water sample by DLLME coupled with UV-VIS spectrophotometry.

Thus, optimization of DLLME method, which has been demonstrated attractive alternative, because of its simplicity, low cost, selectivity and green sample preparation method over the traditional LLE method could also be crucial for analysis of the target analyte environmental water samples.

1.3. Objective of the study.

1.3.1. General objective

- ✓ The main objective of this study was to optimize DLLME for analysis of Pb from water samples by double beam UV-VIS spectrophotometer.

1.3.2. Specific objective

- ✓ To evaluate various experimental parameters influencing the extraction efficiency of DLLME for the analysis of Pb from water samples by UV-VIS.
- ✓ To validate the developed method in terms of linearity, limit of detection, limit of quantification, Intra and Inter-day precision before applying to real water sample.
- ✓ To apply the developed method for the analysis of the target analyte from the real environmental water samples

1.4. Significance of the study

It is well known that uses of water for drinking without asking what chemicals to be it contain in drinking water is very important for controlling heavy metals that may affect the consumers.

However, an intensive and uncontrolled use of water may cause health problems. In general, the findings of this study will be expected to have the following significances:

- The proposed analytical method could be employed for regular monitoring Pb ion residues in waters and other related matrices.
- The obtained results could be used as the background information about the levels of the target Pb residues in environmental waters to which the method will be applied.
- The developed method could be served as resource materials for further study.
- Investigate to human beings in order to pay attention to the quality of the environment.

2. REVIEW LITRATURES

2.1 lead

While Pb can be found in nature as the pure element, it is extremely pricey and it is generally present as Pb (II) in deposits with different origins. In these sources, it is combined with other elements like sulfur and oxygen in a variety of minerals that have a broad range of compositions. There are several minerals such as cruciate (PbCO_3), anglesite (PbSO_4), litharge/massicot (PbO) and minimum (Pb_3O_4) that are regularly used in the production of Pb. Some of the most common impurities found in Pb minerals are zinc, copper, arsenic, tin, antimony, silver, gold and bismuth [22].

Most studies to identify a potential link between Pb exposure and cancer have focused on workers with elevated levels of inorganic Pb exposure in the workplace. Those heavily exposed to Pb in the workplace have much higher blood Pb concentrations than the average blood Pb concentration in the general population [23]. Several studies have investigated the relationship between exposure to Pb in the workplace (primarily in battery workers and smelters) and lung cancer. Some of these studies have shown that the risk of lung cancer has increased slightly. However, most of these studies were limited in that they didn't take into account other factors that might affect lung cancer risk, such as smoking or exposures to arsenic or other heavy metals that typically also occur along with Pb exposures in industrial settings.

Some studies of blood Pb levels in the general population also found a slight increase in the risk of lung cancer in people with higher levels of Pb. A number workplace studies have also examined the risk of stomach cancer. Most studies have shown a higher risk for stomach cancer with higher exposure to Pb [23]. The link between Pb exposure and cancer is obviously of concern, and further research is needed to better define the potential link between Pb exposure and multiple cancers. Nowadays Pb and its derivatives have a very widespread use and world trade in this metal, either impure or refined, as well as in its minerals and compounds has been extensively developed [24]. The large amount of the metal that is produced, the high economic value of its trade and the fact that its production and transformation employs a large number of people, all make Pb an extremely important material. This situation is nothing new and there is evidence of the use of Pb very early, long before the time of the Roman Empire. Pb is one of the

seven metals of ancient times; it was present in all ages of the metal and played a significant role in the progress of humanity [25].

2.2. Use of lead

Lead is currently used for Pb-acid storage batteries, for construction purposes (as sheets and pipes), for cable sheathing, radiation shielding, in alloys and other minor applications. Pb compounds are also present in batteries, PVC additives, pigments and other paint additives, glass, glazes and enamels and functional ceramics. Leaded glass is also used to protect people from the danger of radiation from X- ray equipment or radioactive materials in hospitals, laboratories and nuclear power stations. The incorporation of Pb compounds into PVC gives good stability to heat and UV light, good electrical and mechanical properties and good processing behavior. These reasons make Pb salts, for example, Pb (II) carbonate and a tribasic lead sulfate $3\text{PbO} \cdot \text{PbSO}_4 \cdot \text{H}_2\text{O}$ is the most cost- effective stabilizers and nowadays widely used for rigid PVC products [26].

2.3 Chemical and physical properties of lead

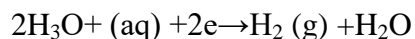
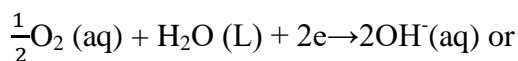
2.3.1. Physical properties

Lead has physical properties common to other metals: it has a metallic luster with shiny freshly cut surfaces, a high density, a low melting point, it is a conductor of electricity and heat and is soft, ductile and malleable [22]. The element has two common oxidation states, Pb(II) (electronic structure: $[\text{Xe}] 4f^{14} 5d^{10} 6s^2$) and Pb(IV) (electronic structure: $[\text{Xe}] 4f^{14} 5d^{10}$). The former dominates the inorganic chemistry of Pb while organolead chemistry is dominated by the latter [27].

2.3.2 Chemical properties

The element the freshly cut metal rapidly loses its metallic shiny luster in moist air due to the formation of a layer of Pb (II) oxide on the surface. The oxide can further react with carbon dioxide to form Pb (II) carbonate. Under normal conditions this surface layer protects the bulk of the metal against further attack. At high temperatures Pb also reacts with sulphur and the halogens. Even though pure deaerated water does not attack Pb, the element is oxidized by the joint action of oxygen and water to give Pb (II).

The metal dissolves: and the electrons are consumed in reactions such as



This corrosion reaction could produce dissolution in water, which in practice is very slow and is often controlled by the reaction products, Pb (II) salts. Such salts in general have low water solubility and can act as protective layers, a phenomenon that will be discussed later. The pH and the presence of other species can affect the solubility of these salts and therefore the solubility of Pb is conditioned by the exact composition of the water [28].

2.4 Sources of lead in our environment

Although Pb is ubiquitous in the environment of industrialized nations, the contribution of natural sources of Pb (II) to concentrations in the environment is low compared to the contribution of human activities. As a result of human activities such as mining, smelting, refining, manufacturing and recycling, Pb ends up in air, water and surface soil. Manufactured products containing Pb (petrol, paint, printing ink, Pb water pipes, Pb-glazed pottery, Pb -welded boxes, battery housings, etc.). The source of greatest concern is old housing, specifically houses once painted with products containing Pb as a pigment [29]. Sanding, scraping or heating painted doors, windows, stairs or fences can release leaded dust into the air, where children and adults may breathe it in. Even suction, sweeping or walking can circulate dust, which eventually collects on the floor where it is accessible to infants and toddlers who engage in hand-mouth activities. Pb -based dust from paint deterioration may also be found in the ground around older houses. A second major source of Pb exposure is from soil contaminated with Pb tailings from leaded gasoline and industrial processes. For many years, Pb smelters, battery manufacturing facilities and automobiles have released hazardous levels of Pb emissions to air and soil. [30].

2.5 Toxicity of lead

Lead is one of the most harmful substances for human health. The Pb (II) cation inhibits biosynthesis and affects the kidneys, brain cells and the permeability of the hepatic membrane, reducing some of its functions. It can collect in the body and cause problems such as anausea,

vomiting, diarrhea, coma and death. Pollution Pb has affected quality of life and can lead to serious health problems for people and animals [31].

2.6 Dispersive liquid-liquid micro-extraction

DLLME is a new and powerful pre-concentration technology that utilizes a ternary solvent system. It has received special attention because of its simplicity, low cost, high recovery, high enrichment factors (EFs), and short extraction time. DLLME was used to determine a wide range of organic and inorganic compounds in various matrices, including clinical, forensic, food and environmental samples [32]. In this technique dispersion of the extractant is achieved by the addition of a third solvent miscible with both phases (the aqueous and the organic phase), known as dispersant or disperser solvent. When the appropriate mixture of extraction and disperser solvent is rapidly injected into a sample, a high turbulence is produced, which causes the formation of small droplets dispersed into the sample. Once this “cloudy solution” is formed, surface area between the extractant and the sample is larger and, thus, the extraction is faster. After extraction, samples are centrifuged in order to separate the two phases. Originally, this micro extraction technique is performed using extraction solvents with higher density than water, such as chlorobenzene, chloroform, tetra chloromethane, tetra chloroethylene and carbon disulfide, and thus, after centrifugation, a sedimented phase is obtained at the bottom of the vial. Typically, the re-formed organic drop is collected with the aid of a micro syringe and led to the analytical instrumentation [33].

2.7 DLLME development and application

The first application of DLLME was performed by Rezaee et al for extraction and preconcentration of polycyclic aromatic hydrocarbons (PAHs) in water samples (surface water, river water, and well water) and their determination by gas chromatography (GC)–flame ionization detection (FID). A mixture of 1.0 mL of acetone (as the dispersed solvent) containing 8.0 μL of C_2Cl_4 (as the extraction solvent) was rapidly injected into 5.0 mL of the sample solution, and the mixture was gently shaken. Then, the cloudy solution (water, acetone, and tetra chloro ethylene) was centrifuged, and 2.0 μL of the sedimented phase was injected into the gas chromatograph for analysis. The technique had a good linear range (approximately 104) and high EFs (603–1,113) and low detection limits (0.007–0.030 $\mu\text{g/L}$) for determination of PAHs. The

results also demonstrated that the method was practically applicable for the determination of the analyte in real water samples. Generally, DLLME is a preferred choice when analyzing samples with a single matrix. After extraction, the samples are centrifuged so that the two phases are separated. However, in recent years, the most reported applications of DLLME have focused on more complex matrices such as wine, fruit and juice, honey, milk, wastewater, and pharmaceutical and biological samples (tablets, urine, plasma, saliva, and sweat). Selected recent DLLME applications for extraction and pre-stressing of organic compounds from different samples [34].

2.8 Principle of DLLME

DLLME is a miniaturized kind of (liquid–liquid extraction) LLE in which micro liter volumes of extraction solvent are used. An appropriate mixture of the extraction solvent and the disperser solvent with high miscibility in both organic and aqueous phases is rapidly injected into the aqueous solution of sample and a cloudy solution is then formed as a result of the formation of fine droplets of the extraction solvent which disperse in the sample solution. The cloudy solution is centrifuged and the fine droplets have settled at the bottom of the conical test tube. The analytes are extracted from the initial solution and concentrated to a small volume of the sediment phase, and the determination of the analytes in the settled phase can then be performed by the conventional a becomes very large due to the formation of cloudy solution, therefore, the equilibrium state is achieved quickly and therefore, the extraction time is very short analytical techniques. In fact, the surface area between the extracting solvent and the aqueous sample becomes very large due to the formation of cloudy solution, therefore, the equilibrium state is achieved quickly and therefore, the extraction time is very short [35]. Figure 2 shows DLLME procedure for the extraction the analyte after addition of a chelating agent into sample solution [36]

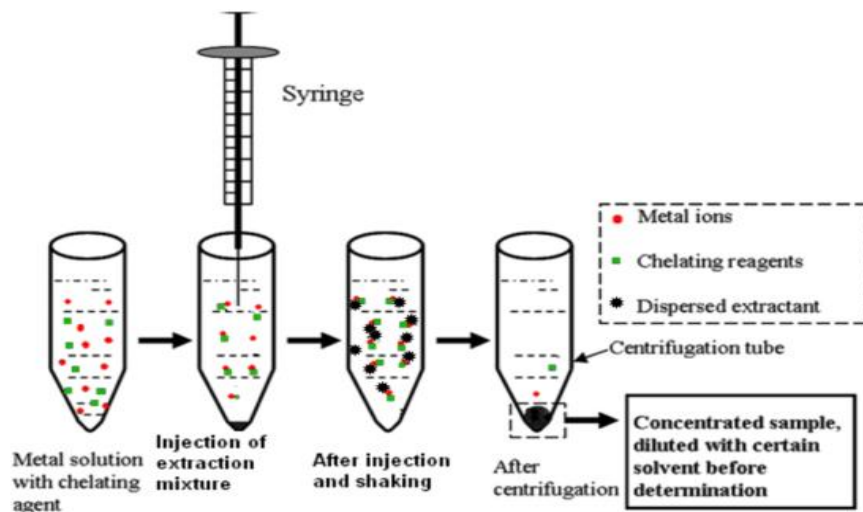


Figure 2: DLLME Procedure for the analyte after addition of a chelating agent [36]

2.9 Spectrophotometric determination of lead

The most important method used to perform the spectrophotometric determination of Pb is the dithizone method. The dithizone (diphenylthiocarbazone) H_2Dz presents a high capacity to form metallic complexes acting as a monobasic acid [38, 37].

2.10. Sample preparation method

Before use, all the water samples were filtered through $0.45\mu m$ micro-pore membranes and stored in brown glass containers under low temperature. Environmental samples for total Pb determination in water samples, it is normal to acidify the sample in order to prevent losses by adsorption on the walls of the sample vessel. Using properly cleaned containers, an acidified natural water sample may be stored at $4^\circ C$, in the dark, for up to 3 months without any measurable changes in the distribution of Pb species [40]. An unresolved question is whether or not the water sample must be filtered. If the sample is not filtered, changes in the distribution of Pb can occur due to adsorption and desorption processes at particle surfaces; moreover the risk of sampling error is increased, due to inhomogeneity in the distribution of particles in the water column. To avoid changes during filtration, the use of bubbling nitrogen through the sample solution in the filter holder unit has been recommended.

3. MATERIALS AND METHODES

3.1. Chemicals and Reagents

Different analytical and reagent grade chemicals and reagents will be used during the research work. These chemicals include Lead nitrate $Pb(NO_3)_2$, Dithizone $(C_{13}H_{12}N_4S)$ were purchased from Beijing chemical reagents company (Beijing china),chloroform, $(CHCl_3)$,Dichloromethane, (CH_2Cl_2) ,tetra chloromethane, (CCl_4) ,Acetonitrile $(C_2H_3N,(99.8\%))$ were purchased from Blulux laboratories LTD ,methanol $(CH_3OH(99.9\%))$,ethanol $(C_2H_5OH,(99.9\%))$ and acetone $(CH_3)_2CO$ were purchased from Sigma Aldrich (Germany),Ammonia solution $(NH_4(25\%w/w))$,merk Germany, Hydrochloric acid $(HCl (37\%))$,obtained from Sigma Aldrich. Dimethyl sulfoxide (DMSO) and Nitric acid $(HNO_3 (65\%))$ were purchased from (Blulux laboratories, LTD). Double distilled water were used in this study during the experimental studies.

3.2 Instruments and Apparatus

Double beam UV-VIS spectrophotometer (Analytic Jena AG Aspect - 1.2.3.617), pH meter, centrifuge machine (1790xg, 4000r/min), falcon tube, weight balance (Kern, ABJ- 220NM, Germany), medical syringe, quartz cuvette (1cm) micropipette, filter paper (whatman® no.542, 110mm, England), and other classical laboratory glassware's and apparatus will be used during the study.

3.3. Sample collection

For application of the optimized procedure in water samples, including tap, bottled water, river and well water samples have been examined to study the effects of sample matrix on the extraction efficiency. Tap water sample was freshly collected from our laboratory, drinking bottled water sample available at the supermarket packed in polymeric container, river water collected from Awitu River, and well water were purposively collected from Jimma city or its vicinity in polyethylene containers. All water samples were stored in glass bottles after filtering through $0.45\mu m$ membrane filter and were kept in the refrigerator at $4^\circ C$ until their analysis.

3.4 Standard and working solution preparation

Stock standard solutions of Pb (II) and dithizone both with a concentration of 1000 mg/L were obtained by will be prepared separately by dissolving appropriate amount of $\text{Pb}(\text{NO}_3)_2$ and dithizone in double distilled water and in ethanol respectively. The stock solutions will be kept in refrigerator when not used. Working standard solutions are then daily prepared by diluting the stock solution.

3.4. DLLME Extraction procedure

DLLME was performed as follows (the optimized conditions): First the PH of the sample was adjusted, at PH 9 then 10.0 mL water sample was placed in 15 mL screw cap polypropylene centrifugation tube. The sample was spiked with the 1 mg/L analyte with the appropriate amount of dithizone reagent its chelating ratio 2:1 and a mixture of 200.0 μL chloroform (CHCl_3) and 800.0 μL Acetone was rapidly injected into the aqueous solution using 1.0 mL micropipette. Immediately after the addition of the extraction mixture, the clear sample solution turned cloudy due to the formation of tiny dispersed droplets of the extraction solvents then 0.3 g NaCl (3%, *w/v*) was added and shaken manually until dissolved. In this step, the metal Pb ion was extracted into the fine droplets of chloroform (CHCl_3). The sample was centrifuged for 3 min at 3000 rpm to enhance phase separation and it formed red color phase at the bottom of the tube. The organic phase chloroform (CHCl_3), being of higher specific gravity than water, was sediment at the bottom of the tube. The sedimented organic phase formed at the bottom was carefully withdrawn using syringe into a quartz cuvette for subsequent analysis. The extraction solvent, chloroform and acetone mixture was used as blank reference during UV-Vis spectrophotometer absorption measurement.

3.6 Optimization parameters

DLLME procedure will be optimized for the extraction of the target metal as its dithizone complex. Organic solvents that have a higher density than water, including dichloromethane, chloroform and tetra chloromethane will be used as a potential extraction solvent. Disperser solvents such as acetonitrile, methanol, acetone, dimethyl sulfoxide and ethanol will be employed as candidates. Various parameters influencing the extraction and preconcentration

efficiency of the method and parameters affecting parameters which include the type and volume of extraction solvent, type and volume of dispersing solvent, extraction time, pH centrifuge time, and salt addition were optimized.

3.7 Method of Validation

Calibration curve construction (determination coefficient, R^2), limit of detection (LOD), limit of quantification (LOQ), intra-day (repeatability) and inter-day (within-lab reproducibility) precisions, and relative recovering (RR %) followed by real sample analysis were used to evaluate the optimum parameters throughout the study.

Limit of detection (LOD): -is the lowest concentration or mass of analyte in a sample that can be detected with a specified level of confidence but not necessarily quantified, under stated condition of the test

Limit of quantification (LOQ): -is the lowest amount of analyte in a sample which can be quantitatively determined with a suitable precision and accuracy

4. RESULT AND DISCUSSION

Many parameters of this approach were considered and optimized, including the enrichment factor (EF). In order to obtain a high-enrichment factor, the effect of different parameters, influencing the complex formation and the extraction conditions, were optimized. These parameters were the type and the volume of the extraction solvent, the type and the volume of the disperser solvent, pH, salt effect and centrifugal time. Finally, these optimal conditions were applied to extract and detect Pb in the real water samples. Enrichment factor of the method was calculated as

$$EF = \frac{C_{sed}}{C_o} \dots\dots\dots (1)$$

Where: C_{sed} is the concentration of the analyte in the sedimented phase and C_o is the concentration of analyte in the sample solution

4.1 UV-Vis absorption spectra of Lead-dithizonate

For maximum absorbance, the UV-Vis absorption spectra of the Lead-dithizonate complex after extraction was recorded in the spectral range of 500 to 550 nm and the observed color of the complex is red color at room temperature. Different literature shows the maximum absorbance of lead-dithizonate complex is usually obtained in between 500 -550 nm [40]. As can be observed from Figure 3 the maximum absorbance of the observed sedimented phase lead-dithizonate complex in chloroform was obtained at 530 nm and thus selected for further experiments.

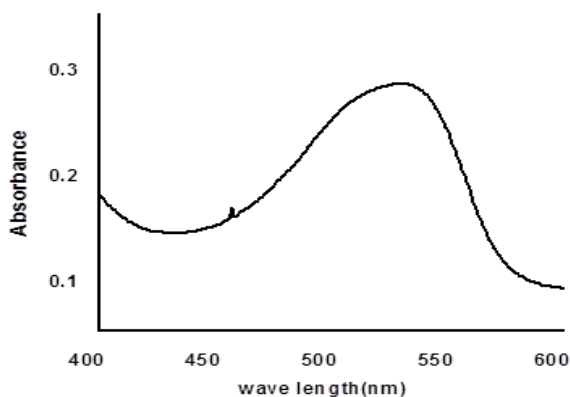


Figure 3: UV-Vis absorption spectra of experimental lead-dithizonate complex

4.2 Optimization of DLLME procedure

Efficiency of DLLME is affected by several parameters which include the type and volume of extraction solvent, type and volume of dispersing solvent, extraction time, pH centrifuge time, and salt addition. All experiments were performed, at least, triplicates.

4.2.1 Effect of the type of Extraction Solvent

Extraction solvent used in DLLME should fulfill several criteria such as: Very low solubility in water, high extraction capability of target analytes, and, higher density than water [41]. In the present work, three organic solvents were tested: carbon tetrachloride, chloroform, and dichloromethane. Thus, the best extraction solvents have been selected good spectrum capability. As can be observed from Figure 4, the highest average absorbance were observed in chloroform solvents so it selected for further experiments.

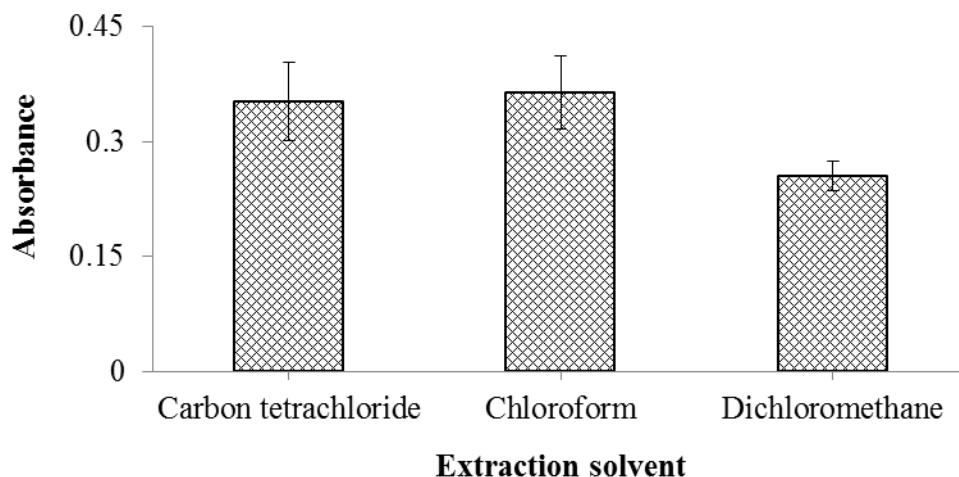


Figure 4: The effect of the type's extraction solvent. Condition: Extraction solvent volume, 300 μL Concentration of Pb, 1 mg/L; volume of acetone, 800 μL ; Sample pH 9; Centrifugation time, 3 min (3000 rpm); and NaCl, 0.3 g.

4.2.2 Effect of the type of the dispersant solvent

Dispersion plays a great role in DLLME. The main criterion for selection of the dispersed solvent is its miscibility in the extraction solvent and aqueous sample [47]. For this purpose, different solvents such as acetonitrile, acetone, methanol, dimethylsulfoxide, and ethanol were tested for their potential use in DLLME as dispersants. As can be seen from Figure 5, acetone had the highest average absorbance comparing others that has higher dispersing efficiency and selected for further experimental study.

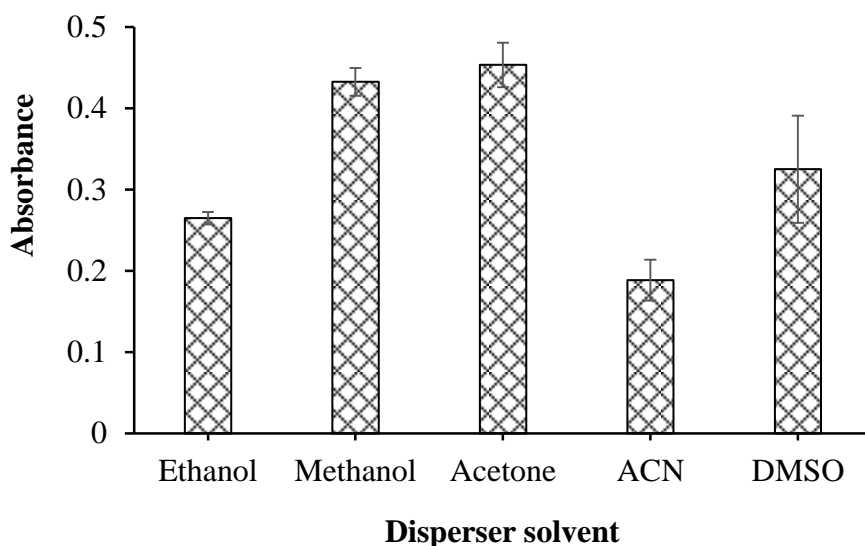


Figure 5: Effect of disperser solvent type. Condition: Similar to Figure 4.

4.2.3 Effect of the volume of extraction solvent

The effect of the volume of chloroform on extraction efficiency was studied in the range 100–600 μL . When the volume chloroform was increased, the average absorbance of the ions increased until 200 μL . In contrast, by further increasing the volume of chloroform, the average absorbance and phase separation of the ions decreased because of the dilution effect [42]. Therefore, 200 μL chloroform was chosen as the optimum extraction solvent volume. The results are shown in Figure 6.

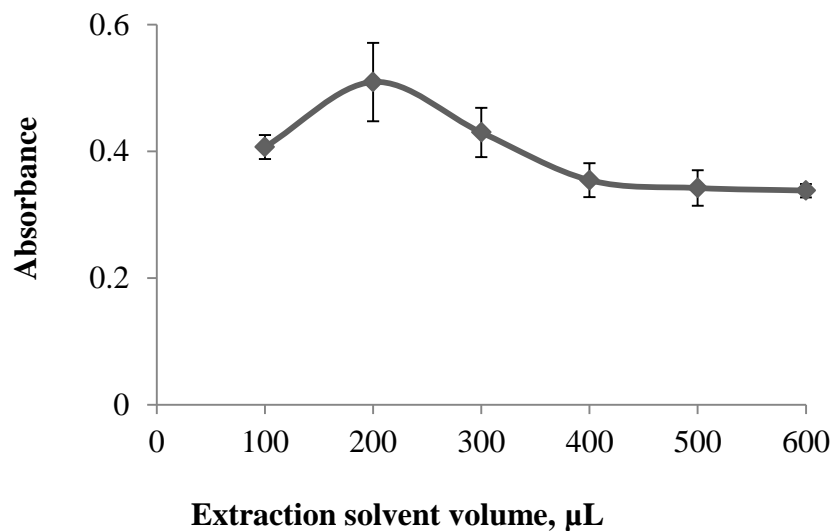


Figure 6: Effect of the extraction solvent volume. Condition: Similar to Figure 4, except for the volume extraction solvent.

4.2.4 Effect of the volume of disperser solvent

The effect of the volume of dispersed solvent on the extraction efficiency was studied in the range 200–1200 μL and the results are shown in Figure 7. At lower volumes of acetone, the average absorbance of these ions was low because the cloudy state was not well formed, resulting in a decrease in absorbance. At higher volumes of acetone, the average absorbance of the ions was low because the solubility of their complexes in the aqueous samples increased. Based on these results, the highest average absorbance was obtained at 800 μL acetone. Therefore, 800 μL acetone was selected as the volume of disperser solvent for achieving better and a more stable cloudy solution. The results are shown in Figure 7.

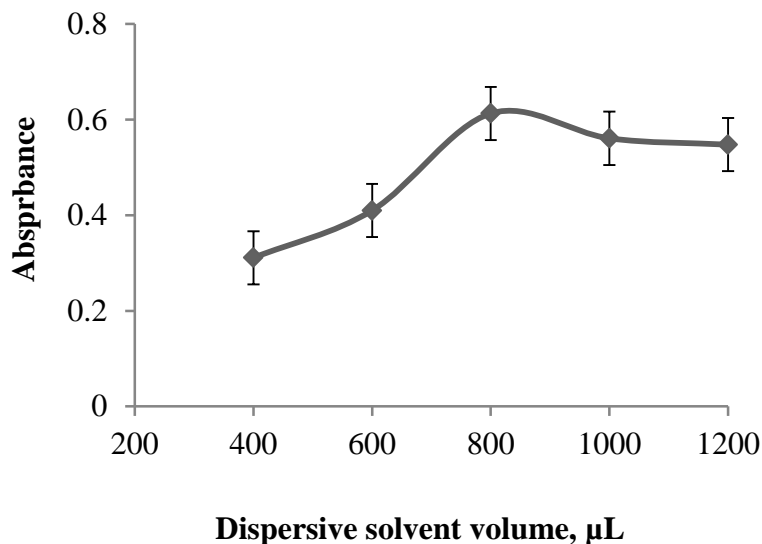


Figure 7: Effect of the disperser solvent volume. Condition: Extraction solvent volume, 200 μL ; Disperser solvent volume, different and other parameters are similar to Figure 4.

4.2.5 Influence of pH

The pH of the aqueous phase has a special role in the extraction efficiency because it affects the metal/chelate formation and its subsequent extraction. The effect of the pH on the complex formation and extraction efficiency was investigated in the pH range 6–11 using hydrochloric acid or ammonium hydroxide. As shown in Figure results, the effect of pH on the extraction efficiency of the metal ions was significant. The highest absorbance was obtained at a pH of 9; hence, this pH level was selected for subsequent work and real-water sample analysis. The results are shown in Figure 8.

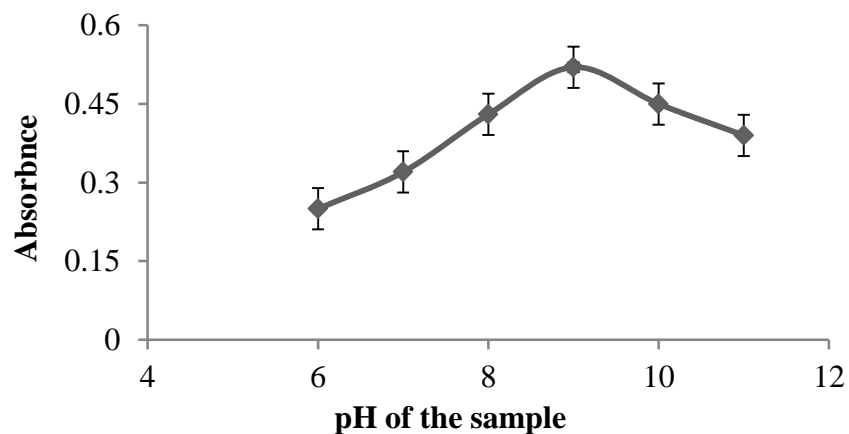


Figure 8: Effect sample pH. Condition: Disperser solvent volume, 800 μL and other parameters are similar to Figure 7.

4.2.6 Effect of salt

In the conventional LLE, the addition of salt may improve the analyte's partition to the organic phase or diminish the solubility of the organic solvent in water phase due to the salting-out effect [43]. Salt concentration was tested in the range of 0–20% (w/v). The results showed that the extraction efficiency of DLLME increased with increasing NaCl concentration up to 3% (w/v), beyond which, the method extraction efficiency, surprisingly, decreased. In this experiment 3% of salt were selected for extraction of lead from water. The results are shown in Figure 9.

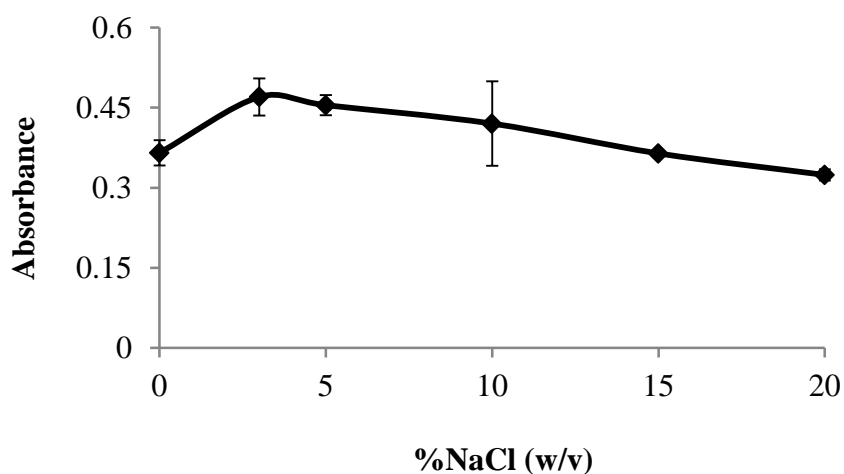


Figure 9: Effect of salt. Condition: Sample pH 9 and other parameters are similar to Figure 8.

4.2.7 Effect of centrifugal time.

Centrifugation is an important parameter in the DLLME method to separate the extraction phase from the aqueous phase [44]. The centrifugation time in the range of (1 - 15 min) with 3 min interval) at maximum speed of 3,000 rpm was used to obtain the highest separation extent. The centrifugal time experimental result was shown in Figure 10 and displayed that the surface area between the extraction solvent droplet and sample was very large. The results are shown in Figure 10 below

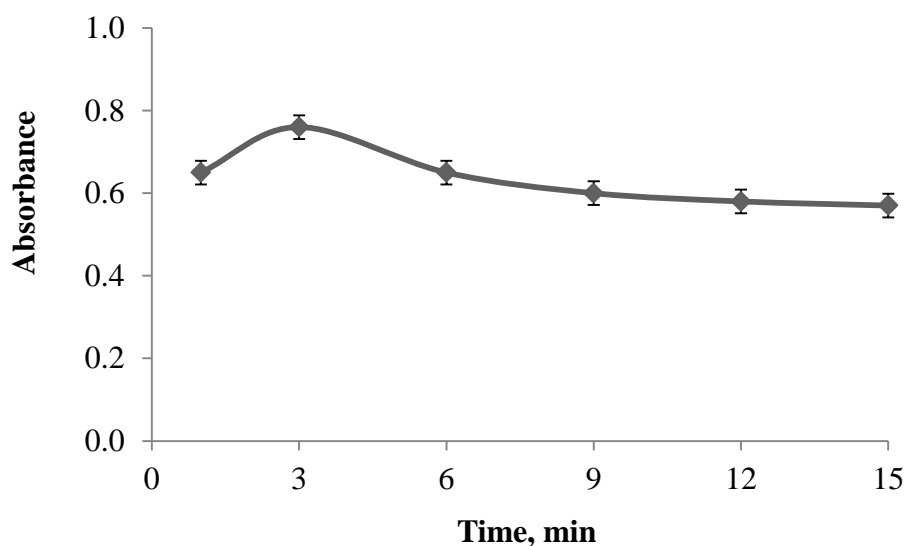


Figure 10: Effect of centrifugation time. Condition: Salt amount, 3% (w/v) & other parameters are similar to Figure 9.

As can be seen from the figure, the transfer of target analyte from aqueous phase to organic phase was fast and the highest absorbance of the target analyte was obtained at 3 min. After three minutes the absorbance became slightly decreased. Considering this result, 3 min was selected as optimal centrifugation time.

4.3 Method of validation

4.3.1 Calibration curve construction

The DLLME method was validated by using a series of 15 mL falcon tube, increasing volumes of working Pb (II) solution are transferred to cover the range of 0.005-2 mg/L Pb (II) levels (blank, 0.005 mg/L, 0.25 mg/L, 0.5 mg/L, 0.75 mg/L, 1 mg/L 1.25 mg/L, 1.5mg/L, and 2 mg/L) by using the developed optimum conditions in 10 mL of sample pH 9 each concentration level was extracted in duplicate (experimental replicates) and each extract was then detected in duplicate (instrumental replicates). Then, the calibration curves were obtained by plotting concentration against corresponding absorbance (Figure 11). The obtained results showed that the curve has wide linearity range, with coefficient of determination (R^2) of 0.9952, which is satisfactory for quantitative analysis of the analyte.

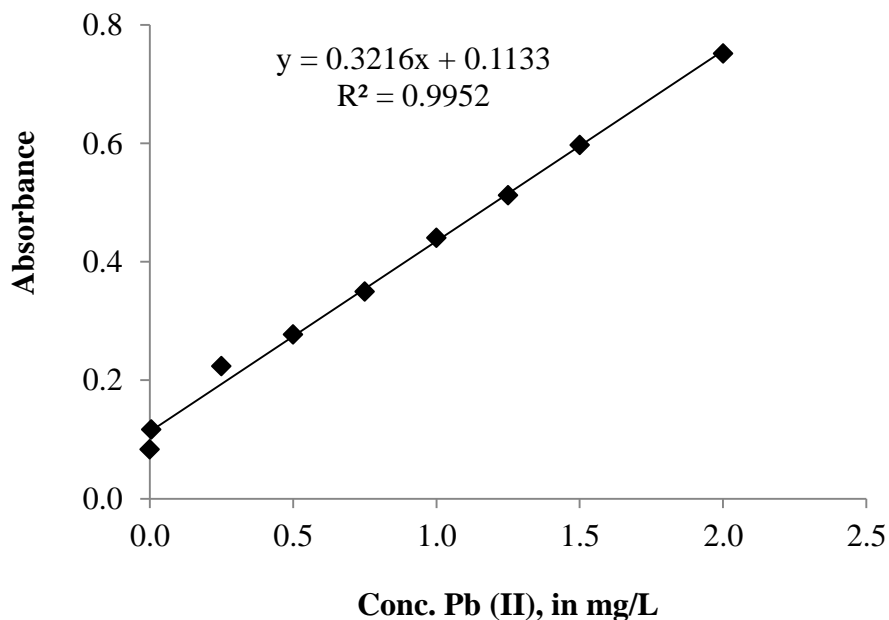


Figure 11: Calibration curve for lead, Pb (II)

4.3.2 Limit of detection and Limit of Quantification

The limits of detection (LOD) and quantification (LOQ) of the method were calculated using 3 and 10 times the ratio of SD of the blank to slope the calibration curve, respectively. In this

study, the values of LOD and LOQ were obtained 0.003 and 0.009 respectively. Details of the figures of the merits of the proposed method are presented in Table 1.

Table 1: Figure of merits of the proposed DLLME method

LDR (mg/L)	r^2	LOD (mg/L)	LOQ (mg/L)	EF
0.005–2.00	0.995	0.003	0.009	14

4.3.3 Precision study

The precision of the proposed DLLME method was investigated in terms of intra-day precision (repeatability) and inter-day precision (within-lab reproducibility). Intra-day precision was studied by extracting the spiked Pb samples at two different concentration levels (0.5 and 1.250 mg/L) and each concentration level was extracted in the duplicates and each extract also was detected in duplicate on the same day (in early morning and evening). Under the same experimental conditions, inter-day precision of the method was also evaluated at both concentration levels earlier used for intra-day precision studies, for five consecutive days with the same time interval. The relative standard deviation (RSD) of the Intra-day (repeatability, n = 8) and inter-day (within-lab reproducibility, n = 10) precisions of the proposed method are shown in Table 2.

Table 2: Intra- and inter-day precisions (RSD) of the proposed method

Analyte	Intra-day		Inter-day	
	0.5 mg/L	1.25 mg/L	0.5 mg/L	1.25 mg/L
Pb ²⁺				
RSD, %	1.8	2.2	1.5	2.6

The %RSD of both intra and inter-day precisions at both concentration levels were below 3%, which indicates the developed method has acceptable precisions for the analysis of the target analyte.

4.3.4 Analysis of real sample and recovery studies

The developed DLLME method was evaluated and applicable by performing relative recovery studies utilizing analysis of the river, tap, bottled, and well water samples. Relative recovery (%RR) studies were performed by spiking at two concentration levels earlier employed for precision studies. At each of the concentration levels, four samples were extracted using the proposed method and each extract were then detected in duplicates. For each water sample, two blank samples (unspiked) were also extracted and analyzed by the method.

The %RR of the analyte was determined by comparing the absorbance of the target analyte extracted from the spiked water sample with unspiked sample. The %RR of the target analyte for each sample are shown in Table 3. The relative recovery (%RR) of the sample was calculated by;

$$\%RR = \frac{\text{Spiked conc} - \text{nonspiked conc}}{\text{conc of standard}} \times 100$$

Table 3: Relative recoveries (%RR) of the method

Sample	Spiked Conc. (mg/L)	%RR	Conc of Pb (II) found in (mg/L)
Tap water	0.5	89.0 ± 4.7	0.014
	1.25	107.0 ± 3.4	
River water	0.5	99.0 ± 4.8	0.04
	1.25	95.1 ± 3.7	
Well water	0.5	79.7 ± 1.7	0.03
	1.25	87.8 ± 3.7	
Bottled water	0.5	108.1 ± 6.0	0.008
	1.25	97.9 ± 0.6	

The observed %RR of the target analyte were in the range of 79.7 – 108.10%, which briefly indicates the proposed method has acceptable relative recoveries for the analysis of Pb from the above different water samples. It was also observed that the contents of Pb in the given water sample except bottled water all are above permissible limits of WHO recommended in drinking water.

4.3.5 Effect of Interfering Ions.

To investigate the effect of coexisting ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Fe^{3+} , Cu^{2+} , Cr^{3+} , Cd^{2+} , NO_3^- , SO_4^{2-} , CO_3^{2-} , and Cl^- different concentrations ranging from 0.05 – 1.2 mg/L were added to a sample containing 0.05 mg/L lead ions. The findings of the study demonstrated that the proposed method has good tolerance towards the studied co-existing ions. From the studied ions Fe^{3+} , Cu^{2+} , Cd^{2+} , SO_4^{2-} and CO_3^{2-} ions are changed the absorbance of the target analyte complex at high concentration with in $\pm 5\%$ [45]. The results obtained are summarized in Table 4.

Table 4: Tolerance limits of interfering ions for the determination of 0.05 mg/L Pb^{2+}

Divers ion	Conc. (mg/L)	Ion: Pb (II) ratio	Recovery (%)
Na^+	1.2	1:24	96
Mg^{2+}	1.2	1:24	98
K^+	1.2	1:24	96
Ca^{2+}	1.2	1:24	95
Fe^{3+}	1.0	1:20	81
Cu^{2+}	1.0	1:20	98
Cr^{3+}	1.2	1:24	104
Cd^{2+}	0.8	1:18	104
Cl^-	1.2	1:24	103
SO_4^{2-}	0.8	1:18	98
NO_3^-	1.2	1:24	98
CO_3^{2-}	1.0	1:20	95

4.3.6 Comparison of the proposed method with other methods

The extraction efficiency of the proposed DLLME method has been compared with related analytical techniques reported in the literatures employed for the extraction of Pb from water sample. The comparison was made in terms of the calibration curve (linear), precision (% RSD), limit of detection (LOD) and solvent type have been used and analytical method was shown below in table 5.

Table 5: Comparison of the proposed DLLME with other methods

Analytical methods	Extraction solvent	LDR ($\mu\text{g/L}$)	R^2	LOD (mg/L)	Precision, RSD	Reference
UA-DLLME- FAAS	1-dodecanol	10-500	0.994	0.002	< 4.0	[46]
DLLME- ETAAS	CCl_4	3- 60	0.9986	0.001	< 6.0	[47]
DLLME- FAAS	CCl_4	0.1- 10	0.9995	0.011	< 4.0	[48]
DLLME- GF AAS	CCl_4	25-75	0.995	0.0002	< 6.0	[49]
DLLME-UV/Vis	CHCl_3	5-2000	0.9952	0.003	< 3.0	This work

As it can be seen from Table 5, it was noted that the method developed in this study has provided better linearity, similar or better LOD and comparable precision relative recovery (% RR and % RSD) cited for comparison and less expensive analytical method have been used than the other methods. The technique also uses conventional laboratory equipment as well as less toxic organic solvents (chloroform) that can be found in common research laboratories. Based on the experimental results, the DLLME methodology developed can be considered one of the preferred alternatives for determining Pb in water.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this work, was proposed for the extraction and/or pre-concentration of Pb in the water before its determination by UV-Vis spectrophotometry. The accuracy of the method expressed by the percentage recoveries calculated from the spiking experiments of different environmental water samples gave analytically acceptable results showing that the various matrices found in environmental waters have insignificant effect on applicability of the developed method. Various optimization parameters influencing the extraction efficiency of the methods have been carefully studied and optimal conditions established. In conclusion, the obtained results demonstrated that the developed method was fully trusted best sample preparation method due to its low cost, simple, rapid as well as its pronounced high enrichment factors for sample extraction, preconcentration and determination of the target analyte Pb from water sample and other related matrices.

5.2 Recommendation

Based on the finding of this study the analyst recommended that:

- ✓ The developments of DLLME method for determining other inorganic analytes.
- ✓ The developed method could be used as alternative method for monitoring the level of Pb in water and other similar matrices.
- ✓ Other more environmentally safe solvents could be investigated to replace the proposed extraction solvent, to totally avoid the use of such toxic organic even at microliter level.

6. REFERENCES

- [1] Ahmad, A.; Ayoob, R.; Ali, E.; Zahra, R.; Ahmad, H. B.; Mahdi, F. Solidified floating organic drop microextraction for pre-concentration and trace monitoring of cadmium ions in environmental food and water samples. *Journal of Iranian Chemical Society*. **2017**, 14, 1725–173.
- [2] Master, Z.; Sturgeon, R. Sample preparation for trace element analysis, 1st Ed.; Professor D. Barcelo.; Barcelona, Spain. **2003**.
- [3] Belay, A. A. Impacts of Chromium from tannery effluent and evaluation of alternative treatment options. *Journal of Environmental Protection*. **2010**, 1, 53-58.
- [4] Park, E.J. Metal speciation, mixtures and environmental health impacts Doctoral dissertation, Harvard, T.H. Chan School of Public Health, **2015**.
- [5] Hamid, S.; Kaveh, S.; Hassen, Z. Determination of trace amount of lead in industrial and municipal effluent water samples based on dispersive liquid-liquid extraction. *Journal of Chemical Society*. **2014**, 58, 137-141.
- [6] Manju, M. Effects of heavy metals on human health. *International Journal of Research Granthaalayah*. **2015**, 35, 1-7.
- [7] Zang, X.H.; Wu, Q.H.; Zhang M.Y.; Xi, G.H.; Wang, Z. Developments of Dispersive Liquid-Liquid Micro Extraction Technique. *Journal of Analytical Chemistry*. **2009**, 37(2), 161-168.
- [8] Ablatif, W.; Anjum, A.; Jawed, A. *Journal of Lead Toxicity*. **2015**, 8, 55-64.
- [9] Liang, P.; Zhao, E.; Li, F. Dispersive liquid-liquid microextraction preconcentration of palladium in water samples and determination by graphite furnace atomic absorption spectrometry. *Talanta*. **2009**, 77, 1854–1857
- [10] Basset, J., Denney. Vogel's textbook of quantitative inorganic analysis; Longman, London: **1983**, 156-159.
- [11] Inglezakis, V.J.; Moustakas, K. Household hazardous waste management. *Journal of Environmental Management*. **2015**, 150, 310-321.
- [12] Guilang, P.; Ying, L.; Qiang, H.; Danel, M.; Guangming, Z.; Junita, C. Dispersive Liquid-Liquid micro extraction using low toxic solvent for the determination of heavy metals in

- water samples by inductively coupled plasma-mass spectrometry. *Journal of AOAC International*, **2016**, 99, 1-7.
- [13] Nezhad, K.G.; Ahmed, M.; Dizajdizi, Z.B. Background corrected Dispersive Liquid–liquid micro extraction of cadmium combined with flame atomic absorption spectrometry. *Journal of The Brazilian Chemical Society* .**2011**,22,1-9.
- [14] Deng,Q.;Chen ,M.; Kong,L.; Zhao,X.;Guo, J.; Wen, X. Novel coupling of surfactant assisted emulsification dispersive liquid–liquid microextraction with spectrophotometric determination for ultra-trace nickel. *Spectrochim Acta Part A*. **2013**, 104, 64-69.
- [15] Zaijun, L.; Yuling, Y.; Jian,T.; Jiaomai, P. Spectrophotometric determination of trace lead in water after preconcentration using mercaptosephadex. *Talanta*. **2003**; 60(1):123-130.
- [16] Khan, H.; Jamludin, M.; Iqbal, B. A simple spectrophotometric method for the determination of trace level lead in biological samples in the presence of aqueous micellar solutions. *Journal of Spectroscopy*. **2006**, 20, 285-297.
- [17] Liang,P.;Sang, H. Determination of trace lead in biological and water samples with dispersive liquid–liquid microextraction preconcentration.*Anal and Biochem*.**2008**, 380, 21–25.
- [18] Nassef, M.; Hannigan, R.; Sayed, K.; Tahawy, M. Determination of some heavy metals in the environment of Sadat industrial city. Proceeding of the 2nd Environmental Physics Conference, Cairo University, Egypt. **2006**. 145-152.
- [19] Naithani,V.; Kakkar. P. Bulletin of Environmental Contamination and Toxicology. *Journal of Environmental Contamination and Toxicology* .**2005**, 75, 197-203.
- [20] JOSÉ, S. C.; JOSÉ, S. Lead Chemistry, Analytical Aspects, Environmental Impact and Health Effects. First Edition Amsterdam, the Netherlands the Boulevard, Langford Lane, Oxford, UK **2006**.
- [21] Sharafi, K. Trace determination of lead in lipsticks and hair dyes using microwave assisted dispersive liquid-liquid micro extraction and graphite furnace atomic absorption spectrometry. *Journal of Cosmetic Science*. **2016**, 37. 4-12
- [22] Farajzadeh, M.; Khoshmaram, L.; Sheykhizadeh, S. A review of application techniques for analysis of chemical compounds and metal ions in food stuffs. *Journal of analytical and bioanalytical chemistry research*. **2014**, 1, 1-19.

- [23] Ghadamali, B.; Mansur, A.; Hassan, S. Determination of copper (II) by flame atomic absorption spectrometry after its preconcentration by a highly selective and environmentally friendly dispersive liquid–liquid microextraction technique. *Journal of Analytical Science and Technology*. **2019**, 10, 1-6.
- [24] Shafa'atu, I.; Muazu, G. A.; Abdullahi, Z. Determination of Lead Concentrations in different water Sources collected From Sokoto Metropolis, Nigeria. *Journal of Applied Chemistry*. **2014**, 7, 53-56.
- [25]. Jiang, H.; Hu, B. Determination of trace Cd and pb in natural waters by direct single drop microextraction combined with electro atomic absorption spectrometry. *Microchim Acta*. **2008**, 161,101-107.
- [26] Deryoush, A.; Maryam, F.;Alimosta, F. Dispersive liquid-liquid micro extraction based on solidification floating organic drop trace amounts of lead in water sample prior to flame atomic absorption spectrometry determination. *Journal of the Chilean Chemical Society*. **2013**, 58, 1-7.
- [27] Lopez,G.;Vicente,M,Y.;Hernandez,C. Determination of lead and cadmium using an ionic liquid and dispersive liquid-liquid micro extraction followed by electrothermalatomic absorption spectrometry. *Talanta*. **2013**, 110, 46-52.
- [28] Mohammadi, V.K.; Mohammadi, A.; Hashemi, M.; Khaskar, R.; Haratian. Microwave-assisted extraction and dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry for isolation and determination of polycyclic aromatic hydrocarbons in smoked fish. *Journal of Chromatography A*, **2012**, 1237: 30 – 36
- [29] Soylak, M.; Yilmaz, E. Ionic liquid dispersive liquid–liquid microextraction of lead as pyrrolidine dithiocarbamate chelate prior to its flame atomic absorption spectrometric determination. *Desalination*. **2011**, 275, 297–301.
- [30] John, R.D. Extraction techniques in analytical sciences .John Wiley and sons; The Graduate School and School of Applied Sciences Northumbria University, Newcastle, UK. **2009**.
- [31] Mohammed, S.; Malihe, K. Recent developments in dispersive liquid-liquid microextraction. *Analytical and Bioanalytical Chemistry*. **2014**, 18 765-773.
- [32] Outeiral, P.J.; Millan, E.; Arrona, G.R. The use of dispersive liquid –liquid micro Extraction and UV-Vis spectrophotometry for the determination of cadmium in water samples. *Journal of Spectroscopy*. **2014**, 4, 1-8.

- [33] Sousa, R.; Homem, V.; Moreira, J.L.; Madeira, L.M.; Alves, A. Optimisation and application of dispersive liquid–liquid microextraction for simultaneous determination of carbamates and organophosphorus pesticides in waters. *Anal Methods*. **2013**; 11: 2736–2745.
- [34] Saidi, H.; Adel, A. The recent developments in dispersive liquid-liquid microextraction for preconcentration and determination of inorganic analyses. *Journal of Saudi Chemical Society*. **2014**, 18, 745-761.
- [35] Sandell, B. Article of Colorimetric Determination of Traces of Metals, Interscience, New York. **1945**, 49, 171-264.
- [36] Thomas, O.; Burgees, C. UV-Visible Spectrophotometry Water and Wastewater, 2nd ed.; Elsevier Science: London, UK, **2017**; p. 2.
- [37] James, S.M.; Bruno, M.S.; Tassia, S.S.; Paula ,D.; Paola, A.M.; Erico, M.M.; Flores, F. Determination of cadmium and lead on sub ppt level in soft drinks; an efficient combination and graphite furnace atomic absorption spectrometry. *Food Chemistry*. **2017**, 221, 907-912.
- [38] Qingxiang, Z.; Nazhao, G. Determination of lead in environmental waters with dispersive liquid-liquid microtomic extraction prior to atomic fluorescence spectrometry. *Journal of Hazardous Materials*. **2011**, 189, 48-53.
- [39] Massimiliano, R.R.; Diana, L.; Lima ,A.; Paula ,F.; Zélia, B. Optimization of a dispersive liquid–liquid microextraction method followed by UHPLC analysis for fluoxetine quantification in environmental water resources. *Journal of Separation Science*. **2018**, 41, 1-7.
- [40] Parveen, N.; Rohan, Y. Spectrophotometric determination of some environmental sample. *Journal of Environmental Research and Development*.**1983**, 6,156-159.
- [41] Habila, M.A.; Yilmaz, E.; Alothman, Z.A.; Soyala, M. Combination of dispersive liquid-liquid micro extraction and multivariate optimization for separation enrichment of traces lead by flame atomic absorption spectrometry. *Journal of Industrial and Engineering Chemistry*. **2016**, 37, 306-340.
- [42] Amelin, V.; Lavrukhin, D.; Tret yakov, A. Dispersive liquid-liquid microextraction for the determination of herbicides of urea derivatives family in natural waters by HPLC. *J. Anal Chem*. **2013**; 68, 822–830.

- [43] Hossen, H.; Abdorreza, M.; Marzih, K. Application and optimization of dispersive liquid-liquid micro extraction coupled with high performance liquid chromatography for the sensitive determination of furfural and hydroxyl methyl furfural in jarred and canned baby foods. *Journal of Nutrition and Food Science Research*. **2017**, 4, 25-32.
- [44] Faraji, H.; Helalizadeh, M. Lead Quantification in Urine Samples of Athletes by Coupling DLLME with UV-Vis Spectrophotometry. *Journal of Biological Trace Element Research*. **2017**, 176, 258–269.
- [45] Mohammad, T.N.; Mohammad, R.M.; Hossieni, Y.A.; Armin, K. Rapid determination of lead in water samples by dispersive liquid-liquid micro extraction coupled with electrothermal atomic absorption spectrometry. *Talanta*, **2008**, 75, 56-62.
- [46] Arpa, C.; Aridasir, I. A method for determination of lead ions in aqueous samples ultrasound-assisted dispersive liquid-liquid microextraction method based on solidification of floating organic drop and back extraction followed by FAAS. *Journal of Analytical Method*. **2018**, 1, 1-7.
- [47] Naseri, M.; Hosseini, M.; Assadi, Y.; Kiani, A. Dispersive liquid-liquid microextraction for the determination of lead by ETAAS. *Talanta*. **2008** 75, 1, 56–62.
- [48] Habila, M.; Alo, Z.; Yilmaz, E. Dispersive liquid- liquid microextraction of lead (II) as tropaeolin ooo chelates from environmental samples prior to micro sampling flame atomic absorption spectrometry. *Journal of Atomic Spectrometry*. **2018**, 39, 3-8.
- [49].Endale, T.; Bezuayehu, T.; Negussie, M. Trace level enrichment of lead from environmental water samples utilizing dispersive liquid-liquid microextraction and quantitative determination by graphite furnace atomic absorption spectrometry. *Journal of Environmental Science and Health* .**2014** 49, 833–842.