GREEN MICROEXTRACTION AND PRECONCENTRATION METHODS FOR HEAVY METAL DETERMINATION COUPLED WITH ATOMIC ABSORPTION SPECTROMETRY

PILAIPAN CHAIKHAN

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
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Thesis entitled "Green microextraction and preconcentration methods for heavy metal determination coupled with atomic absorption spectrometry"
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Title GREEN MICROEXTRACTION AND PRECONCENTRATION METHODS FOR HEAVY METAL DETERMINATION COUPLED WITH ATOMIC ABSORPTION SPECTROMETRY
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ABSTRACT

A novel preconcentration method based on hybrid air-assisted solvent terminated dispersive liquid-liquid microextraction (AA-ST-DLLME) using a domestic fish-tank pump as a bubble generator was developed for the determination of Pb in water and beverage samples using graphite furnace atomic absorption spectrometry (GFAAS). Termination or demulsification was a new approach for centrifuge-less separation in DLLME, based on the demulsification process for emulsion break down. In this approach, the extraction was terminated by the addition of a second portion of the surfactant that acted as a demulsifier and encouraged physical phase separation without centrifugation. The important parameters for the microextraction process (pH, concentration of complexing agent, type of extracting solvent, type of disperser, type of demulsifier, air-assisted time, and extraction time) were optimized. Under the optimized conditions, a sensitivity enrichment factor (EF) of 58.9 was achieved, with the preconcentration factor (PF) being 60.0 and the extraction efficiency of 98.2%. The limit of detection (LOD) and limit of quantitative (LOQ) were 0.025 ng/mL and 0.084 ng/mL, respectively. The relative standard deviation (RSD) was 1.02-3.19%. The proposed method was applied for the determination of trace levels of Pb in water, fruit juice, and beverage samples. The spiked recovery at three different spiked concentrations were 82.0-117.3%. Results from the analysis of standard reference materials (SLRS-6) were not significantly different at the 95% of confidence level.

In the second approach, novel method was proposed to determine the concentration of lead (Pb) in different type of water samples by preconcentration with magnetic dispersive solid phase extraction (MdSPE) using a graphene oxide-magnetite-dithizone (GO-Fe_3O_4-DTZ) sorbent made of synthesized graphene oxide from the used graphite tubes of electrothermal technique, separated by external magnetic field and analyzed with flame atomic absorption spectrometry (FAAS). The synthesized sorbent was evaluated for its surface property, functional group and surface morphology by Zeta potential, Fourier Transform Infrared Spectrophotometer (FTIR), and Scanning Electron Microscope (SEM), respectively. The relevant measurement parameters such as pH, extraction time, type and concentration of eluent, sample volume and reusability were optimized. Under the optimal conditions, preconcentration factor was 13.33. LOD and LOQ obtained were 0.070 and 0.23 mg/L, respectively. %RSD was 3.41% at 1.0 mg/L of Pb. For the accuracy study, percentage recovery values determined using spiked standard solutions in drinking, tap, and river water samples were 90.1-123%. In addition, the robustness of proposed method was reported in terms of the tolerance limit obtained from interference studies.

The third microextraction method involved effervescent tablet assisted-switchable solvent based liquid phase microextraction (EA-SS-LPME) for multi-element sequential determination of Pb and Cd in drinking water, canned tuna, and canned fruit samples by high resolution continuum source flame atomic absorption spectrometry (HR-CS-FAAS). $NaHCO_3$ and KH_2PO_4 were used as effervescent tablets for improving of extraction efficiency. Triethylamine (TEA) as a hydrophobic solvent was synthesized by addition of CO_2 for switching to protonated triethylamine carbonate (P-TEA-C) as a hydrophilic form. The synthesized switchable solvent was applied to extract Pb-dithizone and Cd-dithizone complexes in samples. Under the optimum condition, calibration linearities were obtained from 0.06 to 10.0 mg/L (Pb) and 0.02 to 1.50 mg/L (Cd). For the analytical performance, it was found that LOD was 0.0195 (Pb) and 0.0068 (Cd) mg/L, respectively. LOQ was 0.0649 mg/L (Pb) and 0.0228 mg/L (Cd), respectively. %RSDs were 1.25%-1.69% (Pb) and 1.07%-1.64% (Cd). EA-SS-LPME method was applied for the determination of Pb and Cd in water and canned food samples. The spiked recovery at different spiked concentrations were in the range 82.3-119.0% (Pb) and 81.7-120.0% (Cd). The preconcentration factor of proposed method was 3.3 with enrichment factor of 1.4 (Pb) and 2.6 (Cd) with the analysis time of 8 minutes per extraction.

The fourth approach involved a dispersive liquid-liquid microextraction based deep eutectic solvent with air-assisted technique (AA-DES-DLLME) have been developed and applied to the extraction and preconcentration of Pb and Cd using sequential multi-element determination by HR-CS-FAAS. In this method, mixed Pb and Cd solution were used for optimization and spiked in real samples for evaluation. First, pH 13 buffer and dithizone (DTZ) were added to sample. Pb-DTZ and Cd-DTZ were formed and dispersed by the addition of THF. Then, target complexes were extracted into organic phase by the addition of DES. Extraction efficiency was achieved by small air bubbles addition. This was a simple procedure for air-assisted technique using an inexpensive home fish tank pump. Under the optimum condition, Pb-AA-DES-DLLME were linear in the range 0.017-4.50 mg/L with LOD and LOQ at 0.0050 and 0.0168 mg/L, respectively. %RSD was in the range 1.49-2.43% with the enrichment factor of 2.60. For Cd-AA-DES-DLLME, the calibration curve was in the range 0.009-2.00 mg/L with LOD and LOQ at 0.0028 and 0.0094 mg/L, respectively. %RSDs were 1.39 to 4.19% with the enrichment factor of 3.08. Preconcentration factor was 6.67. Extraction recovery from the spiked standard solution to real samples were in the range (Pb) 84.20-114.6% and (Cd) 84.70-118.0%. AA-DES-DLLME method was applied for preconcentration and extraction of trace Pb and Cd in drinking water and canned food samples with 15 minutes per extraction by HR-CS-FAAS.

In the final work, the determination of Pb in anionic form via hollow fiber liquid phase microextraction (HFLPME) using an electric field with deep eutectic solvent (DES) carrier acceptor and GFAAS detection was firstly reported. Pb-oxyanion or hydrate form of Pb was used as a chemical form in the proposed method. Chemical forms of Pb in samples were converted to anion forms using alkali condition. Electro-assisted technique was applied to facilitate the anion passed thorough the supported liquid membrane (SLM) into the phase of DES mixed with buffer solution so called EM-DES-HFLPME. Therefore, effect of extraction parameters were carefully obtained and evaluated. Under optimum condition, linear range was observed in the range 0.0036-1.00 ng/mL. Limit of detection and limit of quantitation were 0.011 and 0.036 ng/mL, respectively. Precision defined as %RSD was 1.03-3.49%. Enrichment factor and preconcentration factor were 111 and 750, respectively. EM-DES-HFLPME was applied for the determination of Pb in water samples with the acceptable recoveries from 83.9 to 110.9%. The proposed method was successfully applied to the analysis of standard reference materials (SLRS-6) at which found concentration was not significantly different at 95% of confidence level from certified value.

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CHAPTER I

INTRODUCTION

1.1 Introduction

Heavy metals are naturally occurring elements that have specific gravity at least five times greater than water [1] such as cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), and mercury (Hg). All heavy metals are considerably differed in their chemical properties with highly toxic. Their toxicity depends on several factors including the species, route of exposure, and uptake level, as well as the age, gender, and genetics of exposed individuals. Environmentally, heavy metals widely use in covering industries, domestic, agricultural, medical, and technological. This has led to their wide distribution in the environment. Especially, Pb is the one of main raw materials in some industries such as mobile services, cosmetics, paints, batteries, gasoline, pipes, ceramics, and children's PVC toys. Pbs are contaminated in soil, water, honey, and juice by industrial process. Furthermore, Cd is a universal toxic heavy metal that is constantly released into the environment through human economic activities, causing significant harm to human health.

Pb and its associated compounds are also considered as one of the anthropogenic pollutants. Human beings generally are exposed to Pb through soils and dusts, contaminated air, water, food, and stuffs. In the case of drinking water, a major source of Pb contamination comes through the use of Pb-pipes that carry the water. Foods that are stored in containers which are coated with Pb-based paints also present yet another source of Pb contamination. Principally, the most of inorganic Pb species are found in orange and yellow colored paints [2]. Pb is the most systematic toxicity that affects many organs in the body including the liver, kidneys, central nervous system, endocrine system, hematopoietic system, and reproductive system. There are many published studies that one of the major mechanisms by which Pb utilizes, its toxic effect is inhibit the actions of calcium and to interact with proteins [1]. Pb may compete with essential metallic cations for binding sites, inhibiting enzyme activity, or altering the transport of essential cations such as calcium [3]. Pb contamination has been detected in drinking water, tap water (For drink) and surface water in Thailand, at levels above the maximum allowable concentration of 0.05 mg/L [4] set by the Pollution Control Department (PCD) of Thailand. In food products, Pb has been detected at levels higher than the maximum residue limit of 1.0 mg/kg [5] set by the Thailand Food and Drug Administration (FDA). European Union sets the maximum allowable concentration in fruit juices (or concentrated fruit juices as reconstituted and fruit nectars) at 0.05 mg/kg and 0.1 mg/kg for vegetables and fruit [6].

Cd is generally used in several industrial activities. The significant industrial utilizations of Cd include the production of alloys, stabilizers, pigments, cigarette, textiles, insecticides, solders, television sets and batteries [7]. The major routes of exposure to Cd are via inhalation and ingestion. Skin absorption is quite rare. Human being exposures to Cd are possible through the Cd contaminated workplace sources including employment in primary metal industries, eating contaminated food, and smoking cigarettes. Besides occupational exposure to Cd, the general population can be exposed environmentally. Environmental Cd contamination can be found in air, water, and sediment from industrial releasing. Additionally, Cd also presents in trace amounts in some food such as leafy vegetables, potatoes, grains, and seeds. The foodstuffs are rich Cd concentration such as liver, mushrooms, shellfish, mussels, cocoa powder, and dried seaweed [1]. Cd as original cause which damages to multiple organs and tissues such as the kidneys, liver, lungs, bones and brain, and leads to cells carcinogenesis in these organs. Pollution Control Department (PCD) of Thailand concerns about Cd poisoning and has recommended the Maximum Allowable Concentration at 0.005 mg/L or not have at all in drinking waters, 0.01 mg/L or not have at all for tap water (For drink), and 0.05 mg/L for surface water [4] along with World Health Organization (WHO) sets the guideline value for Cd to 3 μg/L in drinking water [8]. European Union sets the maximum allowable concentration in vegetables and fruit at 0.05 mg/kg [6].

Nowadays, it is well known that both Pb and Cd are found in environmental, food, stuff, and body at low level contamination. Moreover, components in these sample are very complex with high organic and inorganic matters. Preconcentration and separation are the priorities to evaluate for cleanup sample. In order to separate analytes from other matrixes, development of new preconcentration technique can completely increase the analytes concentration that are interesting research criteria. Previously, large number of publications reported the extraction method which can simultaneously preconcentrate and separate such as solid phase extraction (SPE) [9] or solid phase microextraction (SPME) [10], co-precipitation [11], cloud point extraction (CPE) [12], liquid-liquid extraction (LLE) [13] or liquid phase microextraction (LPME) [14], hollow fiber liquid phase microextraction (HF-LPME) [15], and dispersive liquid-liquid microextraction (DLLME) [16].

LPME is a basic method for exhaustive cleaning. LPME is a cost-effective and simple technique that uses a minimum solvent volume to extract the maximum amount of analyte. Differently, normal LPME is limited by type of extracting solvents that are high toxicity and quite low efficiency at microvolumes. However, researchers are continued to develop the interesting green methods for Pb extraction using novel extracting solvent types. For example, the accurate monitoring of Pb in water samples using a new amine based microextraction with N,N-dimethyl-n-octylamine as extraction solvent and chelating agent for Pb [14]. Green solvent as switchable-polarity solvents (SPS) is considered to be extracting solvent since 2010 by Samorì and coworker [17]. Switchable solvent is proposed to extract hydrocarbons from dried and water-suspended samples of the microalga *Botryo-coccus braunii*. Fundamentally, switchable solvent is defined as a system which a hydrophobic liquid can convert to a hydrophilic liquid under the existence of carbon dioxide and then change back to its hydrophobic form when the gas is purged from the solution [18]. The possibility use of carbon dioxide leads the method a low-cost and eco-efficient as well as the main advantage of switchable solvents is environmentally friendly which allow the extraction of the analytes in a homogeneous phase without disperser and easy phase separation without centrifugation.

DLLME is developed from liquid phase microextraction [19] to improve the extraction efficiency via the formation of small droplets of an organic extracting solvent in sample solution using a dispersive solvent which is thoroughly miscible in both aqueous and organic phases. The high contact surface with both the aqueous and organic phases causes the rapid and efficient extraction of target analytes. The disperser (dispersive solvent) acts as an essential solvent for the application of two-immiscible phases [20] in situations where organic solvents such as methanol [21], ethanol [22], and acetonitrile [23] would be used. In addition, one of the main advantages of the extracting solvent is that it fully retains the target analyte after extraction is completed. Hence, there is a great interest in DLLME among researchers and many developments in this area are continually being published. Importantly, there is a growing demand for the development of more environmentally friendly extraction. Nowadays, deep eutectic solvent (DES) begins a alternative green solvent type in some application such as the development of liquid phase microextraction used for Pb determination using DES in milk samples [24], using of DES with vortex assisted liquid-liquid microextraction for trace curcumin analysis in food and herbal tea samples [25], Applying of DES for three-phase hollow fiber liquid phase microextraction for extraction and preconcentration of steroidal hormones [26] and using of DES for ultrasound assisted dispersive liquid-liquid microextraction of arsenic speciation in water and environmental samples [27]. DES is defined as the mixture of at least two components. In the part of synthesis, eutectic mixtures are obtained by hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) which are able to self-associate to form a new eutectic phase (Melting point below 100 ºC) [28]. Some publication defines two or three components of HBA and HBD which mostly are the associating of hydrogen bonding, electrostatic force and Van der Waals interaction [29]. DES have been prepared using HBA such as choline chloride, quaternary ammonium salt (QAS: tetramethylammonium chloride, tetrabutylammonium chloride, tetraethylammonium chloride), *N*,*N*-diethyl-2-hydroxy ethanamidium chloride, methyltriphenylphosphonium bromide, *N*-benzyl-2-hydroxy-*N*,*N*-dimethyl ethan-amidium chloride and 2-Acetate-*N*,*N*-trimethyl- ethanamidium chloride and HBD such as urea, thiourea, acetamide, benzamide, glycerol, imidazole, malonic acid and glucose [28]. Generally, DES has the good characteristic in terms of water-compatibility, non-flammability, non-toxicity, biocompatible, biodegradable and environmentally friendly solvent [29]. Hence, the potential applications of DES are still interesting for LPME and DLLME. In addition, there have a wide range of advantages and efficiency. However, the LPME and DLLME procedures require long separation times [30] for the centrifugation of the dispersed solvent during phase separation, and these disadvantages have inspired research into new developments in the DLLME set-up that could simplify and speed up the extraction process. As mentioned earlier, the solvent-terminated (ST) technique or solvent-based demulsification (SD) [31] is a new approach for centrifuge-less extraction. Combined with DLLME, this then becomes solvent-terminated dispersive liquid-liquid microextraction (ST-DLLME). ST-DLLME is based on the de-emulsification (or demulsification) process [32] that proceeds with emulsion break down. In this approach, the extraction is ended by the addition of a second portion of the disperser that acts as a de-emulsifier (demulsifier or demulsifying agent) and encourages physical phase separation without centrifugation. The stability of small extracting droplets in the dispersed system depends on the nature of the emulsion interface, surface electrical charge, and Van der Waals forces [33].

HF-LPME is a mode of LPME that is designed to reduce the volume of extracting solvent and increase the extraction efficiency. HF-LPME uses a porous polypropylene (or polytetrafluoroethylene) hollow fiber for immobilization of organic solvent in the pores of hollow fiber as supported liquid membrane (SLM). Fundamentally, its porous of membrane are dipped with organic solvent. Thus, the organic channel inside the immobilized membrane is introduced and acts like permeable membrane. The lumen of the hollow fiber is filled with acceptor solution as acceptor phase and sample vial containing the sample is donor phase. During the extraction, analyte in the donor phase passes thorough the SLM to the acceptor via passive diffusion (High to low concentration). Generally, stirrer is applied in normal-HF-LPME to enhance the mass transfer. Differentiations of acceptor types in HF-LPME can be classified for two-phase and three-phase modes; (1) two-phase mode: the acceptor solution is organic solvent and (2) three-phase mode: the acceptor phase is an acidic or alkaline aqueous solution [26]. HF-LPME greatly presents in many developments for example the speciation of arsenic (III) and arsenic (V) in fresh waters and human hair extracts [27], determination of mercury traces in water samples [28], and analysis of steroidal hormones from biological fluid samples [21]. This method has high enrichment factor, high sensitivity and selectivity, analytical precision, low volume of consumed reagents, and reducing rate of wastes. However, the HF-LPME requires a long extraction time to reach the equilibrium and slow diffusion across the SLM. Therefore, the electromembrane extraction (EME) is developed to overcome the reduction of the long extraction time by applying the electric field that so called an electro-enhanced HF-LPME or electromembrane-based hollow fiber liquid phase microextraction (EM-HF-LPME). Electro-enhanced HF-LPME or EM-HFLPME also uses hollow fiber with SLM formation as a selective membrane. Mass transfer in this method is both of passive diffusion and electro-migration [29]. The method is relatively rapid because of the introduction of the electric field as well as sample clean-up is obtained due to the nature of the SLM and the electric field. Applications of EME or HF-LPME are widely used for preconcentration and separation of analytes in various samples. For example, determination of bismuth in human plasma and pharmaceutical samples [30], Cr in water samples [31] and environmental samples [32], acidic drugs in complex samples [33], Hg in water samples [34, 35], pyridine derivatives in urine [36], As in water samples [37] are published.

SPE is a branch of sample pretreatment technique combined with extraction, preconcentration and sample introduction in one step. The SPE has several advantages over liquid phase extraction: faster operation, easier handing, higher preconcentration factor and ready linkage to analytical instruments such as AAS, ICP-MS, HPLC, and spectrophotometry. SPE is done in four steps; (1) conditioning: preparation of sorbent as stationary phase, (2) loading: addition of sample solution, (3) washing: elimination of matrixes in sample, and (4) eluting: selection of analyte in sample. Developments in SPE are still ongoing, using a variety of adsorbents of different sizes to apply the method in several different chemical processes *e.g.*, the use of 5 -Phenylazo-8 - hydroxyquinoline (5Ph8HQ) as chelating resins [9], the application of octadecyl silica cartridge with 4-bpdb (1,4-bis(4-pyridyl)-2,3-diaza-1,3-butadiene) as a sorbent and ligand [38], the modification of the polypyrrole/carbon nanotube/1,10-phenanthroline as a composite sorbent [39] and the applicability of dithizone-functionalized materials as sorbents in solid phase extraction/displacement [40]. Moreover, the magnetic property of the material used can be a substitute for the centrifugation or filtration steps and the solid phase can easily separate the analyte via an external magnetic field, in a technique that is called magnetic dispersive solid phase extraction (MdSPE). Usually, small size materials are also applied as selective sorbent for MdSPE [41]. This technique is generally used to prepare liquid samples, extract semi volatile, and nonvolatile analytes. The MdSPE procedure involves preconcentration of samples in solution and make it free of interfering matrix components. Applications of MdSPE are widely used for preconcentration and separation of analytes in various samples. For example, the determination of Pb in beverages using magnetic ion-imprinted polymer [42], Cd in water samples using magnetite schiff bases [43], Zn in water, flour, celery and egg using GO/Fe_3O_4@polythionine [44], Tl in environmental samples using magnetic multi-walled carbon nanotubes/zeolite nanocomposite [45], and Hg and organic-Hg in water and caprine blood using carboxyl-functionalized nanoporous graphene.

General methods for determination of trace heavy metals are x-ray photoelectron spectroscopy (XPS) [23], voltammetry [46], UV-Vis spectrophotometry [47] [20], colorimetry [48], and inductively coupled plasma mass spectrometry (ICP-MS) [49] as well as inductively couple plasma-optical emission spectrometer (ICP-OES) [45]. Among these techniques, in the present study, atomic absorption spectrometry (AAS) both electrothermal atomic absorption spectrometry (GFAAS) [50] and flame atomic absorption spectrometry (FAAS) [51] were selected in the present method for evaluation and determination of Pb and Cd based on the separated techniques; LPME, DLLME, SPE, HF-LPME for preconcentration and extraction.

1.2 Objective

The objectives of this studies are to develop green microextraction methods by ST-DLLME, MdSPE, SS-LPME, DES-LPME, and EM-DES-HFLPME for Pb and Cd determination using FAAS, GFAAS, and HR-CS-FAAS. Different types of assisted-extraction techniques such as effervescent tablet-assisted, air-assisted, electro-enhanced, solvent terminated, and centrifuge-less methods for the increasing of the extraction efficiency, decreasing extraction time and proving the analytical figure of merits are also investigated. Finally, the developed methods are practically applied for determination of trace Pb and Cd in different types of environmental and food samples.

CHAPTER II

LITERATURE REVIEWS

In the present green microextraction method, dissertation focused on using the remarkable preconcentration techniques such as AA-ST-DLLME, MdSPE, EA-SS-LPME, AA-DES-DLLME and EM-DES-HFLPME for determination of trace Pb and Cd by atomic adsorption spectrometry. Literature reviews of these methods were summarized as followed.

2.1 Solvent termination and dispersive liquid-liquid microextraction

2.1.1 The fundamental theory for DLLME

In the conventional DLLME, the standard or sample solution was mixed with chelating agent and extraction solvent for the separation of analyte into the organic phase. For well distribution, the extraction system was added by dispersive solvent to disperse the analyte to organic extractant. The main point of DLLME was the use of dispersive solvent which could be the compromised solvent for both partitioning in the aqueous and the organic phases. At the same time, the dissolution of dispersive solvent in both phases can caused the emulsion. Thus, centrifugation was an important step for complete separation and the physical emulsion-breakage in normal DLLME.

Example of normal DLLME application are summarized as followed, Li and coworker [52] determined Hg in water samples using DLLME and back extraction along with capillary zone electrophoresis (CZE or CE). In the part of DLLME procedure, the aqueous sample solution of 10 mL containing Hg, boric acid buffer, and 1-(2-Pyridylazo)-2-naphthol (PAN; Chelating reagent) was placed in a conical centrifuge tube. The mixed solution of chlorobenzene (Extraction solvent) with ethanol (Dispersive solvent) was placed in the conical centrifuge tube and was shaken well (Approximately 3 sec). A cloudy solution was formed in the tube. After the centrifugation at 3000 rpm for 3 min, the dispersed fine droplets of chlorobenzene were sedimented in the bottom of conical tube. Next, back extraction was started from the sedimented chlorobenzene was removed and injected into a new centrifuge tube with L-Cys solution and then ultrasonicated for 5 min. In this step, L-Cys displaced PAN to form the stable Hg-L-Cysg complex in aqueous phase. Finally, the upper phase of aqueous phase was prepared for CE assays. Martínez and coworker [53] determined Cd and Pb in wine samples using DLLME-ETAAS procedure. First, 2 mL of wine sample or analyte standard were placed on a centrifuge tube with 100 µL of the suitable buffer solution. Then, 100 µL of NaCl and 100 µL of the DDTC solutions were added. Next, a mixture of BmimPF6 (extractant) and methanol (disperser) was injected using a 1.00 mL syringe and the resulting system was shaken for 2 minutes using a vortex. To separate the two phases generated (water/ionic liquid), the mixture was centrifuged for 5 minutes at 3500 rpm. The lower layer of the ionic liquid was removed with a micropipette and then diluted with methanol (BmimPF6/methanol 1:1 ratio) prior to ETAAS analysis. Tuzen and Pekiner [54] reported the determination of Se in foods and beverages using UA-IL-DLLME-GFAAS procedure. 10 mL of an aqueous sample solution containing 0.5 µg/L Se(IV) and Se(VI) were introduced in a 25 mL centrifuge tube. This solution was adjusted to pH 2 using dilute HCl, then 1 mL (0.1%) 1-Phenylthiosemicarbazide and 100 µL ([C_6MIM][Tf_2N] as an extractive solvent were added. The mixture was vortexed for 1 min and immediately removed to ultrasonic bath for 10 min. The extractant was dispersed into solution and cloudy solution was formed. The analytes were extracted into the fine droplets. The cloudy solution was centrifuged at 3000 rpm for 5 min in order to archive phase separation. The content of the tube was cooled in an ice bath to increase the viscosity of the enriched phase. The bulk aqueous phase was decanted carefully by inverting the tube. The ionic liquid enrich phase was treated with 100 µL of 0.1 mol/L HNO_3 in ethanol (1:1, v/v) and determined by GFAAS.

2.1.2 Solvent termination for ST-DLLME

Because of the emulsion problem and centrifugation of DLLME, the ST-DLLME was developed for elimination of these disadvantages. ST-DLLME extraction steps were similar to conventional DLLME via extraction solvent and dispersive solvent, but the cloudy solution (sample/extraction solvent/dispersive solvent) was injected with demulsified solvent to break the emulsion and induced phase separation. Demulsification process was started by flocculation of the dispersed microdroplets by coalescence to form larger droplets. Then, the extractant either floats or drops (depended on its density as compared with the aqueous sample) (Fig. 1). ST-DLLME procedure did not required a centrifugal step, making it rapid and efficient. For deeply understanded, Mansour and Danielson [55] explained that the ideal demulsified solvent should fulfill the following requirements:

1) The demulsified solvent should be reasonable cost to make the cost effective in extraction process

2) The demulsified solvent should be safe and environmetally friendly

3) The demulsified solvent must had high surface activity and low surface tension in order to disperse over a large area and break the emulsion

4) A small amount of demulsified solvent can break the emulsion for the lessening solvent consumption

5) The demulsified solvent should not be totally miscible with the extraction solvent

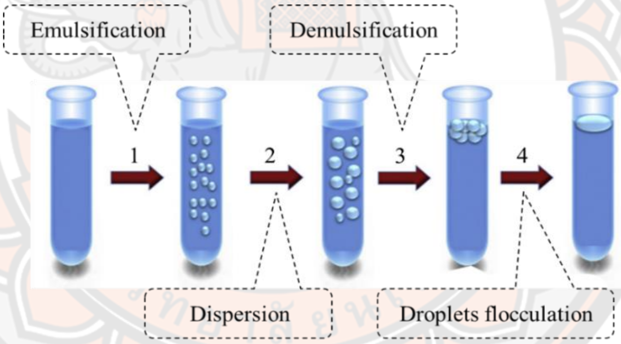


Figure 1 *Steps of ST-DLLME sample preparation*

Source: *[55]*

For example of ST-DLLME, Liang and coworker [56] proposed the high-density extraction solvent-based solvent de-emulsification dispersive liquid–liquid microextraction (HSD-DLLME) for detection of chlorophenols in water samples. The procedure of the SD-DLLME, 5 mL water sample were placed in a glass centrifuge tube. The mixed solution of 55 µL chloroform (Extraction solvent) with 0.5 ml acetone (Dispersive solvent) were injected into glass centrifuge tube and set extraction for 3 min. To break down the emulsion, acetonitrile (demulsified solvent) was spiked into the extraction tube. Then the emulsion was separated into two phases quickly. Lower phase was collected into a new glass tube and purged with N_2 for drying. After that, the extracting phase was dissolved in 0.1 mL water containing 5 mM SDS and analysis with CE.

2.1.3 Assisted-technique for ST-DLLME

The extraction efficiency was improved by the several assistant techniques in microextraction such as ultrasonic-assisted technique [22, 57], vortex-assisted technique [20, 58, 59], and air-assisted technique [60, 61]. These techniques were effective and easy to develop as the new method. Especially, air-assisted technique in liquid phase microextraction were reported as simple procedure. For example, Farahmand and coworker [60] reported the development of air-assisted technique via the use of glass syringe to push in and pushed out into the extraction tube repeatedly for ten times using syringe needle before applied to solidification of floating organic drop (SFOD) for the spectrometric determination of some drugs in biological samples. Lamei and coworker [62] studied the emulsification liquid-liquid microextraction using deep eutectic solvent (DES) for preconcentration of methadone in water and biological samples and applied the air-assisted technique via the pushed out the syringe into tube (repeated 10 times) using syringe needle. Li and coworker [61] developed DES-LPME for determination of rare ginsenosides and N_2 was injected into the extraction tube as air generator. Ezoddin and coworker [63] reported the development of LPME using switchable-hydrophilicity solvent for the determination of palladium in environmental samples and the extraction was rapidly withdrawn and pushed out into the tube (5 times) using a syringe. Molaey and coworker [64] also determined Ni, Cu, and Zn waste activated sludge using the air-assisted ultrasonication.

2.1.4 The application of solvent terminated method for sample extraction

Nowadays, the solvent-terminated dispersive liquid-liquid microextraction (ST-DLLME) or solvent-based de-emulsification dispersive liquid-liquid microextraction (SD-DLLME) or centrifugation-free extraction was still new as interesting topic. The application of ST-DLLME can be modified in many steps such as the properties of extraction solvent, dispersive solvent, and demulsifier. There are the compatibility of method and analytes in several matrixes was in noticeable development as shown in Table 1.

In the part of this work, the first dissertation was to develop the cost-effective solvent-terminated dispersive liquid-liquid microextraction (ST-DLLME) coupled with simple air-assisted microextraction using commercial bubble generator for determination of Pb in environmental samples by GFAAS. This work consumed a small volume of extraction solvent, dispersive solvent, and demulsified solvent as well as non-centrifugation in AA-ST-DLLME. In addition, a home fish-bubble generator was used as air generation device for the first time.

Table 1 *Application and extraction condition of ST-DLLME for the determination of analytes in various types of samples*

Technique	**Analyte**	**Complexing agent**	**Sample**	**Extraction solvent**	**Dispersive solvent**	**Demulsified solvent**	**Linearity (µg/L)**	**LOD (µg/L)**	**LOQ (µg/L)**	**%RSD**	**%Recovery**	**EF or PF**	**Ref**
SD-DLLME with ETAAS	Pd	TMK	- roads dust - sea water - river water - mineral water	1-octanol	Acetonitrile	Acetonitrile	0.025–0.50	0.007	-	3.68	90-106.5	231	[65]
HSD-DLLME with CE	Chlorophenols	MECK	- wastewater - tap water	Chloroform	Acetone	Acetonitrile	20-4000	3.5-4.0	-	8.2-9.3	89.5–101.9	233-797	[56]
SPE-SD-DLLME with FAAS	Cd	Neocuproine	- sea water - river water - wastewater - tap water - lake water	1-octanol	Acetonitrile	Acetonitrile	0.1–50	0.03	-	5.1	97-102	165	[66]
VLDS–SD–DLLME with HPLC	Pesticides	-	- tap water - surface water - agricultural water	Dodecanol	Acetonitrile	Acetonitrile	0.5–500	0.25–1	0.6–3.5	2.4–5.3	78-106	72-79	[67]
SD-DLLME with HPLC	- 2,4-D - MCPA	-	- water - urine	1-octanol	Acetonitrile	Acetonitrile	2-400 1-300	0.2-1.6	-	4.1–5.6	97.5-98.2	123-131	[68]
AALLME with HPLC	Ginsenosides	-	*Kang'ai*	DES (ChCl:Ph)	THF	THF	250-100,000	10.2-137.8	55.5-428.5	1.2-4.5	91.3-106.7	-	[61]

Remarks;

SD-DLLME	Solvent-based de-emulsification dispersive liquid-liquid microextraction
HSD-DLLME	High-density solvent-based solvent de-emulsification dispersive liquid–liquid microextraction
SPE-SD-DLLME	Solid phase extraction combined with solvent-based de-emulsification dispersive liquid–liquid microextraction
VLDS–SD–DLLME	Vortex-assisted low-density solvent based solvent demulsified dispersive liquid–liquid microextraction
AALLME	Air-assisted liquid-liquid microextraction

2.2 Magnetic dispersive solid phase microextraction and its application

2.2.1 Basis of solid phase extraction and its development

Sample pretreatment was a fundamental step to enhance the separation of matrixes and increased the selectivity of analytes. Especially, when enrichment of trace level was highly required for quantitative analysis. Accuracy, precision, and quantitative limits of the method could be increased. Solid phase extraction (SPE) was one of simplest, fastest, and most suitable for application in several fields by reason of its high adaptability.

General procedures of SPE were described by Alexandra and Piletska [69]. The extraction steps complied with the purification and preparation of samples; (1) conditioning (to contain the solvent into column), (2) loading (to fill the sample solution), (3) washing (to eliminate the matrixes with washing solvent), and (4) elution (to elute the analyte by eluent). Nowadays, magnetic solid phase extraction (MSPE) or magnetic dispersive solid phase extraction (MdSPE) was a new mode of SPE that proofs of the high selectivity and sensitivity according to decrease the use of solvent volume, SPE-cartridge, and centrifugation. MdSPE consisted of two main steps: (1) dispersion of the solid magnetic sorbent particles into the sample solution and (2) phase separation by external magnetic field. Examples of MdSPE were following concluded.

Magnetic dispersive micro solid-phase extraction (MDMSPE) was studied for trace mercury preconcentration and determination in water, hemodialysis solution and fish samples by Es'haghi and coworker [70]. In this method, a mixture of chloroform as an extraction solvent, methanol as a dispersive solvent and nanomagnetite (Fe_3O_4)/ chelating agent 1-(2-ethoxyphenyl)-3-(4-ethoxyphenyl) triazene functionalized multi-walled carbon nanotubes with silica shell (MWCNTs-Fe_3O_4 MNPs-silica-EET), as an adsorbent injected rapidly into Hg solution and cloudy solution was produced after sonication for 2 min. Then, the adsorbents were isolated by external magnet from the outer of extraction tube. Finally, the collected adsorbents were evaporated and eluted by HNO_3 before detected by cold vapor atomic absorption spectrometry.

Vortex-assisted dispersive micro-solid phase extraction (VA-d-µ-SPE) was investigated by Moyakao and coworker [71] for preconcentration of trace neonicotinoid insecticide residues in natural surface waters using HPLC detection. In the VA-d-μ-SPE step (Fig. 2), 13 mL of standard/sample solution was mixed with 0.03 g of Na_2SO_4 before being placed in a centrifuge tube containing 30 mg of MMT Cloisite 10A clay sorbent. In this step, the suspension was formed. After that, the centrifuge tube was placed the vortex agitation for 1 min and centrifuged at 3500 rpm for 10 min to enhance the adsorption. Finally, the clay sorbents were extracted by acetonitrile and this mixture was vortexed for 2 min and centrifuged at 3500 rpm for 5 min until the clear supernatant was formed.

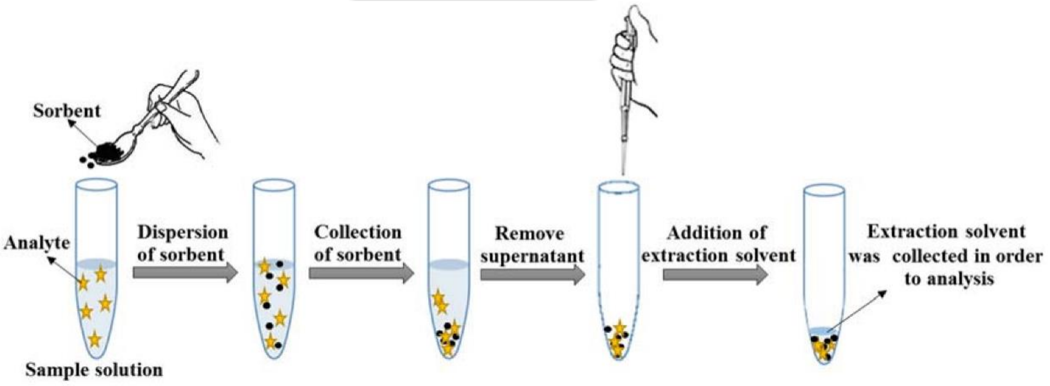


Figure 2 *Extraction diagram of the VA-d-μ-SPE method*

Source: *[71]*

2.2.2 The modified sorbent for magnetic solid phase extraction

Generally, some traditional solvents in the extraction were more toxic than the target compound. Nevertheless, the development of a new sorbent can be improved the selectivity in a wide range of applications without the less of resulting quality, sensitivity, accuracy, and precision. So, the modification of MdSPE from previous works were summarized.

A novel and simple SPE-HPLC-ICP-MS method was developed by Yin and coworker [40] for determination of inorganic mercury (IHg), methylmercury (MeHg), and ethylmercury (EtHg) in water samples. Dithizone-functionalized C18 column was used in the extraction procedure. Standard/sample solution was slowly loaded into the functionalized-column with buffer solution and the mercury-dithizone complexes were retained in column. Then the column was dried with air for 2 min to remove water. For eluting, $Na_2S_2O_3$ solution was used to elute the trapped mercury species on the column as the hydrophilic mercury species-$Na_2S_2O_3$ complexes and injected into HPLC-ICP-MS for mercury speciation.

Magnetic dispersive solid phase extraction (MdSPE) with graphene/$ZnFe_2O_4$ nanocomposite adsorbent (G/$ZnFe_2O_4$) was introduced by Yavuz and coworker [72] for the sensitive determination of Hg in water and fish samples by CVAAS. In this experimental step, Hg solution, buffer solution, and G/$ZnFe_2O_4$ sorbent were contained into centrifuge tube and vortexed for 3 min. Then the sorbent was separated by a strong magnet and the supernatant was decanted carefully. HCl was used for elution of Hg within the 3 min of vortex.

Li and coworker [73] reported the modification of dithizone functionalized magnetite-reduced graphene oxide (rGO). They proposed the mechanism of Fe_3O_4 and dithizone in sorbent that were absorbed on rGO as shown in Fig. 3.

Figure 3 *Modification of dithizone functionalized magnetite-rGO*

Source: *[73]*

The work of Babaei and coworker [74], new graphene oxide/Fe_3O_4/ polythionine (GO/Fe_3O_4/PTh) nanocomposite sorbent was introduced for magnetic dispersive solid-phase extraction (MdSPE) to detect of Zn in water, flour, celery and egg. The experimental extraction setup (Fig. 4), a mixed solution was of samples/ standard solution, ammonium/ammonia buffer (pH 8.0), and phenanthraquinone monophenyl thiosemicarbazone (PPT) as a masking agent). Then, the GO/Fe_3O_4/PTh sorbent was added into the tube and sonicated for 7 min. After that, the sorbent was separated from aqueous by a strong magnet and eluted with HNO_3 in ethanol as well as shaken for 2 min since the clear solution was appeared.

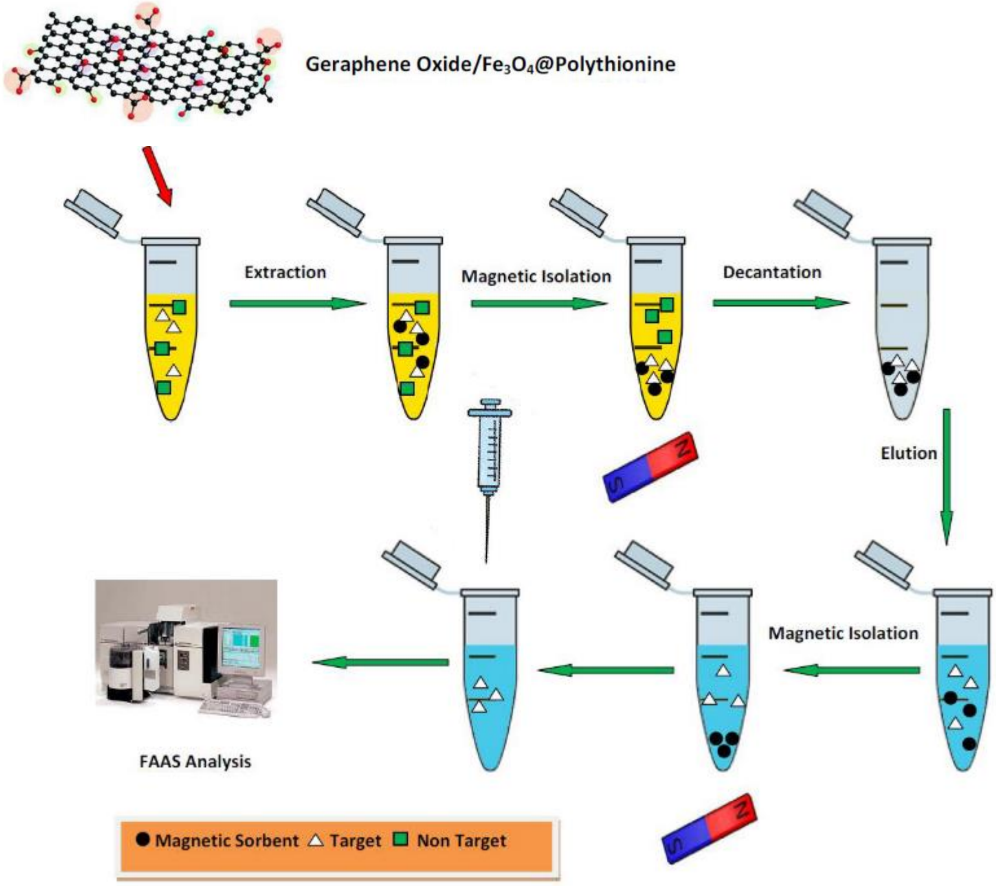


Figure 4 *Extraction diagram of MdSPE-FAAS for the determination of Zn*

Source: *[74]*

Nonetheless, surfactant was one choice of the modified adsorbent. Eventually, Faraji and coworker [75] suggested the sodium dodecyle sulphate (SDS)-coated magnetite nanoparticles (SDS-coated Fe_3O_4 NPs) as a new extractant for extraction of trace amounts of Hg by SPE-FI-ICP-OES. This advantage technique, sample solution was transferred into glass beaker and sequentially added with Michler's thioketone (TMK) as a complexing agent, Fe_3O_4 NPs, and SDS solution. The mixtures were shaken and allowed to complete the extraction process for 1 min. Then a strong magnet was placed at the bottom to isolate sorbent from the solutions for 10 min. After supernatant solutions were decanted, the sorbent was eluted by 1-propanol.

2.2.3 The application of MdSPE for separation and preconcentration

Recently, MdSPE was apparent that method was clearly separated for preconcentration. The process of MdSPE can be modified into two main steps, the sorbent modification and the features of separation. Various types of sorbents and eluents in MdSPE were illustrated in Table 2.

This work was to combine the carbon-based materials (graphene oxide (GO)) with iron-based magnetic nanoparticles to form a new sorbent. GO had impressive physicochemical properties such as high polarizability, strong adsorptive donor, or acceptor properties, and π-π electron interactions which made it an ideal extracting sorbent. In addition, GO can be easily interacted with various types of adsorbates and functional groups on various materials [76]. Common forms of iron oxide such as hematite (α-Fe_2O_3), magnetite (Fe_3O_4), and maghemite (γ-Fe_2O_3) were extensively used in wide applications. Magnetite (Fe_3O_4) was one of most abundant with high magnetization ability and the point of zero charges in aqueous solution close to 7 [77]. Examples of magnetic sorbents that can be used in MdSPE were TiO_2 nanoparticles [10], graphene/$ZnFe_2O_4$ [72], graphene oxide/Fe_3O_4@ polythionine [44], sodium dodecyl sulphate- Fe_3O_4 nanoparticles [75], multi-walled carbon nanotubes-Fe_3O_4@1-(2-ethoxyphenyl)-3-(4-ethoxyphenyl) triazene [70], graphene oxide/Fe_3O_4@ ternary hydrosulphonyl-based deep eutectic solvent [78], and pyrocatechol violet impregnated magnetic graphene oxide [79]. Dithizone (DTZ) was a well-known and efficient chelating agent. It have been reported as a ligand for liquid-liquid extraction [80, 81] and solid-phase extraction [39, 82]. DTZ was modified onto the graphene oxide/Fe_3O_4 as an efficient adsorbent and solid phase chelating agent. Due to its high specific surface area and adsorption sites of graphene oxide/Fe_3O_4 [82], the dithizone modified-particles represented high adsorption efficiency and short adsorption time. Therefore, in the second work of dissertation was carried out to use a used-graphite tube as a graphene oxide core-sorbent modified with the magnetite (Fe_3O_4) and dithizone. The composite of graphene oxide/ Fe_3O_4/dithizone (GO/Fe_3O_4/DTZ) as a novel sorbent for SPE pretreatment have been applied to investigate trace Pb in environmental water samples using FAAS detection.

Table 2 *Application and extraction condition of MdSPE in different analytes and various types of samples*

Technique	Analyte	Sample	Sorbent	Eluent	Linearity (µg/L)	LOD (µg/L)	LOQ (µg/L)	%RSD	%Recovery	PF	Ref.
SPE with ICP-OES	Hg	- mineral water - tap water - well water	SDS-coated Fe_3O_4 NPs	1-propanol	0.2-100	0.04	-	< 5	95-103	12 30	[75]
MDMSPE	Hg	- fresh water - spring water - Caspian sea - canned tuna fish - hemodialysis solution	MWCNTs-Fe_3O_4-MNPs-silica-EET	HNO_3	9-1000	1.5	5.0	2.07-5.01	96.6-103.44	-	[70]
US-D-IL- µ-SPE with FI-CV-AAS	Hg (II), Organic-Hg, and total-Hg	- wastewater - river water - well water	NG-COOH	HNO_3	0.03-6.3	0.0098	-	< 3	95-103	10.4	[83]
THS-DES/M-GO based MSPE with AFS	Hg	wastewater	THS-DES/M-GO	EDTA	-		--	1.55	93-97	-	[78]
MDSPE with FAAS	Zn	- wheat flour - celery - egg yolk and white - mineral water - tap water	GO/Fe_3O_4/ polythionine	HNO_3	0.5-30	0.08	0.5	2.6	87.4-96.2	60	[74]
MdSPE with CVAAS	Hg	- wastewater - tap water - well water - fish	G/$ZnFe_2O_4$	HCl	0.25-10	0.01	0.04	2.7-3.8	91-107	30	[72]
VA-d-µ-SPE with HPLC	Neonicotinoid insecticides	fruit juice and natural surface water	MMT Cloisite 10A clay	Acetonitrile	0.5-1000	0.005-0.065	0.008-0.263	7.17	8–176	-	[71]

Remarks;

MDMSPE	Magnetic dispersive micro solid phase extraction
MWCNTs-Fe_3O_4 MNPs- silica-EET	Multi-walled carbon nanotubes-magnetic nanoparticles modified with silica and 1-(2-ethoxyphenyl)-3-(4- ethoxyphenyl) triazene
US-D-IL- µ-SPE	Ultrasound assisted-dispersive-ionic liquid-micro-solid phase extraction
MSPE	Magnetic solid phase extraction
THS-DES/M-GO	Ternary hydrosulphonyl-based deep eutectic solvent modified magnetic graphene oxide
MdSPE or MDSPE	Magnetic dispersive solid phase extraction
VA-d-µ-SPE	Vortex-assisted dispersive micro-solid phase extraction

2.3 Switchable solvent based liquid phase microextraction

Switchable solvent liquid phase microextraction was an alternative method of green chemistry for extraction the analytes via switchable solvent as extracting solvent. Switchable solvents (SS) or switchable-polarity solvents (SPS) or switchable-hydrophilicity solvents (SHS) were non-volatile liquids at which hydrophilicities can be reversibly switched from one form to another. For example, similar behavior have been observed for (1) single component; amines, primary amines, diamines, hydroxyamines and hydroxyguanidines [84-87] including (2) mixing component; guanidine/alcohol, amidine/primary amine, and guanidine/acidic alcohol mixtures [88]. In normal experiment, ionic analyte in sample matrices were complexes with chelating agent that complex as a hydrophobic substrate can be extracted to solvent phase using switchable solvent (Fig. 5). The extracting phase were observed on the bottom or below of centrifuge tube which depended on its density.

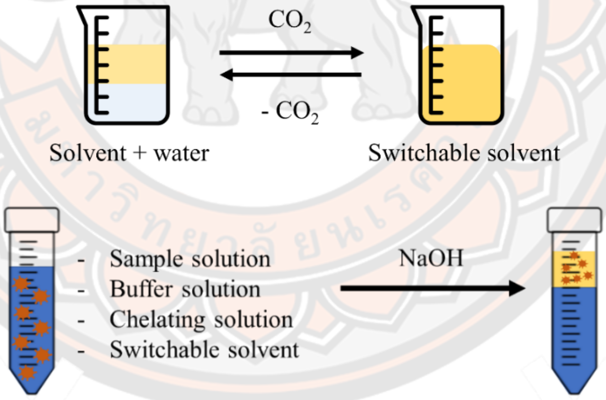


Figure 5 *Synthesis of switchable solvent and normal SS-LPME procedure*

Recently, the different types of SS-LPME were being developed to establish high extraction efficiency as summarized below;

2.3.1 The synthesis of switchable solvents

A new type of solvent called as switchable solvents (SS) have been received attention because of its reversibly conversion physical properties. Most of literature reviews, these systems can be changed between hydrophobic-hydrophilic forms by the addition or removal of CO_2 or N_2 from the system. The miscibility of switchable solvent was provided by an acid-base reaction between hydrated CO_2 or N_2 and the solvent.

Reclo and coworker [89] reported the synthesis method for switchable solvent based liquid phase microextraction that preparation used the same portion (v/v) of *N*,*N*-dimethylcyclohexylamine (DMA) and water in a glass bottle. Next, a piece of dry ice was added while the solution was stirred. Dry ice was added until homogeneous solution was observed because of hydrophilic bicarbonate salt of the protonated amine occurring. The addition of dry ice generated the acidic-base reaction between amine and either hydrated CO_2 in this work.

Shahraki and coworker [90] developed the homogeneous liquid-liquid microextraction based on an acid-base reaction using switchable-hydrophilicity solvents (SS-HLLME). *N*,*N*-dipropylamine (DPA) was added with water and hydrochloric acid (HCl) for drop-by-drop addition. A homogeneous solution was formed after agitated using a vortex mixer for 10 s.

Naeemullah and coworker [84] applied green switchable dispersive liquid-liquid microextraction for cadmium determination. For the preparation of switchable water (SW), were added a mixture of H_2O and Tetraethylenepentamine (TEPA) for 10:1 mL and bubbled CO_2 for a time of 2-50 min, while stirring for 200 to 600 rpm. The zwitterion mechanism (eq. 1 and eq. 2) was one of the most acceptable mechanisms between CO_2 and amine that consisted of two steps; (1) The formation of a zwitterion using CO_2 and (2) The deprotonation of the zwitterion using H_2O.

$$NH_2(CH_2)_2NH(CH_2)_2NH_2 + CO_2 \rightleftharpoons NH_2(CH_2)_2NH(CH_2)_2NH_2COO^-RNH_3^+ \text{ ....(1)}$$

$$NH_2(CH_2)_2NH(CH_2)_2NH_2COO^-RNH_3^+ + H_2O \rightleftharpoons RNH_3^+HCOO^- + RNH_3^+HCOO^- \text{ ....(2)}$$

Source: *[84]*

Memon and coworker [51] developed switchable solvent hyphenated liquid phase microextraction (SS-LPME). Synthesis of switchable solvents using *N*,*N* dimethyl-n-octylamine and Milli-Q water. Two phases of solvent and water were broken by the addition of dry ice for phase homogenization. Complete protonation was achieved by stirring the solvents for 3 h at room temperature.

Ezoddin and coworker [63] developed air assisted liquid phase microextraction based on switchable-hydrophilicity solvent (SHS-LPME). The switchable solvent (P-TEA-C) was synthesized in an equal volume mixture of triethylamine (TEA) and water with 10 g of dry ice. In this work, NaOH addition was used for final separation via CO_2 elimination to change from P-TEA-C to TEA (eq. 3)

$$NH_3 + H_2O \underset{+\,NaOH}{\overset{+\,CO_2}{\rightleftharpoons}} HNR_3^+ + HCO_3^- \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

*NR_3 was triethylamine (TEA)

2.3.2 Assisted-extraction technique for SS-LPME

New version of SS-LPME was the assisted-techniques coupled with SS-LPME such as ultrasonic assisted switchable solvent based liquid phase microextraction (UA-SS-LPME) [91], air assisted liquid phase microextraction based switchable-hydrophilicity solvent (AA-SHS-LPME) [63], vortex-assisted switchable solvent based green liquid phase microextraction (VA-SSLPME) [51], and effervescence assisted emulsification microextraction with switchable solvent (EA-EME-SS) [92]. In the proposed approach, effervescence assisted SS-LPME as EA-SS-LPME was focused. Recently, many publications were reported on the purpose of effervescence assistant for extraction improvement as followed;

The use of CO_2-effervescence assisted-extraction was published by Hemmati and Rajabi [92]. The principal necessities of an effective effervescent process were an effervescency agent (CO_2 source) and a proton donor agent, which was weak alkali compounds (Na_2CO_3, $NaHCO_3$, etc.) as well as mineral acids (H_2SO_4, H_3PO_4, HCl, etc.), respectively. Theoretically, the effervesce process desired the same valences of acids and bases. Practically, an emulsification microextraction was assured by an excessive amount of the proton donor. In the experiments, they selected the greatest bivalent effervescency and proton donor agents from sodium carbonate (Na_2CO_3) and sulfuric acid (H_2SO_4) prior to HPLC-UV for efficient analysis of toxic azo dyes in foodstuffs.

Jing and coworker [93] reported effervescent-assisted dispersive liquid-liquid microextraction based on the solidification of a floating organic droplet. Effervescent agent was sodium bicarbonate introducing bubbles into extraction system.

Arpa and coworker [94] developed an effervescence-assisted dispersive liquid-liquid microextraction (EA-DLLME) technique for determination of Cu using DES as extraction solvent. Six different effervescence powders were examined to achieve maximum extraction efficiency. Finally, sodium dihydrogen phosphate and sodium carbonate were selected as an appropriate type.

Guillermo and coworker [95] developed an effervescence assisted dispersive liquid-liquid microextraction (EA-DLLME). The effervescence occurred from the addition of sodium carbonate aqueous solution.

Wang and coworker [96] represented the novel and simple effervescence tablet-assisted magnetic ionic liquids-based microextraction (ETA-MILs-ME). By the effervescence tablet, sodium carbonate and sodium dihydrogen phosphate were chosen for their experiments to press as a tablet before use.

Li and coworker [97] studied magnetic effervescent tablet-assisted ionic liquid-based dispersive liquid-liquid microextraction (META-IL-DLLME). Magnetic effervescent tablet in this work obtained with sodium dihydrogen phosphate, sodium carbonate, ionic liquid (IL) and magnetic Fe_3S_4 nanoparticles. Subsequently, all precures powder was compressed by a T5 Single Punch Press as seen in Fig. 6.

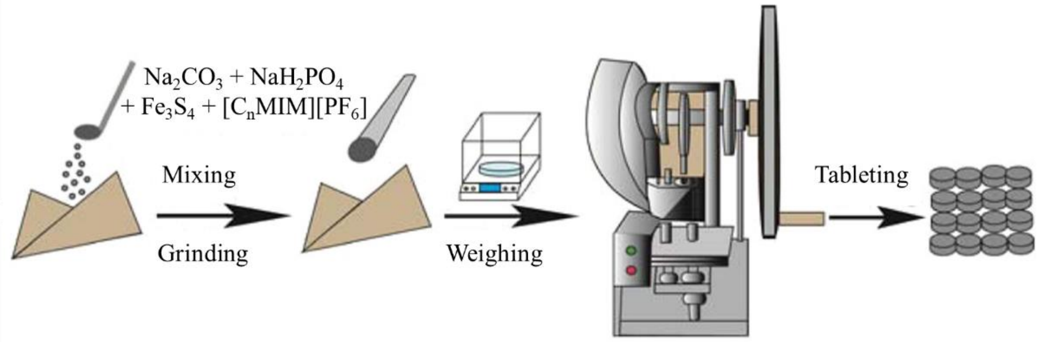


Figure 6 *Preparation of the magnetic effervescent tablets*

Source: *[97]*

2.3.3 The application of switchable solvents for microextraction

Nowadays, switchable solvent (SS) was the popular extracting solvent for liquid phase microextraction. The SS was regarded as a green class of extraction media that not only overcame the separation of a polar miscible organic solvent, but also provided high interface between two immiscible phases, which rapidly improved the extraction efficiency of the proposed method. Moreover, the use of CO_2 had many advantages; low cost, environmentally friendly, non-accumulating in the extraction system, and easy removing. Many literature reviews demonstrated the application of switchable solvents in various targets, type of switching agent, type of complexing agent, and evaluation of analytical performance such as limit of detection, limit of quantitation, working range, precision, and accuracy (Table. 3).

Therefore, the third work was to develop the alternative approach for the enrichment of extraction efficiency using triethylamine (TEA) as a switchable solvent based on liquid phase microextraction for significant preconcentration and separation. The alternative method for enrichment efficiency applied the effervescent-tablet assisted technique to advocate the extraction as the effervescent-tablet assisted switchable solvent based liquid phase microextraction (EA-SS-LPME) coupled with HR-CS-FAAS for determination of toxic Pb and Cd in environmental and food samples.

Table 3 *Application of switchable green solvent based on LPME for the analysis of various types of samples*

Techniques	**Analyte**	**Complexing agent**	**Sample**	**Switching agent**	**Switchable solvent**	**Linearity (μg/L)**	**LOD (μg/L)**	**LOQ (μg/L)**	**%RSD**	**%Recovery**	**EF or PF**	**Ref.**
SSLLME with FAAS	Pd	5-Br-PADAP	- automotive catalytic converter - roadside dust - sea water - river water	CO_2	*N,N*-dimethylcyclohexylamine	15-1600	4.28	-	0.16	92	37.5	[89]
LL-SHS-LPME with FAAS	Ni	-	- tea - cigarette - cauliflower - balsam - eggplant - pomegranate - rosemary	CO_2	1- ethylpiperidine	17–500	5.2	17.2	6.0	94-98	-	[98]
SWDLLME with GFAAS	Cd	PAN	- canal water - tap water - tomato - spinach - lettuce	CO_2	Tetraethylenepentamine	0.05-5.0	0.00038	0.00128	4.5	>97	22	[84]
SS-HLLME with differential pulse voltammetry	Nitrazepam	-	- urine	HCl	*N,N*-dipropylamine	0.03-450	0.009	0.03	7.4	87.00-91.02	-	[90]

Table 3 *(Cont.)* …

Techniques	Analyte	Complexing agent	Sample	Switching agent	Switchable solvent	Linearity (µg/L)	LOD (µg/L)	LOQ (µg/L)	%RSD	%Recovery	EF or PF	Ref.
SS-LPME with FAAS	Co	1-Nitroso-2-Naphthol	- tea - tobacco - thyme - cherry - black paper - bread	CO_2	*N,N* dimethyl-n-octylamine	-	3.2	10.6	-	92-102	80	[51]
SPs-LLME with FAAS	Cd	APDC	- dam water - tomato - green pepper - cigarette - dem water - peach - parsley	CO_2	Triethylamine	0.53-157	0.16	-	5.4	97-103	30	[18]
UA-SS-LPME with FAAS	Cd	Dithizone	- tap water	CO_2	Triethylamine	0.7-100	0.24	0.69	1.3	100.2	106.5	[91]
	Ni		- bottled water			2.5-250	0.76	2.24	3.7	99.8	102.5	
	Co		- urine			2.5-250	0.72	2.40	2.9	99.5	101.1	
	Pb		- tea infusion			2-200	0.63	2.10	3.8	96.1	101.6	
AA-SHS-LPME with ETAAS	Pd	PAN	- converter - roads dust - sea water - river water - tap water - wastewater	CO_2	Triethylamine	0.16–2.5	0.07	-	3.5	98.3-103.2	64	[63]

Table 3 *(Cont.)* …

Techniques	**Analyte**	**Complexing agent**	**Sample**	**Switching agent**	**Switchable solvent**	**Linearity (µg/L)**	**LOD (µg/L)**	**LOQ (µg/L)**	**%RSD**	**%Recovery**	**EF or PF**	**Ref.**
CO_2-EA-EME-SS with HPLC	Azo dyes	-	- Turmeric - Saffron - Pepper	Na_2CO_3 and H_2SO_4	Hexanoic acid (Fatty acid)	4.0-3000	1.0-5.0	5.0-15.0	4.5-6.7	83-88	62-66	[92]
SS-Oil extraction	Microalgae oil	-	- Biodiesel	CO_2	*N*,*N*-dimethylcyclohexylamine (DMCHA), *N*-ethyl-butylamine (EBA), and dipropylamine	-	-	-	-	-	-	[99]
SPS-LPME with spectrophotometry	Hg	Dithizone	- Hair - Dam water - Wastewater - Sea water	CO_2	*N*,*N*-dimethyl cyclohexylamine	10-300	0.19	0.62	1.06	95-105	40	[100]

Remarks;

SSLLME	Switchable solvent-based liquid–liquid microextraction
LL-SHS-LPME	Ligandless switchable solvent based liquid phase microextraction
SWDLLME	Switchable water dispersive liquid-liquid microextraction
SS-LPME	Switchable solvent based liquid phase microextraction
SPs-LLME	Switchable-polarity solvent based liquid-liquid microextraction
UA-SS-LPME	Ultrasound-assisted switchable solvent based liquid phase microextraction
AA-SHS-LPME	Air-assisted switchable-hydrophilicity solvent based liquid phase microextraction
CO_2-EA-EME-SS	CO_2 effervescence ameliorated emulsification microextraction-switchable solvent
SPS-LPME	Switchable-polarity solvent based liquid-liquid microextraction

2.4 Deep eutectic solvent based liquid phase microextraction

2.4.1 Deep eutectic solvent (DES)

DES was a subclass of ionic liquids (ILs), as a type of green solvents. As mentioned about ILs, ILs were salts that generally composed of a delocalized charge and the poorly coordinated ions with melting points below 100ºC [101]. ILs had a high chemical and electrochemical stability, water soluble, low vapor pressure, and wide range of viscosity. Nonetheless, DES showed comparable characteristics with ILs at which they were easier synthesis, lower cost of raw materials, less toxic, and often biodegradable, but higher viscosity. DES usually provided a cooperation of hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). The HBA was often a quaternary ammonium salts, whereas HBD comprises urea, thiourea, acetamide, benzamide, glycerol, imidazole, malonic acid, glucose, amine, carboxylic acid, alcohols, and carbohydrates [23, 102] as illustrated in Fig. 7. Production of DESs involved the simple mixing of the two components in suitable amounts, generally with moderate heating. Behaviors of the solid-liquid phase diagrams for these DESs were summarized in Fig. 8. It should be noted that the eutectic composition was a single value (Eutectic point) that related to the minimum melting temperature in the phase diagram. The very low melting points of DES in comparison with those for the salts (HBA) and HBD forming them was one of their characteristic properties. For example, in the case of choline chloride (ChCl) with urea, which form a DES at 1:2 (salt:urea) molar ratio [103], the melting point of DES is 12 ºC whereas those for pure ChCl and urea were 303 ºC and 134 ºC, respectively. Melting point upon mixing arised from the selection of strong HBA-HBD intermolecular interactions, which were optimal for each composition of eutectic mixture. The melting points temperatures of selected DES were summarized in Table 4.

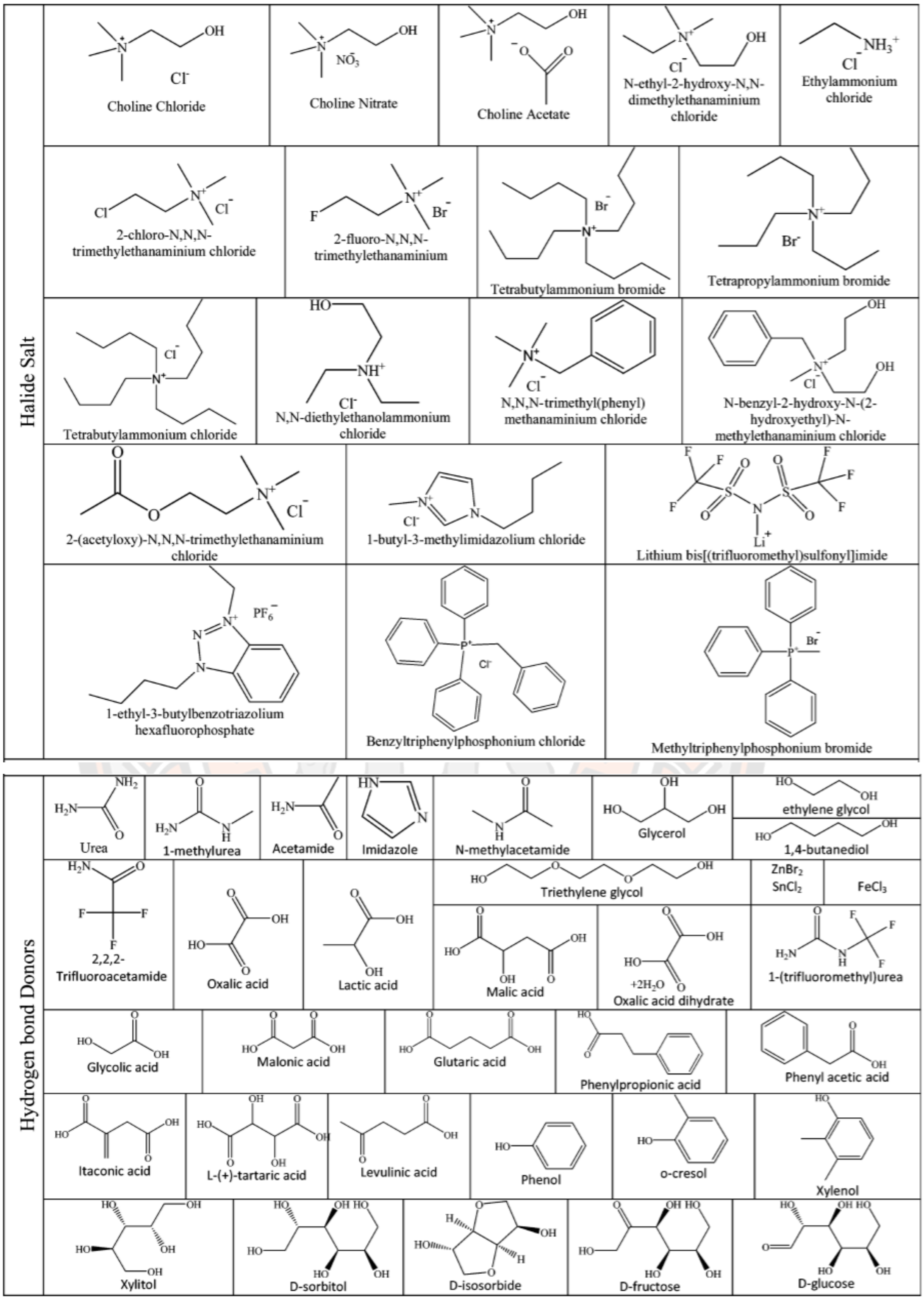


Figure 7 *Structures of some HBA (Halide salt) and HBD for the formation of DES*

Source: *[104]*

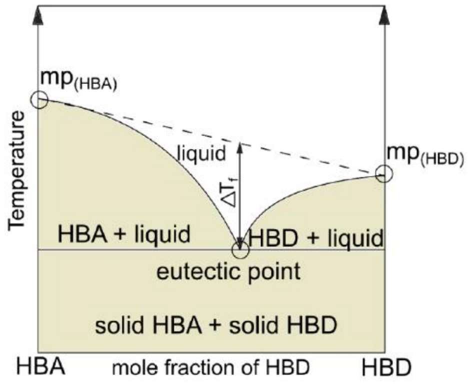


Figure 8 *Phase diagram of a eutectic point on a two-component (HBA and HBD)*

Source: *[102]*

Table 4 *Melting points temperatures of selected DES (by molar ratio)*

HBA	**THBA (ºC)**	**HBD**	**THBD (ºC)**	**HBA:HBD**	**TDES (ºC)**	**Ref.**
ChCl	303	Urea	134	1:2	12	[105]
ChCl	303	Thiourea	175	1:2	69	[103]
ChCl	303	Acetamide	80	1:2	51	[103]
ChCl	303	Banzamide	129	1:2	92	[103]
ChCl	303	Phenol	40	1:2	18-58	[106]
ChCl	303	Benzoic acid	122	1:1	95	[105]
ChCl	303	Citric acid	149	1:1	69	[105]
ChCl	303	Malonic acid	134	1:1	10	[105]
ChCl	303	Oxalic acid	190	1:1	34	[105]
ChCl	303	Succinic acid	185	1:1	71	[105]
MTPB	(-44)-(-42)	Glycerol	17.8	-	-4.03	[107]
BzTPPCl	70-72	Glycerol	17.8	-	50.36	[107]
$ZnCl_2$	20	Urea	134	-	9	[108]
$ZnCl_2$	20	Ethylene glycol	-12.9	-	-30	[108]

Remarks;

MTPB was methyltriphenylphosphonium bromide

BzTPPCl was benzyltriphenylphosphonium chloride

2.4.2 DES coupled with liquid phase microextraction and its development

As mentioned earlier, the most liquid phase microextraction required the contrast of incompatibility solutions that were hydrophilic aqueous and hydrophobic solvent. Since, normal-LPME, DLLME, SFODME, EME, HF-LPME, and CPE used several organic solvent or hydrophobic solution as extracting solvent, the finding new type of solvent was also subject of interest. The traditional extracting solvents such as chloroform [109, 110], dichloromethane [111, 112], methanol [113, 114], hexane [115], heptane [116], and undecanol [117, 118] are used. Besides, the alternative extracting medias were also reported such as switchable solvent (SS), ionic liquids (ILs), and DES. By the outstanding choices of DES, many publications presented the important procedure to produce DES that were concluded and summarized as follow;

Shafie and coworker [119] reported the preparation of DES by mixing of the quaternary ammonium salt (choline chloride: ChCl) and HBD (Citric acid monohydrate) at optimum ratios 1:1 by molar. The mixture of compounds was stirred at 80 °C until a colorless liquid was shown and maintained as a liquid after leaving about 24 h at room temperature (Fig. 9). The DES were then stored in a desiccator before various characterizations were carried out to prevent moisture absorption.

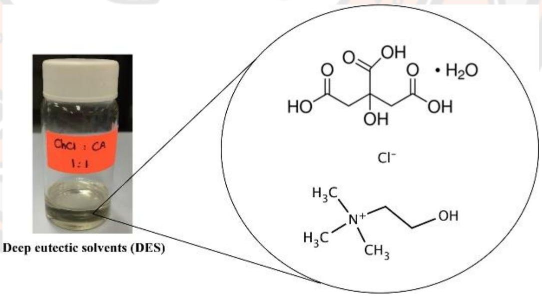


Figure 9 *Schematic presentation of synthesized DES by ChCl and citric acid monohydrate*

Source: *[119]*

Thongsaw and coworker [120] presented the step of ultrasound-assisted deep eutectic solvent liquid phase microextraction (UA-DES-LPME) for preconcentration and speciation of Hg^{2+} and CH_3Hg^+ combined with ETAAS. The two components of ChCl and phenol (1:3 molar ratio) were stirred at room temperature until homogeneous water-immiscible liquids were formed. The freshly prepared of DESs were stored in precleaned polypropylene bottles (Fig. 10).

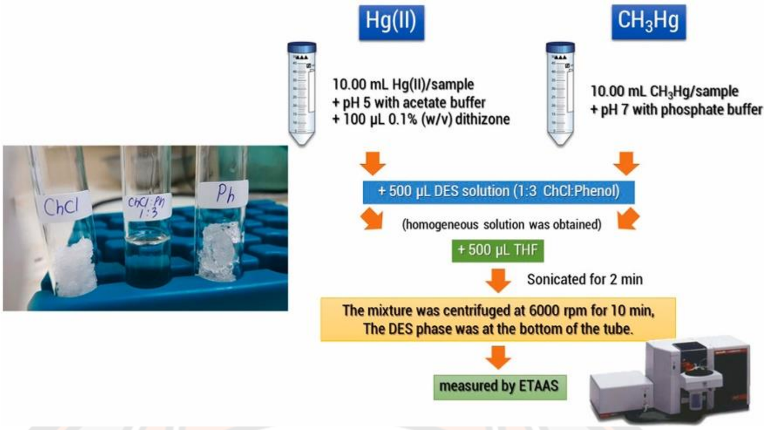


Figure 10 *Mixture of ChCl/Phenol as DES for application of Hg speciation analysis*

Source: *[120]*

Soylak and Koksal [121] proposed DES-LPME of Pb, Co, Ni and Mn ions for the separation and preconcentration in some oil samples using FAAS detection. The DES were formed by mixing of ChCl with urea (1:2 mol). Mixtures were prepared on the water bath at 80 °C with constant stirring by magnetic stirrer for 2 min until a homogeneous liquid were formed.

El-Deen and Shimizu [122] have been reported the application of DES as a novel disperser in dispersive liquid-liquid microextraction based on solidification of floating organic droplet (DLLME-SFOD) for preconcentration of steroids in water samples. DESs were synthesized by mixing tetrabutylammonium bromide (TBABr) as HBA with different HBD (Formic acid, acetic acid, propinoic acid, and ethylene glycol), as indicated in Fig. 11, at a molar ratio of (1:2) at 80 °C with constant stirring until forming a transparent liquid. The mixture was allowed to cool and used at the microextraction procedure.

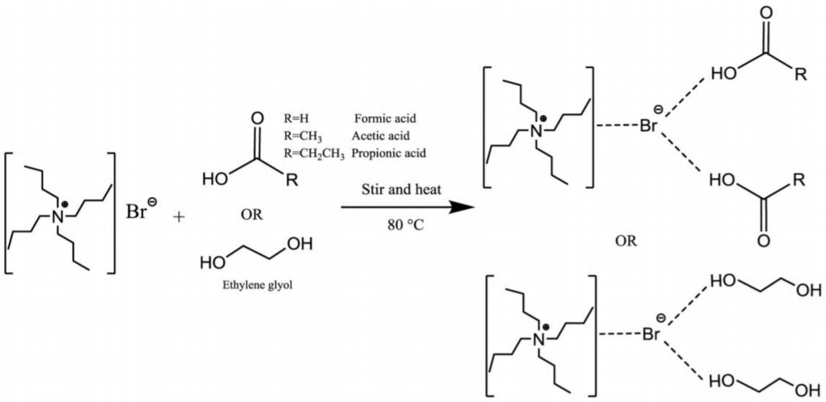


Figure 11 *Structure of the prepared DESs (Tetrabutylammonium bromide; TBABr with HBDs)*

Source: *[122]*

Tekin and coworker [123] described that preparation of DES from their proposed method were the simply mixing of ChCl and phenol in 50 mL centrifuge tubes at 1:2, 1:3, 1:4 and 1:5 M ratios by vortexing (3000 rpm) at room conditions until a colorless and homogeneous liquid was observed. The prepared DES was left in the ultrasonic bath for 15-20 sec to get rid of air bubbles. Analytical method based on vortex assisted deep eutectic solvent-liquid phase microextraction (VD-DES-LPME) have been applied for the determination of cobalt by SQT-FAAS detection.

Azizi and coworker [124] cited the work of Mamajanov et. al. [125] that the used DES was synthesized by the mixtures of ChCl (100 mmol) with urea (200 mmol). The DES mixture was stirred and heated until a clear liquid appears. They proposed the mechanism of DES preparation as shown in Fig. 12.

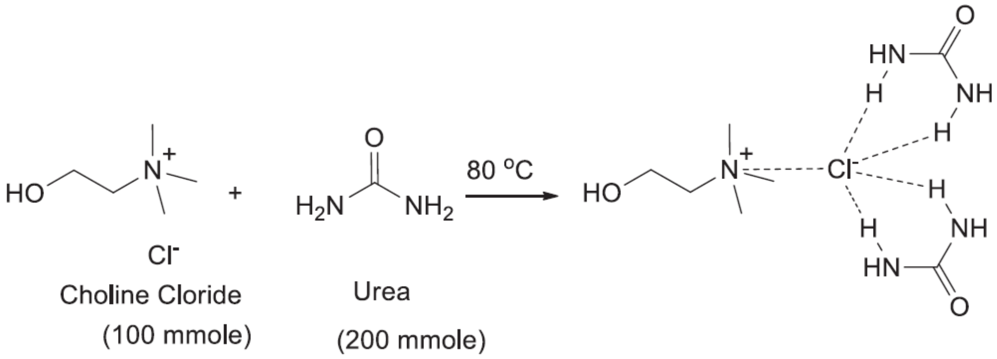


Figure 12 *Mechanism of DES (ChCl/urea) preparation*

Source: *[124]*

Verma and Banerjee [126] reported that the HBA (DL-menthol) was combined with HBD (lauric acid) with a molar ratio of 2:1. They were added in a flat-bottom flask which was fitted with a reflux condenser for 1 h at 50 °C with magnetic stirring until a clear liquid was formed (Fig. 13).

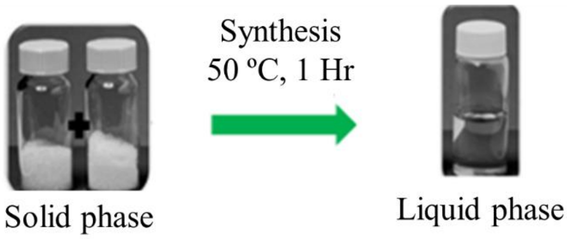


Figure 13 The mixtures of DL-menthol with lauric acid in the DES synthesis

Source: *[126]*

Xingquan and coworker [127] described the application of deep-eutectic solvents in green organic synthesis with some advantages of DES compared with the traditional ILs, DES were greener, cheaper and more accessible. Furthermore, the preparation methods of deep-eutectic solvents were easy, and the components of DES were biodegradable as mentioned in Fig.14 .

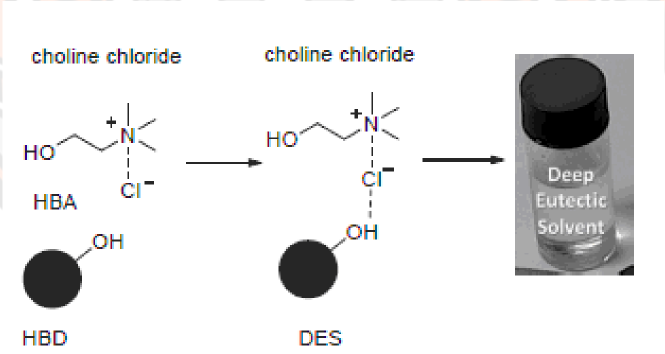


Figure 14 *The common reaction of ChCl-based DES synthesis*

Source: *[127]*

Eutectic mixtures, formed from choline chloride and HBD, have been of interest due to their ability to solvate several species of transition metals, including chlorides and oxides [108]. A range of HBD have been studied with deep eutectic solvents formed using amides, carboxylic acids, and alcohols (Fig. 7). These eutectic mixtures were simple to prepare, and unreactive with water. Many types were biodegradable and low cost. The various HBD available mean that this class of DES was particularly adaptable.

Most of the DES reviewed in the present work were based on the quaternary ammonium cation as choline (or as cholinium cation). This cation was nontoxic and had a comparatively low cost (when compared with imidazolium and pyridinium) [108]. In the present dissertation, DES from choline chloride and phenol was focused as an environmentally friendly solvent to combine with DLLME using air-assisted enhancement for the effective preconcentration and determination of Pb and Cd in food samples in the fourth work of dissertation.

2.4.3 Summarized application of DES in microextraction techniques

Numerous published articles devoted to the development of DES, its composition, preparation and properties, extraction and separation were summarized in Table 5. A wide range of DES composition was noticeable choices for the development of microextraction and the modification of alternative solvents under the condition of high extraction efficiency and friendly for human and environment.

In the present study, trace determination of Pb and Cd was carried out on the basis of the extraction of Pb and Cd complexes in the presence of contaminated samples using DES via DLLME and detection by HR-CS-FAAS. The dispersion of phases was achieved by air-assisted technique. A simple, rapid, selective, sensitive, and eco-friendly air-assisted deep eutectic solvent dispersive liquid-liquid microextraction (AA-DES-DLLME) method was used for the fast sequential multi elements analysis in food samples.

Table 5 *DES (or NADES) composition and its application for microextraction methods*

Techniques	Detection	Analyte	Sample	Ligand	DES composition	LOD	%RSD	Ref.
THS-DES/M-GO-MSPE	AFS	Hg	wastewater	EDTA		-	1.55	[78]
DES-UA-ELPME	Spectrophotometry	Malachite green	fish water	-	Choline chloride /Phenol	3.6 µg/L	2.7	[128]
VA-DES-ELLME	Spectrophotometry	Curcumin	food and herbal tea	-	Choline chloride /Phenol	2.86 µg/L	1.8	[20]
DES-LPME	GFAAS	Pb Cd	edible oils	-	Choline chloride /Urea	8.0 ng/kg 0.2 ng/kg	3.5 4.5	[129]
DES-AAELLME	GC-FID	Methadone	water, urine, and plasma	-	Choline chloride /TNO	0.7 µg/L	5.4	[62]
DES-LPME	SQT-FAAS	Pb	milk samples	Dithizone	Choline chloride /Phenol	8.7 µg/L	3.1	[19]
CL-DES-MNF-AALLME	GFAAS	Cd Pb Cu As	walnut, rice, tomato paste, spinach, orange juice, black tea, and water	Dithizone	Choline chloride /Phenol	4.2 ng/L 3.0 ng/L 3.5 ng/L 3.6 ng/L	2.5–3.1	[130]

Table 5 *(Cont)…*

Techniques	Detection	Analyte	Sample	Ligand	DES composition	LOD	%RSD	Ref.
NADESs-HF-LPME	HPLC-UV	Caffeic acid	coffee, green tea, and tomato	-	Serine /Lactic acid	0.3 µg/L	4.1	[131]
UAµE-DES	FAAS	Cd	lipsticks and eye shadows	APDC	$ZnCl_2$/Acetamide	0.86 µg/L	<5	[57]
		Pb				0.66 µg/L		
DES-UA-DLLME	FAAS	Cd	wine	Dithizone	Lactic acid/Aliquat 336	0.080 µg/L	2.9-4.5	[132]
		As				0.30 µg/L		
DES-DLLME	FAAS	Cd	milk samples	Menthol/ Mandelic acid	Menthol/Mandelic acid	0.38 µg/L	3.6	[133]
		Cu				0.42 µg/L	3.4	
		Pb				0.40 µg/L	4.1	
NADESs-UA-DLLME	FAAS	Cu	honey	Methyl green	Citric acid/ Sucrose	0.077 µg/L	1.7-3.2	[134]
		Pb				0.29 µg/L	2.7-3.1	
		Cd				0.16 µg/L	2.5-3.8	
VA-DES-LPME	FAAS	Pb	oil samples	-	Choline chloride /Urea	2.4 µg/L	0.9-4.3	[121]
		Co				4.6 µg/L		
		Ni				7.5 µg/L		
		Mn				1.0 µg/L		
UA-DES-LPME	GFAAS	Hg	water and fish	Dithizone	Choline chloride/Phenol	0.073 µg/L	<4.1	[120]

Remarks;

THS-DES/M-GO-MSPE	Ternary hydrosulphonyl-based deep eutectic solvent-magnetic graphene oxide- magnetic solid phase extraction
AFS	Atomic fluorescence spectrometry
DES-UA-ELPLME	Deep eutectic solvent based ultrasound-assisted emulsification liquid phase microextraction
VA-DES-ELLME	Vortex assisted deep eutectic solvent-emulsification liquid-liquid microextraction
DES-AAELLME	Deep eutectic solvent air-assisted-emulsification liquid-liquid microextraction method
CL-DES-MNF-AALLME	Centrifuge-less deep eutectic solvent based magnetic nanofluid-linked air-agitated liquid–liquid microextraction
NADESs-HF-LPME	Natural deep eutectic solvents hollow fiber-liquid microextraction
UAμE-DES	Ultrasonic assisted microextraction method based on deep eutectic solvent
DES-UA-DLLME	Deep eutectic solvent-based ultrasonic-assisted dispersive liquid-liquid microextraction

2.5 Hollow fiber liquid phase microextraction and electromembrane

2.5.1 The fundamental theory of hollow fiber liquid phase microextraction and electromembrane

Hollow fiber liquid phase microextraction (HF-LPME) was a mode of LPME that used a porous polypropylene hollow fiber for immobilization of organic solvent in the pores of hollow fibers as supported liquid membrane (SLM). This method was based on the use of hollow fibers as membrane, filled with a micro volume of extracting solution as acceptor phase and submerged into an aqueous solution as donor phase. SLM acted as the permeable membrane then analyte through passed the SLM by mass transfer via stirring. HF-LPME can be carried out using different modes were described that [135, 136]: (1) two-phase HF-LPME (Fig. 15(1)) was effective of extracting uncharged ions or hydrophobic analytes because of the both of HF-pores and HF-lumen as the organic solvent ($\text{Donor}_{\text{aqueous}}/\text{SLM}_{\text{organic}}/\text{Acceptor}_{\text{organic}}$). (2) three-phase HF-LPME (Fig. 15(2)) was suitable for the charged ions or hydrophilic analytes because of the HF-pores as supported liquid membrane (SLM) as well as HF-lumen as the aqueous phase ($\text{Donor}_{\text{aqueous}}/\text{SLM}_{\text{organic}}/\text{Acceptor}_{\text{aqueous}}$). High preconcentration ratio was an outstanding advantage of this method. On the other hand, the HF-LPME system should be concerned about air bubbles in the surface of the hollow fiber during the extraction. Since 2006, electromembrane extraction (EME) was founded by Pedersen-Bjergaard and Rasmussen [137]. EME was a subclass of three-phase-HF-LPME when hollow fiber was used as membrane. EME coupled with HF-LPME was encouraged for elimination of long-time extraction in normal-HF-LPME via the use of electro-enhancement. In this specific microextraction technique, charged analytes were extracted across the SLM with the application of an electrical field. The application of an electrical field over the SLM improved the extraction speed significantly [35].

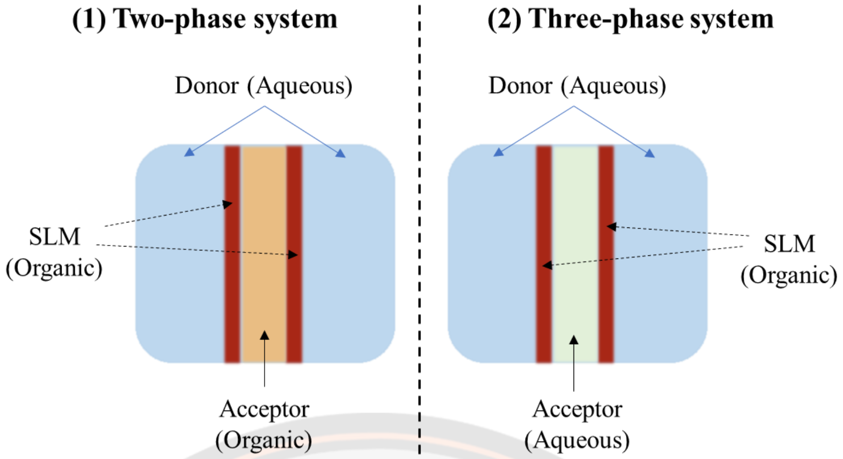


Figure 15 *Two modes of HF-LPME*

2.5.2 Fundamental aspects of electromembrane-hollow fiber liquid phase microextraction

In electromembrane-hollow fiber liquid phase microextraction (EM-HFLPME), the knowledge on fundamental aspects of this method as mass transfer, electrolysis/pH shift, and SLM modification were summarized.

Mass transfer Mass transfer via passive diffusion (High to low concentration) of normal membrane extraction and the use of stirring method in HF-LPME was required the long extraction time for equilibrium. Recently, EM-HFLPME or EE-HFLPME were modified with external electric system to overcome the long extraction times. Mass transfers across the SLM in EM-HFLPME related to a time-dependent transient model. Chuixiu Huang [29] have been proposed this model that mainly based on assumptions:

1) SLM-dependent lag time, the mass transfer was enhanced by modifying the properties of the SLM. For example, the modification of HF through carbon nanotubes, polymers, and silver nanoparticles.

2) The efficient convection in sample and acceptor solution, the mass transfer was enhanced by modifying the properties of sample. For example, surfactant-assisted and ultrasound-assisted method.

3) Direction of migration, the mass transfer could be enhanced by modifying the electric field to drive the unidirectional ions movements.

Electrolysis The electrolytic traction generally occurred at the cathode and anode electrodes during the EM-HFLPME. Especially, at high voltages applying, the electrolysis might be generated the critical current, bubble formation [30], and joule heating [35] which affected the extraction performance significantly. Due to the increasing of ionic contents in sample and acceptor solution might be led to excess electrolysis [29]. In addition to boundary layer formation of analyte ions at the SLM/donor phase interfaces [36] and resistance at the interfaces at both sides of the SLM [35] were considered. Moreover, acceptor solutions with strong buffer capacity were recommended for the pH stabilization and the decreasing of pH shift as well. Thus, the acceptor solution in EM-HFLPME should be carefully avoided the high applied voltages.

Supported liquid membrane SLM was one of the most important factors for the successful electro-kinetic migration of ionic compounds in EM-HFLPME which prepared by the dipping of hollow fiber into organic solvent. The membrane was impregnated with organic solvent that shown in Fig. 16. The criteria for good SLM included; low water solubility, low vapor pressure, high polarity-polarizability, high hydrogen bonding capability (high hydrogen-bond basicity for cations and high hydrogen-bond acidity for anions), and reasonable hydrophobicity [29]. The utilized solvent should have certain characteristics such as good extraction efficiency, high selectivity for the target analytes, water-immiscibility, and electrical conductivity [138]. Nowadays, non-polar (toluene, cumene, and nitrophenyloctyl ether (NPOE)) and polar (1-pentanol, 1-dodecanol, 1-octanol, and 1-hexanol) organic solvents [31] were used as SLM in the EME, HFLPME, and EM-HELPME for heavy metals analysis. When solvent polarity increased, the current levels also increased. Thereby, the acceptor phase volume can be changed because of the high occurred current value. In contrast, a poor extraction efficiency or lack of metal ion transportation witnessed when a nonpolar solvent was used. Therefore, the modification of SLM with carriers such as di-(2-ethylhexyl) phthalate (DEHP) [34], tris-(2-ethylhexyl) phosphate (TEHP) [139], NPOE [140], Aliquat 336 [141], and cetyl trimethyl ammonium bromide (CTAB) [142] was the option to improve the extraction efficiency.

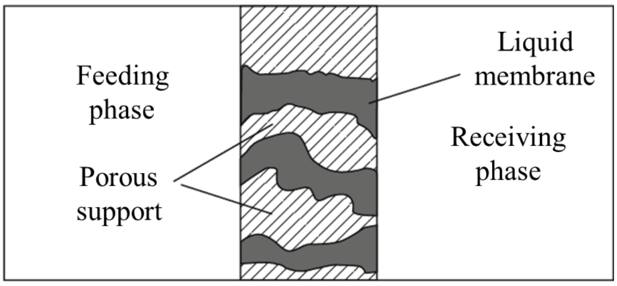


Figure 16 *Configurations of liquid membrane in SLM system*

Source: *[143]*

EM-HFLPME method gave the crucial advantages such as the higher efficiency, easier to control, smaller volume, faster extraction, quicker equilibrium, better mass transfer, and cheaper cost operation than conventional method. Different design of extraction system was set under the appropriate purposes.

In the part of cation analysis, the determination of heavy metals mostly used this design. Since, most of metal species were in cation forms. This technique successfully used for the extraction and determination of Hg^{2+} [34], Pb^{2+} [144], Ag^{+}, Cd^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} [139], Cr^{3+} and Cr^{6+} [32], and Th^{4+} [145] in different samples. The extraction system for cation setting as Fig. 17, anode electrode was inserted in acceptor phase inside hollow fiber and cathode electrode was directly submerged in donor phase. Cationic analytes were transferred from donor to acceptor.

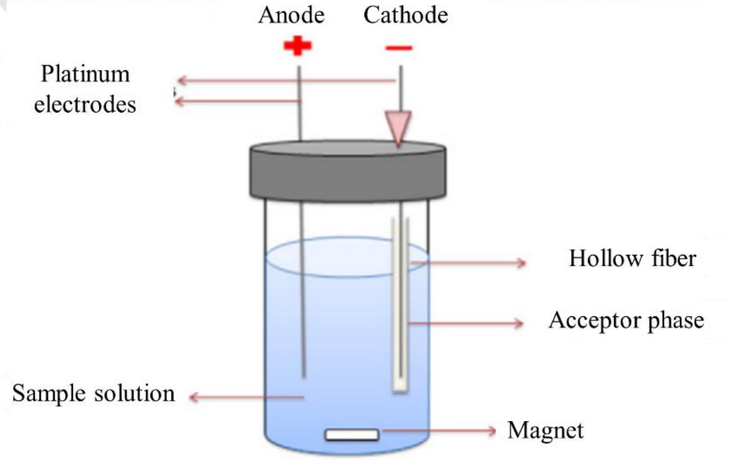


Figure 17 *Schematic setting system for cation analysis by EM-HFLPME*

Source: *[34]*

In the part of anion analysis, heavy metals were rarely used this design. Recently, only a publication of Chanthasakda and coworker [141] reported the analysis of anion of Cr (VI). Chromate ions (CrO_4^{2-}) as Cr (VI) oxoanions in donor phase were extracted. As demonstrated in Fig. 18, one of the electrodes (anode) was inserted inside the lumen of the fiber and the other (cathode) was directly placed inside the sample solution. Anionic analytes were transferred from donor to acceptor.

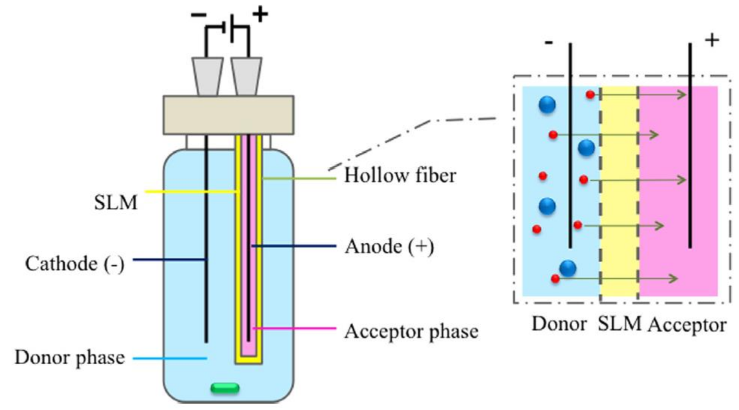


Figure 18 *Schematic setting system for anion analysis*

Source: *[141]*

2.5.3 Development of EME or electro-enhanced-HF-LPME

Many publications pay attention to electromembrane and electro-enhanced-HF-LPME. Modification of SLM, type of acceptor, optimized voltage, and mode of extraction via the selecting of complexing agent were summarized in Table 6. According to determination of different analytes, its analytical of merits from different targets and samples were considered.

From this concept, EM-HFPLME was applied in the semi-three-phase mode via the modification of acceptor using DES to determine contaminated Pb in form of anion. The proposed EM-DES-HFLPME was novel and this was the first time to report the determination of Pb in Pb-oxyanion forms. Successfully, the proposed method was applied in drinking and tap water samples by GFAAS detection.

Table 6 *The application of electromembrane-based hollow fiber liquid phase microextraction for various types of samples*

Technique	**Analyte**	**Sample**	**Complexing agent**	**Acceptor**	**SLM**	**Voltage (V)**	**Linearity (µg/L)**	**LOD (µg/L)**	**LOQ (µg/L)**	**%RSD**	**%Recovery**	**EF or PF**	**Ref.**
HF-EME with FAAS	- Ag - Cd - Co - Cu - Zn	- nano silver suspension - wastewater	-	HCl (pH 1.2)	Mixtures of 1-octanol/ DEHP/TEHP	60	-	-	-	-	-	-	[139]
EME with spectrophotometry	Hg	- water - fish	PAN	pH 3 buffer	1-octanol and DEHP	70	2.3-950 40-9500	0.7 12	2.3 40	5.6 6.2	94.7 89.3	176 130	[35]
EME with CE	- Methamphetamine - Ephedrine - Methadone	- hair - urine	-	HCl (pH 1)	NPOE	125	-	0.90 - 2.42	-	3.7-4.3	70-82	140-164	[140]
EE-HF-LPME with spectrophotometer	Cr (VI) oxoanions	- MilliQ - drinking water	DPC	NaOH	Aliquat-336 in 1-heptanol	30	3–15	1.0–3.5	-	<12	96–101	200	[141]
EME with spectrophotometer	Bi	- human plasma - water	xylenol orange	H_2SO_4	1-octanol and DEHP	70	2.1–800	0.64–1.47	21.-4.9	4.1-5.9	93.8-102.8	151–187	[30]
EME with GFAAS	Hg	- tap water - river water	-	HNO_3	1-octanol and DEHP	60	0.5–10	0.5	-	6.2–7.1	41-43	102–108	[34]
DEME with HPLC	Cr (III), Cr (VI)	- tap water - river water - mineral water	APDC	pH 7	1-octanol	30	20-500	5.4	-	9.8-13.7	31.1-47.2	21.8-33	[32]

Table 6 *(Cont)…*

Technique	Analyte	Sample	Complexing agent	Acceptor	SLM	Voltage (V)	Linearity (µg/L)	LOD (µg/L)	LOQ (µg/L)	%RSD	%Recovery	EF or PF	Ref.
EME with spectrophotometer	As (V)	- tap water - under-ground water - gelatin powder	Molybdenum	pH 7.3 buffer	1-octanol with DEHP	70	5–300	1.5	-	3.4–7.6	60-65	30.0-32.5	[37]
CM-HFLPME-GFAAS	Pb	blood	-	DES containing $KClO_4$	CTAB with 1-octanol	-	1–200	0.1	0.4	2.3-4.4		33	[142]
EME-EA-LLME with GC-FID	Antidepressant drugs	- urine - wastewater	-	NaOH	2-ethyl hexanol	75	0.5–750	0.15	-	6.9-12.2	47.3-64.1	561.4 and 770.3	[146]

Remarks;

HF-EME	Hollow fiber based electromembrane extraction
EME	Electromembrane extraction
CE	Capillary electrophoresis
EE-HF-LPME	Electro-enhanced hollow fiber membrane liquid phase microextraction
DEME	Dual electromembrane extraction
CM-HFLPME	Carrier-mediated hollow fiber liquid phase microextraction
EME-EA-LLME	Electromembrane extraction- electro-assisted liquid-liquid microextraction

2.6 Atomic absorption spectrometry

Spectrometric methods were a large group of analytical methods based on atomic and molecular spectroscopy. Spectroscopy was a general term for the science that dealt with the interactions of various types of radiation with matter. These parts also considered concepts and instrumental components of absorption process and its spectrometry. When radiation passed through a layer of solid, liquid, or gas, certain frequencies may be selectively removed by absorption. The process in which electromagnetic energy was transferred to atoms, ions, or molecules composing the samples. Absorption promoted these particles from their ground state, to one or more higher energies excited state. For absorption of radiations to occur, the energy of exciting photon exactly matched the energy difference between the ground state and one of the excited state of the absorbing species [147]. Quantitative absorption methods required for two measurements: beams before passed through the medium (P_0) and after (P). Transmittance (T) and absorbance (A) were related to the ratio of P_0 and P as below.

$$T = \frac{P}{P_0} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$A = \log_{10} T = \log \frac{P_0}{P} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

For monochromatic radiation, absorbance was directly proportional to the path length (b) through the medium and the concentration (c) of the absorbing species. These relationships were given by Beer's Law

$$A = abc \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

where a was an absorptivity (Constant, L/g/cm), b was path length (cm), and c was concentration (g/L)

or $$A = \varepsilon bc \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

where ε was a molar absorptivity (Constant, L/mol/cm), b was path length (cm), and c was concentration (mol/L)

There were three major types of atomic absorption spectrometry for identifying and determining the elements in samples in this dissertation: flame atomic absorption spectrometry, high resolution continuum source flame atomic absorption spectrometry, and graphite furnace (Electrothermal) atomic absorption spectrometry.

2.6.1 Flame atomic absorption spectrometry (FAAS)

In this section, theoretical basis of flame atomic absorption spectrometry (FAAS) and its importance characteristics were briefly considered. FAAS was a widely used technique for trace metal analysis. Sample solution was introduced to burn via flame on a head of burner. While the aerosol of solution was decomposed to atom forms in the atomization, the characteristic radiation was absorbed by the generated atoms. Absorbances was magnified and detected via the spectrometer parts then the results of analysis were processed. Perceptively, FAAS instrumental components were demonstrated in Fig. 19.

Light source or radiation source The most common source for FAAS was the hollow cathode lamp (HCL) which consisted of a tungsten anode and a cylindrical constructed metal cathode. In the ionization, while a potential was applied into electrodes, the electrons at electrode was migrated. Hence, the metal atoms from constructed cathode surface were dislodged via the sputtering of the ionized gaseous cations (Sputtering). Next, the sputtered metal atoms were excited (Excitation) and thus emitted their characteristic radiation as they returned to ground state (Emission).

Sample introduction Sample must be introduced in the form of a fine aerosol. This was achieved using a nebulizer (Produced an aerosol) and spray chamber (Homogenized the aerosol with gases) prior to the burner (Compound decomposition and atomization).

Atomizer In a flame atomizer, aerosol of sample was nebulized by a flow of fuel (Natural gas, hydrogen, and acetylene) mixed with oxidant (Air, oxygen, and nitrous oxide), and carried into the flame where atomization occurs. In dissertation, air/acetylene flame was selected for Pb (283.3 nm) analysis that flame produced temperatures from 2100-2400 ºC.

Monochromator Monochromator was a wavelength selector that employed slits (Entrance and exit slits), lenses, mirrors, windows, and prism or gratings (Reflection grating and Echelle grating). The prisms or gratings was designed for spectral scanning. The slit played an important role in performance characteristics. A single line wavelength was set by slit width that affected on the resolution of monochromator.

Detector and processor Photomultiplier tube (PMT) was detector in the used FAAS. The tube maintained with dynodes for accommodation of incident radiation. Several electrons were emitted for each electron that hit the surface. Numerous electrons (10^6 -10^7 electrons for each incident photon) have been performed at anode then the current was electronically amplified and measured.

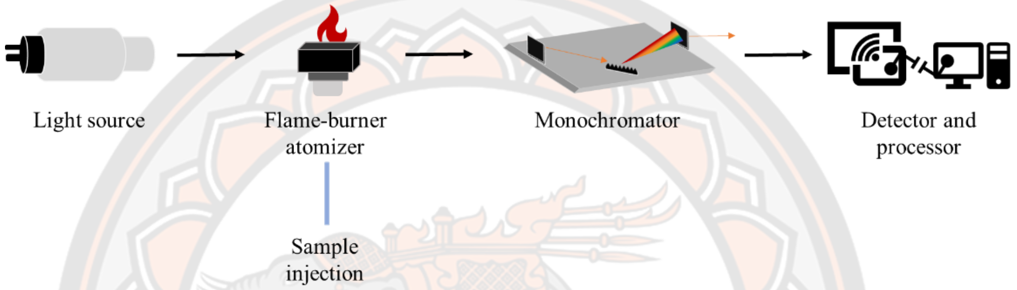


__Figure 19__ Block diagram of FAAS spectrometer

There were many applications for FAAS, most requiring the sample were in solution. Typical preconcentration extraction methods, detection limits, and precision for the determination of metals in different samples were summarized in Table 7.

Table 7 *Application of flame atomic absorption spectrometry for microextraction*

Spectrometry	**Analyte**	**Sample**	**Preconcentration**	**%RSD**	**LOD**	**Ref.**
FAAS	Pb	Milk samples	Vortex assisted deep eutectic solvent based liquid phase microextraction	3.1	8.7 µg/L	[19]
FAAS	Cu	Water samples	On-line sequential injection dispersive liquid-liquid microextraction	2.1	0.04 µg/L	[148]
	Pb			1.9	0.54 µg/L	
FAAS	Ni	Chocolate powder	In-syringe dispersive liquid-liquid microextraction	4.8	0.1 mg/kg	[149]
FAAS	Cd	Water samples	Solvent-based de-emulsification dispersive liquid–liquid microextraction	5.1	0.03 µg/L	[66]
FAAS	Fe	Hair samples	Switchable solvent liquid phase extraction	4.7	2.6 µg/L	[58]
FAAS	Cd	Water, urine and tea	Ultrasonic assisted switchable solvent based on liquid phase microextraction	1.3	0.24 µg/L	[91]
	Ni			3.7	0.76 µg/L	
	Pb			2.9	0.72 µg/L	
	Co			3.8	0.63 µg/L	
FAAS	Co	Tobacco and food	Switchable solvent based green liquid phase microextraction	-	3.2 µg/L	[51]

Table 7 *(Cont)*…

Spectrometry	Analyte	Sample	Preconcentration	%RSD	LOD	Ref.
FAAS	Cu	Oil samples	Ligandless reversed-phase switchable-hydrophilicity solvent liquid–liquid microextraction	4.7-9.4	6.9 µg/L	[150]
FAAS	Pb	Water samples	Switchable dispersive liquid–liquid microextraction	3.5	0.25 µg/L	[151]
FAAS	Pd	Water and dust	Switchable solvent based liquid phase microextraction	0.9-8.0	4.28 µg/L	[89]
FAAS	Ni	Cigarette	Ligandless switchable solvent based liquid phase microextraction	3.0	5.2 µg/L	[98]
FAAS	Cd Zn	Water and fruit juice	Effervescence-assisted dispersive liquid-liquid microextraction	1.2 2.5	0.21 µg/L 0.14 µg/L	[152]
FAAS	Cd	Water, vegetable, fruit, and cigarette	Switchable polarity solvent for liquid phase microextraction	5.4	0.16 µg/L	[18]
FAAS	Mn	Soil samples	Switchable solvent based liquid phase microextraction	4.9	0.71 µg/L	[153]

Table 7 *(Cont)*…

Spectrometry	Analyte	Sample	Preconcentration	%RSD	LOD	Ref.
FAAS	Cd	Tap and well water	Magnetic solid-phase extraction	1.9-9.3	0.0015 mg/L	[43]
	Pb			1.9-7.7	0.01 mg/L	
FAAS	Zn	water, flour, celery and egg	Magnetic dispersive solid-phase extraction	2.4-5.8	0.08 µg/L	[44]
FAAS	Pb	Red pepper	Magnetic nanoparticle based sonication assisted dispersive solid phase extraction	11	10 µg/L	[154]
FAAS	Pb	River water	Magnetic dispersive solid-phase extraction	3.8	50.2 µg/L	[155]
FAAS	Pb	Black tea	Magnetic particles based dispersive solid-phase microextraction	-	7.7 µg/L	[156]
FAAS	Pb	Water and sediment	Flow injection-on-line preconcentration	3.4	2.0 µg/L	[157]

Table 7 *(Cont)*…

Spectrometry	Analyte	Sample	Preconcentration	%RSD	LOD	Ref.
FAAS	Cu, Cd, Pb, Zn, Ni, and Co	Tomato source, canned tuna, tobacco, and chickpea	Multiwalled carbon nanotubes solid phase extraction	<5	0.30-0.60 µg/L	[158]
FAAS	Cu	Water, black tea, and diet stuffs	Magnetic solid phase microextraction	4.93	13.3 µg/L	[79]

2.6.2 High resolution continuum source flame atomic absorption spectrometry (HR-CS-FAAS)

This section was devoted to high resolution continuum source flame atomic absorption spectrometry (HR-CS-FAAS), the best of standard FAAS instruments for automatic single-element and sequential multi-element analysis. Theoretically, atomization and instrumental partway of this method were not different from FAAS. However, resolution, technology, and performance were better excellent than normal FAAS. Instrumental accessories of HR-CS-FAAS were demonstrated in Fig. 20.

Light source or radiation source The used HR-CS-FAAS was obtained with xenon short arc lamp as a single light source for all applications which light intensity was significantly higher than with traditional AAS light sources (or HCL). The continuum emission spectrum of a xenon short arc lamp covered the complete spectral range of AAS. The lamp provided an excellent signal-to-noise ratio resulting in improved detection limits. Any element in the applicable spectral range can be analyzed on primary or secondary wavelength.

Sample introduction Sample must be introduced in a fine aerosol as in traditional AAS using the part of nebulizer and spray chamber as well.

Atomizer In a flame atomizer, aerosol of sample was together homogenized with fuel and oxidant. For fast-sequential multi-elements analysis, ratio of fuel and oxidant should be a compromised condition for the effective switching line.

Monochromator High-resolution spectrometer was built using a double-monochromator such as a prism/echelle monochromator and a double-echelle monochromator as a high performance for spectral scanning and focusing.

Detector and processor Charge-coupled device (CCD) was a detector which offered the advantages of greater sensitivity to low light levels. CCD detectors consisted of a two-dimensional array of silicon as pixels by *p*-type silicon and *n*-type silicon. The movement of formed electrons induced many charges on circuit that charges were transferred to an amplifier and readout.

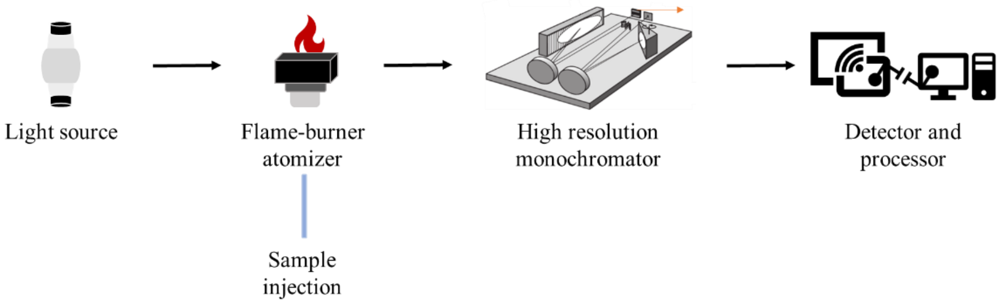


Figure 20 *Block diagram of HR-CS-FAAS spectrometer*

There were many available publications for HR-CS-FAAS, reporting for atomic or molecular analysis. Applications of HR-CS-FAAS for a variety of targets and samples were summarized in Table 8.

Table 8 *Application of high-resolution flame atomic (or molecular) absorption spectrometry*

Spectrometry	Analyte	Sample	Preconcentration	%RSD	LOD	Ref.
HR-CS-FAAS	Cu	Automotive gasoline	Emulsion breaking	5-7	3.2 µg/L	[159]
	Fe				5.2 µg/L	
	Pb				14 µg/L	
HR-CS-FAAS	Si	Food products	Water-in-oil-in-water emulsions	4.5-11	0.04-0.11 mg/L	[160]
HR-CS-FAAS	Zn	Alcoholic and non-alcoholic drinks	Direct analysis	3.2-7.2	0.040 mg/L	[161]
	Cu			1.7-4.6	0.016 mg/L	
	Pb			2.1-4.3	0.099 mg/L	
HR-CS-FAAS	Cu	Brazilian red wines	Direct analysis	<5	0.04 mg/L	[162]
	Li				0.005 mg/L	
	Rb				0.02 mg/L	
	Na				0.9 mg/L	
	K				4.4 mg/L	
	Mn				0.04 mg/L	
	Co				0.06 mg/L	
	Ca				0.9 mg/L	
	Sr				0.02 mg/L	
	Be				0.02 mg/L	

Table 8 *(Cont)…*

Spectrometry	Analyte	Sample	Preconcentration	%RSD	LOD	Ref.
HR-CS-FAAS	S (as CS)	Coal samples	Microwave-assisted digestion	-	0.01 %w/w	[163]
HR-CS-FAAS	B	Plant leaves	Wet digestion	<5.7	0.001 µg/g	[164]
	Ca				0.15 µg/g	
	Cu				0.58 µg/g	
	Fe				1.6 µg/g	
	K				0.12 µg/g	
	Mg				0.48 µg/g	
	Mn				0.96 µg/g	
	Mo				0.016 µg/g	
	P				0.014 µg/g	
	S				0.11 µg/g	
	Zn				0.66 µg/g	
HR-CS-FAAS	Zn	Yogurt	Slurry sampling	2.08	0.10 µg/g	[165]
HR-CS-FAAS	S	Food samples	Microwave-assisted digestion	-	1.5 mg/g	[166]
HR-CS-FAAS	Ca	Yogurt, cow milk and milk powder	Slurry sampling	2.7-2.9	0.038 mg/g	[167]
	Mg				0.016 mg/g	
HR-CS-FAAS	Pb	Gasoline	Dilution	-	0.1-0.2 mg/L	[168]

Table 8 *(Cont)…*

Spectrometry	**Analyte**	**Sample**	**Preconcentration**	**%RSD**	**LOD**	**Ref.**
HR-CS-FAAS	Ca	Teas and tisanes made	Acid digestion	0.4-3.6	29.4 µg/L	[169]
	Co			-		
	Cu			0.2-5.7		
	Fe			1.3-15.1		
	Mn			1.1-2.6		
	Ni			-		
	Na			0.3-1.6		
	Zn			3.2-3.3		

2.6.3 Graphite furnace atomic absorption spectrometry (GFAAS)

Graphite furnace (Electrothermal) atomic absorption spectrometry (GFAAS or ETAAS) was a flame-less AAS technique. Briefly, a few microliters of sample were evaporated at low temperature (Drying: 80-200ºC) then ashed at higher temperature in an electrically heated graphite tube (Ashing or pyrolysis: 300-1500ºC). After that, atomization of the sample occurred in a period of a few milliseconds to seconds (Atomization: 1600-3000ºC). Finally, the graphite tube was heated for cleaning. The absorbing of generated atoms was measured as peak area absorbance. Parts of GFAAS instrumental apparatus were schemed in Fig. 21.

Light source or radiation source The used GFAAS constructed with HCL as light source.

Sample introduction Sample must be introduced in a form of solution (or slurry) using plastic capillary. Injector carrying sample was settled on graphite tube and sample was released on platform (as called L'vov platform).

Atomizer In a flame-less (or electrothermal) technique, graphite tube with pyrolytic coating was used as a sample holder and atomizer. In this device, atoms occurred in a cylindrical graphite tube that was opened at both end sides. Surface of graphite was coated with a thin layer of pyrolytic carbon, which served to seal the pores of tube to prevent the reducing of porosity of graphite tube. Pyrolytic graphite tube was to overcome the effect of degeneration for extending the tube life and good reproducibility.

Monochromator Slits (Entrance and exit slits), lenses, mirrors, windows, and prism or gratings (Reflection grating and echelle grating) were a monochromator for GFAAS as mentioned in FAAS.

Detector and processor The most widely used PMT was a detector in this instrument.

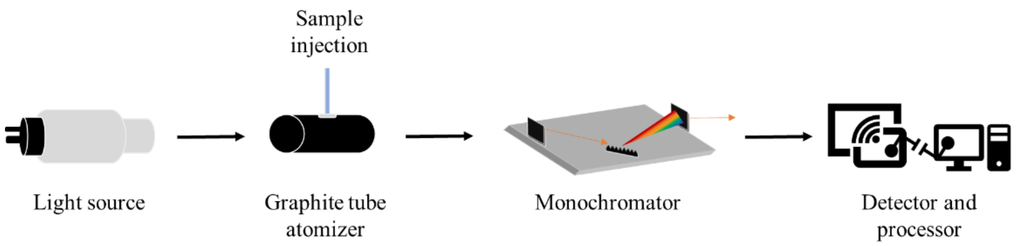


Figure 21 *Block diagram of GFAAS spectrometer*

GFAAS or ETAAS was one type of high sensitivity with low detection limit AAS instruments. Hence, the determination of trace analyte in natural, food, environmental, clinical, and biological samples were applied by the detection of GFAAS as demonstrated in Table 9.

Table 9 *Application of graphite furnace atomic absorption spectrometry for microextraction*

Spectrometry	**Analyte**	**Sample**	**Preconcentration**	**%RSD**	**LOD**	**Ref.**
GFAAS	Cd	Water and food	Switchable dispersive liquid–liquid microextraction	4.5	0.38 ng/L	[84]
GFAAS	Pb	Beverage	Magnetic ion-imprinted polymer	4.1	1.7 µg/L	[170]
GFAAS	Pb	Water, tea, and hair	Switchable solvent based liquid phase microextraction	4.2	16.0 ng/L	[171]
	Cd			6.2	3.9 ng/L	
GFAAS	Pb	Water and formula base powder	Solidification of a floating organic drop microextraction	8.8	0.058 µg/L	[172]
GFAAS	Cd	Water samples	Dispersive liquid-liquid microextraction	3.5	0.6 ng/L	[173]
GFAAS	V	Food and water	Micropipette tip syringe- switchable hydrophilicity microextraction	2.91	0.0075 µg/L	[174]
GFAAS	Cd	Seawater	Hollow fiber supported liquid membrane extraction	2.5%	0.8 ng/L	[15]
GFAAS	Bi	Water, urine, and hair	Cloud point extraction	<5	0.02 µg/L	[81]
GFAAS	Cu	Water samples	Magnetic solid-phase extraction	-	0.18 µg/L	[82]

Table 9 (*Cont*)…

Spectrometry	Analyte	Sample	Preconcentration	%RSD	LOD	Ref.
GFAAS	Cr	water samples	Electromembrane extraction through novel nanostructured hollow fibers	8.9	0.006 µg/L	[31]
GFAAS	Hg	water samples	Electromembrane extraction	6.2-7.1	0.50 µg/L	[34]
GFAAS	Cd	food, beverage and water	Ultrasonic assisted deep eutectic solvent liquid-phase microextraction	3.1	0.023 ng/L	[50]
GFAAS	As	water and environmental samples	Ultrasonic assisted deep eutectic solvent liquid-phase microextraction	4.3	10.0 ng/L	[22]
GFAAS	Se	food samples	Ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction	4.2	12 ng/L	[54]
GFAAS	Cd Pb	wine	Dispersive liquid–liquid microextraction	-	0.01 µg/L 0.08 µg/L	[53]
GFAAS	Pd	environmental samples	Air-assisted switchable-hydrophilicity solvent liquid-liquid microextraction	3.5	0.007 µg/L	[63]
GFAAS	Pd	environmental samples	Solvent-based de-emulsification dispersive liquid-liquid microextraction	3.68	0.007 µg/L	[65]

Table 9 *(Cont)*…

Spectrometry	Analyte	Sample	Preconcentration	%RSD	LOD	Ref.
GFAAS	Pb	herbs	Microwave digestion	8.8	0.45 ppb	[175]
	Cd			7.1	0.03 ppb	
	Cr			3.3	0.20 ppb	
	As			3.4	0.64 ppb	
GFAAS	Cd	liver and eggs	Wet digestion	2.7-10.9	0.0001 mg/kg	[176]
	Pb			2.8-9.9	0.0010 mg/kg	
GFAAS	Hg	water samples	Hollow fiber based liquid-phase microextraction	3.2	0.06 µg/L	[28]
GFAAS	Pb	fish and shellfish samples	Microwave digestion	3.21	4.35×10^{-5} mg/kg	[177]
	Cd			2.62	1.58×10^{-5} mg/kg	
	Sn			2.14	1.36×10^{-5} mg/kg	
GFAAS	Pb	edible oils	Deep eutectic liquid organic salt liquid-phase microextraction	3.5	8.0 ng/kg	[129]
	Cd			4.5	0. 2 ng/kg	

Remarks;

GFAAS or ETAAS was a flameless atomic absorption spectrometry via graphite furnace or electrothermal technique.

CHAPTER III

AIR-ASSISTED SOLVENT TERMINATED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION

The first research of this dissertation was related to the development of preconcentration method based on a novel hybrid of air-assisted solvent terminated dispersive liquid-liquid microextraction (AA-ST-DLLME) for determination of Pb in water and beverage samples using GFAAS. The proposed method combined a domestic fish-tank pump for bubble generator with demulsification to analyze trace Pb using a surfactant as a green demulsifier for the termination of the miscible solvent. In this chapter, the experimental procedures were described. The important parameters for the microextraction process (effect of pH, concentration of complexing agent, type of extracting solvent, type of disperser, type of demulsifier, air-assisted time and extraction time) were evaluated. Analytical performance and method validation were investigated as followed;

3.1 Experiment

3.1.1 Instrumentation

A graphite furnace atomic absorption spectrometer (Varian, Model SpectrAA 220Z, Germany) equipped with Zeeman background correction was employed with a Pb hollow-cathode lamp (Agilent technologies, part number 5610102900, Germany) as the light source and operating at 10 mA, using a wavelength of 283.3 nm and a slit width of 0.5 nm as default settings. Pyrolytic-coated graphite tubes (Agilent technologies, part number 6310001200, Germany) were used for the analysis. The operating instrumental temperature program for the graphite atomizer was given in Table 10. The sample volume was 10 µL and the modifier volume was 5 µL in all experiments. A pH meter (Metrohm, 827 pH lab, Switzerland) with a combined glass electrode was used for pH measurements. A bubble generator (Big boy air pump, BB-8000, Thailand) was purchased from a local store in Phitsanulok, Thailand and was used as the air-assisted source. A domestic microwave oven (Samsung, Trible distribution system (TDS), Thailand) was used for sample digestion.

3.1.2 Reagents

The chemical reagents used were 30% ammonia solution (Loba Chemie, India), 99.5% ammonium chloride, (Loba Chemie, India), 98% 1-(2-Pyridylazo)-2-naphthol (PAN), (Acros Organics, India), chloroform (RCI Labscan, Thailand), sodium dodecyl sulfate, SDS ≥99.0% (Merck, Germany), triton X-100, ≥99.0% (Fluka, Switzerland), 65% nitric acid (RCI Labscan, Thailand), hydrogen peroxide, >30% (VWR International, USA), and ethanol (RCI Labscan, Thailand). The standard solution of 1000 mg/L lead was purchased from Loba Chemie and was prepared for stock solution of 1000 ng/mL, while the 10.0 g/L palladium matrix modifier solution was obtained from Merck. Deionized water was used for the preparation of all solutions. Argon gas with 99.999% purity was used in GFAAS. All glassware used in these experiments was soaked in 10% nitric acid overnight and washed with deionized water before being used, to reduce contamination.

Table 10 *Temperature program of GFAAS for Pb determination by AA-ST-DLLME*

Step	**Temperature (ºC)**	**Time (s)**	**Ar-flow rate (L/min)**	**Read**
Drying	85	40.0	3.0	No
Pyrolysis	400	5.0	3.0	No
Atomization	2100	2.0	0.0	Yes
Cleaning	2100	2.0	3.0	No

3.1.3 Optimization

In AA-ST-DLLME-GFAAS, the important parameters for microextraction *e.g.* effect of pH, type of extracting solvent, disperser, and demulsifier as well as its concentration, were investigated by univariate optimization method.

Effect of pH 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of buffer solution from pH 3-11, 0.5 mL of 1.0 mM PAN (complexing agent), 0.5 mL of chloroform (extracting solvent), 0.5 mL of 0.5% v/v Triton X-114 (disperser), and 0.3 mL of 0.5% v/v Triton X-114 (demulsifier) were used under the pre-optimized condition with the air-assisted time for 5 sec and extraction time for 3 min following AA-ST-DLLME-GFAAS procedure.

Effect of concentration of PAN 30 mL of 1 ng/mL Pb solution was adjusted with 0.5 mL of pH 9 buffer solution. 1.0 mL of different concentration of PAN (complexing agent) for final concentration in the range of 0.0-3.0 mM were studied. 0.5 mL of chloroform (extracting solvent), 0.5 mL of 0.5% v/v Triton X-114 (disperser), and 0.3 mL of 0.5% v/v Triton X-114 (demulsifier) were used under the pre-optimized condition with the air-assisted time for 5 sec and extraction time for 3 min following AA-ST-DLLME-GFAAS procedure.

Type of extracting solvent 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution and 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent). 0.5 mL of different extracting solvents (chloroform, dichloromethane, and dimethyl sulfoxide) were studied. 0.5 mL of 0.5% v/v Triton X-114 (disperser) and 0.3 mL of 0.5% v/v Triton X-114 (demulsifier) were used under the pre-optimized condition with the air-assisted time for 5 sec and extraction time for 3 min following AA-ST-DLLME-GFAAS procedure.

Type of disperser 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution, 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent), and 0.5 mL of chloroform (extracting solvents). 0.5 mL of 0.5 %v/v different types of dispersers (Triton x-100, Triton x-114, and SDS) were studied. 0.3 mL of 0.5% v/v Triton X-114 (demulsifier) were used under the pre-optimized condition with the air-assisted time for 5 sec and extraction time for 3 min following AA-ST-DLLME-GFAAS procedure.

Concentration of disperser 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution, 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent), and 0.5 mL of chloroform (extracting solvents). 0.5 mL of the different concentration of SDS (disperser) in the range of 0.00-0.17 %w/v (final concentration) were studied. 0.3 mL of 0.5% v/v Triton X-114 (demulsifier) were used under the pre-optimized condition with the air-assisted time for 5 sec and extraction time for 3 min following AA-ST-DLLME-GFAAS procedure.

Type of demulsifier disperser 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution, 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent), 0.5 mL of chloroform (extracting solvents), and 0.5 mL of 9.18% w/v (final concentration 0.14% w/v) SDS (disperser). 0.3 mL of different types of demulsifiers (Triton x-100, Triton x-114, SDS, methanol, ethanol, acetone, acetonitrile, and tetrahydrofuran (THF)) were optimized. Air-assisted time for 5 sec and extraction time for 3 min were applied to the AA-ST-DLLME-GFAAS procedure.

Concentration of demulsifier 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution, 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent), 0.5 mL of chloroform (extracting solvents), and 0.5 mL of 9.18% w/v (final concentration 0.14% w/v) SDS (disperser). 0.3 mL of Triton x-100 (demulsifier) in the range of 0.000-0.055 %v/v (final concentration) were optimized. Air-assisted time for 5 sec and extraction time for 3 min were applied to the AA-ST-DLLME-GFAAS procedure.

Effect of salt addition 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution, 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent), 0.5 mL of chloroform (extracting solvents), 0.5 mL of 9.18% w/v (final concentration 0.14% w/v) SDS (disperser), and 0.3 mL of 4.0 %v/v Triton x-100 (demulsifier) (final concentration 0.037 %v/v). 0.3 mL of the different concentrations of NaCl (Final concentration 0.0-12.8 mM) were optimized. Air-assisted time for 5 sec and extraction time for 3 min were applied to the AA-ST-DLLME-GFAAS procedure.

Air-assisted time 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution, 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent), 0.5 mL of chloroform (extracting solvents), 0.5 mL of 9.18% w/v (final concentration 0.14% w/v) SDS (disperser), and 0.3 mL of 4.0 %v/v Triton x-100 (demulsifier) (final concentration 0.037 %v/v). Air-assisted time from 0-5 sec was optimized under extraction time for 3 min following the AA-ST-DLLME-GFAAS procedure.

Extraction time 30 mL of 1 ng/mL Pb solution was added with 0.5 mL of pH 9 buffer solution, 1.0 mL of 0.0656 M (final concentration 2.0 mM) PAN (complexing agent), 0.5 mL of chloroform (extracting solvents), 0.5 mL of 9.18% w/v (final concentration 0.14% w/v) SDS (disperser), and 0.3 mL of 4.0 %v/v Triton x-100 (demulsifier) (final concentration 0.037 %v/v). Air-assisted bubbles were applied for 5 sec. The extraction times were optimized in the range of 0-20 min following the AA-ST-DLLME-GFAAS procedure.

3.1.4 Analytical performance

Analytical performances of the proposed AA-ST-DLLME-GFAAS method were investigated under the optimum conditions obtaining experiments.

Linear range Different concentrations of stock Pb solution in the range of 0.00-8.00 ng/mL were applied to AA-ST-DLLME-GFAAS procedure under the optimum condition.

Limit of detection (LOD) and limit of quantitative (LOQ) 0.00 ng/mL Pb solution was sample blank solution that was applied to AA-ST-DLLME-GFAAS procedure. Peak and absorbance of blank were analyzed for 20 times. LOD and LOQ were calculated by eq. 8 and eq. 9.

$$LOD = \frac{3 \times SD_{blank}}{m} \quad \text{...} \quad (8)$$

$$LOQ = \frac{10 \times SD_{blank}}{m} \quad \text{...} \quad (9)$$

where SD was standard deviation of blank and m was slope from calibration curve

Relative standard deviation (RSD) Using three interested concentrations of Pb solution (low, medium, and high concentration of calibration) applied into AA-ST-DLLME-GFAAS procedure and repeatedly measured the signal. %RSD or coefficient of variation have been calculated by eq. 10.

$$\%RSD = \frac{SD \times 100}{\bar{x}} \quad \text{...} \quad (10)$$

where SD was standard deviation and $\bar{x}$ was average of signal (Peak area or peak height)

Preconcentration factor (PF) PF was the parameter that represented the preconcentration scale via volume. This factor have been calculated as demonstrated in eq. 11.

$$PF = \frac{V_i}{V_f} \quad \text{...} \quad (11)$$

where V_i was the initial volume of the extraction system and V_f was the final volume of the extraction system

Enrichment factor (EF) EF was the parameter that represented the preconcentration scale via slope. This factor can be calculated as demonstrated in eq. 12.

$$EF = \frac{A_{extraction}}{A_{without\ extraction}} \quad \text{..................................} \quad (12)$$

where $A_{extraction}$ was the slope of calibration with extraction and $A_{without\ extraction}$ was the slope of calibration without extraction

Extraction efficiency (EE) %EE was percentage of ability of the developed extraction method. This factor has been calculated as demonstrated in eq. 13.

$$\%EE = \frac{EF \times 100}{PF} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(13)}$$

where EF was the enrichment factor and PF was the preconcentration factor

Characteristic mass (M_0) M_0 was defined as the absolute mass of analyte giving a peak area of 0.0044 absorbance in GFAAS technique as demonstrated in eq. 14.

$$m_0\,(pg) = \frac{Vol.(\mu l) \times [Conc.(pg.\mu l^{-1})] \times 0.0044\ Abs}{Abs.} \ldots\ldots\ldots\ldots\ldots \text{(14)}$$

where Vol. was the sample volume, Conc. was the concentration of standard solution, Abs. was absorbance, and 0.0044 was 1% absorption

Percentage of recovery (%R) In this experiment, the pretreated real sample solution was added with standard Pb solution at 0.0, 1.0, and 3.0 ng/mL and prepared for AA-ST-DLLME-GFAAS preconcentration method. The recovering value have been calculated as demonstrated in eq. 15.

$$\%R = \frac{C_{found} - C_0}{C_{added}} \times 100 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(15)}$$

%Relative absorbance in the optimization, %relative absorbance was calculated from eq. 16 where ; $Abs_{(i)}$ was the measured absorbance and $Abs_{(max)}$ was the maximum absorbance.

$$\%Rel.Abs. = \frac{Abs_{(i)}}{Abs_{max}} \times 100 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(16)}$$

3.1.5 Method validation

River water certified reference material for trace metals and other constituents (SLRS-6) that contained with concentration of Pb 0.170 ± 0.026 µg/L was used for method validation. 30 mL of reference material was extracted with AA-ST-DLLME-GFAAS procedure and measured the result of Pb concentration from this method. Finally, the measured result was compared with the certified value using statistically tested at 95% confidence level.

3.1.6 Determination of Pb in real samples

The developed AA-ST-DLLME was applied for determination of trace level of Pb in water, beverage, and fruit juice samples.

Water sample preparation Drinking water (or bottled water), beverage, and fruit juice samples were purchased from local stores. Tap and well water samples were collected from different sources around Naresuan university, Phitsanulok, Thailand using clean plastic bottles. All samples were passed through a Whatman™ No.1 filter paper before analysis using AA-ST-DLLME-GFAAS.

Beverage and fruit juice preparation Both beverage and fruit juice samples were passed through the Whatman™ No.1 filter paper before 5 mL of the sample was mixed with 5 mL concentrated HNO_3 and 1 mL of 30% H_2O_2 in a microwave vessel before placing in the microwave oven for 10 minutes at the power of 600 W for the complete digestion. Thereafter, each sample was diluted with 2.0 M NaOH to a final volume of 30 mL, and then Pb content was measured using the AA-ST-DLLME-GFAAS procedure.

AA-ST-DLLME-GFAAS procedure The extraction method for Pb determination as AA-ST-DLLME (Fig. 22) involves the mixing of 30 mL of Pb solution (standard or sample solution), 0.5 mL of pH 9 ammonia/ammonium buffer solution, and 0.5 mL of 2.0 mM PAN solution for Pb-PAN complexation. Then, the extraction was started by the addition of 0.5 mL of chloroform to extract the Pb-PAN complex from aqueous to organic solvent. The dispersion step was the main point of DLLME as the use of 0.5 mL of 0.14% w/v SDS to disperse an analyte between two immiscible phases. After that, the air generator was applied for 4 sec to help a spreading of analyte. To increase the higher efficiency, the extraction tube was stayed with extraction time for 5 min at room temperature. For the centrifugal elimination, 0.3 mL of 0.037% v/v Triton X-100 as demulsifier was consumed for the emulsion breaking. After this step, the aqueous phase and extracting phase were completely separated without centrifugation in AA-ST-DLLME. Finally, 0.5 mL of the extracting phase was selected and directly injected to GFAAS detection.

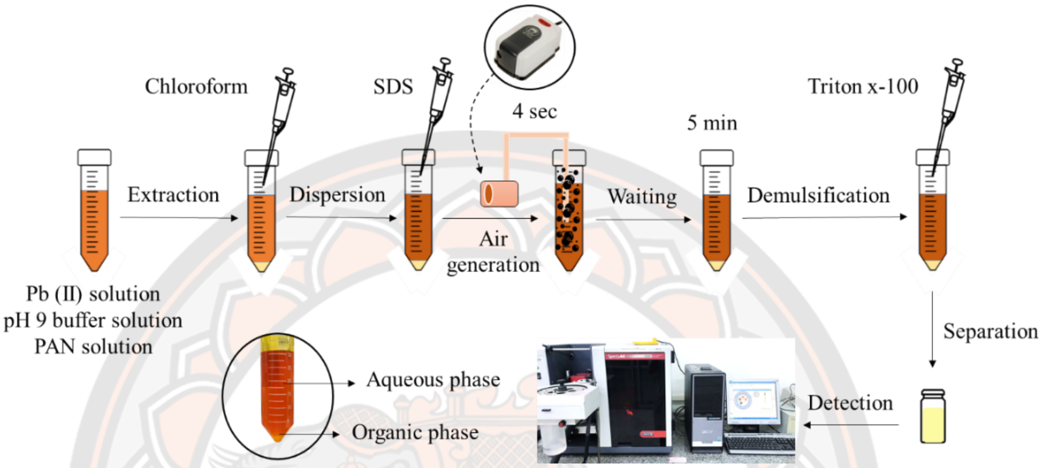


Figure 22 *Procedure of AA-ST-DLLME-GFAAS for Pb determination*

3.1.7 Investigation of robustness

Solution of Na^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, Fe^{3+}, and Cu^{2+} were added to Pb solution for interfering ion study of the proposed method. These metals were investigated in ratios of 1:1, 1:50, 1:100, and 1:200 of the target analyte to interference ions. Using 0.5 µg/L Pb solution was mixed with the interfering metal solution and applied into AA-ST-DLLME-GFAAS under the optimum condition. %Recovery was calculated to demonstrate the interfering effect.

3.2 Results and discussion

3.2.1 Optimization

Effect of pH For the AA-ST-DLLME system, the pH had an important impact on the metal complexation efficiency of the Pb-PAN complex. The pH effect was studied using various buffer solution ranging in pH from 5 to 11 as seen in Fig. 23. The results showed that the relative absorbance gradually increased with increasing pH until reaching a maximum at pH 9. This suggested that the Pb-PAN complex was less stable in acidic conditions and gained stability when the solution turned basic and provided a high relative absorbance. After pH 9, the relative absorbance significantly decreased, indicating a weakening stability of the complex. Thus, pH 9 (using ammonia/ammonium buffer solution) was utilized as optimum pH value for all experiments.

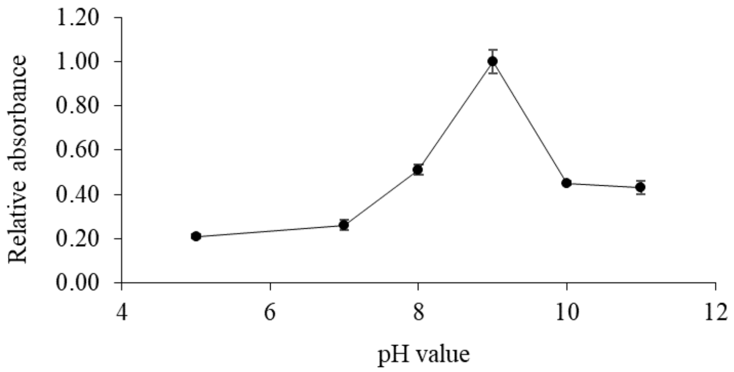


Figure 23 *Effect of pH in AA-ST-DLLME*

Effect of concentration of PAN 1-(2-Pyridylazo)-2-naphthol or PAN was a complexing agent which formed the Pb-PAN complex. This form was a very stable hydrophobic complex which allowed the solubility of the metal complex in the extracting solvent. The stoichiometric composition of the chelate was in 1:2 (Pb:PAN) [178]. The result from the study of the effect of the concentration of PAN solution on the extraction system was given in Fig. 24, it was studied in various concentrations. Aliquots of 1.0 mL of PAN solution to reach final concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mM were used in the study. The relative absorbance of Pb-PAN complex increased as PAN concentration increased. The amount of PAN was not sufficient for the complex formation within this range. The highest absorbance was obtained for 2.0 mM PAN which was the optimum concentration, at which the probable structure of the Pb-PAN complex was as proposed in Fig. 25. At PAN concentrations higher than optimum, the absorbance was reduced. This may be because high amounts of PAN which may be caused carbon residues and high background during GFAAS measurement.

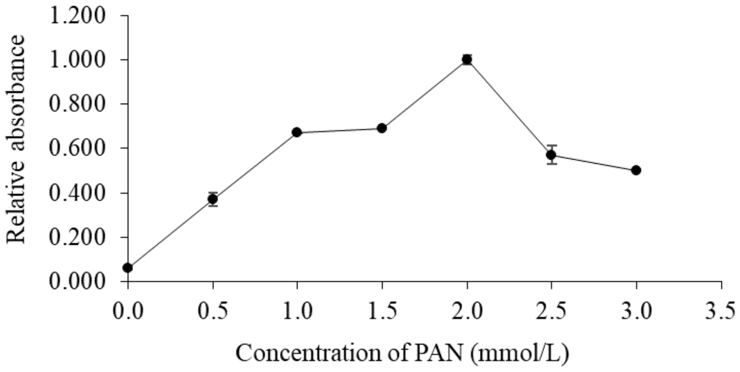


Figure 24 *Effect of concentration of PAN in AA-ST-DLLME*

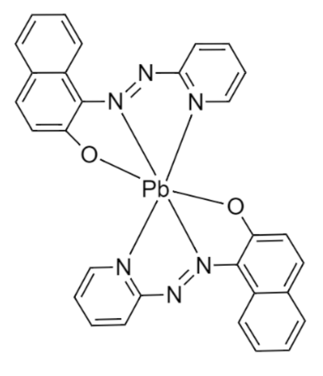


Figure 25 *Structure of the Pb (II)-PAN complex*

Type of extracting solvent The selection of the most appropriate extracting solvent was a very important aspect of the proposed method. Extracting solvents with high interfacial tension made it easier to form tiny droplets for improving the extraction efficiency, and therefore made it easy to extract the target analyte in a like-dissolves-like manner. Experimentally, high density solvents such as chloroform, dichloromethane, and dimethyl sulfoxide were investigated in AA-ST-DLLME. Based on the results in Fig. 26, chloroform provided the highest efficiency. Hence, it was selected as the suitable solvent for further experiments.

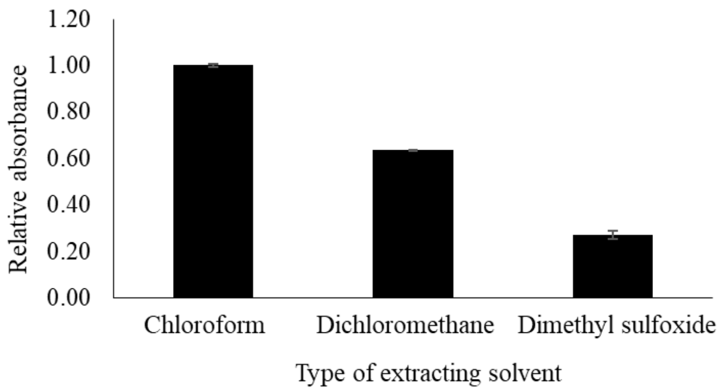


Figure 26 *Type of extracting solvent*

Type of disperser In this study, the surfactant acted as a disperser, and therefore it must be highly miscible with both aqueous and organic phases. Principally, the surfactant played a necessary role in decreasing the interfacial tension between the aqueous and extracting solvent and thus made the droplet size smaller. Triton X-100, Triton X-114, and SDS were investigated. As shown in Fig. 27, the highest relative absorbance was obtained with SDS. Accordingly, SDS was selected as the disperser.

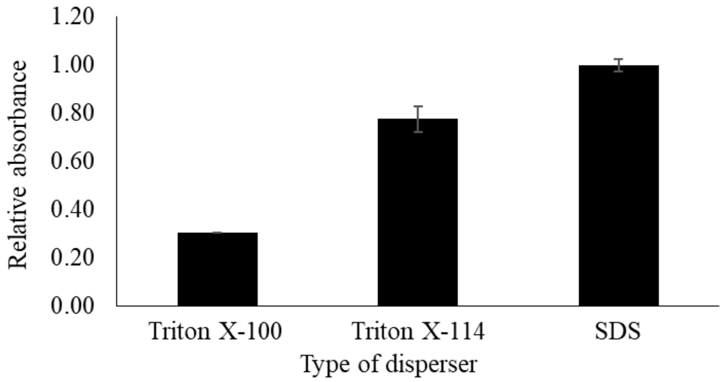


Figure 27 *Type of disperser*

Concentration of disperser The concentration of SDS was studied afterwards, while its volume was fixed at 0.5 mL throughout this experiment. The extraction system was adjusted to final SDS concentrations of 0.05, 0.07, 0.09, 0.12, 0.14, 0.16, and 0.17% w/v. The results in Fig. 28 observed that the 0.14% w/v final concentration of SDS provides a higher relative absorbance than all the other concentrations. The suggested reason was that low concentrations of the disperser lower the interfacial tension necessary for the droplet splitting, thereby reducing emulsification. Also, low disperser concentration might be resulted in droplet sizes that were too small, and thereby affected a decline in the phase separation.

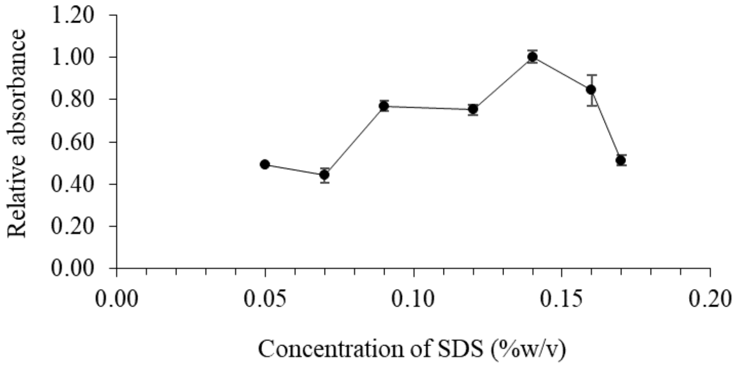


Figure 28 *Effect of concentration of SDS*

Type of demulsifier Demulsification was a significant step to achieve quick isolation of the extracting solvent droplets from the aqueous phase. In some cases, the disperser solvent may be served as a terminating solvent or demulsifier which broken up the emulsion and induced phase separation. In this study, surfactants (Triton X-100, Triton X-114, and SDS), methanol, ethanol, acetone, acetonitrile, and tetrahydrofuran (THF) were examined as the demulsifier. The results obtained in Fig. 29 indicated that the maximum relative absorbance signal was obtained with Triton X-100. Comparison of the no demulsifier bar with the Triton X-100 bar showed that the isolation efficiency improved by approximately 50%. Even though Triton X-100 provided the highest signal, Triton X-114 also provided a comparable signal intensity because both were nonionic surfactants that vary only in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups. Since Triton X-100 proved to be the most effective for this method, it was chosen as the optimal demulsifier.

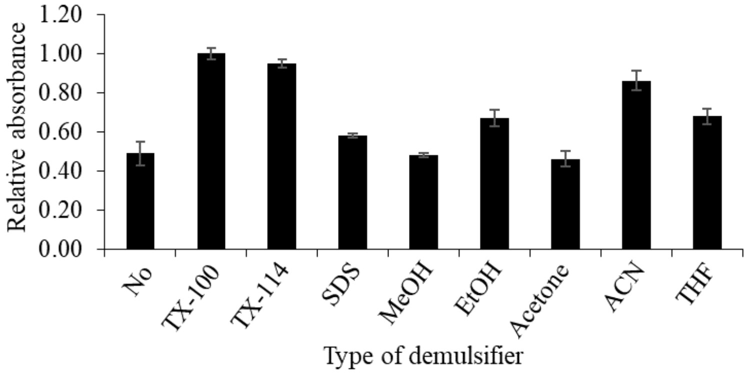


Figure 29 *Type of demulsifier*

Concentration of demulsifier In the next step of this experiment, the final concentration of Triton X-100 in the extraction tube was evaluated at 0.000, 0.009, 0.018, 0.037, 0.046, 0.055% v/v with a total volume of 0.3 mL throughout the study. As indicated by Fig. 30, the relative absorbance was slightly increased by increasing the concentration of demulsifier up to 0.018% v/v, held steady until 0.037% v/v and then decreased for concentrations of demulsifier higher than 0.037% v/v. Concentrations of Triton X-100 within the range 0.018-0.037% v/v gave the maximum signal. The decrease in signal with high amounts of demulsifier was the result of excess coalescence of droplets in addition to dispersing them back to the aqueous phase again. The 0.037% v/v Triton X-100 was selected as demulsifier for termination in this experiment.

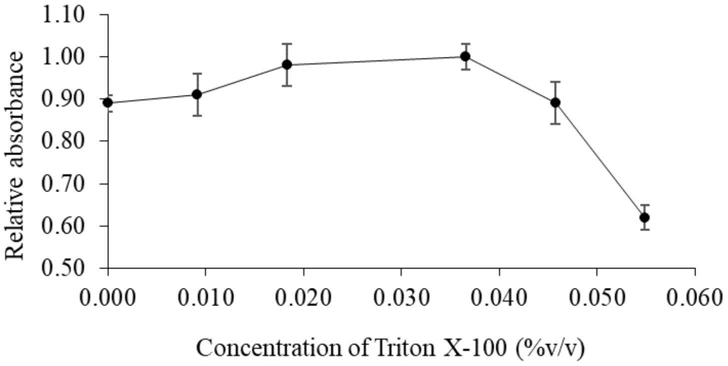


Figure 30 *Effect of concentration of Triton X-100*

Hence, AA-ST-DLLME-GFAAS method have been described about the possible process that shown in Fig. 31. Chloroform as an extracting solvent with high interfacial tension can made the formation of tiny droplets in this extraction system. Next, the addition of SDS as disperser which played an important role in decreasing the interfacial tension between water and extracting solvent and thus made the droplet size smaller. Finally, the addition of Triton X-100 as demulsifier in this work helped to break up the emulsion and formed the coagulation of droplets.

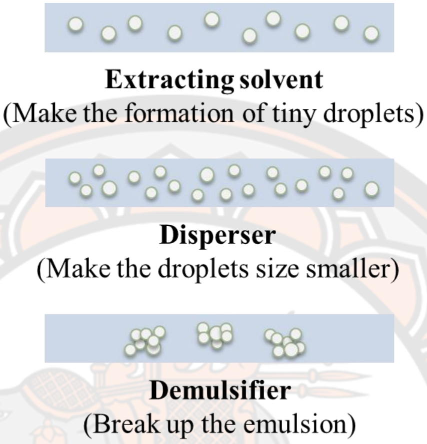


Figure 31 *Terminating process in this AA-ST-DLLME*

Effect of salt addition Effect of salt addition was evaluated because the addition of salt helped to increase the ionic strength and to break the emulsion during extraction in some cases. NaCl was a popular choice of salt. Final concentrations of NaCl in the 0.00-12.85 mM range were studied. The obtained results were shown in Fig. 32. The signal showed a sharp decline with the introduction of salt and then held steady at this low signal with rising concentration of salt. This was because the termination in the proposed method using demulsifier was sufficient to break the emulsion and induced phase separation, whereas addition of salt may be promoted the re-merging of the extracting solvent into the aqueous again, making separation harder. So, there was no need for salt addition in this work.

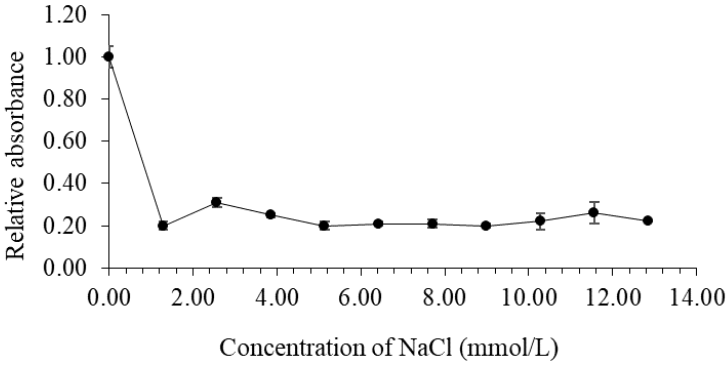


Figure 32 *Effect of NaCl-salt addition*

Air-assisted time The use of a home fish-tank pump in AA-ST-DLLME for the first time was carefully considered with regards to the extraction stage. The air-generated system consisted of an air generator tube that was a 1 mL long plastic dropper with 3 mm inner diameter. This tube was made of polyethylene and inert to the solvent and the reagent used for the microextraction process. For the air assisted DLLME, this tube was inserted into the 50 mL extraction centrifuge tube containing the sample solution and positioned 3 cm above the sample solution. A Big boy air pump (BB-8000) at maximum power (Frequency at 50 Hz) was selected as the bubble generator and its performance was investigated at time intervals of 0, 1, 2, 3, 4, and 5 seconds to generate the air in extraction tube. As the results in Fig. 33, the relative absorbance with increasing air-assisted time up to 4 seconds and after that the signal decreased. As can be seen that increasing of air bubbles breaking resulted in higher extraction efficiency. The surface area of each bubble was surrounded by the tiny droplets of organic solvent. When the bubble continually grown, it can be helped to increase the opportunities of contact between the extracting solvent and the Pb-PAN complexes before it cleaves off, and thereby caused rapid extraction. On the other hand, excessive air-assisted times affected the coexistence of the aqueous solvent, extracting solvent, and disperser and lead to a decline in the relative absorbance of Pb-PAN complexes.

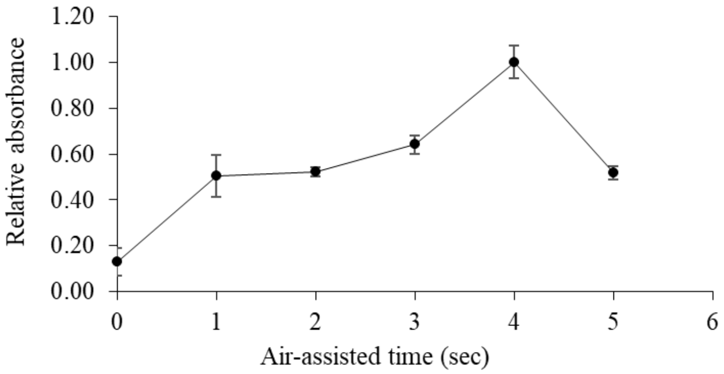


Figure 33 *Effect of air-assisted time*

Extraction time Extraction time was the time of waiting for complete isolation of target complexes. In this work, the results of studying extraction times of 0, 3, 5, 10, 15, and 20 minutes were shown in Fig. 34. The signal showed a steep raising up to 5 min and then declined beyond that time interval. After the addition of disperser and air bubbles, the isolation increased due to the mixing, while an extraction equilibrium was also being established. This explains the increase in the time required for complete extraction in this method. Extraction times beyond 5 minutes gave lower signals because as the two immiscible phases were kept in the extraction tube for a longer time, there can be over spread of target analyte thereby reducing separation and therefore the preconcentration factor.

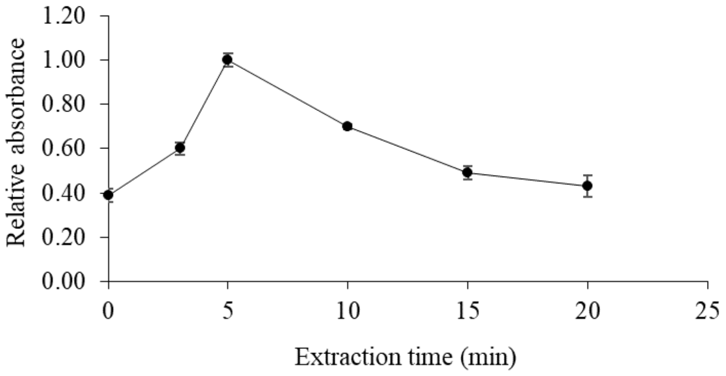


Figure 34 *Effect of extraction time*

3.2.2 Analytical performance

The performance of the developed method was investigated under the optimum conditions established in earlier experiments. Calibration curve was linear in

0.084-8.0 ng/mL. The slope of the calibration curve was established using deionized water for comparison. The ratio of the slopes (Fig. 35) from the preconcentrated method to the slope without preconcentration calculated from eq. 12 in term of the enrichment factor (EF) was 58.9. The preconcentration factor calculated from the initial volume and the final volume as described in eq. 11 was 60. The extraction efficiency calculated from eq. 13 was 90%. The limit of detection (LOD) at S/N=3 and limit of quantitation at S/N=10 (LOQ) were 0.025 ng/mL and 0.084 ng/mL, respectively. The LOD and LOQ were far below the PCD recommendation [4]. Figures of merit of the proposed method was summarized in Table 11. The accuracy (% recovery) of spiked Pb standard solution for 1 ng/mL and 3 ng/mL in different types of samples were 82.0-117.3% (water samples) and 88.0-115.7% (beverage and juice samples) as shown in Table 12. The precision (%RSD) of the proposed method was acceptable according to international guidelines established by the Association of Official Analytical Chemists (AOAC). Intraday (n=3) and interday precision values (n=5) were approximately 1.02-2.62% and 2.77-3.19%. Calculated characteristic mass (M_0) from eq. 14 represented the improving sensitivity which was better than 63 times ($M_{0\ (GFAAS)}$ was 93.2 pg and $M_{0\ (AA\text{-}ST\text{-}DLLME\text{-}GFAAS)}$ was 1.5 pg).

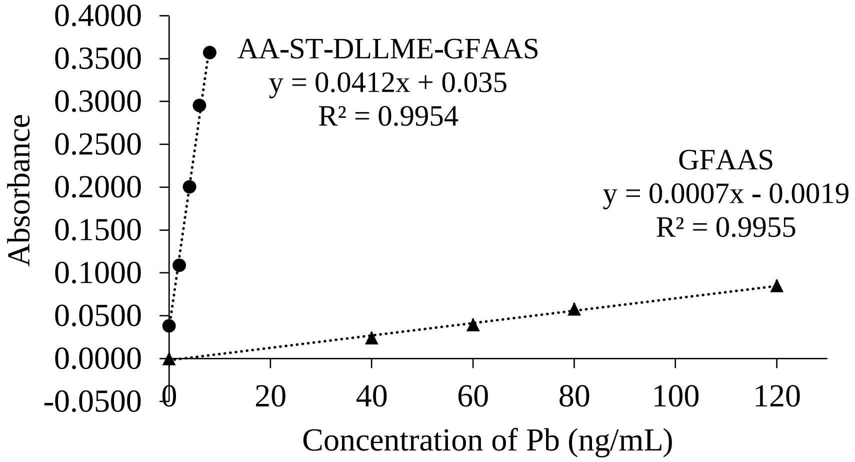


Figure 35 *Calibration curves from AA-ST-DLLME-GFAAS and GFAAS methods*

Table 11 *Analytical figures of merit from AA-ST-DLLME method*

Figures of merit	**Value**
Linearity (ng/mL)	0.08-8.0
LOD (ng/mL)	0.025
LOQ (ng/mL)	0.084
%RSD	1.02-2.62
%Recovery	82.70-117.3
EF	58.9
PF	60.0
%EE	98.2

3.2.3 Method validation

To validate the accuracy of the developed method, river water certified reference material for trace metals and other constituents (SLRS-6) as standard reference material was used for method validation. The reference material contained Pb at a certified concentration value of 0.170±0.026 µg/L. The found concentration of Pb in reference material obtained by the proposed method was 0.148±0.025 µg/L. These values were comparable and were not significantly different at the 95% confidence level.

3.2.4 Determination of Pb in environmental and foods samples

AA-ST-DLLME was applied for determination of Pb in water, beverage, and juice samples. The results from Table. 12 showed quantitative analysis that obtained non-detectable (N.D.) values in drinking water (1), drinking water (2), and lemon tea. These samples were reported as not detectable because they were contaminated with trace Pb less than 0.084 ng/mL, which cannot be detected quantitatively with this method. However, the contamination in other samples were reported with the acceptable percentage recovery in the range 82.0-117.3%.

The comparative results between our method (AA-ST-DLLME) and previous works on the preconcentration of Pb were summarized in Table. 13. The results indicated that the AA-ST-DLLME method provided a higher precision for repeatability and reproducibility along with lower LOD, and the LOQ of this method was far below the Pollution Control Department recommendation (Maximum allowable concentration of 0.05 mg/L).

Table 12 *Results of Pb determination in real samples (n=3)*

Sample	**Pb concentration (ng/mL±SD)**		**%Recovery±SD**
	Added	**Found**	
Drinking water (1)	-	N.D.	-
	1.0	1.03±0.06	103.0±5.7
	3.0	2.79±0.01	93.0±0.5
Drinking water (2)	-	N.D.	-
	1.0	1.07±0.06	107.0±5.7
	3.0	3.43±0.18	114.3±5.9
Drinking water (3)	-	0.30±0.02	-
	1.0	1.39±0.08	109.0±8.5
	3.0	3.82±0.04	117.3±1.2
Tap water (1)	-	1.29±0.03	-
	1.0	2.22±0.03	93.0±3.5
	3.0	4.31±0.05	100.7±1.5
Tap water (2)	-	1.33±0.03	-
	1.0	2.35±0.02	102.0±2.1
	3.0	3.94±0.04	87.0±1.2
Tap water (3)	-	1.87±0.09	-
	1.0	2.81±0.04	94.0±4.0
	3.0	4.65±0.12	92.7±4.1
Well water (1)	-	1.94±0.16	-
	1.0	2.76±0.02	82.0±1.5
	3.0	5.25±0.17	110.3±5.7
Well water (2)	-	1.35±0.10	-
	1.0	2.39±0.04	104.0±4.2
	3.0	4.47±0.06	104.0±1.9
Well water (3)	-	1.37±0.03	-
	1.0	2.51±0.02	114.0±2.1
	3.0	4.57±0.11	106.7±3.8

Table 12 *(Cont.)* …

Sample	**Pb concentration (ng/mL±SD)**		**%Recovery±SD**
	Added	**Found**	
Energy drink	-	0.12±0.04	-
	1.0	1.08±0.01	96.0±0.7
	3.0	3.10±0.04	99.3±1.4
Lemon tea	-	N.D.	-
	1.0	0.88±0.03	88.0±2.8
	3.0	3.22±0.13	107.3±4.2
Sparking drink	-	0.11±0.01	-
	1.0	1.08±0.01	97.0±0.7
	3.0	3.58±0.01	115.7±0.2
Vegetable juice	-	0.32±0.03	-
	1.0	1.41±0.06	109.0±6.4
	3.0	3.18±0.02	95.3±0.7
Purple carrot juice	-	0.21±0.02	-
	1.0	1.20±0.01	99.0±1.0
	3.0	3.36±0.04	105.0±1.4
Carrot juice	-	0.27±0.03	-
	1.0	1.20±0.01	93.0±0.6
	3.0	3.28±0.02	100.3±0.6
Orange juice	-	0.20±0.01	-
	1.0	1.35±0.01	115.0±1.4
	3.0	2.79±0.03	86.3±0.9

N.D. was not detectable in AA-ST-DLLME-GFAAS (less than 0.084 ng/mL)

Table 13 *Comparison of proposed method (AA-ST-DLLME) with previous works on Pb determination.*

Technique	Detection	Sample	Linearity (µg/L)	LOD (µg/L)	LOQ (µg/L)	%RSD	%Recovery	PF	Ref.
CPE	FAAS	- soil - wastewater	20-300	8	20	2.7	95-111.1	35	[179]
DLLME	FAAS	- lake water - ground water - sea water - wastewater	-	2.6	7.7	1.8	98-99	70	[14]
UAµE-DES	FAAS	- eye shadow - lipstick	1-10	0.66	-	2.3	97.0-98.7	71.6	[57]
UA-SS-LPME	FAAS	- tap water - bottled water - urine - tea infusion	2-200	0.63	2.10	3.8	96.1	101.6	[91]
SFODME	GFAAS	tap water	0.2–10	0.058	0.2	8.8	92-95	113	[172]

Table 13 (*Cont.*) …

Technique	**Detection**	**Sample**	**Linearity (µg/L)**	**LOD (µg/L)**	**LOQ (µg/L)**	**%RSD**	**%Recovery**	**PF**	**Ref.**
DES-LPME	FAAS	raw milk	50-1000	8.7	29	3.1	102.5-103.2	-	[19]
Paper-based analytical device	Colorimetric detection	- tap water - drinking water - wastewater	10–100	10	-	<20	80.5-117.2	-	[180]
DLLME	GFAAS	wine	-	0.08	-	-	96-100	-	[53]
AA-ST-DLLME with GFAAS	GFAAS	- waters - beverages - fruit juices	0.084-8.0	0.025	0.084	1.02-3.19	82.0-117.3	58.9	This method

Remarks;

CPE	Cloud point extraction
UAµE-DES	Ultrasonic assisted micro- extraction method based on deep eutectic solvent
UA-SS-LPME	Ultrasound-assisted switchable solvent based liquid phase microextraction
SFODME	Solidified floating organic drop microextraction
DES-LPME	Deep eutectic solvent-based liquid phase microextraction
HF-LLSME	Hollow-fiber liquid–liquid–solid micro-extraction

3.2.5 Robustness

Interfering study was a measure of the robustness of any proposed method. When AA-ST-DLLME was used for the treatment of real samples, potentially interfering ions might be presented. The effect of other ions on the intensity signal was studied under optimum experimental conditions. According to the composition of common environmental waters and beverages, the metal ions that interfere in these samples are Na^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, Fe^{3+}, and Cu^{2+}, and these were investigated. The ratios 1:1, 1:50, 1:100, and 1:200 of the target analyte to interference ions were studied. Experimentally, 0.5 ng/mL of Pb standard solution was spiked with various concentrations of interfering ions followed by the studied ratio before its determination by the AA-ST-DLLME method. The robustness of the results was determined by the percent recovery which gave values in the acceptable range of 80-120%. To summarize the robustness of this method, tolerance limits of Pb investigation in different ratio of interfering ions were presented in Table 14. The result indicate that this method was robust for high-level contamination of Na^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, and Fe^{3+} at tolerance levels of approximately 200 and 100. However, the contamination of Cu^{2+} may limit the robustness of this method because the stability of Cu-PAN complex can be disturbed the Pb-PAN complex through competitive complexation and caused the recovery of Pb to reduce, given that the tolerance limit of Cu^{2+} was 50.

Table 14 *Influence of interfering ions for ST-DLLME-GFAAS procedure*

Interfering ions	Tolerance limit	Interfering ions	Tolerance limit
Na^+	200	Cd^{2+}	100
Ca^{2+}	200	Hg^{2+}	100
Mg^{2+}	200	Fe^{3+}	100
Ni^{2+}	200	Cu^{2+}	50

3.3 Conclusions

The proposed AA-ST-DLLME method was successfully developed for the determination of Pb in water samples, environmental samples, beverages, and fruit juice samples by GFAAS. The experimental parameters were optimized by univariate optimization method. Under the optimum condition, the analytical performances of the proposed method were investigated and summarized in Table 11. In addition, effect of interfering ions was evaluated as shown in Table14. The found concentration of Pb in drinking water (3) sample was 0.30 µg/L, tap water samples were 1.29-1.87 µg/L, well water samples were 1.35-1.94 µg/L, beverages were 0.11-0.12 µg/L, and vegetable and fruit juices were 0.20-0.32 µg/. However, the contamination of Pb in drinking water (1), drinking water (2), and lemon tea were not detectable. Meanwhile, the precision of the proposed method was validated by SRM.

CHAPTER IV

MAGNETIC DISPERSIVE SOLID PHASE EXTRACTION

In the second work, a selective magnetic dispersive solid phase extraction (MdSPE) as a new sorbent from used GFAAS-graphite tubes with the magnetic nanoparticles as graphene oxide composited with magnetite (Fe_3O_4) and functionalized-dithizone (DTZ) was emphasized. The synthesized sorbent (GO-Fe_3O_4-DTZ) was then applied in MdSPE for the preconcentration and determination of trace Pb in water samples using FAAS detection. In this chapter, the characterizations of the new synthesized sorbent were evaluated by Zeta potential analyzer, FTIR and SEM. The synthesis and extraction procedures were emphasized. Important parameters (effect of pH, extraction time, type and concentration of eluent, centrifugation time, sample volume and reusability), adsorption isotherm with adsorption capacity and analytical performance were studied as followed;

4.1 Experiment

4.1.1 Instrumentation

Pb determination was performed by flame atomic absorption spectrometer (FAAS, SpectrAA 220, Varian, USA) utilizing an air/acetylene flame with a Pb hollow-cathode lamp at 217.0 nm. The pH of solutions was measured using a pH meter (HI98107, Hanna instrument, USA). Filter paper (No 1, Whatman™, Thailand) was used for filtration of samples. A super magnet (50×50×5 mm) (Neodymium, MagnetDD, Thailand) was purchased from a magnet store in Bangkok. A scanning electron microscope (SEM, Leo1455VP, Leo, Germany) was used to observe surface morphology. A Zeta potential analyzer (Zetasizer Nano ZS90, Malvern Panalytical, UK) with folded capillary cell (DTS1060) was utilized for the measurement of surface charge properties. FTIR spectral data were recorded by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR, Spectrum GX, Perkin Elmer, USA).

4.1.2 Reagents

All reagents used in this work were of analytical grade. These were: 30% ammonia solution, (Loba chemie, India), 99.5% ammonium chloride, (Loba chemie, India), 99% dithizone, (Ajax Finechem, Australia), dimethyl sulfoxide, 99.9% (RCI Labscan, Thailand), sodium nitrate, 99.5% (AnalaR, UK), nitric acid, 65% (RCI Labscan, Thailand), sulfuric acid, 96% (RCI Labscan, Thailand), hydrochloric acid, 37% (RCI Labscan, Thailand), hydrogen peroxide, 30% (VWR International, USA), ethanol, (RCI Labscan, Thailand), and iron (II, III) oxide, 95% (Sigma-Aldrich, Singapore). The lead (II) standard solution, 1000 mg/L (Loba Chemie, India) was diluted to a 100 mg/L stock solution with deionized water. Working solutions were prepared with deionized water and the dithizone solution was prepared with dimethyl sulfoxide (DMSO). Ammonia/Ammonium buffer was used to adjust the pH to a value of 9.

4.1.3 Synthesis of GO-Fe_3O_4-DTZ sorbent

A novel magnetic dispersive solid phase extraction (MdSPE) method has been designed by utilizing used-graphite tubes from the GFAAS as a graphene oxide sorbent modified with magnetite (Fe_3O_4) and dithizone (GO-Fe_3O_4-DTZ sorbent) (Fig. 36(1)). The GO synthesis process was modified from Hummers' method for the magnetic dispersive solid-phase extraction based on a nanocomposite of graphene oxide/Fe_3O_4@polythionine [74]. The used-graphite tubes were washed with HNO_3, dried at 100 ^{o}C in a thermostatic oven, and then ground with a mortar and pestle. Graphite powder (3 g) was mixed with 1.5 g $NaNO_3$ and 69 mL H_2SO_4 was added thereafter. The mixture was cooled in an ice bath. Then, 9 g of $KMnO_4$ was added as an oxidizing agent for the oxidation process. The solution was held at 20 ^{o}C for 5 minutes using a thermostat. Next the solution was heated and stirred at 35 ^{o}C for 10 hours. After the solution was cooled to 25 ^{o}C, it was digested with 3 mL of 30% v/v H_2O_2 in an ice bath. During this step, the used-graphite was changed to GO completely. To separate the suspended phase, the solution was filtered with No.1, Whatman™ filter paper. The resulting solid phase was washed and dried with 5%v/v HCl at 120 ^{o}C.

To synthesize the magnetic sorbent (GO-Fe_3O_4), 0.3 g of the previously prepared GO was dispersed in 150 mL of deionized water through ultrasonication and adjusted to pH 10 with 25%v/v NH_4OH. Then 1.147 g of iron (II, III) oxide was dissolved in 25 mL deionized water, and this was added into the GO solution at room temperature. The temperature of the mixture was raised to 80 °C and stirred for 50 minutes. The solution was filtered and washed three times with deionized water and dried at 100 °C in an oven.

To synthesize GO-Fe_3O_4-DTZ, 0.3 g of the GO-Fe_3O_4 was put in 150 mL in deionized water and shaken with the mixture of 0.1 g $FeCl_3.6H_2O$ as a catalyst and 0.2 g dithizone (DTZ) with 100 mL slowly added DMSO for 10 min. Next, 2 mL of H_2O_2 as an oxidizing agent was added dropwise during strong shaking and stirring at 50 °C for 1 hour. Iron (III) chloride was a good Lewis acid and was effectively used as a catalyst in a variety type of chemical reactions [181]. Practically, the DTZ powder was mixed with iron (III) chloride and dissolved in DMSO and thoroughly mixed with GO-Fe_3O_4. In this step, iron (III) chloride act as the intermediate agent for the effective interaction from GO to be a GO-Fe_3O_4. Hydrogen Peroxide was one of the most powerful oxidizers. The addition of oxidizing agent was to increase amount of (-O-) group in the surface of GO for the raised active sites. During this step, DTZ was formed on the GO-Fe_3O_4. The sedimented phase was separated using a strong magnet and washed four times with deionized water and dried at 25 ^{o}C in an oven.

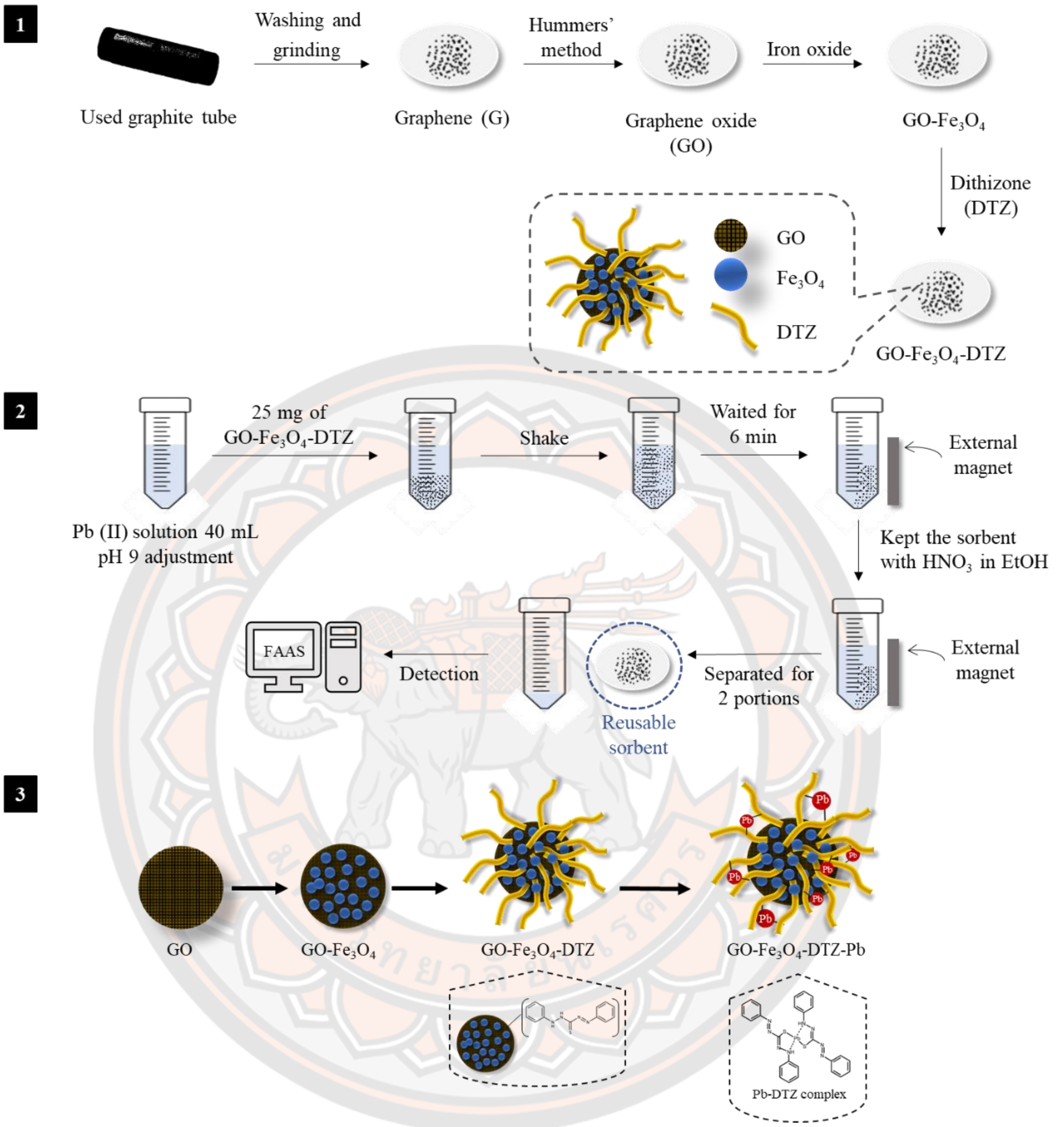


Figure 36 *Process of GO-Fe$_3$O$_4$-DTZ synthesis*

4.1.4 Characterization of GO-Fe$_3$O$_4$-DTZ sorbent

Zeta potential analyzer In this experiment, the GO-Fe$_3$O$_4$-DTZ sorbent was prepared as suspension by using the different buffer solutions with pH values from 2-10. The suspension was contained in specific cuvette and analyzed with Zetasizer Nano ZS90.

ATR-FTIR The powder of G, GO, GO-Fe$_3$O$_4$, and GO-Fe$_3$O$_4$-DTZ were directly investigated by Spectrum GX-ATR-FTIR at mid-IR range.

SEM The powder of G, GO, GO-Fe_3O_4, and GO-Fe_3O_4-DTZ were coated with gold and placed on the white adhesive tape on the septum. The surface morphology of sorbents at several magnification was photographed by SEM (Leo1455VP).

4.1.5 Optimization

In MdSPE-FAAS, the important parameters *e.g.,* effect of pH, extracting time, type and concentration of eluent, sample volume, and reusability of sorbent were studied by univariate optimization method.

Effect of pH 30 mL of 3 mg/L Pb solution was added with pH buffer solution in the range of 2-10 from different types of buffers. 25 mg of GO-Fe_3O_4-DTZ sorbents was dispersed. Under the pre-optimized condition, extracting time for 7 min and 3 mL of 2.0 M HNO_3 in ethanol (eluent) were applied following MdSPE-FAAS procedure.

Effect of sonication time 30 mL of 3 mg/L Pb solution was added with pH 9 ammonia/ammonium buffer solution, and 25 mg of GO-Fe_3O_4-DTZ sorbents. Sonication was applied from 1 to 12 min. Under the pre-optimized condition, extracting time for 7 min and 3 mL of 2.0 M HNO_3 in ethanol (eluent) were applied following MdSPE-FAAS procedure.

Effect of extracting time 30 mL of 3 mg/L Pb solution was added with pH 9 ammonia/ammonium buffer solution, and 25 mg of GO-Fe_3O_4-DTZ sorbents. Extracting times in the range of 1-30 min were studied with the use of 2.0 M HNO_3 in ethanol, 3 mL as eluent following MdSPE-FAAS procedure.

Type of eluent 30 mL of 3 mg/L Pb solution was added with pH 9 ammonia/ammonium buffer solution, and 25 mg of GO-Fe_3O_4-DTZ sorbents. Extracting for 6 min was applied. 3 mL of HNO_3 in ethanol, HNO_3, HCl, and H_2SO_4 were optimized following MdSPE-FAAS procedure.

Concentration of eluent 30 mL of 3 mg/L Pb solution was added with pH 9 ammonia/ammonium buffer solution, and 25 mg of GO-Fe_3O_4-DTZ sorbents. Extracting for 6 min was applied. 3 mL of the different concentration of HNO_3 in ethanol in the range of 0-1.0 M was optimized following MdSPE-FAAS procedure.

Sample volume Volume of 3 mg/L Pb solution in the range of 0, 15, 20, 25, 30, 35, 40, 45 and 50 mL were optimized obtaining with pH 9 ammonia/ammonium buffer solution, and 25 mg of GO-Fe_3O_4-DTZ sorbents. Under the pre-optimized condition, extracting time for 6 min and 3 mL of 0.3 M HNO_3 in ethanol (eluent) were applied following MdSPE-FAAS procedure.

Reusability 40 mL of 3 mg/L Pb solution was added with pH 9 ammonia/ammonium buffer solution. 25 mg of GO-Fe_3O_4-DTZ sorbents were dispersed for 6 min. 3 mL of 0.3 M HNO_3 in ethanol was used as eluent following MdSPE-FAAS procedure. The sorbents after extraction were washed with HNO_3 and deionized water before using in the next extraction procedure. Reused process was carried out for 8-10 times.

4.1.6 Analytical performance

The analytical performances of MdSPE-FAAS method were investigated under the optimum conditions. The parameter namely linear range, LOD, LOQ, enrichment factor (EF), preconcentration factor (PF), and percentage extraction efficiency (%EE) were investigated using the same equation as previously described in section 3.1.4. In addition, %RSD was evaluated by 1.0 mg/L Pb solution. For investigation of recoveries, 0.0, 1.0, and 2.0 mg/L of Pb solution were spiked with real samples.

Adsorption capacity 20.0 mg/L Pb solution was mixed with 25 mg sorbent and extracted with MdSPE-FAAS method under the optimum condition. Supernatant was analyzed and calculated amount of Pb in GO-Fe_3O_4-DTZ via eq. 17.

$$The\ amount\ of\ metal\ ion\ adsorbed\ (mg\ g^{-1}) = \frac{(c_0-c)\times v}{m\times 1000} \quad \ldots\ldots\ldots\ldots(17)$$

were C_o being concentration of Pb before adsorption, C was concentration of Pb after adsorption, v was volume (mL), and m was mass of sorbent (g)

Adsorption isotherm In this experiment, 0.00-20.0 mg/L of Pb solution was mixed with 25 mg sorbent and extracted with MdSPE-FAAS method under the optimum condition. Supernatant was analyzed and the curve of Langmuir and Freundlich isotherm were plotted following eq. 18 and eq.19, respectively.

The Langmuir model was:

$$\frac{X_2}{X_1} = \frac{X_2}{X_m} + \frac{1}{(K)(X_m)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (18)$$

where X_1 was = amount of Pb adsorbed per mass unit of sorbent (mg/g), X_2 was residual concentration of Pb after adsorption (mg/L), X_m was amount of Pb for saturated monolayer adsorption, and K was equilibrium constant

The Freundlich model was:

$$log\frac{X}{m} = \log k + \frac{1}{n}\log C_e \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (19)$$

where $\frac{X}{m}$ being amount of Pb adsorbed per mass unit of sorbent, C_e was concentration of Pb in solution after adsorption, and K and n were constant value

4.1.7 Application for Pb analysis in water samples

The developed MdSPE-FAAS was applied for determination of trace amount of Pb in water samples.

Water sample preparation Drinking (or bottled water) water samples were purchased from local stores. Tap and surface water samples were collected from different sources around Naresuan university, Phitsanulok, Thailand using clean plastic bottles. All samples were passed through a Whatman™ No.1 filter paper before analysis using MdSPE-FAAS.

MdSPE-FAAS procedure 40 mL of sample solution or standard solution was transferred to a 50 mL centrifuge tube and adjusted to pH 9 with ammonia/ammonium buffer, Then 25 mg of the previous synthesized GO-Fe_3O_4-DTZ powders were dispersed into the solution and shaken for a few minutes to make the dispersive particle solution. The dispersive solution was allowed to sit for 6 minutes' extraction time. Then the magnetic sorbents were separated using the external super magnet. Afterwards, the supernatant phase was discarded. The separated sorbent was eluted with 3 mL of 0.3 M of HNO_3 in ethanol and used separated with the external super magnet separation again. To ensure the small size elimination, the eluted solution was filtered with filter paper. Finally, the clear solution was introduced into the FAAS for analysis (Fig. 36(2)).

4.1.8 Robustness

1.00 mg/L of Pb solution was spiked with interfering ions, Na^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, and Cd^{2+} at ratios of 1:1, 1:5, 1:10, 1:50, 1:100 and 1:200 (Pb : interfering ion). Then, MdSPE extraction procedure under the optimum condition was carried out. %Recoveries were calculated to evaluate the interfering effect.

4.2 Results and discussion

4.2.1 Characterization

Zeta potential analyzer Zeta potential is a measurement technique based on the charges on the particles in an emulsion or suspension. In this experiment, the results (Fig. 37) indicated that the charge value at the surface of sorbent provided a negative maximum zeta potential value of -27.0 mV at pH 8. This zeta potential result was due to the effect of pH. The result showed a negatively charge surface which was suitable for Pb ions analysis in this work. Since the metal ions have positive charges, it is easy for them to react with the negatively charged sorbent. Furthermore, the size distribution of synthesized sorbent was investigated by Zetasizer Nano ZS90 and it was observed that the diameter of synthesized Go-Fe_3O_4-DTZ sorbent used in solid phase extraction were in the range 14.29-31.56 nm.

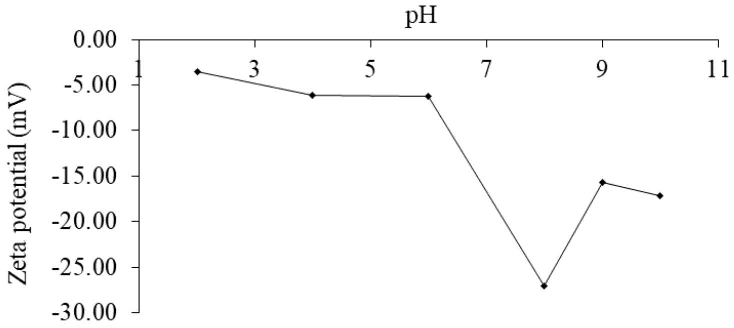


Figure 37 *Zeta potential profile of GO-Fe_3O_4-DTZ sorbent*

ATR-FTIR This spectrometric technique was selected for characterization of the chemical identity of a sample molecule. The technique gave both functional group information (4,000-1,500 cm^{-1}) and fingerprint region (1,500-400 cm^{-1}) of GO-Fe_3O_4-DTZ. The GO-Fe_3O_4-DTZ powder sorbent was directly detected with the Spectrum GX-ATR-FTIR (4,000-400 cm^{-1}). The spectral patterns of GO, GO-Fe_3O_4 and GO-Fe_3O_4-DTZ were summarized in Fig. 38. The chemical structure information of the GO was obtained with the oxygen-containing groups of alcohol, carboxylic acid, aromatic ring, and epoxy ring. The results indicated that the peak at 3,147.45 cm^{-1} showed the O-H stretching of alcohol or carboxylic acid. Peaks at 1,696.09 cm^{-1} and 1,554.83 cm^{-1} showed the C=C stretching of aromatic ring, while peaks at 1,561.91 cm^{-1} showed the C=O stretching of carboxylic acid and the peak at 1,116.10 cm^{-1} showed the C-H bending of aromatic ring. The peak at 1,010.95 cm^{-1} showed the C-O stretching of the aromatic ring. The chemical structure of DTZ have been confirmed with the aromatic ring, amine, and thioketone. The peak at 2,682.84 cm^{-1} showed the N-H stretching in DTZ. Peaks at 1,208.65 cm^{-1} and 1,114.89 cm^{-1} showed the C-N and C=S stretching. The Fe-O bond of Fe_3O_4 was shown at 542.68 cm^{-1}. From the FTIR spectral interpretation, the synthesis of the GO-Fe_3O_4-DTZ sorbent was successful.

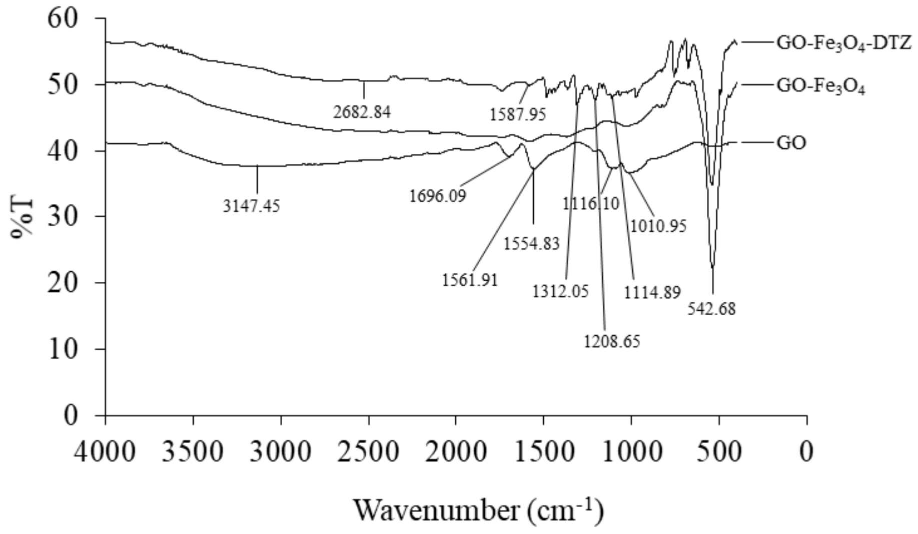


Figure 38 *FTIR spectral profile of the functional groups of GO-Fe_3O_4-DTZ*

SEM Surface morphology of G, GO, GO-Fe_3O_4, and GO-Fe_3O_4-DTZ were evaluated via SEM (Fig. 39). SEM images represented the surface morphology of materials at 5000-times magnification. In Fig. 39(1) the surface area of pure graphene (G) was a porous area. Fig. 39(2) was the surface of graphene oxide (GO) and showed the pliable sheet-like structure obtained from the oxygen-contents after the graphene-oxidation. Fig. 39(3) detailed the morphology of graphene magnetite (GO-Fe_3O_4) represented by the bright spherical shape of magnetite coated on the GO surface, and Fig. 39(4) was for the graphene magnetite composited with DTZ (GO-Fe_3O_4-DTZ). The surface areas appeared to be in the stick shape of DTZ which was co-plated on the GO-Fe_3O_4 as a GO-Fe_3O_4-DTZ sorbent.

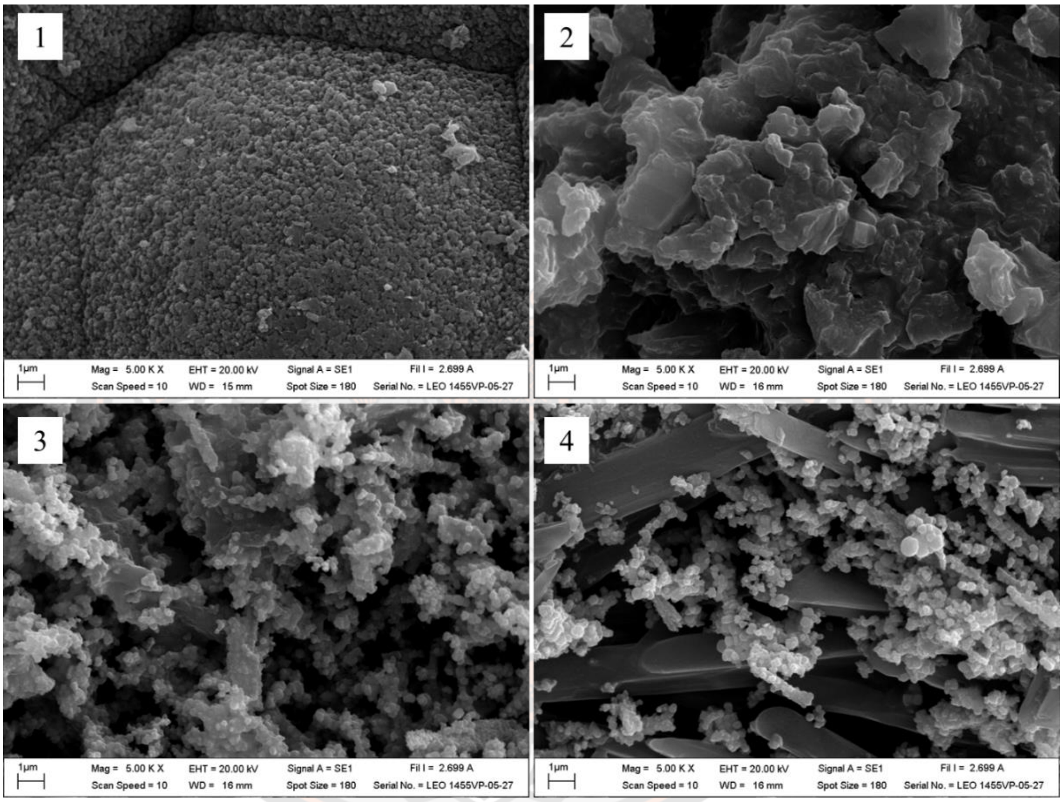


Figure 39 *Surface morphologies from SEM image at 5000X magnification of: (1) G, (2) GO, (3) GO-Fe_3O_4, and (4) GO-Fe_3O_4-DTZ*

4.2.2 Optimization

Effect of pH The effect of pH on the MdSPE extraction system was investigated using a 3.0 mg/L Pb solution and 25 mg of GO-Fe_3O_4-DTZ for the sorption of Pb ions at varying pH values. The pH influence was shown in the rising trend of the graph in Fig. 40. The relative absorbance of Pb increased with increasing pH values up to pH 9. For the next operation, the suitable value was pH 9. At the pH values greater than 9, the Pb signal intensity reduced. This observation was explained by the precipitation and sorbent structure. At pH 10, $Pb(OH)_2$ was precipitated, and its presence influenced the Pb adsorption. Active sites were responsible for the adsorption of ions on the active functional groups of DTZ. At low pH values, these were protonated and therefore cannot attached to the Pb.

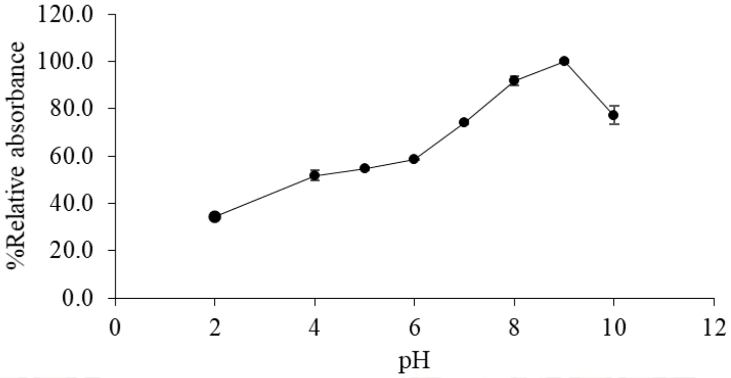


Figure 40 *Effect of pH in MdSPE-FAAS*

Effect of sonication time Sonication would be applied in extraction to toughly mix the reagents and to distribute analyte. In this focus (Fig. 41), the use of sonication has been affected in the method because of sonicating force. Its vibration made the over-spreading out of Pb and damaged the weak attraction force between GO-Fe_3O_4-DTZ and Pb. The signal has been decreased with the increased sonication time. Hence, Extraction under sonication was unworkable in this method.

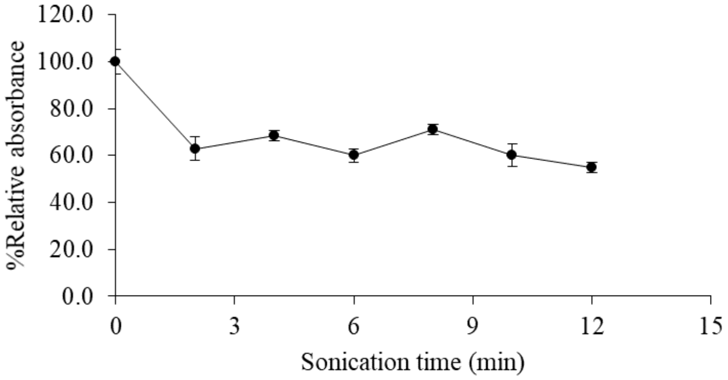


Figure 41 *Effect of sonication*

Effect of extracting time The extraction time was important in an equilibrium adsorption process. To determine the time required for an extraction condition and the contact of adsorption, the extraction time of the proposed MdSPE was investigated in the range 0-10 minutes using 3.0 mg/L Pb solution and 25 mg of GO-Fe_3O_4-DTZ (Fig. 42). It was observed that the relative absorbance continually increased until 6 minutes and then the absorbance decreased. This means that at 6 minutes extraction time, an equilibrium was established for maximum adsorption. The mechanism of the synthesized sorbent was that dithizone and Fe_3O_4 were adsorbed onto GO surfaces. Then, Pb was interacted with DTZ to form Pb-dithizone complexes (Fig. 36(3)). The existence of the hydroxyl group (-OH) and (-COOH) groups in the magnetic adsorbent caused the interaction of Pb on GO-Fe_3O_4-DTZ surfaces. After 6 min, the relative absorbance was decreased. This may be due to the fact that when the excessive extraction time for the adsorption procedure was applied, it could be led to the difficulty to leach out Pb from the magnetic adsorbent during the desorption process by the eluent (2.0 M HNO_3 in ethanol). Therefore, the extraction time of 6 minutes was selected as the adsorption contact time in all experiments.

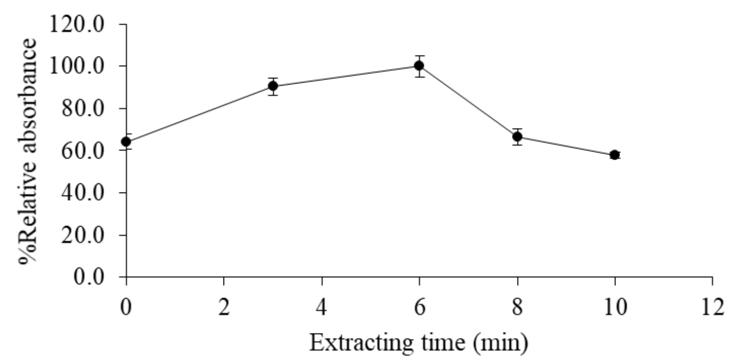


Figure 42 *Effect of extracting time in MdSPE-FAAS*

Type of eluent Strong acid solutions including nitric acid (HNO_3), sulfuric acid (H_2SO_4), and hydrochloric acid (HCl) were chosen for the study of the effect of eluent types. The results (Fig. 43) indicated that the maximum relative absorbance for Pb ions was obtained for 2.0 M of HNO_3 in ethanol. Having of ethanol in the elution system provided a positive effect on the dispersal of the particles in solution as well as to increase the ability of dissolution. Therefore, HNO_3 in ethanol was used as eluent for all subsequent experiments. Determination of the optimal concentration of HNO_3 in ethanol was the next focus.

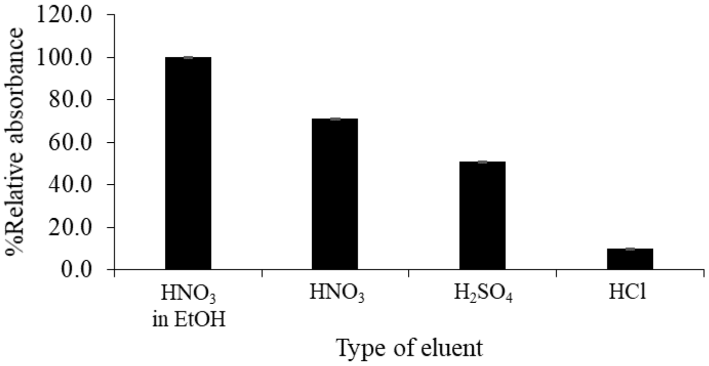


Figure 43 *Type of eluent*

Concentration of eluent Concentrations of HNO_3 at in the range 0.0-3.0 M were diluted with ethanol and used to desorb Pb ions from sorbents. The results (Fig. 44) showed that the increased relative absorbance was depended on the increased of concentration of acids. The absorbance signal increased up to 0.3 M of HNO_3 in ethanol and decreased slightly when the acid concentration increased beyond that. Thus, 0.3 M HNO_3 in ethanol was selected as the optimized eluent in the next experiments.

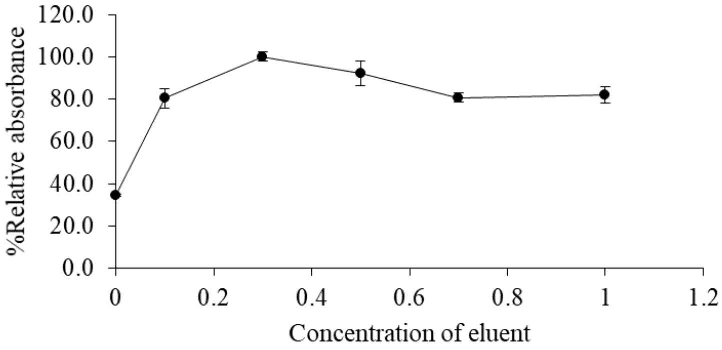


Figure 44 *Effect of concentration of eluent*

Sample volume The result was summarized in Fig. 45, showing that the maximum relative absorbance was obtained with 40 mL sample volume. Under the optimum condition, sample volume between 10 to 35 mL obtained the slightly increased in the range of 10-35 mL because of low preconcentration factor at low sample volume and all reagent in extraction system can be interacted with high amount of Pb until 40 mL. Then, the overload amount of Pb in this system which higher than 40 mL was shown the low signal via the complex competition and obscuring themselves. Hence, 40 mL of sample was selected as the optimum volume with preconcentration factor at 13.33.

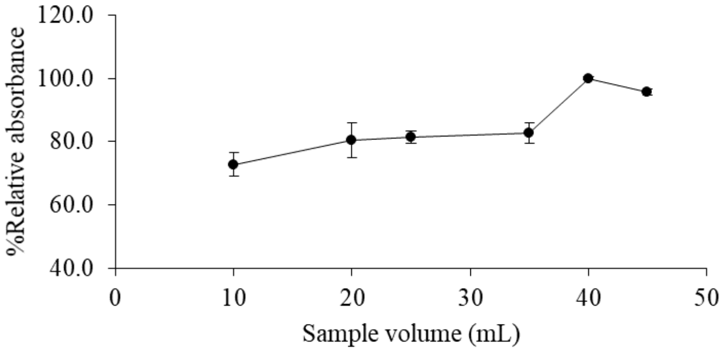


Figure 45 *Effect of sample volume*

Reusability The reusability of the synthesized GO-Fe_3O_4-DTZ was studied to determine its recycling capability and lifetime of sorbent for MdSPE. In this study, GO-Fe_3O_4-DTZ was investigated for several adsorption and elution cycles under the optimum conditions described earlier. After elution, the used sorbent was washed with 5 mL of 2.0 M HCl and deionized water. The results (Fig. 46) showed that the adsorption efficiency of the synthesized GO-Fe_3O_4-DTZ sorbent did not significantly changed up 4 times for Pb analysis (relative absorbance was higher than 80%). When the sorbent was used more than 4 times, the relative absorbance decreased significantly because GO-Fe_3O_4-DTZ sorbent was not stable owing to the deterioration of Fe_3O_4 or DTZ composition on GO. In addition, the proposed method gave the cost saving for preconcentration of Pb.

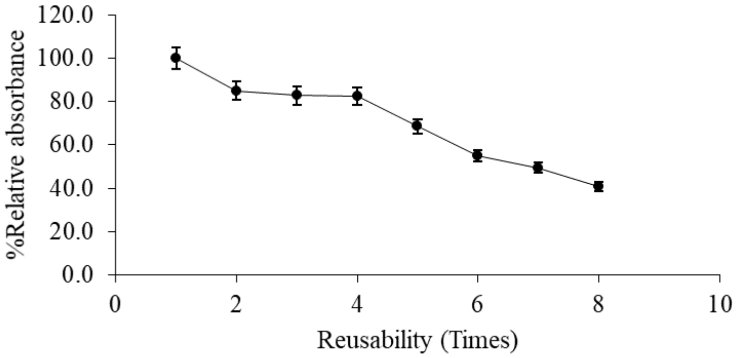


Figure 46 *Reusability of GO-Fe_3O_4-DTZ*

4.2.3 Analytical performance

The analytical performance of the proposed MdSPE-FAAS was evaluated under the optimum conditions.

Under the optimum conditions, Linearity of conventional FAAS was observed in the range 0.440-20.0 mg/L with the linear equation of Y = 0.0149X + 0.0061, R^2 0.9942. For MdSPE-FAAS, the linear range of 0.070-3.0 mg/L with the linear equation for Y = 0.1149X + 0.0198, R^2 0.9861 were obtained as demonstrated in Fig. 47. LOD and LOQ were calculated by eq. 8 and eq. 9 from signal-to-noise ratio of 3 and 10 which were 0.070 and 0.23 mg/L, respectively. %RSD was determined from a 1.0 mg/L Pb solution and found to be 3.41%. Recoveries were studied with spiked standards of 1.00 and 2.00 mg/L of Pb solutions to samples, and these gave recoveries in the range 90.1-123.0%. Preconcentration factor (PF) was the one performance parameters of any preconcentration method. In this work, the preconcentration factor calculated by eq. 11 was 13.33. Enrichment factor (EF) calculated from ratios of slopes as described in eq. 12 which gave a measure of the authenticity of the preconcentration method. EF calculated was found to be 7.71. Percentage of extraction efficiency (%EE) calculated from PF and EF by eq. 13 was 57.8%. Figures of merit of the proposed method was summarized in Table 15.

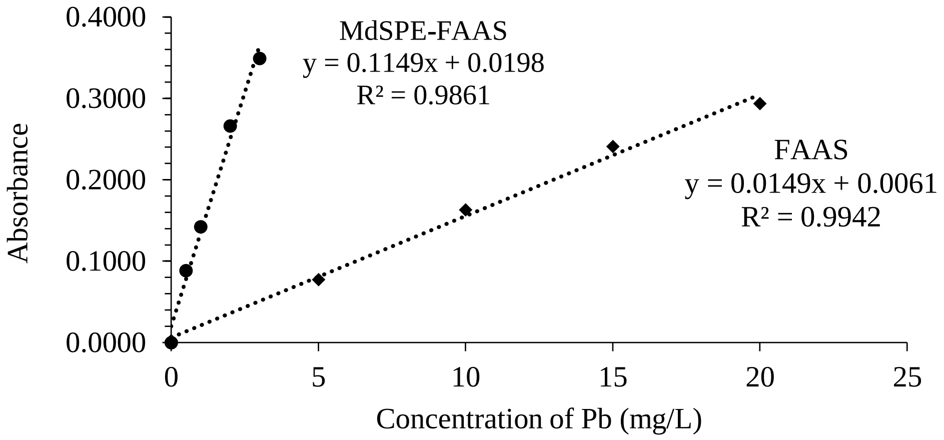


Figure 47 *Linearity of MdSPE-FAAS method*

Adsorption capacity Adsorption capacity was investigated by the adsorption study of Pb on the GO-Fe_3O_4-DTZ sorbent. As a result, the proposed method provided adsorption capacity of 30.53 mg/g. The result mean that 1.0 mg of GO-Fe_3O_4-DTZ sorbent can adsorb 30.53 mg of Pb.

Adsorption isotherm Two adsorption isotherms frequently used Langmuir and Freundlich isotherms. The Langmuir model described adsorption of solution species onto solid sorbents including homogeneous adsorption surface. The Freundlich isotherm model was applicable to adsorption processes that occurred on heterogonous surfaces [182]. The results in Fig. 48 and Fig. 49 indicated that the adsorption of Pb using GO-Fe_3O_4-DTZ sorbent fitted with Freundlich adsorption isotherm more than Langmuir adsorption isotherm. Relationship between log $\frac{x}{m}$ and log C_e was consistency in linear line with the correlation coefficient of 0.9864. The result described that the adsorption of Pb using GO-Fe_3O_4-DTZ sorbent was the heterogonous adsorption surface that had a complicated absorbed molecule, non-monolayer absorbate, multi-type of surface, and difficult adsorption mechanism. Eventually, adsorption isotherm result can explain that there were the several shapes of molecules in SEM surface morphology.

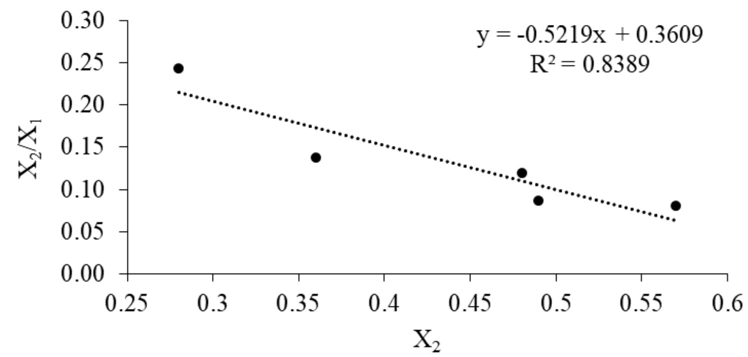


Figure 48 *Langmuir equation plotting for the adsorption study of Pb on the GO-Fe_3O_4-DTZ sorbent*

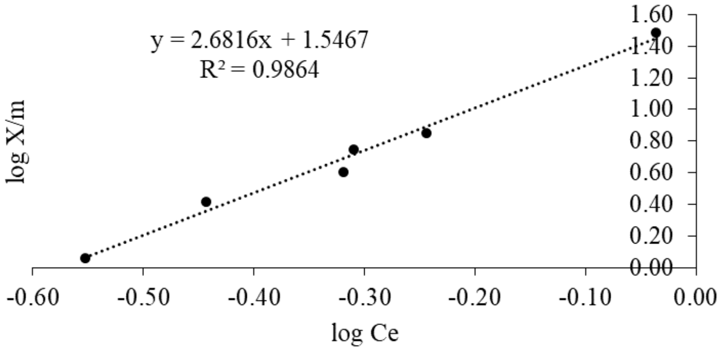


Figure 49 *Freundlich equation plotting*

Table 15 *Figures of merit from MdSPE method*

Figures of merit	**Value**
Linearity (ng/mL)	70.00-3000
LOD (ng/mL)	70.0
LOQ (ng/mL)	230
%RSD	3.41
%Recovery	88.60-118.8
EF	7.7
PF	13.3
%EE	57.8

4.2.4 Determination of Pb in water samples

The proposed MdSPE-FAAS method was applied to Pb analysis in water samples. Drinking water, tap water, and surface water samples were analyzed, and the results were shown in Table. 16. Recoveries were in the range 90.1 to 123.0%.

The comparison of reported preconcentration methods for Pb analysis was presented in Table 17. Among variety of sorbents, the results showed the strong point of the method in terms of the precision and accuracy. This method gave enrichment factor and adsorption capacity greater than those of other methods [42, 158] which used identical FAAS detection.

Table 16 *Analysis of Pb by standard addition method in different types of water samples using MdSPE-FAAS (n=3)*

Sample	**Pb concentration (mg/L)**		**%Recovery±SD**
	Added	**Found**	
Drinking water (1)	-	N.D.	-
	1.0	0.93	92.1±1.3
	2.0	2.02	100.5±0.3
Drinking water (2)	-	N.D.	-
	1.0	0.98	97.4±0.2
	2.0	2.01	100.2±1.4
Drinking water (3)	-	N.D.	-
	1.0	0.99	99.0±1.3
	2.0	2.00	100.0±0.9
Drinking water (4)	-	N.D.	-
	1.0	1.05	104.4±0.5
	2.0	1.99	99.2±0.7
Drinking water (5)	-	N.D.	-
	1.0	1.12	110.8±1.5
	2.0	2.01	99.9±0.4
Tap water (1)	-	N.D.	-
	1.0	1.06	106.2±1.6
	2.0	1.98	99.1±1.0

Table 16 *(Cont.)*

Sample	**Pb concentration (mg/L)**		**%Recovery±SD**
	Added	**Found**	
Tap water (2)	-	N.D.	-
	1.0	0.90	90.6±1.0
	2.0	1.99	99.8±0.9
Tap water (3)	-	N.D.	-
	1.0	1.23	123.0±0.5
	2.0	2.02	101.0±1.7
Surface water (1)	-	N.D.	-
	1.0	0.99	100.6±2.5
	2.0	2.01	101.3±0.4
Surface water (2)	-	N.D.	-
	1.0	0.89	90.1±2.2
	2.0	2.01	101.1±1.4
Surface water (3)	-	N.D.	-
	1.0	1.12	112.1±1.9
	2.0	1.99	99.6±1.1

N.D. was not detectable by MdSPE-FAAS (less than 0.070 mg/L)

Table 17 *Comparison of MdSPE and previous works for the determination of Pb in various types of samples*

Technique	**Sample**	**Sorbent**	**Linearity (mg/L)**	**LOD (mg/L)**	**LOQ (mg/L)**	**%RSD**	**%Recovery**	**PF**	**Adsorption capacity (mg/g)**	**Ref.**
SQT-FAAS	red pepper samples	SAC-MNPs	0.03-0.25	0.010	0.034	11.0	102.6-106.6	-	-	[154]
FAAS	water	Fe_3O_4-VSB	0.058-0.30	0.017	0.058	1.9-7.7	58.1-63.3	7.3-11.4	-	[43]
FAAS	river water	PHE-MAC	0.05-2.50	0.05		3.8	112-118	10	2.01	[170]
FAAS	food and environmental samples	MWNTs-APDC	-	0.60		<5	95-98	80	10.30	[158]
FAAS	drinking and environmental water	GO-Fe_3O_4-DTZ	0.07- 3.00	0.07	0.23	3.41	90.1-112.1	13.33	30.53	This method

Remarks;

SQT-FAAS	slotted quartz tube mounted flame atomic absorption spectrometry
SAC-MNPs	stearic acid coated magnetic nanoparticle

VSB	(E)-2-methoxy-4-(((3-(triethoxysilyl)propyl) imino)-methyl)phenol
PHE-MAC	the ion imprinted poly (hydroxylethyl methacrylate-N-methacryloyl-(L)-cysteine)
MWNTs-APDC	multiwalled carbon nanotubes coupled with ammonium pyrrolidine dithiocarbamate

4.2.5 Robustness

To evaluate the robustness and selectivity of the proposed method, the influence of ions in extraction system was studied. Different concentrations of interfering ions in 1.00 mg/L Pb solution at ratios of 1:1, 1:5, 1:10, 1:50, 1:100 and 1:200 (Pb : other ions in mass ratio) were extracted using the proposed method under the optimum conditions. According to the interfering study results (Table 18), the 80-120% range of recovery was achieved across all ratios. The maximum robustness of up to 200 was observed in the contamination of anion species (chloride and sulfate). Alkali and alkaline earth metals (Na^+, Ca^{2+}, and Mg^{2+}) had interfered the extraction system when their concentration ratio was greater than 10 for Na^+ and Ca^{2+}, or 5 for Mg^{2+}. This may be due to the possibility of the adsorption of these ions on the porous surfaces of the synthesized sorbent. This effect can be caused the reducing of adsorption capacity and recovery of Pb. Dithizone was a high selective ligand which hardly reacted with alkali and alkali earth metal ions. Tolerance ratios of these metals were expected to be high in this work. However, the results indicated that the tolerance ratio of alkali and alkali earth metal ions were quite lower than expected. The method can be also withstood for transition metals (Ni^{2+}, Zn^{2+}, and Cd^{2+}) up to a ratio of 5. Due to complex formation competition, the strong complex between the interfering ion and DTZ may gave a larger formation constant (K_f) than that of the Pb-DTZ, which consequently reduced the %recovery and tolerance limit.

Table 18 *Robustness of proposed method via the addition of interfering ions with the tolerance limit and %recovery results (n=3)*

Interfering ions	Tolerance limit	%Recovery ± SD
Cl^-	200	90.5±3.0
SO_4^{2-}	200	85.2±0.9
Na^+	50	97.8±1.5
Ca^{2+}	10	84.9±1.8
Mg^{2+}	5	93.6±1.5
Ni^{2+}	5	92.3±1.2
Zn^{2+}	5	81.7±0.2
Cd^{2+}	5	95.9±2.1

4.3 Conclusions

The proposed GO-Fe_3O_4-DTZ based MdSPE method was successfully applied for the determination of Pb in water samples and environmental samples by FAAS. The important parameters were optimized by univariate optimization method. Under the optimum condition, the analytical performances of the proposed method were examined and concluded in Table 15. In addition, effect of interfering ions was investigated as shown in Table 18. The concentrations of Pb in drinking water and environmental water samples were not detectable.

CHAPTER V

EFFERVESCENT TABLET-ASSISTED SWITCHABLE SOLVENT BASED LIQUID PHASE MICROEXTRACTION

In this chapter, switchable solvent based liquid phase microextraction (SS-LPME) was developed using the switching solvent (Triethylamine : TEA) as a single extraction solvent for Pb and Cd determination by HR-CS-FAAS. TEA can be switched to hydrophilic form as the protonated triethylamine carbonate (P-TEA-C). This solvent was used for dispersion and extraction of Pb and Cd in aqueous solution into enrichment phase for preconcentration. In addition, effervescent assisted technique (EA) was introduced in SS-LPME procedure. The tablets of effervescence consisting of two compositions (CO_2 source and H donor agents) were pressed into tablets using a mould for compression. For EA-SS-LPME procedure, effect of pH, concentration of DTZ, type and optimum ratio of effervescent tablet, volume of switchable solvent, volume of NaOH, centrifugation speed, time of centrifugation and volume of sample were optimized. Analytical performance parameters of the proposed method and analysis of real samples (Water and canned food samples) were evaluated as follows;

5.1 Experiment

5.1.1 Instrumentation

Pb and Cd determination were performed by high resolution continuum source flame atomic absorption spectrometry (HR-CS-FAAS, ContrAA 800, Analytik-jena, Germany) utilizing an air/acetylene flame with other operation parameters as given in Table 19. The pH of solutions was measured using a pH meter (HI98107, Hanna instrument, USA). Filter paper (No 1, Whatman™, Thailand) was used for sample filtration. Clinical centrifugation (DM0412, DLAB, Thailand) was used for phase separation. Stirrer (PMC Beware, IOWA 52001, U.S.A) with magnetic bar were used to stir the synthesized switchable solvent. Microwave digestion system (SK-15eT, Milestone, Italy) was used for acid-digestion sample pretreatment.

Table 19 *Instrumental parameters for multielement Pb and Cd analysis of HR-CS-FAAS*

Line	Wavelength (nm)	Read time (s)	C_2H_2/Air (L/h)	Burner height (mm)	Eval. Pixels
Cd	228.8018	3	50	6	3 pixels
Pb	217.0005	3	65	6	3 pixels

5.1.2 Reagents

All reagents used in this work were of analytical grade. These were: Nitric acid, 65% (RCI Labscan, Thailand), 99% dithizone, (Ajax Finechem, Australia), sodium hydroxide, 97% (RCI Labscan, Thailand, ethanol (RCI Labscan, Thailand), triethylamine, 99.5% (Loba chemie, India), sodium acetate 99% (VWR International, England), 99.7% acetic acid (RCI Labscan, Thailand), hydrochloric acid, 37% (RCI Labscan, Thailand), tetrahydrofuran, 99.8% (RCI Labscan, Thailand), hydrogen peroxide, 30% (VWR International, USA), potassium dihydrogen phosphate, 99.5% (Loba chemie, India), sodium bicarbonate (Chemipan, China), and dry ice (Phitsanulok ice, Thailand). 1000 mg/L Pb (II) standard solution (PanReac AppliChem, Germany) and 1000 mg/L Cd (II) standard solution (Qrecqchemical, New Zealand) were diluted to a 100 mg/L stock solution with deionized water.

Working solutions were prepared with deionized water. Phosphate buffer was used to adjust the pH to 9.

5.1.3 Synthesis of switchable solvent

Triethylamine (TEA) for 200 mL was mixed with 200 mL of deionized water. Then, the mixed portion was stirred via stirrer and added with 2 kg of dry ice until the homogeneous phase was appeared and kept stirring for 3 hrs. Finally, TEA (Hydrophobic solvent) was switched to the protonated-TEA-carbonate (P-TEA-C: hydrophilic solution) as seen in Fig. 50(1).

5.1.4 Preparation of effervescent tablet

The effervescent tablets were prepared by $NaHCO_3$ and KH_2PO_4 at ratio of 1:4 by mole. The powder of two compositions were mixed and filled into the mould and pressed to tablets as illustrated in Fig. 50(2).

5.1.5 EA-SS-LPME procedure

The extraction procedure was schematically presented in Fig. 50(3). First, the mixed of Pb and Cd standard solution for 10 mL was obtained into 50 mL centrifuge tube and adjusted with buffer to pH 9. Then, 1 mL of DTZ (Final concentration at 1.5 mM) was added into extraction tube. Next, 1 mL of P-TEA-C and an effervescent tablet were added, consecutively. During this process, the complexes of Pb-DTZ and Cd-DTZ were thoroughly dispersed in aqueous solution. Next, 2 mL of 10 M NaOH were added for switching of P-TEA-C back to TEA. This step, Pb-DTZ and Cd-DTZ complexes were extracted into TEA phase and the enriched phase was separated by centrifugation at 2000 rpm for 5 min and dissolved with 0.1 M HNO_3 before measurement by HR-CS-FAAS.

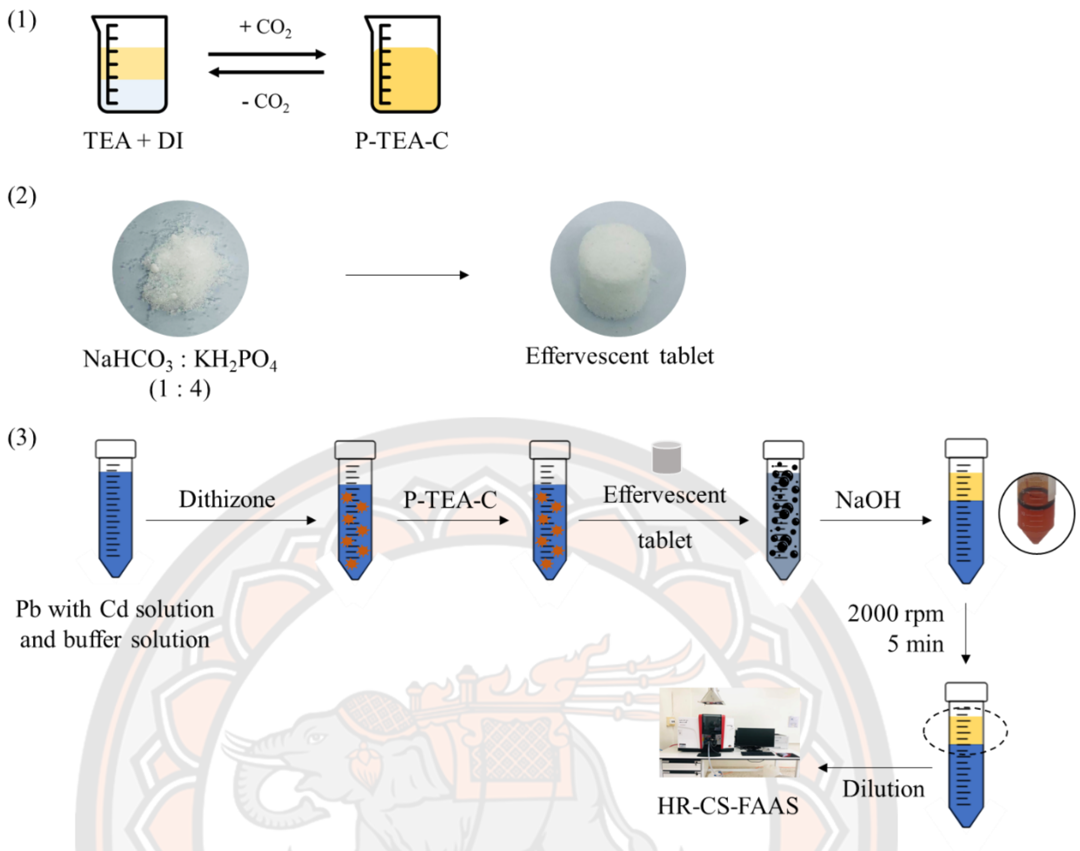


Figure 50 *Schematic diagram for EA-SS-LPME procedure*

5.1.6 Optimization

In EA-SS-LPME, the important parameters *e.g.*, effect of pH, concentration of DTZ, type and optimum ratio of effervescent tablet, volume of switchable solvent, volume of NaOH, rate of centrifuge speed, time of centrifugation and volume of sample were optimized by univariate method.

Effect of pH 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were adjusted to pH 5-13 using different types of buffer solution (acetic/acetate buffer, phosphate buffer, and ammonia/ammonium buffer). Then, 1.0 mL of 0.3 mM DTZ (final concentration) as complexing agent, 1.0 mL of P-TEA-C (extracting phase), one of effervescent tablet, and 2.0 mL of 10 M NaOH (switching-back agent) were added. After extraction, centrifugation at 4000 rpm was introduced for 4 min. The solution was diluted by 0.1 M HNO_3 for dissolution.

Concentration of DTZ 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were adjusted to pH 9 using ammonia/ammonium buffer. Then 1.0 mL of DTZ (complexing agent) in the range of 0.3, 0.7, 1.0, 1.5, 2.0 and 2.5 mM (final concentration) were optimized. Next, 1.0 mL of P-TEA-C (extracting phase), one of effervescent tablet, and 2.0 mL of 10 M NaOH (switching-back agent) were added. After extraction, centrifugation at 4000 rpm was introduced for 4 min. The solution was diluted by 0.1 M HNO_3 for dissolution.

Type of effervescent tablet 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were adjusted to pH 9 using ammonia/ammonium buffer. Then 1.0 mL of DTZ at 1.5 mM (final concentration) and 1.0 mL of P-TEA-C (extracting phase) were added. One of effervescent tablet at the ratios of 1:3 (Na_2CO_3 : $C_6H_8O_7$, Na_2CO_3 : $C_6H_8O_6$, Na_2CO_3 : KH_2PO_4, Na_2CO_3 : K_2HPO_4, $NaHCO_3$: $C_6H_8O_7$, $NaHCO_3$: $C_6H_8O_6$, $NaHCO_3$: KH_2PO_4, and $NaHCO_3$: K_2HPO_4) were optimized. Next, 2.0 mL of 10 M NaOH (switching-back agent) was applied. After extraction, centrifugation at 4000 rpm was introduced for 4 min. The solution was diluted by 0.1 M HNO_3 for dissolution.

Ratio of effervescent tablet 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were adjusted to pH 9 using ammonia/ammonium buffer. Then 1.0 mL of DTZ at 1.5 mM (final concentration) and 1.0 mL of P-TEA-C (extracting phase) were added. One of effervescent tablet ($NaHCO_3$: KH_2PO_4) at the ratio of 1:1 to 1:7 was optimized. Next, 2.0 mL of 10 M NaOH (switching-back agent) was applied. After extraction, centrifugation at 4000 rpm was introduced for 4 min. The solution was diluted by 0.1 M HNO_3 for dissolution.

Volume of switchable 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were added with pH 9 buffer and 1.0 mL of DTZ at 1.5 mM (final concentration). Then P-TEA-C (extracting phase) in the range of 1.0 to 10.0 mL were optimized. One of effervescent tablet ($NaHCO_3$: KH_2PO_4 at 1:4) and 2.0 mL of 10 M NaOH (switching-back agent) were applied. After extraction, centrifugation at 4000 rpm was introduced for 4 min. The solution was diluted by 0.1 M HNO_3 for dissolution.

Volume of NaOH 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were added with pH 9 buffer, 1.0 mL of DTZ at 1.5 mM (final concentration), and 1.0 mL of P-TEA-C (extracting phase). Then one of effervescent tablet($NaHCO_3$: KH_2PO_4 at 1:4) were applied into the extraction. Next, 10 M NaOH (switching-back agent) was optimized in the range of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mL. After extraction, centrifugation at 4000 rpm was introduced for 4 min. The solution was diluted by 0.1 M HNO_3 for dissolution.

Rate of centrifugation speed 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were added with pH 9 buffer, 1.0 mL of DTZ at 1.5 mM (final concentration), and 1.0 mL of P-TEA-C (extracting phase). Then one of effervescent tablet ($NaHCO_3$: KH_2PO_4 at 1:4) and 10 M NaOH (switching-back agent) were added for the extraction procedure. After that, centrifugation speed in the range of 1000-4000 rpm was introduced for 4 min. Then, the solution was diluted by 0.1 M HNO_3 for dissolution.

Centrifugation time 10 mL of 1.0 mg/L Pb and 0.5 mg/L Cd solution were added with pH 9 buffer, 1.0 mL of DTZ at 1.5 mM (final concentration), and 1.0 mL of P-TEA-C (extracting phase). Then, one of effervescent tablet ($NaHCO_3$: KH_2PO_4 at 1:4) and 10 M NaOH (switching-back agent) were applied for the extraction. After that, centrifugation time at 2000 rpm was optimized in the range of 1-6 min. The solution was diluted by 0.1 M HNO_3 for dissolution.

Sample volume Sample volume of 1.0 mg/L Pb and 0.5 mg/L Cd solution were optimized in the range of 10-40 mL and added with pH 9 buffer, 1.0 mL of DTZ at 1.5 mM (final concentration), and 1.0 mL of P-TEA-C (extracting phase). Then one of effervescent tablet ($NaHCO_3$: KH_2PO_4 at 1:4) and 10 M NaOH (switching-back agent) were applied for the extraction. After that, centrifugation at 2000 rpm for 5 min was introduced. The solution was diluted by 0.1 M HNO_3 for dissolution.

5.1.7 Analytical performance

The analytical performances of EA-SS-LPME-HR-CS-FAAS method were investigated under the optimum conditions. The parameter namely LOD, LOQ, enrichment factor (EF), preconcentration factor (PF), and percentage extraction efficiency (%EE) were investigated using the same equation as previously described in section 3.1.4. In addition, linearities were investigated in the range of 0.0, 1.0, 5.0, 10.0, 15.0 and 20.0 mg/L of Pb solution and 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L of Cd solution. %RSD was evaluated by 0.1, 1.0 and 10.0 mg/L (Pb) and 0.05, 0.5 and 1.0 mg/L (Cd). For the investigation of recoveries, 0.1 and 10.0 mg/L of Pb solution and 0.05 and 1.00 mg/L of Cd solution were spiked into the real samples.

5.1.8 Application for Pb and Cd analysis

The developed EA-SS-LPME-HR-CS-FAAS was applied for simultaneous determination of trace amount of Pb and Cd in water and canned food samples.

Water sample preparation Drinking (or bottled water) water samples were purchased from local stores, Naresuan university, Phitsanulok, Thailand. Drinking water samples were extracted by EA-SS-LPME procedure.

Canned food sample preparation Canned food samples (Canned tuna and fruits) were purchased from local stores. All samples were weighed for 0.50 g and 9.0 mL of 65% HNO_3 and 1.0 mL of H_2O_2 were added. Samples were digested by microwave digestion system at 210 °C for 35 min. Next, Digested samples were diluted to 50 mL with deionized water before extraction.

5.1.9 Robustness

0.10 mg/L of Pb and Cd solution was spiked with interfering ions, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Hg^{2+}, Co^{2+}, Sn^{2+}, NO_3^-, CO_3^{2-}, PO_4^{3-} and Cl^- at the ratios of 1:1, 1:50, 1:100, 1:500 and 1:1000 (Analyte: interfering ion). Then, EA-SS-LPME extraction procedure under the optimum condition was carried out. %Recoveries were calculated to evaluate the interfering effect.

5.2 Results and discussion

5.2.1 Optimization

Effect of pH The effect of pH in the EA-SS-LPME was investigated using a 1.0 mg/L Pb with 0.5 mg/L Cd solution and the solution was adjusted to pH 5-13 by various types of buffer solution. The influence of pH was shown in the graph (Fig. 51). It was observed that the relative absorbance of Pb and Cd increased with increasing pH values from 5 to 9 for Cd and 9-11 for Pb. pH 9 was the optimized value that gave the maximum signal for Cd. At pH in the range 9-11, it was observed that signal for Pb gave the highest %relative absorbance. This can be explained that pH affected the complex stability of Pb-DTZ and Cd-DTZ. By using the compromised condition, pH 9 was selected for multi-element analysis by the proposed method. Therefore, the pH of solution was adjusted to 9 as the suitable pH for the next study.

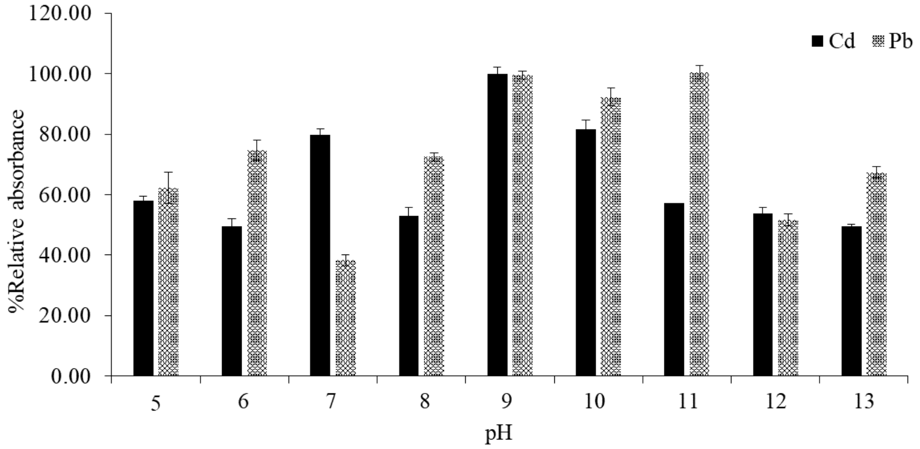


Figure 51 *Effect of pH for determination of Pb and Cd using EA-SS-LPME*

Effect of concentration of dithizone In this study, dithizone (DTZ) was selected as complexing agent to form complex with Pb and Cd ions. DTZ was a highly selective ligand. The optimum concentration of DTZ for Pb and Cd analysis was studied. After extraction, Pb-DTZ and Cd-DTZ were extracted to TEA phase giving high relative absorbance as shown in Fig. 52. Absorbance of Cd was increased and remained constant for the concentration of DTZ in the range of 0.7-2.5 mM. Absorbance of Pb increased to 1.5 mM and decreased after that. At low concentration of DTZ, these was not enough to complete the reaction. In the excess amount of DTZ, the absorbance decreased because the extraction system containing high alkali condition. Therefore, the excess-DTZ could be precipitated and reduced the dispersion and phase separation by EA-SS-LPME. For the next experiment, 1.5 mM of DTZ was selected as an optimum condition.

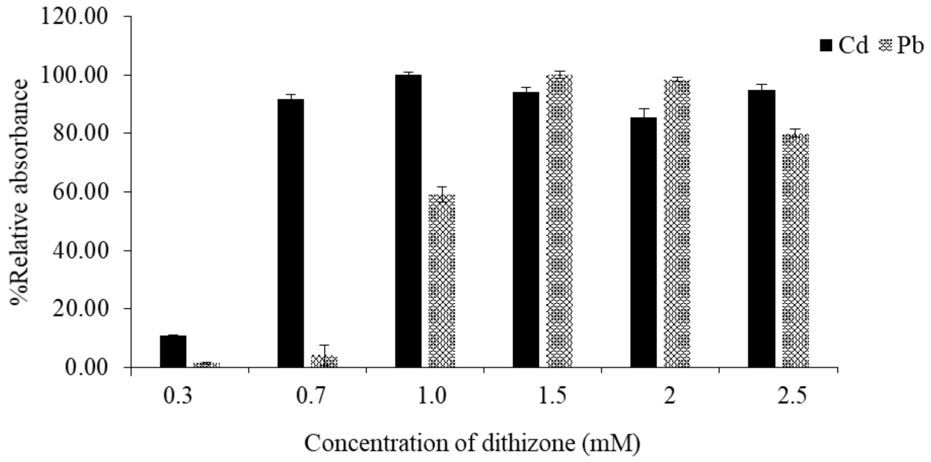


Figure 52 *Effect of dithizone concentration for the determination of Pb and Cd using EA-SS-LPME*

Composition of effervescent tablet In the proposed EA-SS-LPME, effervescence technique was based on the formation of CO_2 gas by reaction of a CO_2 source and a proton donor agent was carried out. By means of small bubble generation of CO_2, P-TEA-C and complexes were easily and homogeneously dispersed on the aqueous sample. In this study, an effervescent tablet was made by Na_2CO_3 and $NaHCO_3$ as CO_2 source with different types of weak acid namely $C_6H_8O_7$, $C_6H_8O_6$, KH_2PO_4 and K_2HPO_4 as proton donor at 1:3 mole ratio of CO_2 source : proton donor. As shown in Fig. 53, the relative absorbances of Cd at different compositions were not significant different and represented the maximum signal for Na_2CO_3 and KH_2PO_4. Composition of $NaHCO_3$ and KH_2PO_4 provided the highest relative absorbance for Pb. In the part of different combinations, %relative absorbance obtained by fixing of CO_2 source (Na_2CO_3) and varying the proton donor ($C_6H_8O_7$, $C_6H_8O_6$, KH_2PO_4 and K_2HPO_4) was demonstrated in Fig. 53. It was found that KH_2PO_4 was the best proton donor (Both for Pb and Cd). By fixing of KH_2PO_4 as proton donor and varying the CO_2 source (Na_2CO_3 and $NaHCO_3$), $NaHCO_3$ was a great CO_2 source. By these reasons, the effervescent tablets made of $NaHCO_3$ and KH_2PO_4 were applied for the next experiment following this reaction.

$$3KH_2PO_4 + 6NaHCO_3 \rightarrow K_3PO_4 + 2Na_3PO_4 + 6CO_2 + 6H_2O$$

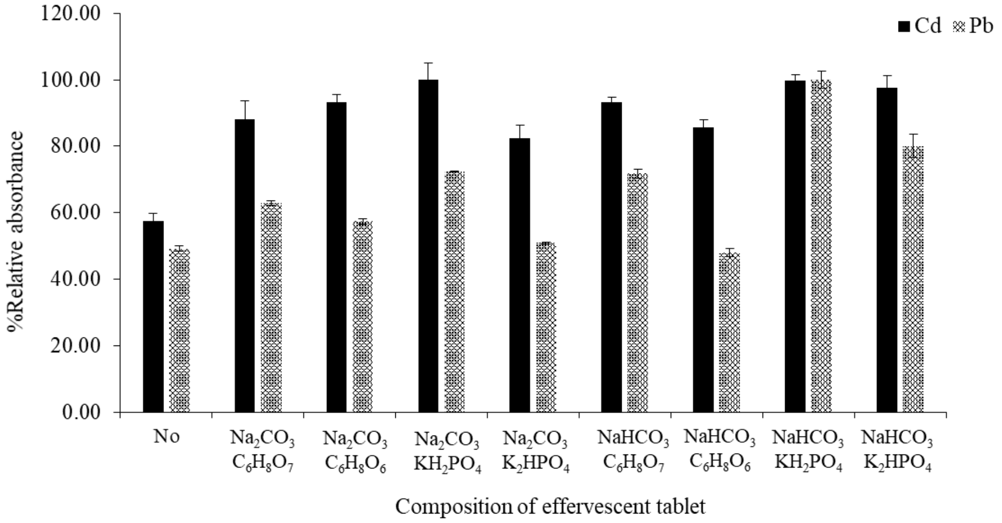


Figure 53 *Composition of effervescent tablet*

Ratio of effervescent composition The effervescent reaction between CO_2 source and proton donor strongly depended on the mole ratios of these components. Therefore, studying the appropriate ratio was very important to achieve maximum relative absorbance of Pb and Cd. In order to investigate this effect, different types of effervescent tablets consisting of different ratios of $NaHCO_3$: KH_2PO_4 were prepared at 1:1, 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7 mole ratio (Fig. 54). It was observed that maximum absorbance was obtained by 1:4 ratio of $NaHCO_3$ and KH_2PO_4 (Both for Pb and Cd). Hence, mole ratio between $NaHCO_3$ and KH_2PO_4 was selected as 1:4 for further experiments.

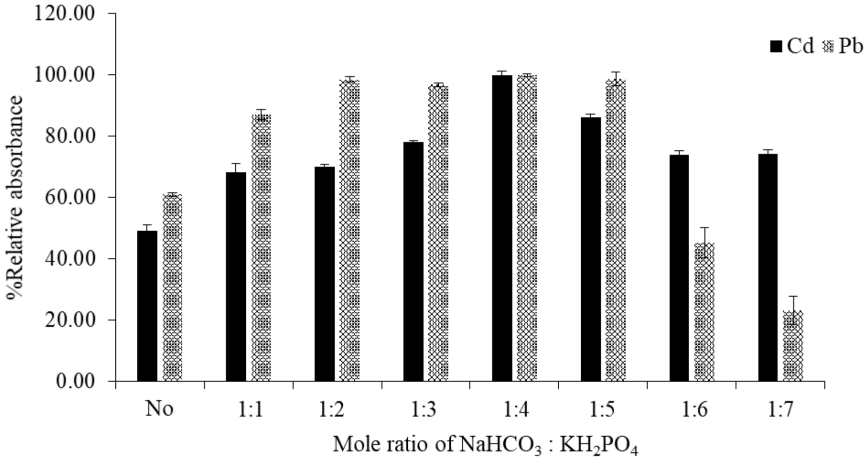


Figure 54 *Optimum ratio of effervescent tablet composition*

Volume of switchable solvent After selecting triethylamine (TEA) as the switchable solvent for this microextraction studies, it was necessary to optimize the volume of P-TEA-C (switching form of TEA), which provided homogenous extraction phase for the Pb and Cd extraction. The effect of the volume of P-TEA-C as the extraction solvent on the relative absorbance was investigated. The studies were performed at different volume of P-TEA-C ranging from 1.0-10.0 mL as detailed in the Fig. 55. It can be concluded from the result that there was a gradual increase in the percent relative absorbance of Pb from 1.0 mL until 2.0 mL of switchable solvent volume and gradually decreased after that. For Cd, %relative absorbance was not significant change and showed the maximum signal at 2.0 mL of switchable solvent volume. At volume of P-TEA-C 2.0 mL represented the preconcentration factor of 3.3. The volumes of solvent over 2.0 mL gave comparable signal but the preconcentration factor was lower. Therefore, 2.0 mL of switchable solvent was used in further studies.

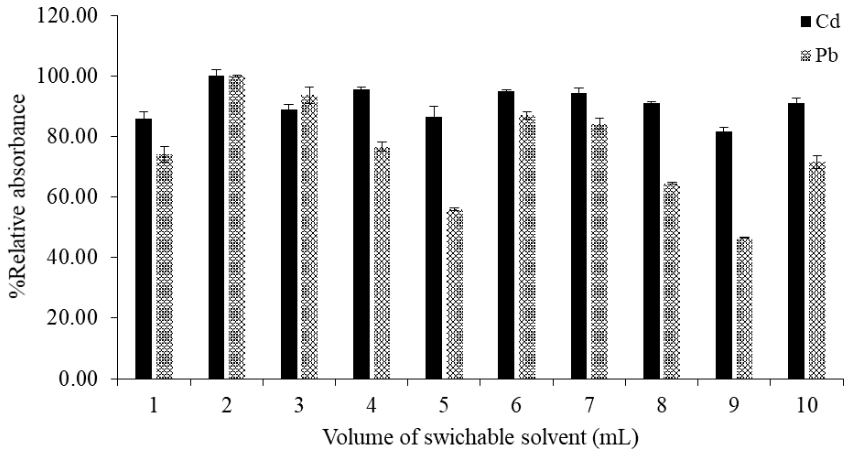


Figure 55 *Effect of switchable solvent volume*

Volume of NaOH As 10 M NaOH was found to be the most frequently used switching agent for removing of CO_2 from P-TEA-C back to TEA from previous report [18, 63, 91]. In order to convert the hydrophilic form of switchable solvent to hydrophobic form and also to extract the complexes of Pb-DTZ and Cd-DTZ from aqueous sample solution, the proposed method was carried out at different volume of 10 M of NaOH ranging from 1.5-3.5 mL as shown in Fig. 56. The result showed that the relative absorbance for Pb was raised up to 2.0 mL of 10 M NaOH while relative absorbance for Cd slightly changed from 1.5-3.0 mL. Existence of high volume of NaOH might affect the interaction of complex formation inter-extraction. Meanwhile, the relative absorbance of Pb was decreased after 2.0 mL and that decreased of Cd after 3.0 mL of NaOH as shown in Fig. 56. As a consequence, 2.0 mL of 10 M NaOH was used for further experiments.

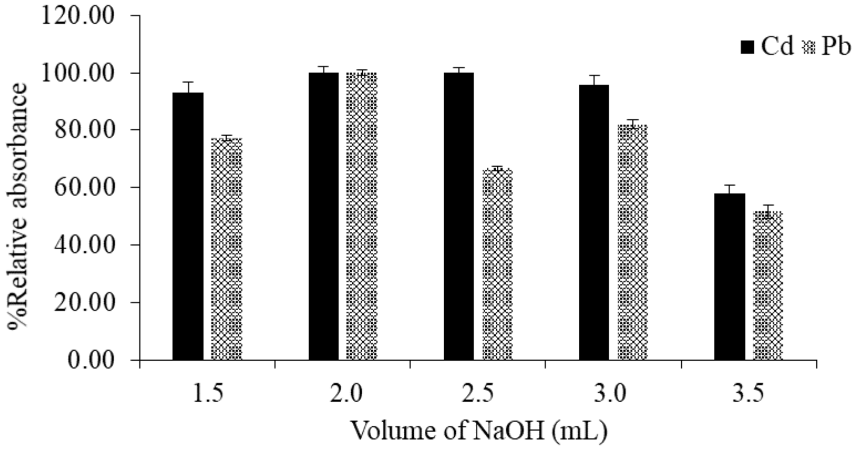


Figure 56 *Effect of NaOH volume*

Rate of centrifugation For EA-SS-LPME, centrifugation speed plays an important role in organic-aqueous phase separation. The completely separated phase affected to increase extraction efficiency and preconcentration. To investigate the effect of centrifuge speed, 1.00 mg/L of Pb and 0.5 mg/L of Cd were used for this study. The extraction was carried out with centrifuge speed at 1000-4000 rpm for 4 min. The result in Fig. 57 showed that the highest relative absorbance was observed at 2000 rpm then the signals were slightly decreased. It was found that with no-centrifugation and at 1000 rpm centrifugal speed provided the relative absorbance approximate 70-85% of Pb and 82-90% for Cd because the switching of P-TEA-C to TEA using 10 M NaOH was relatively effective, which represented the clear phase separation. Therefore, with no-centrifugation and 1000 rpm, these still provided high absorbance. After increasing of speed for 3000 and 4000 rpm, the relative absorbance was decreased because some portion of TEA phase was back-distribution into aqueous phase by the strong-centrifugal force. As a consequence, this effect led to low relative absorbance and enrichment factor. Therefore, rate of centrifugal speed at 2000 rpm was selected for further works.

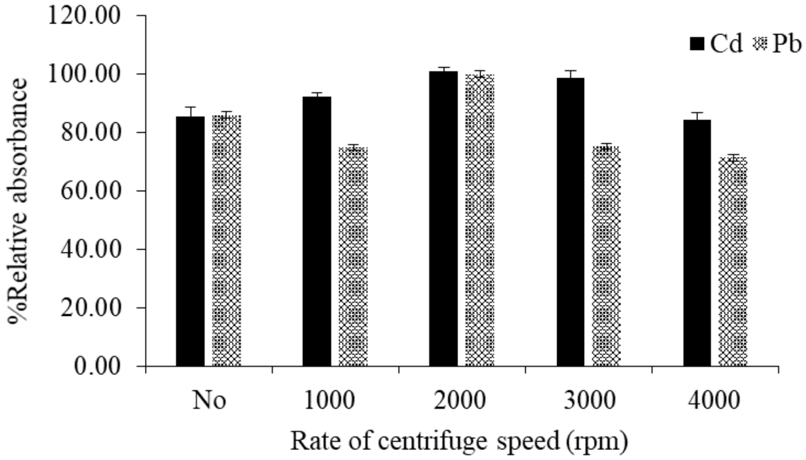


Figure 57 *Rate of centrifuge speed for phase separation in EA-SS-LPME*

Centrifugation time In this experiment, 1.00 mg/L of Pb and 0.5 mg/L of Cd were used for studied. AA-SS-LPME was applied with centrifuge speed at 2000 rpm for 1-6 min. The result in Fig. 58 showed that centrifugation time was not influenced on Cd signal as indicated with steady high signal. For Pb, relative absorbance was increased while the time was increased from 1 min to 5 min. As the reason of back-distribution of TEA, relative absorbance after 5 min was lowered. As a consequence, centrifugation time in EA-SS-LPME at 2000 rpm was applied for 5 min for the next study.

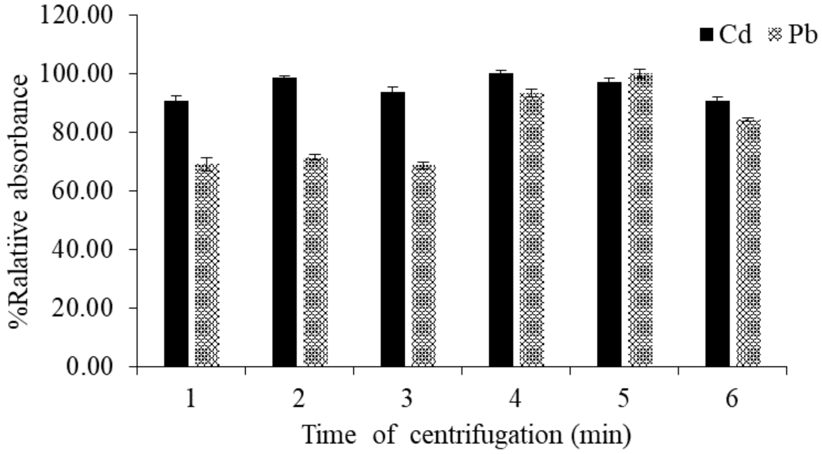


Figure 58 *Centrifugation time for phase separation in EA-SS-LPME*

Sample volume Volume of sample affected to the preconcentration factor following eq. 11. The results (Fig. 59) were obtained with the apparent observation. Only 10 mL of sample was shown the separated phases. 20-40 mL of sample solution was not appeared the separated phases of TEA enriched with analytes by the inadequate of NaOH or switchable solvent in the extraction system. Therefore, 10 mL of sample was selected as the optimized volume for the next apparatus.

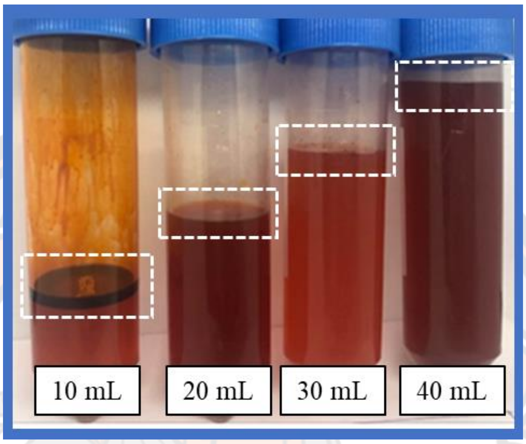


Figure 59 *Effect of sample volume*

5.2.2 Analytical performance

Under the optimized condition, analytical performance of proposed method was investigated. Calibration curves were plotted for the determination of Pb and Cd according to the conventional procedure. Linear equation of HR-CS-FAAS without EA-SS-LPME was Y = 0.1856X + 0.014, with a correlation coefficient (R^2) of 0.9928 and the linear range of 0.11-2.00 mg/L Cd. Linear equation of HR-CS-FAAS with EA-SS-LPME was Y= 0.4829X + 0.0422, with R^2 of 0.9936 with the working range of 0.04-1.50 mg/L Cd as shown in Fig. 60. The EF calculated from eq. 12 using the slope of calibration curve was 2.6 for Cd analysis. The LOD and LOQ calculated by eq. 8 and eq. 9 were 0.0068 mg/L and 0.0228 mg/L, respectively. %RSDs of the method were in the range 1.07-1.64 % (At 0.05, 0.50 and 1.00 mg/L of Cd). For Pb determination as can be seen from Fig. 61, the linear equation of HR-CS-FAAS with EA-SS-LPME and HR-CS-FAAS without EA-SS-LPME were Y= 0.0308X + 0.0199, with a R^2 of 0.9900 (0.06-10.00 mg/L) and Y= 0.0222X + 0.0014, with a R^2 of 0.9910 (0.09 to 24.0 mg/L,) respectively. LOD and LOQ were 0.0195 and 0.0649 mg/L, respectively. EF for preconcentration of Pb was 1.4. %RSDs of the method were in the range 1.25-1.69 % (At 0.10, 1.0 and 10.00 mg/L of Cd). Preconcentration factor of Pb and Cd was. In order to demonstrate the performance of the method, the proposed EA-SS-LPME-HR-CS-FAAS was applied for determination of Pb and Cd in water and canned food samples by standard addition method and the accuracy of method was evaluated as %recovery. Samples were spiked with different concentrations of Pb and Cd before extraction and preconcentration. The results were shown in Table 21. It was observed that the percentage recoveries of Cd (0.05 and 1.00 mg/L) and Pb (0.10 and 10.0 mg/L) were acceptable in the range 81.67-120.00% and 82.30-119.00%, respectively. Figures of merit of the proposed method were summarized in Table 20.

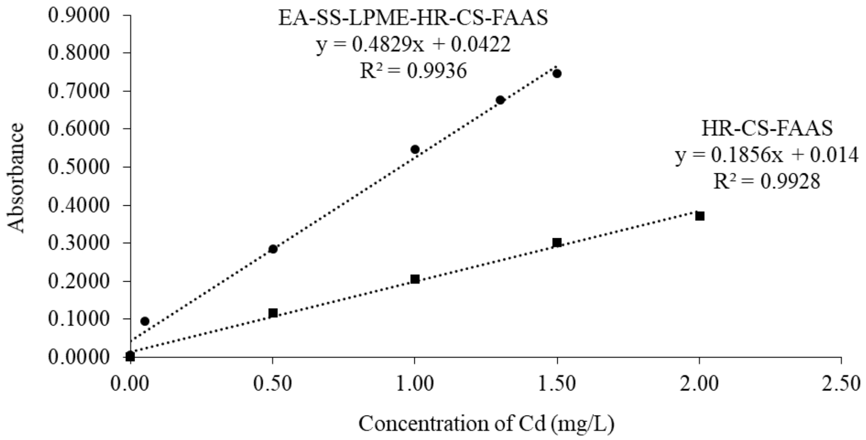


Figure 60 *Calibration curves of Cd by normal HR-CS-FAAS and the proposed method*

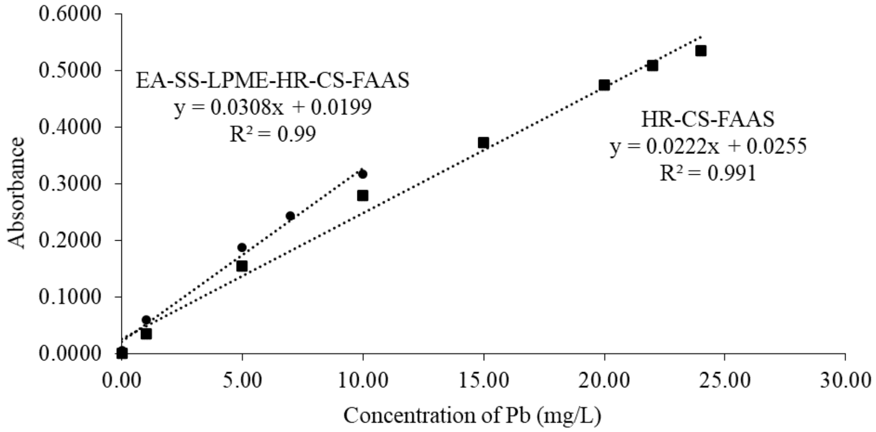


Figure 61 *Calibration curve of Pb by normal HR-CS-FAAS and the proposed method*

Table 20 *Figures of merit from EA-SS-LPME method*

Figures of merit	**Value**
Linearity (ng/mL)	(Pb) 64.90-1500
	(Cd) 22.80-10000
LOD (ng/mL)	(Pb) 19.50
	(Cd) 6.80
LOQ (ng/mL)	(Pb) 64.90
	(Cd) 22.80
%RSD	(Pb) 1.25-1.69
	(Cd) 1.07-1.64
%Recovery	(Pb) 82.30-119.0
	(Cd) 81.67-120.0
EF	(Pb) 1.4
	(Cd) 2.6
PF	3.3

5.2.3 Application of EA-SS-LPME for Pb and Cd analysis

This proposed method was applied to sequentially determine Pb and Cd in drinking water, canned tuna, and canned fruit samples. The complicated matrix samples were digested with microwave digestion as represented in section 5.1.8

before extraction by EA-SS-LPME. The results were given in Table 21 showing satisfactory recoveries for Pb and Cd analysis. The content of Pb and Cd in were not detectable in all samples.

Comparison of the analytical performance with the previous reports was concluded in Table 22. The comparative results of %RSD was found that the proposed SS-LPME-HR-CS-FAAS gave better precision than other methods for HR-CS-FAAS [159], normal FAAS [183], SS-HR-CS-GFAAS [184], SPs-LPME-µS-FAAS [18], and VA-SHS-LPME-GFAAS [171]. The proposed method gave high preconcentration factor as compared with similar method for HR-CS FAAS [159]. A superior performance with respect to LOD and LOQ was achieved as compared to HR-CS FAAS [159], normal FAAS [183], and spectrophotometry [185].

Table 21 *Result for the determination of Pb and Cd in drinking water and canned food sample using EA-SS-LPME-HR-CS-FAAS*

Samples	**Analyte**	**Concentration (mg/L)**		**%Recovery ± SD**
		Added	**Found**	
DW-1	Cd	0.00	N.D	-
		0.05	0.053	98.00 ± 2.80
		1.00	1.153	115.30 ± 2.40
	Pb	0.00	N.D	-
		0.10	0.119	119.00 ± 0.00
		10.00	10.05	100.49 ± 3.15
DW-2	Cd	0.00	N.D	-
		0.05	0.047	89.00 ± 1.40
		1.00	1.051	105.10 ± 6.70
	Pb	0.00	N.D	-
		0.10	0.112	111.67 ± 5.77
		10.00	9.739	97.39 ± 2.30
DW-3	Cd	0.00	N.D	-
		0.05	0.054	107.20 ± 4.00
		1.00	1.030	102.90 ± 3.60
	Pb	0.00	N.D	-
		0.10	0.115	115.67 ± 2.89
		10.00	9.825	98.25 ± 1.11
Tuna-1	Cd	0.00	N.D	-
		0.05	0.047	99.30 ± 0.00
		1.00	0.972	97.00 ± 1.70
	Pb	0.00	N.D	-
		0.10	0.108	107.70 ± 2.80
		10.00	10.190	101.90 ± 1.50
Tuna-2	Cd	0.00	N.D	-
		0.05	0.046	82.00 ± 0.00
		1.00	0.822	82.40 ± 0.28
	Pb	0.00	N.D	-
		0.10	0.102	101.30 ± 11.10

Table 21 *(Cont)…*

Samples	Analyte	Concentration (mg/L)		%Recovery ± SD
		Added	Found	
		10.00	9.790	97.70 ± 0.10
Tuna-3	Cd	0.00	N.D	-
		0.05	0.055	118.70 ± 1.20
		1.00	0.908	90.80 ± 1.10
	Pb	0.00	N.D	-
		0.10	0.089	88.30 ± 5.70
		10.00	10.17	101.40 ± 0.60
Fruit-1	Cd	0.00	N.D	-
		0.05	0.054	107.33 ± 5.66
		1.00	0.819	81.82 ± 1.06
	Pb	0.00	N.D	-
		0.10	0.099	99.00 ± 11.06
		10.00	9.199	91.99 ± 1.44
Fruit-2	Cd	0.00	N.D	-
		0.05	0.042	81.67 ± 1.41
		1.00	0.844	84.33 ± 2.68
	Pb	0.00	N.D	-
		0.10	0.084	84.00 ±0.00
		10.00	9.615	96.13 ± 0.36
Fruit-3	Cd	0.00	N.D	-
		0.05	0.046	91.33 ± 1.15
		1.00	1.044	104.37 ± 2.05
	Pb	0.00	N.D	-
		0.10	0.109	108.50 ±7.78
		10.00	9..020	90.20 ± 2.74

N.D was not detectable in EA-SS-LPME-HR-CS-FAAS (less than 0.0228 mg/L (Cd) and 0.0649 mg/L (Pb))

__Table 22__ Comparison of the proposed EA-SS-LPME-HR-CS-FAAS method with the previous works for the preconcentration of Pb and Cd in different samples

Method	**Analyte**	**Sample**	**Linearity (mg/L)**	**LOD (mg/L)**	**LOQ (mg/L)**	**%RSD**	**%Recovery**	**EF**	**PF**	**Ref.**
emulsion breaking-HR-CS FAAS	Pb	gasoline	-	0.014	0.048	5.0	-	-	2.0	[159]
- FAAS - RP-DLLME-SQT-FAAS	Cd	fish oil syrup	0.100-2.00 0.00377-0.1620	0.03 0.00075	0.1003 0.00248	4.3-6.7	-	-	-	[183]
SS-HR-CS-GFAAS	Pb	rice grains	-	2.3 µg/kg	-	15	-	-	-	[184]
Spectrophotometric associated with silver nanoparticle	Pb	honey	0.0500-0.167	0.0135	0.0451	1.2-4.2	-	-	-	[185]
SPs-LPME- µS-FAAS	Cd	water, vegetable, fruit and cigarette	0.00053-0.157	0.00016	0.00053	5.4	99-105	28.1	-	[18]

Table 22 *(Cont)*…

Method	Analyte	Sample	Linearity (mg/L)	LOD (mg/L)	LOQ (mg/L)	%RSD	%Recovery	EF	PF	Ref.
VA-SHS-LPME-GFAAS	Pb	water, tea and human hair	0.000040-0.00200	0.0000160	0.000045	4.2	94.9-110	49	-	[171]
	Cd		0.000010-0.000025	0.0000039	0.000010	6.2	98.5-106	52	-	
EA-SS-LPME-HR-CS-FAAS	Pb	water and canned food	0.06-10.00	0.0195	0.0649	1.25-1.69	82-30-119.0	1.4	3.3	This work
	Cd		0.02-1.50	0.0068	0.0228	1.07-1.64	81.67-120.0	2.6		

Remarks;

RP-DLLME-SQT-FAAS	reverse phase dispersive liquid–liquid microextraction coupled to slotted quartz tube flame atomic absorption spectrometry
SS-HR-CS-GFAAS	solid sampling high-resolution continuum source atomic absorption spectrometry
SPs-LPME-µS-FAAS	switchable-polarity solvent based liquid-liquid microextraction with micro syringe-flame atomic absorption spectrometry
VA-SHS-LPME	vortex assisted switchable hydrophilicity solvent based liquid phase microextraction

5.2.4 Robustness

The effect of potentially coexisting ions in the selected samples on the preconcentration of Pb and Cd was examined under the optimum experimental conditions. In this experiment, 0.1 mg/L of Pb and Cd standard solution was spiked with different concentrations of interfering ions and preconcentrated by EA-SS-LPME method. %Recovery of target analyte was focused on the range 80-120%. Ratio of interfering ions were studied at 1:1, 1:50, 1:100 1:500 and 1:1000 mass ratio of 0.1 mg/L of Pb and Cd. Tolerance limits of interfering ions were defined as the largest number of matrixes in the presence of which microextraction recoveries of Pb and Cd could be maintained in the acceptable range. As shown in Table 23, it was found that the maximum robustness of proposed method was represented for the analysis of Pb and Cd with phosphate (PO_4^{3-}) and chloride (Cl^-) at 500. Next, existing of nitrate (NO_3^-) had less effect for both analytes as seen the tolerance limit at 100. Nonetheless, tolerance ratio at 50 equally was affected to carbonate (CO_3^{2-}), Fe^{3+}, Cu^{2+}, Co^{2+}, and Sn^{2+}. For K^+, Na^+, Mg^{2+}, and Hg^{2+}, it was found that the robustness of these coexisting ions had significant effect to Pb analysis more than Cd except for Ca^{2+}. Form these results, it was indicated that the proposed EA-SS-LPME was interfered by Mg^{2+} because Mg^{2+} could be coprecipitated with Pb and Cd in the extraction system. Therefore, the precipitates can be settled down to the bottom of centrifuge tube, which affected to the abundance of Pb-DTZ and Cd-DTZ complexes in TEA upper phase. Therefore, the tolerance ratio and recovery were decreased as demonstrated in Table 23.

Table 23 *Effect of interfering ions by the proposed method*

Interfering ions	**Cd**		**Pb**	
	Tolerance ratio	**%Recovery ± SD**	**Tolerance ratio**	**%Recovery ± SD**
K^+	100	110.00 ± 0.00	50	80.00 ± 0.00
Na^+	100	111.67 ± 1.53	50	102.33 ± 3.51
Mg^{2+}	50	116.67 ± 1.15	1	83.67 ± 2.31
Ca^{2+}	50	103.33 ± 1.53	100	97.00 ± 2.80
Fe^{3+}	50	98.33 ± 4.73	50	88.50 ± 2.10
Cu^{2+}	50	93.70 ± 4.50	50	91.67 ± 2.89
Hg^{2+}	100	117.00 ± 1.41	50	87.00 ± 1.70
Co^{2+}	50	92.67 ± 3.06	50	88.50 ± 2.10
Sn^{2+}	50	110.00± 0.00	50	90.00± 0.00
NO_3^-	100	118.00 ± 0.00	100	90.30 ± 2.90
CO_3^{2-}	50	83.50 ± 0.00	50	105.30 ± 5.10
PO_4^{3-}	500	99.00 ± 0.00	500	83.00 ± 1.40
Cl^-	500	97.00 ± 1.00	500	92.70 ± 4.00

5.3 Conclusions

The proposed EA-SS-LPME method was successfully developed for the sequential determination of Pb and Cd in drinking water samples and canned food samples by HR-CS-FAAS. The related parameters were optimized by univariate optimization method. Under the optimum condition, the analytical performances of the proposed method were investigated and concluded in Table 20. In addition, effect of interfering ions was examined as shown in Table 23. The concentrations of Pb and Cd in drinking water, canned tuna, and canned fruit samples were not detectable.

CHAPTER VI

AIR-ASSISTED DEEP EUTECTIC SOLVENT BASED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION

The fourth research work was related to air-assisted deep eutectic solvent dispersive liquid-liquid microextraction (AA-DES-DLLME). Liquid phase microextraction method using dispersive solvent and the modification of a simple air-assisted technique for preconcentration enhancement was carried out. The extraction efficiency was enhanced be applying air bubbles which provided small droplets the extraction system. Furthermore, influencing parameters on the extraction including effect of pH, concentration of ligand, ratio of DES composition, volume of DES, volume of dispersive solvent, air-assisted extraction time, centrifugation time and speed, and volume of sample were investigated. Analytical characteristics such the accuracy, precision, limit of detection, limit of quantitative, and dynamic range were also evaluated. Eventually, the proposed AA-DES-DLLME was applied for the preconcentration of Pb and Cd in drinking water and canned vegetable samples while the absorbances of theses analytes were sequentially measured by HR-CS-FAAS as described in the following details.

6.1 Experiment

6.1.1 Instrumentation

A high resolution continuum source flame atomic absorption spectrometry (HR-CS-FAAS, ContrAA 800, Analytik-jena, Germany) was used for the determination of Pb and Cd. Absorbance measurements of Pb and Cd were performed using a compromised stoichiometric flame composed of air/acetylene mixture as given in Table 19. pH of solutions was measured using a pH meter (HI98107, Hanna instrument, USA). Clinical centrifugation (DM0412, DLAB, Thailand) was used for separation. Stirrer (PMC Beware, IOWA 52001, U.S.A) with magnetic bar were applied to stir the synthesized DES. A bubble generator (Big boy air pump, BB-8000, Thailand) was purchased from a local store in Phitsanulok, Thailand as the air bubble source. Microwave digester (SK-15eT, Milestone, Italy) was used for sample pretreatment.

6.1.2 Reagents

All reagents used in this work were of analytical grade. These were: nitric acid, 65% (RCI Labscan, Thailand), 99% dithizone, (Ajax Finechem, Australia), sodium hydroxide, 97% (RCI Labscan, Thailand), ethanol (RCI Labscan, Thailand), tetrahydrofuran, 99.8% (RCI Labscan, Thailand), hydrogen peroxide, 30% (VWR International, USA), choline chloride, 99.8% (Sigma life Science, China), and phenol crystalline, 99% (PanReac AppliChem, Germany). 1000 mg/L Pb (II) standard solution (PanReac AppliChem, Germany) and 1000 mg/L Cd (II) standard solution (Qrecqchemical, New Zealand) were diluted to a 100 mg/L stock solution with deionized water. Working solutions were prepared with deionized water. Phosphate buffer was used to adjust the pH to a value of 13.

6.1.3 Synthesis of deep eutectic solvent

Preparing of 20.0 g of choline chloride and 27.0 g of phenol were mixed in a beaker and stirred with magnetic stirrer at room temperature until homogeneous phase of DES was appeared as shown in Fig. 62(1).

6.1.4 AA-DES-DLLME procedure

The developed AA-DES-DLLME was applied for sequential multi-elements analysis of Pb and Cd (Fig. 62(2)). Sample or mixed standard solution (Pb and Cd mixed solution) for 20 mL was adjusted to pH 13 with phosphate buffer solution.

Then, 0.5 mL of DTZ was added into the solution for final concentration at 0.3 mM and consecutively added with 1.5 mL of DES as extracting solvent and 1.5 mL of THF as disperser. In this step, Pb-DTZ and Cd-DTZ complexes were formed and thoroughly dispersed in DES phase via the addition of THF. Two phases (aqueous and DES phases) were good separated after addition of THF. After that, air-assisted technique was introduced into extraction tube to increase the dispersion and extraction efficiency using a simple air generator, home fish-tank pump. The generated tiny bubbles were applied for 30 sec. The Pb-DTZ and Cd-DTZ complexes were distributed better to DES phase and led to enhance the greater preconcentration. DES phase that enriched of Pb-DTZ and Cd-DTZ complexes was centrifuged at 1000 rpm for 10 min before the separated phase was diluted to 3.0 mL with 1% HNO_3 in ethanol. HR-CS-FAAS was used for measuring absorbance of Pb and Cd sequentially.

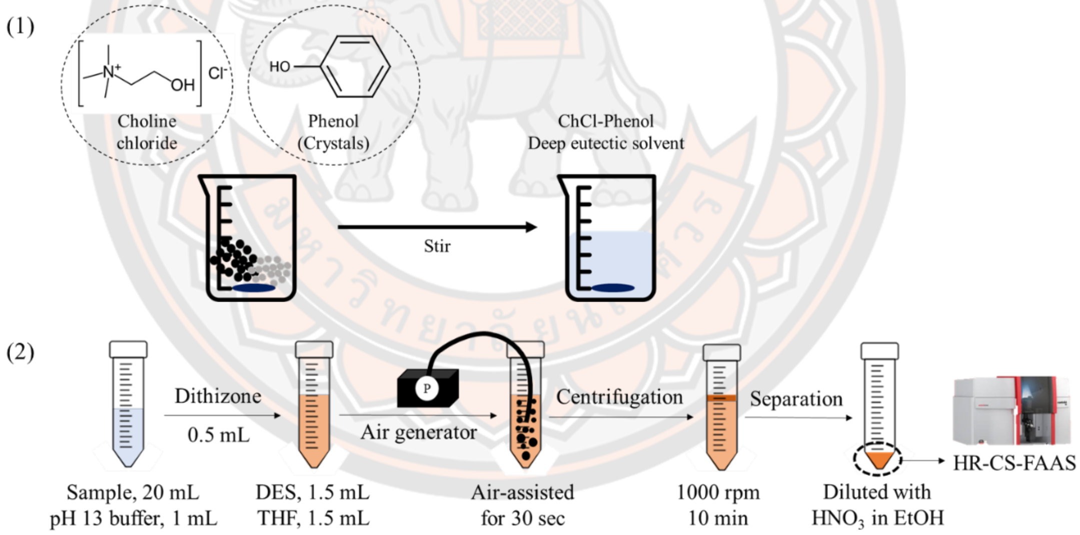


Figure 62 *Schematic diagram for AA-DES-DLLME procedure; (1) Synthesis of DES and (2) AA-DES-DLLME-HR-CS-FAAS*

6.1.5 Optimization

In AA-DES-DLLME, the extraction parameters *e.g.*, effect of pH, concentration of DTZ, Ratio of DES composition, volume of DES, volume of disperser solvent, air-assisted time, speed and time of centrifugation, and an effect of sample volume were studied by univariate optimization.

Effect of pH 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was adjusted to pH 6 to 14 using different types of buffer solution. 0.5 mL of 0.5 mM (final concentration) DTZ (complexing agent), 1.0 mL of DES (extracting solvent), and 1.0 mL of THF (dispersive solvent) were added. Big boy pump at maximum power (50 Hz) was applied as air bubble generator for 30 sec. After extraction, centrifugation at 3000 rpm was applied for 8 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Concentration of dithizone 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was adjusted to pH 13 using phosphate buffer solution. 0.5 mL of dithizone was varied from 0.1 to 4.0 mM (final concentration). Then, 1.0 mL of DES (extracting solvent) and 1.0 mL of THF (dispersive solvent) were added. Air-assisted extraction was introduced using big boy pump at maximum power (50 Hz) for 30 sec. After extraction, centrifugation at 3000 rpm was applied for 8 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Ratio of DES composition 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was added with pH 13 buffer solution and 0.5 mL of 0.3 mM (final concentration) DTZ. 1.0 mL of DES (extracting solvent) was optimized in the different compositions from 1:1 to 1:5 mole ratio (Choline chloride : Phenol). Then 1.0 mL of THF (dispersive solvent) was applied. Air-assisted extraction was introduced using big boy pump at maximum power (50 Hz) for 30 sec. After extraction, centrifugation at 3000 rpm was applied for 8 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Volume of DES 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was added with pH 13 buffer solution and 0.5 mL of 0.3 mM (final concentration) DTZ. The volume of DES (1:2 of Choline chloride : Phenol) was optimized in the range of 0.5 to 4.0 mL. Then 1.0 mL of THF (dispersive solvent) was applied. Air-assisted extraction was introduced using big boy pump at maximum power (50 Hz) for 30 sec. After extraction, centrifugation at 3000 rpm was applied for 8 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Volume of disperser 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was added with pH 13 buffer solution , 0.5 mL of 0.3 mM (final concentration) DTZ, and 1.5 mL of DES (extracting solvent). The volume of THF (disperser) was optimized from 0.5 to 3.0 mL. Air-assisted extraction was introduced using big boy pump at maximum power (50 Hz) for 30 sec. After extraction, centrifugation at 3000 rpm was applied for 8 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Air-assisted time 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was added with pH 13 buffer solution , 0.5 mL of 0.3 mM (final concentration) DTZ, 1.5 mL of DES (extracting solvent), and 1.5 mL of THF (disperser). Air-assisted extraction times were optimized using big boy pump at maximum power (50 Hz) in the range of 10-120 sec. After extraction, centrifugation at 3000 rpm was applied for 8 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Time of centrifugation 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was added with pH 13 buffer solution , 0.5 mL of 0.3 mM (final concentration) DTZ, 1.5 mL of DES (extracting solvent), and 1.0 mL of THF (disperser). Air-assisted extraction was introduced using big boy pump at maximum power (50 Hz) for 30 sec. After extraction, centrifugation at 3000 rpm was optimized for 2-20 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Speed of centrifugation 20 mL of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was added with pH 13 buffer solution , 0.5 mL of 0.3 mM (final concentration) DTZ, 1.5 mL of DES (extracting solvent), and 1.0 mL of THF (disperser). Air-assisted extraction was introduced using big boy pump at maximum power (50 Hz) for 30 sec. After extraction, centrifugation was optimized in the range of 500-4000 rpm for 10 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

Sample volume Volume of 1.0 mg/L (Pb) and 0.5 mg/L (Cd) mixed standard solution was optimized in the range of 10-40 mL. The standard solution was added with pH 13 buffer solution , 0.5 mL of 0.3 mM (final concentration) DTZ, 1.5 mL of DES (extracting solvent), and 1.0 mL of THF (disperser). Air-assisted extraction was introduced using big boy pump at maximum power (50 Hz) for 30 sec. After extraction, centrifugation at 1000 rpm was applied for 10 min. The solution was diluted to 3 mL using 1.0 % HNO_3 in ethanol and detected by HR-CS-FAAS.

6.1.6 Analytical performance

The analytical performances of AA-DES-DLLME-HR-CS-FAAS method were investigated under the optimum conditions. The parameter namely LOD, LOQ, enrichment factor (EF), preconcentration factor (PF), and percentage extraction efficiency (%EE) were investigated using the same equation as previously described in section 3.1.4. In addition, linearities were investigated in the range of 0.00-10.0 mg/L of Pb solution and 0.00-5.0 mg/L of Cd solution. %RSD was evaluated by 0.5, 1.5 and 2.0 mg/L (Pb) and 0.5, 1.0 and 1.5 mg/L (Cd). For the investigation of recoveries, 0.5 and 1.5 mg/L of Pb solution and 0.5 and 0.7 mg/L of Cd solution were spiked into the real samples.

6.1.7 Application for Pb and Cd analysis

The developed AA-DES-DLLME-HR-CS-FAAS was applied for sequential multi-element determination of Pb and Cd in drinking water and canned food samples.

Water sample preparation Drinking (or bottled water) water samples were purchased from local stores, Naresuan university, Phitsanulok, Thailand. Drinking water samples were extracted following AA-DES-DLLME procedure.

Canned food sample preparation Canned vegetables samples were purchased from local stores. All samples were weighed for 0.50 g and added with 9.0 mL of 65% HNO_3 and 1.0 mL of H_2O_2. Samples were digested by microwave digestion at 210 °C for 35 min. Next, Digested samples were diluted to 50 mL with deionized water before use.

6.1.8 Robustness

0.10 mg/L of Pb and Cd solution was spiked with interfering ions, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Hg^{2+}, Co^{2+}, Sn^{2+}, PO_4^{3-}, and NO_3^- at the ratios of 1:1, 1:50, 1:100, 1:500 and 1:1000 (Analyte: interfering ion). Then, AA-DES-DLLME extraction procedure under the optimum condition was carried out. %Recoveries were calculated to evaluate the interfering effect.

6.2 Results and discussion

6.2.1 Optimization

Effect of pH Effect of pH was investigated from 6-14 using the different types of buffers. Under this study, Pb-DTZ and Cd-DTZ complexes were occurred and preconcentrated by AA-DES-DLLME-HR-CS-FAAS. pH of solution affected to the stability of complex formation. The results (Fig. 63) showed that %relative absorbance from Pb and Cd analysis reached highest relative absorbance at pH 13 (Pb) and pH 14 (Cd). The alkaline aqueous solution was obtained with high absorbance for sequential multi-element analysis. The reaction between DTZ and metal ions in alkaline solution was agreed with the report by Fouad J. Ismael [186]. DTZ ligand containing S and N active group can interacted with metal ion in alkaline condition as seen in Fig. 64. Therefore, the compromised value at pH 13 was selected in the next experiment using phosphate buffer for sequential Pb and Cd determination.

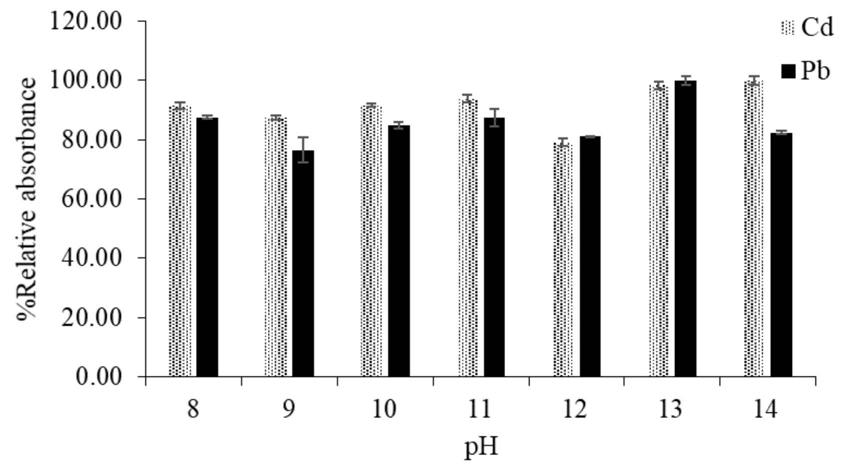
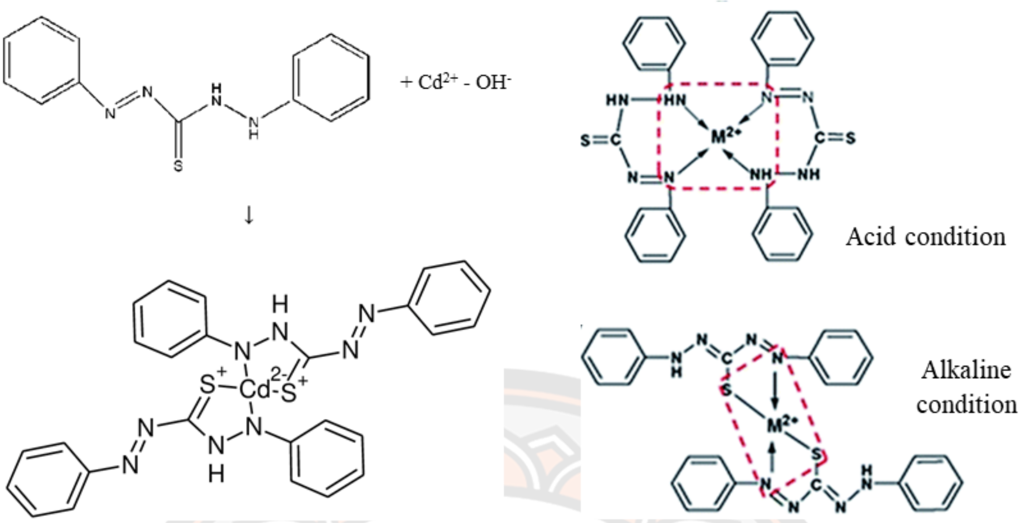


Figure 63 *Effect of pH for the sequential determination of Pb and Cd using AA-DES-DLLME*



(1) (2)

Figure 64 *The proposed reaction of DTZ with metal ion; (1) DTZ with Cd in alkaline condition by Ismael and (2) DTZ with cation (M^{2+}) in acid and alkaline condition by Fu and coworker*

Source: [186] and [187]

Effect of concentration of complexing agent 1,5-diphenylthiocarbazone or dithizone (DTZ) was used as complexing agent in the proposed method. An effect of DTZ concentration was important for complex formation of Pb-DTZ and Cd-DTZ forms as reported by Fu and coworker [187]. Concentration of DTZ were varied from 0.1-4.0 mM (final concentration). As detailed in the Fig. 65, %relative absorbances increased from 0.1 mM to 0.3 mM and were relatively constant from 0.3-1.5 mM. After 1.5 mM, the signal of Pb and Cd decreased. At low concentration of DTZ, this was the inadequate concentration for complex formation and caused low absorbances of Pb and Cd. At high concentration of DTZ, this made low extraction efficiency. The excess amount of DTZ may cause the barrier of phase dispersion and separation in AA-DES-DLLME procedure as clearly seen at the concentration of DTZ greater than 1.5 mM. Therefore, 0.3 mM of final concentration of DTZ was applied for the next experiments.

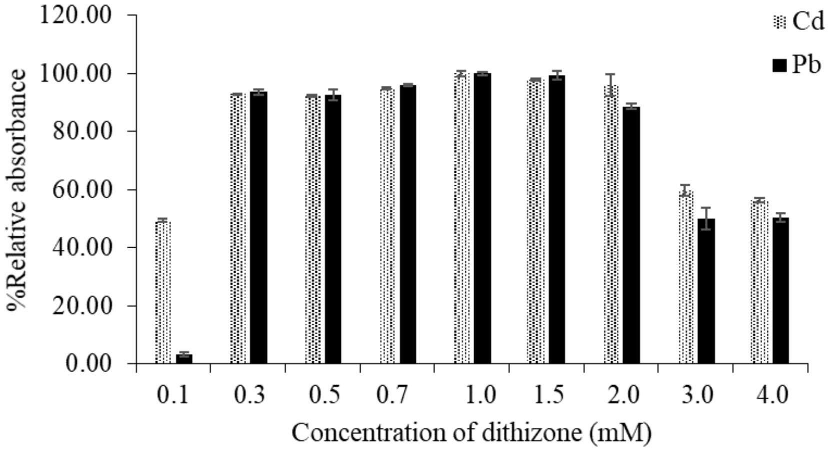


Figure 65 *Effect of dithizone concentration for the sequential determination of Pb and Cd using AA-DES-DLLME*

Effect of DES ratio In order to find the most suitable mole ratio of the DES from choline chloride as organic salt (hydrogen bond donor, HBA) and phenol as hydrogen bond donor (HBD), DES was prepared by simple mixing the choline chloride and phenol crystal at different ratios with magnetic stirrer at room temperature. The colorless and clear liquid was obtained and applied to AA-DES-DLLME procedure for Pb and Cd analysis. Ratio of Choline chloride : Phenol at 1:1, 1:2, 1:3, 1:4, and 1:5 was evaluated. As shown in Fig. 66, it was observed that good composition was obtained with 1:2 and 1:3 mole ratios of DES. The ratio of organic salt : HBD had significant impact on its density. From the result, DES at 1:1 mole ratio had low density and lead to easily spread in aqueous phase and the phase separation became difficult. As a consequence, the amount of Pb and Cd complexes in DES phase was lower with the low relative absorbance. On the other hand, DES at 1:4 and 1:5 mole ratio had high densities. Despite the fact that the separated DES phase containing analytes was diluted with diluter, but the high density of this phase also affected into injector and nebulizer of HR-CS-FAAS. Therefore, %relative absorbances of Pb and Cd were decreased at these ratios. Consequently, suitable ratio of DES was selected as 1:2 (Choline chloride : Phenol) for time-saving and low-cost preparation and used in all experiments.

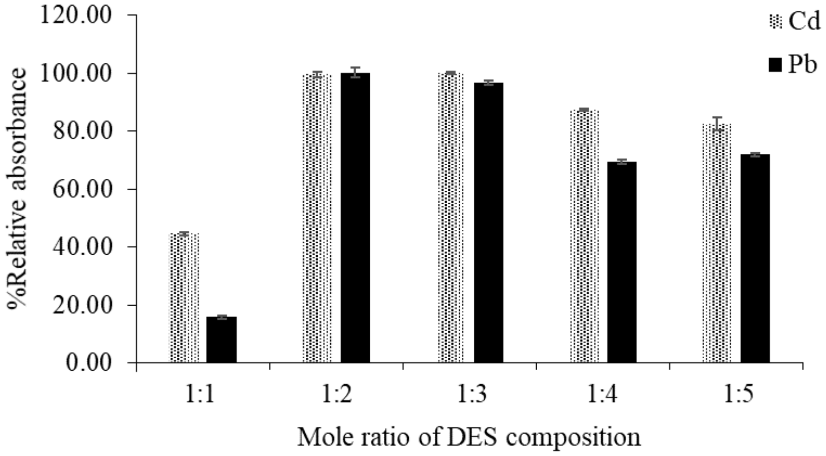


Figure 66 *The study of mole ratio of DES composition*

Volume of extracting solvent DES from choline chloride and phenol (1:2 mole ratio) was used as extracting solvent. The Pb-DTZ and Cd-DTZ complexes were interacted with DES via strong H-bonding and π-π interactions [128]. Volume of DES was used in the extraction system in the range 0.5-4.0 mL. The results (Fig. 67) demonstrated that the best separation was achieved by using 1.5 mL of DES. The higher volume of DES affected to dilute concentration of target analytes and eventually reduced the absorbance and preconcentration factor. Hence, 1.5 mL of synthesized DES was applied for further work.

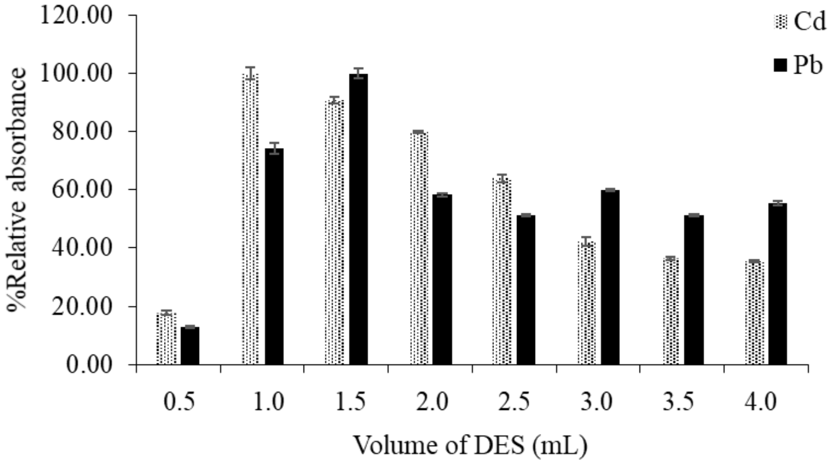


Figure 67 *Volume of DES as extracting solvent in AA-DES-DLLME*

Volume of disperser solvent In this experiment, tetrahydrofuran (THF) was used as dispersive solvent (Disperser). THF had an impact to separate DES containing target analytes from aqueous phase. The emulsion from the aggregation of DES and aqueous was formed in the extraction system. The addition of THF was introduced for emulsion break down. The volume of THF was estimated in the range of 0.5-3.0 mL (Fig. 68). The maximum relative absorbance of Pb and Cd were observed by the addition of 1.5 mL. Trend of absorbances were decreased at volume 1.5 mL of THF because the high volume of disperser might be affected to reduce the preconcentration factor and analytes signals. Therefore, 1.5 mL of THF was selected for all experiments.

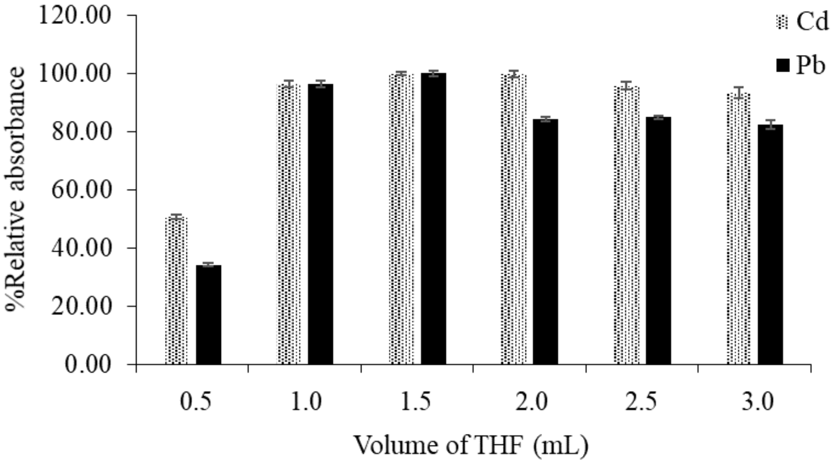


Figure 68 Influence of volume of THF as disperser solvent

Effect of air-assisted time Small bubbles were introduced into extraction tube via air bubble generator for air-assisted DES-DLLME. Time of air insertion was the most importance parameter for mass transfer process. The aggregation of DES in aqueous solution was achieved by the addition of THF and air bubbles. The dispersive efficacy was increased which led to increase the relative absorbance and reduced the extraction time. In addition, the extraction efficiency was improved via air-assisted technique. As demonstrated in Fig. 69, the highest efficiency was obtained at 30 min of air assisted extraction times. However, excess time of air bubbles insertion can cause the dissolution of the DES layer in the aqueous phase. Therefore, the longer air-assisted time caused the decreasing signal of Pb and Cd. As a consequence, the home fish tank pump as air bubble generator was selected for 30 sec for further studies.

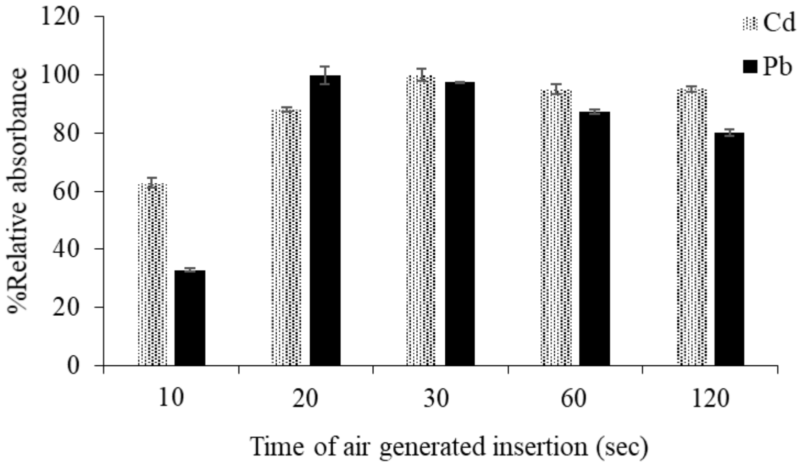


Figure 69 *Effect of air-assisted time*

Rate of centrifugation The extracting solution was applied to the centrifugation at the different speed varied from 500-4000 rpm. Centrifugation was used to separate the two phases. The results in Fig. 70 showed that a complete phase separation could be achieved from 500-1000 rpm. Complete phase separation and the maximum relative absorbance were reached at the centrifugal speed was 1000 rpm. Rate of centrifugation more than 1000 rpm provided slightly decreasing in relative absorbance caused by strong centrifugal force. Therefore, 1000 rpm of centrifugation speed was selected as an optimized value.

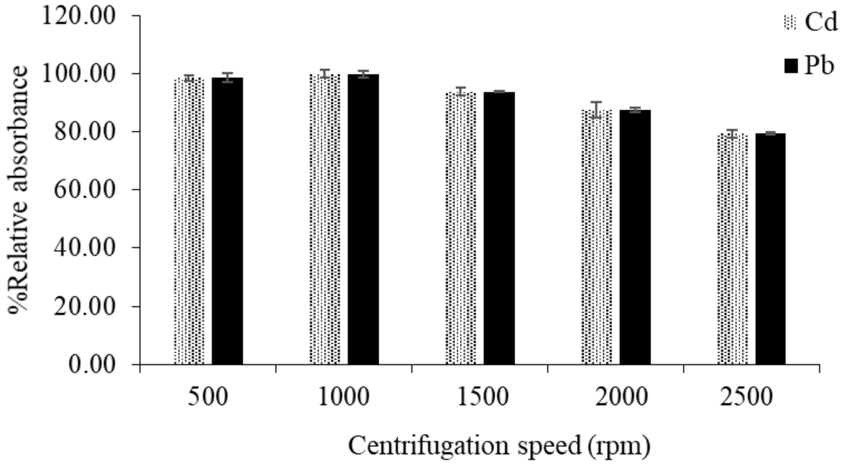


Figure 70 *Rate of centrifuge speed for phase separation in AA-DES-DLLME*

Time of centrifugation The time of centrifugation was subsequently estimated. The result (Fig. 71) showed that a complete phase separation could not be occurred at the time more than 10 min. The maximum absorbance was obtained the point at 10 min and decreased after 10 min. At 10 min of centrifugal time was sufficient to spend less time on Pb and Cd extraction using AA-DES-DLLME. Consequently, centrifugal time at 10 min was chosen for further experiment.

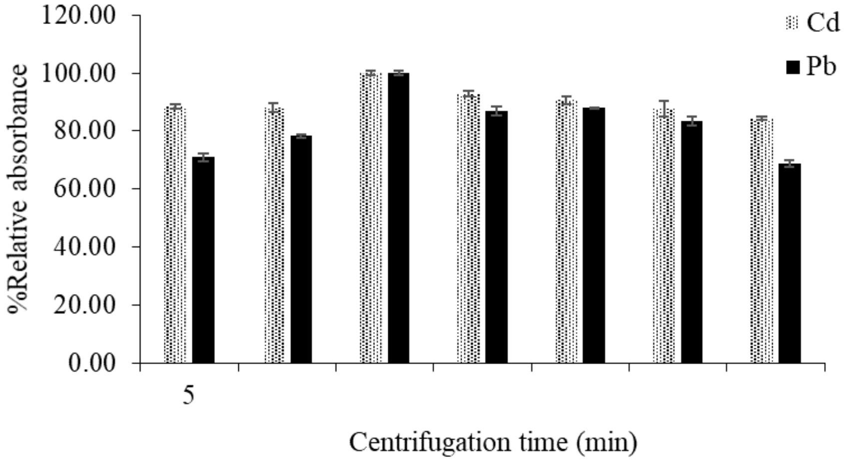


Figure 71 *Effect of centrifugation time*

Volume of sample The effect of volume of sample play an importance role of preconcentration factor, enrichment factor, and extraction efficiency. In this experiment, AA-DES-DLLME was carried out at different sample volume in the range 10-30 mL. It was observed from Fig. 72 that the maximum relative absorbance can be obtained by using of sample volume at 20 mL. The lower and higher volume were not suitable observing from low absorbance and extraction efficiency. Hence, AA-DES-DLLME was achieved by using of sample volume at 20 mL with the preconcentration factor for 6.67.

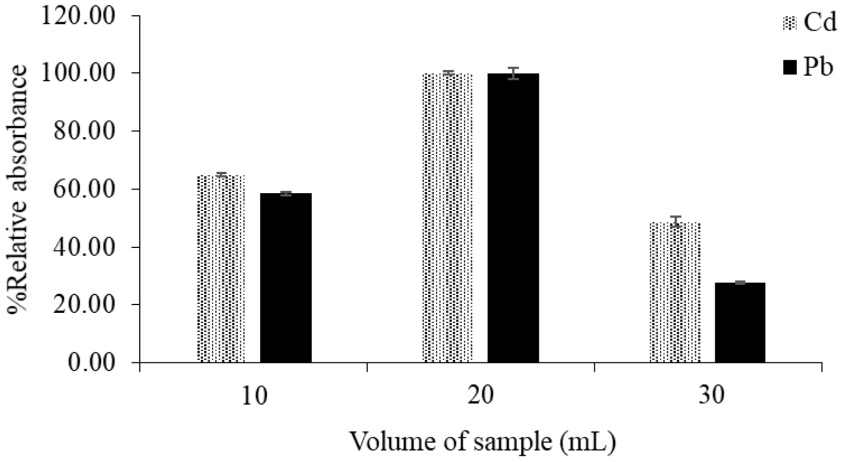


Figure 72 *Effect of sample volume for AA-DES-DLLME*

6.2.2 Analytical performance

Under optimized condition of AA-DES-DLLME-HR-CS-FAAS, figures of merit of the method including linearity, limit of detection (LOD), limit of quantitative (LOQ), relative standard deviation (%RSD), extraction recovery (%Recovery), enrichment factor (EF), and preconcentration factor (PF) were investigated. The equations of the calibration curves were established by linear regression of absorbance versus the concentration of analyte. The results (Fig. 73) of Pb-AA-DES-DLLME-HR-CS-FAAS were linear in the range 0.017-4.50 mg/L ($Y = 0.0676X + 0.0231$, R^2 0.9904). The calibration curve without extraction as Pb-HR-CS-FAAS were linear in the range 0.039-9.00 mg/L ($Y = 0.026X – 0.006$, R^2 0.9915). It was found that, EF calculated from slope ratio was 2.60. LOD and LOQ were calculated by eq. 8 and eq. 9, respectively. LOD and LOQ of the proposed procedure were 0.0050 mg/L and 0.0168 mg/L, respectively. Precision defined as the %RSD evaluated by replicate measurements of three concentrations were in the range 1.49-2.43%. The results (Fig. 74) of Cd-AA-DES-DLLME-HR-CS-FAAS were linear in the range 0.009-2.00 mg/L (Y = 0.5565X + 0.1107, R^2 0.9902) and that of Cd-HR-CS-FAAS were in the range 0.006-2.50 mg/L (Y = 0.1804X + 0.017, R^2 0.9903). EF of proposed method was 3.08. The LOD and LOQ were calculated by eq. 8 and eq. 9 using three sigma and ten sigma criteria were 0.0028 and 0.0094 mg/L, respectively. %RSD was in the range 1.39-4.19%. The results indicated that Cd and Pb can be efficiency extracted with preconcentration factors of 6.67 which corresponding %recovery, of Cd at 84.7-118.0% in drinking water samples and 84.7-102.3% in canned vegetable samples, and that of Pb at 84.2-111.3% in drinking water samples and 91.4-114.6% in canned vegetable samples. Figures of merit were summarized in Table 24.

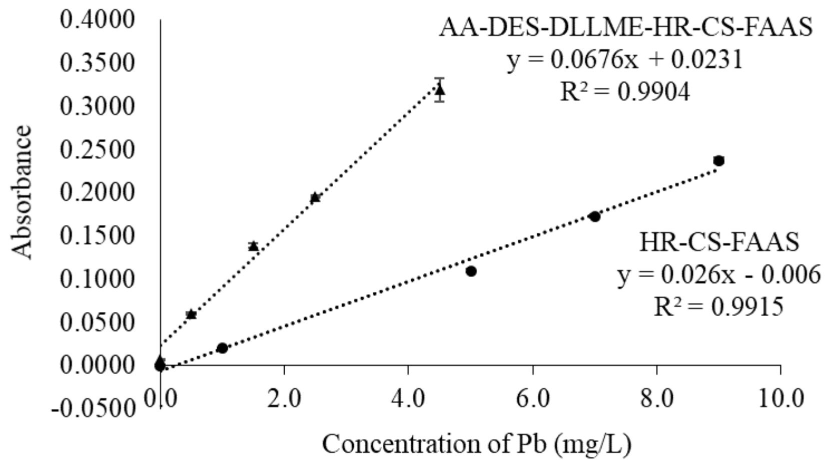


Figure 73 *Calibration curve of Pb from proposed method compared to conventional method*

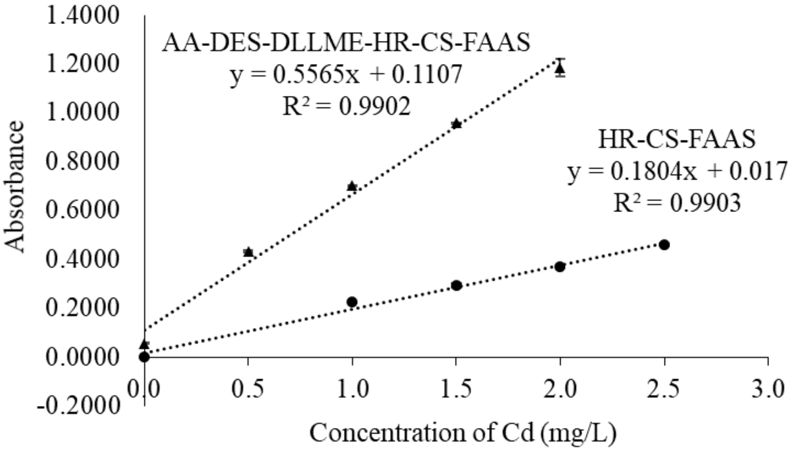


Figure 74 *Calibration curve of Cd from proposed method compared with the conventional method*

Table 24 *Figures of merit from AA-DES-DLLME method*

Figures of merit	**Value**
Linearity (ng/mL)	(Pb) 16.79-4500
	(Cd) 9.43-2000
LOD (ng/mL)	(Pb) 5.07
	(Cd) 2.83
LOQ (ng/mL)	(Pb) 16.79
	(Cd) 9.43
%RSD	(Pb) 1.49-2.43
	(Cd) 1.39-4.19
%Recovery	(Pb) 84.20-114.6%
	(Cd) 84.70-118.0%
EF	(Pb) 2.60
	(Cd) 3.08
PF	6.67

6.2.3 Application of AA-DES-DLLME for Pb and Cd analysis in sample

In order to apply the applicability of the developed AA-DES-DLLME, the method was applied to determine Pb and Cd determination in different water and food samples. Drinking water were directly extracted via AA-DES-DLLME but canned vegetable samples were digested using microwave digestion before extraction. These samples were spiked with different concentration of standard Pb and Cd solution. The extraction recoveries were acceptable in the range 80-120% as seen in Table 25. Concentrations of Pb and Cd in real samples were not found with acceptable recovery.

The comparison between the proposed AA-DES-DLLME method with other existing microextraction methods for the determination of Pb and Cd was presented in Table 26. It was observed that, the developed method as compared with previously reported procedures has some advantages such as its simplicity to use, better EF [159] good repeatability [159, 184], comparable LOD to ICP-AES [188], and better than HR-CS-FAAS [159].

Table 25 *Results for the determination of Pb and Cd in drinking water (DW1- DW3) and canned vegetable (CV1- CV2) samples by AA-DES-DLLME-HR-CS-FAAS*

Sample	**Concentration of Cd**			**Concentration of Pb**		
	Added (mg/L)	**Found (mg/L)**	**%Recovery ± SD (n=3)**	**Added (mg/L)**	**Found (mg/L)**	**%Recovery ± SD (n=3)**
DW-1	0.00	N.D	-	0.00	N.D	-
	0.50	0.55	110.8 ± 0.87	0.50	0.68	107.8 ± 2.53
	0.70	0.60	84.7 ± 0.41	0.70	1.48	103.4 ± 1.06
DW-2	0.00	N.D	-	0.00	N.D	-
	0.50	0.56	112.0 ± 2.07	0.50	0.59	84.2 ± 2.44
	0.70	0.70	100.0 ± 0.47	0.70	1.51	100.6 ± 2.29
DW-3	0.00	N.D	-	0.00	N.D	-
	0.50	0.59	118.0 ± 1.15	0.50	0.71	101.4 ± 0.0
	0.70	0.80	114.2 ± 0.82	0.70	1.67	111.3 ± 1.92
CV-1	0.00	N.D	-	0.00	N.D	-
	0.25	0.23	92.4 ± 4.10	0.25	0.46	91.4 ± 7.9
	0.70	0.70	84.7 ± 1.60	0.70	0.73	104.5 ± 9.4
CV-2	0.00	N.D	-	0.00	N.D	-
	0.25	0.23	102.3 ± 3.2	0.25	0.51	102.5 ± 2.1
	0.70	0.70	94.9 ± 0.9	0.70	0.80	114.6 ± 1.9

*N.D was not detectable in AA-DES-DLLME-HR-CS-FAAS (less than 0.0094 mg/L (Cd) and 0.0168 mg/L (Pb))

Table 26 *Comparison of the proposed method with previous works for the determination of Pb and Cd*

Method	Analyte	Sample	Linearity (mg/L)	LOD (mg/L)	LOQ (mg/L)	%RSD	%Recovery	EF	PF	Ref.
MIL-DLLME-ETAAS	Pb	bee product	0.00001-0.00300	3.0 ng/L	-	3.6-3.9	94.8-101.0	107	-	[189]
emulsion breaking-HR-CS FAAS	Pb	gasoline	-	0.014	0.048	5.0	-	-	2.0	[159]
- FAAS - RP-DLLME-SQT-FAAS	Cd	fish oil syrup	0.100-2.00 0.00377-0.1620	0.03 0.00075	0.1003 0.00248	4.3-6.7	-	-	-	[183]
SS-HR-CS-GFAAS	Pb	rice grains	-	2.3 μg/kg	-	15	-	-	-	[184]
Microcolumns preconcentration-ICP-AES	Cd	water	0.005-0.100	0.0011-0.0043	-	1.1-2.5	-	-	86	[188]
AA-DES-DLLME-HR-CS-FAAS	Pb Cd	drinking water and canned vegetable	0.017-4.50 0.009-2.00	0.0050 0.0028	0.0168 0.0094	1.49-2.43 1.39-4.19	84.2-114.6 84.7-118.0	2.60 3.08	6.67	This report

Remarks;

MIL-DLLME-ETAAS	magnetic ionic liquid dispersive liquid-liquid microextraction electrothermal atomic absorption spectrometry
RP-DLLME-SQT-FAAS	reverse phase dispersive liquid–liquid microextraction coupled to slotted quartz tube flame atomic absorption spectrometry
SS-HR-CS-GFAAS	solid sampling high-resolution continuum source atomic absorption spectrometry
ICP-AES	Inductively couple plasma atomic emission spectrometry

6.2.4 Robustness

In order to evaluate the robustness of proposed method, the effect of added interfering ions in target ions were brought to represent in the term of the tolerance limit or tolerance ratio. 0.10 mg/L of mixed Pb and Cd solution was spiked with Na^+, Ca^{2+}, Mg^{2+}, Hg^{2+}, Sn^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, phosphate (PO_4^{3-}), and nitrate (NO_3^-) at 1:1, 1:50, 1:100, 1:500 and 1:1000 (Analyte : other ions). The tolerance limit was presented as acceptable recovery under 80-120%. The result in Table 27 showed that the extraction of Pb and Cd via AA-DES-DLLME-HR-CS-FAAS was high robustness from anions such p PO_4^{3-} and NO_3^- for 100-1000 times. In addition, Na^+, Mg^{2+}, Hg^{2+}, Sn^{2+}, and Fe^{3+} did not had high effect for Cd analysis (Tolerance limit in 100-500) while Ca^{2+} ion had moderate effect (Tolerance limit at 50). Low robustness was observed for interfering of Cu^{2+} and Co^{2+} for Cd analysis. According to these results for Pb, there was significant suffering for Na^+ and Mg^{2+} (Tolerance limit at 100) with moderate interfered from Ca^{2+}, Hg^{2+}, and Sn^{2+} (Tolerance limit at 50). Fe^{3+}, Cu^{2+}, and Co^{2+} ions interfered for Pb determination in the studied method (Tolerance limit at 1). In conclusions, the preconcentration of Cd and Pb via AA-DES-DLLME-HR-CS-FAAS can tolerate at low level of Fe^{3+}, Cu^{2+}, and Co^{2+} ions. These may be owing to the competition of complex formation between Cd-DTZ and Pb-DTZ with Fe-DTZ, Cu-DTZ, and Co-DTZ causing the absorbances decreased in sequential analysis of Pb and Cd.

Table 27 *Effect of interfering ions for sequential Cd and Pb determination using AA-DES-DLLME-HR-CS-FAAS (Using 0.10 mg/L mixed standard of Cd and Pb)*

Interfering ions	**Cd**		**Pb**	
	Tolerance limit	**%Recovery ± SD**	**Tolerance limit**	**%Recovery ± SD**
Na^{+}	500	88.1±1.0	100	116±2.5
Ca^{2+}	50	81.2±0.0	50	105.7±3.0
Mg^{2+}	100	88.1±1.0	100	88.1±1.0
Hg^{2+}	500	117.3±1.5	50	86.5±1.6
Sn^{2+}	500	83.8±2.2	50	100.9±2.7
Fe^{3+}	100	83.7±1.8	1	84.1±3.3
Cu^{2+}	1	103.8±1.1	1	100±2.2
Co^{2+}	1	110.1±1.1	1	87.8±3.0
PO_4^{3-}	1000	85.4±2.7	1000	102.7±3.3
NO_3^{-}	100	105.7±4.1	500	116.5±2.0

6.3 Conclusions

The proposed AA-DES-DLLME method was developed for the fast sequential determination of Pb and Cd in drinking water samples and canned vegetable samples by HR-CS-FAAS. The important effects were optimized by univariate optimization method. Under the optimum condition, the analytical performances of the proposed method were optimized and investigated in Table 24. In addition, effect of interfering ions was studied as demonstrated in Table 27. The concentrations of Pb and Cd in drinking water and canned vegetable samples were not detectable.

CHAPTER VII

ELECTROMEMBRANE-BASED HOLLOW FIBER LIQUID PHASE MICROEXTRACTION

This chapter involved the application of electromembrane method (EM) with hollow fiber liquid phase microextraction (HF-LPME) as EM-HFLPME for Pb determination. Deep eutectic solvent in buffer solution was used as extracting solvent for anion of Pb detection. Pb ions in sample were converted to anion form as oxyanion. Electro-assistant extraction was applied to enhance the anion passed thorough the supported liquid membrane (SLM) into the DES phase mixed with buffer solution. The new proposed method was defined as a semi-two and three phase HFLPME. The optimum Pb extraction conditions were elucidated using multivariate optimization methods. Effect of pH in donor and acceptor phase, the modification of SLM, effect of applied voltage, extraction time, and volume of sample were investigated. Analytical figures of merit were obtained using the optimized extraction conditions. In addition, the proposed method was applied to the determination of Pb in drinking and tap water samples.

7.1 Experiment

7.1.1 Instrumentation and materials

Zeeman background correction equipped with graphite furnace atomic absorption spectrometry (Varian, Model SpectrAA 220Z, Germany) was employed with a Pb hollow-cathode lamp (Agilent technologies, part number 5610102900, Germany) as the light source and operating at 10 mA, using a wavelength of 283.3 nm and a slit width of 0.5 nm as default settings. Pyrolytic-coated graphite tubes (Agilent technologies, part number 6310001200, Germany) were used for the analysis. The operating instrumental temperature program was given in Table 28. pH of solutions was measured using a pH meter (HI98107, Hanna instrument, USA) and pH-Indicator strips (Merck, Germany). Stirrer (PMC Beware, IOWA 52001, U.S.A) with magnetic bar were applied to stir the synthesized DES. A DC power supply (NPS605W, Wanptex, China) was purchased from an official online store with a mechanical pencil lead for inner diameter at 0.5 mm with length of 60 mm (2B, Quantum, Korea) was selected as graphite electrodes. Q3/2 Accurel PP was used as hollow fiber (Membrana, Wuppertal, Germany). 0.60 x 25.00 mm needle (Neolus, Terumo, Thailand) and 1 mL syringe (Tuberculin, Nipro, Thailand) were used as the setting equipments.

Table 28 *Temperature program of GFAAS for Pb determination in EM-DES-HFLPME-GFAAS method*

Step	**Temperature (ºC)**	**Time (s)**	**Ar-flow rate (L/min)**	**Read**
Drying	100	40.0	3.0	No
Pyrolysis	450	5.0	3.0	No
Atomization	2100	2.0	0.0	Yes
Cleaning	2100	2.0	3.0	No

7.1.2 Reagents

All reagents used in this work were of analytical grade. These were: Sodium hydroxide, 97% (RCI Labscan, Thailand), choline chloride, 99.8% (Sigma life Science, China), phenol crystalline, 99% (PanReac AppliChem, Germany), N-Cetyl-

N,N,N-trimethyl ammonium bromide (CTAB), 99% (Loba Chemie, India), and 1-octanol, 98% (Acros Organics, India). 1000 mg/L Pb (II) standard solution (PanReac AppliChem, Germany) was diluted to a 1000 µg/L stock standard solution with deionized water. Working solutions were prepared with deionized water. Ammonia/ammonium buffer was used to adjust the pH to a value of 8 and 9.

7.1.3 Preparation of supported liquid membrane on wall-hollow fiber

A 20 cm of polypropylene hollow fiber was dipped into a 250 mL beaker containing 1-octanol for 1 min as shown in Fig. 75(1). The dipped hollow fiber was used as supported liquid membrane. Then, acceptor phase (DES: pH 8buffer solution at 25:75 by %volume) was introduced into the hollow fiber to wash remaining 1-octanol. End of hollow fiber was plugged with stainless steel needle and acceptor was filled with acceptor solution after that.

7.1.4 EM-DES-HFLPME procedure

A novel HFLPME method was presented by using electro-assisted extraction and green solvent. In EM-DES-HFLPME procedure, 30 mL of sample/standard solution in 50 mL beaker were set with hollow fiber and electric system (Fig. 75(2)). One of graphite electrode using 2B pencil leads was inserted into acceptor phase in hollow fiber as anode (+) and another piece was dipped into donor phase containing in beaker as cathode (-). Sample/solution was added with pH 9 buffer and adjusted to desired pH by with NaOH as the donor phase. Power supply was used to introduce the voltage at 30 volts for 25 min throughout the extraction. Then, the acceptor phase was drawn out using needle and syringe. The obtained solution was measured by GFAAS.

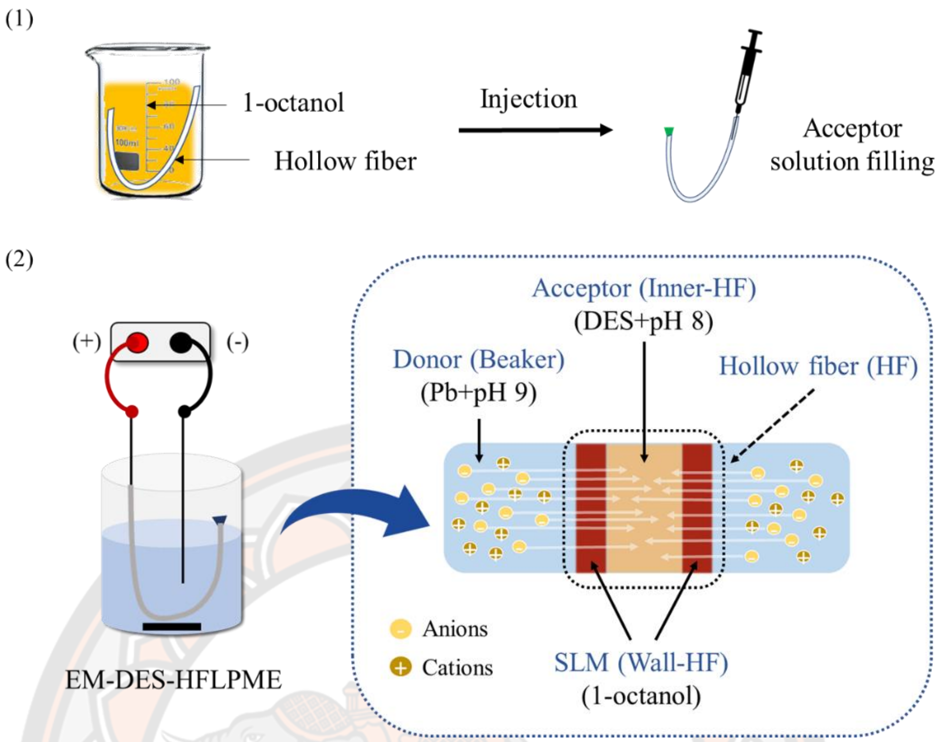


Figure 75 *Experimental procedure of EM-DES-HFLPME; (1) Preparation of SLM on wall-HF and (2) Step of EM-DES-HFLPME-GFAAS*

7.1.5 Optimization

In EM-DES-HFLPME, the extraction parameters *e.g.*, effect of pH, concentration of DTZ, Ratio of DES composition, volume of DES, volume of disperser, air-assisted time, speed and time of centrifugation, and an effect of sample volume were optimized by univariate optimization method.

Modification of SLM 1-octanol was mixed with N-Cetyl-N,N,N-trimethyl ammonium bromide (CTAB) with varied concentration in the range of 0-5 %v/v of CTAB in 1-octanol. 25 mL of 0.1 µg/L Pb solution was adjusted to pH 9 using ammonia/ammonium buffer solution as donor phase. DES (1:2 of ChCl:Phenol) mixed with pH 10 ammonia/ammonium buffer solution was filled into hollow fiber as acceptor phase. For extraction, DC power supply was applied at 20 V for 30 min under the stirring of 500 rpm.

Effect of pH on donor phase 25 mL of 0.1 µg/L Pb solution was adjusted to pH in the range of 8-14 using different types of buffer solution (ammonia/ammonium buffer and phosphate buffer) as donor phase. DES (1:2 of ChCl:Phenol) mixed with pH 10 ammonia/ammonium buffer solution (1:1 by volume) was filled into hollow fiber as acceptor phase. For extraction, DC power supply was applied at 20 V for 30 min under the stirring of 500 rpm.

Composition of DES as accepter media 25 mL of 0.1 µg/L Pb solution was adjusted to pH 9 using ammonia/ammonium buffer as donor phase. DES composition (ChCl:Phenol) in the ratios of 1:1, 1:2, 1:3, 1:4, and 1:5 by mole ratio was mixed with pH 10 ammonia/ammonium buffer solution (1:1 by volume) and filled into hollow fiber as acceptor phase. For extraction, DC power supply was applied at 20 V for 30 min under the stirring of 500 rpm.

Effect of pH on acceptor phase 25 mL of 0.1 µg/L Pb solution was adjusted to pH 9 using ammonia/ammonium buffer as donor phase. 1.0 mL of DES (1:2 of ChCl:Phenol) was mixed with 1.0 mL of different types of buffer solution (ammonia/ammonium buffer and phosphate buffer) in the range of 7-14 as acceptor phase. For extraction, DC power supply was applied at 20 V for 30 min under the stirring of 500 rpm.

Volume of DES in acceptor phase 25 mL of 0.1 µg/L Pb solution was adjusted to pH 9 using ammonia/ammonium buffer as donor phase. DES (1:2 of ChCl:Phenol) and pH 8 ammonia/ammonium buffer solution was mixed in the ratio of 0:25, 25:75, 50:50, 75:25, and 100:0 by %volume as acceptor phase. For extraction, DC power supply was applied at 20 V for 30 min under the stirring of 500 rpm.

Effect of applied voltage 25 mL of 0.1 µg/L Pb solution was adjusted to pH 9 using ammonia/ammonium buffer as donor phase. 3.75 mL of DES (1:2 of ChCl:Phenol) was mixed with 1.25 ml of pH 8 ammonia/ammonium buffer solution and filled this solution into hollow fiber as acceptor phase. For extraction, DC power supply was optimized in the range of 10-50 V for 30 min under the stirring of 500 rpm.

Effect of stirring speed 25 mL of 0.1 µg/L Pb solution was adjusted to pH 9 using ammonia/ammonium buffer as donor phase. 3.75 mL of DES (1:2 of ChCl:Phenol) was mixed with 1.25 ml of pH 8 ammonia/ammonium buffer solution and filled this solution into hollow fiber as acceptor phase. For extraction, DC power supply at 30 V was applied under the stirring speed in the range of 0-1000 rpm for 30 min.

Effect of extraction time 25 mL of 0.1 µg/L Pb solution was adjusted to pH 9 using ammonia/ammonium buffer as donor phase. 3.75 mL of DES (1:2 of ChCl:Phenol) was mixed with 1.25 ml of pH 8 ammonia/ammonium buffer solution and filled this solution into hollow fiber as acceptor phase. For extraction, DC power supply at 30 V and stirring rate at 500 rpm was applied in the range of 10-40 min.

Sample volume Volume of 0.1 µg/L Pb solution was optimized from 10-50 mL. The standard solution was added with pH 9 buffer (donor phase). The mixed solution of DES and pH 8 buffer solution (75:25 by %volume) was filled into hollow fiber as acceptor phase. For extraction, DC power supply was applied at 30 V for 25 min under the stirring of 500 rpm.

7.1.6 Method validation

River water certified reference material for trace metals and other constituents (SLRS-6) consisting of Pb at concentration 0.170 ± 0.026 µg/L was used for method validation. 30 mL of reference material was extracted with EM-DES-HFLPME-GFAAS method and Pb concentration was determined from this method. Finally, the measured result was compared with the certified value and statistically tested at 95% confidence level.

7.1.7 Analytical performance

The analytical performances of EM-DES-HFLPME-GFAAS method were investigated under the optimum conditions. The parameters namely LOD, LOQ, enrichment factor (EF), preconcentration factor (PF), and percentage extraction efficiency (%EE) were investigated using the same equation as previously described in section 3.1.4. In addition, linear range was investigated in the range of 0.036-1.00 µg/L of Pb solution. %RSD was evaluated by 0.3 and 0.5 µg/L of Pb solution. For the investigation of recoveries, 0.3 and 0.5 µg/L of Pb solution were spiked into the real samples.

7.1.8 Application of proposed method for Pb analysis

The developed EM-DES-HFLPME-GFAAS was applied for determination of trace Pb in drinking water and tap water samples.

Water sample preparation Drinking water (or bottled water) samples were purchased from local stores, Naresuan university, Phitsanulok, Thailand. Sampling of tap water were selected from department and resident. All water samples were extracted and detected by EM-DES-HFLPME-GFAAS.

7.1.9 Robustness

0.30 µg/L of Pb solution spiked with the solution of Na^+, Ca^{2+}, K^+, Cd^{2+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, chloride, and sulfate at the ratios up to 2000 (Analyte: interfering ion) were investigated for the effect of interfering ions by EM-DES-HFLPME-GFAAS under the optimum condition.

7.2 Results and discussion

7.2.1 Optimization

Modification of SLM SLM act as a medium layer between the donor and acceptor solutions to enhance the electro-kinetic migration of the analytes. In this study, porous hollow fiber was impregnated with organic solvent (1-octanol) as SLM. The use of CTAB in 1-octanol might increase the transportation of Pb anions (Plumbite and plumbate forms) from donor to acceptor. Therefore, 1-octanol was mixed with CTAB at concentrations in the range 0.0-5.0 %w/v. CTAB was a cationic surfactant that can help to increase the mass transfer via ion exchange in some cases. As the shown in Fig. 76, the addition of CTAB mixed in SLM affected to the decreasing of Pb anions signals. When, HF was dipped into 1-octanol for 1 min, this represented high effective SLM on porous support of membrane as shown in Fig. 77. Since, 1-octanol was a polar solvent which gave a good extraction efficiency without carriers. By using carrier, it can affect the high solvent polarity, unwanted electric current, and changing of acceptor volume. Then, relative absorbance after CTAB addition was also decreased. Therefore, further experiments were performed under 1-octanol conditions with no CTAB addition.

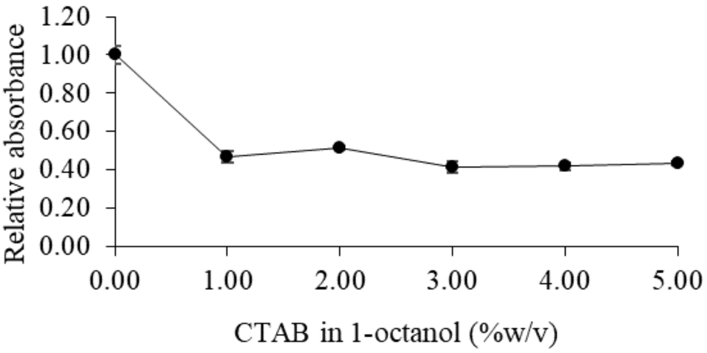


Figure 76 *Modification of SLM via carrier addition*

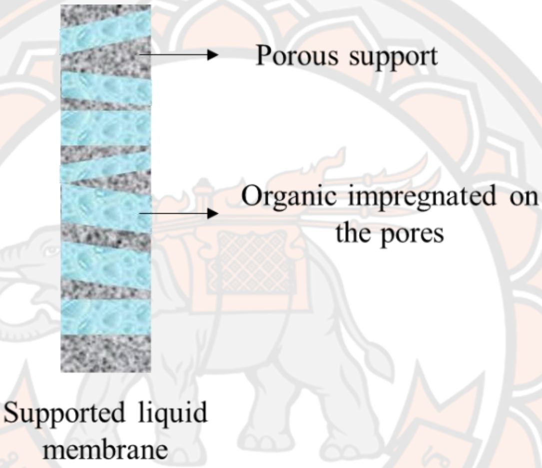


Figure 77 *Supported liquid membrane for EM-DES-HFLPME*

pH of donor phase pH values of donor phase affected the predominant form of Pb anions in the aqueous solution. Pb species can had the anionic forms of Pb-plumbite (PbO_2^{2-}) and Pb-plumbate (*e.g.*, PbO_3^{2-}, $HPbO_2^{-}$, $Pb(OH)_6^{2-}$, and $Pb(OH)_6^{4-}$) as oxyanion or hydrate forms of Pb based on the alkali solution. While in alkali media ($pH > 8$) (Fig. 78), the Pb anions mainly existed as plumbite and plumbate. When pH was 9, most forms of Pb in donor phase was changed to Pb-oxyanion (Plumbite and plumbate) forms. At pH 9 of donor, it was observed that the maximum relative absorbance was obtained. Therefore, pH of donor at 9 was chosen for further experiment.

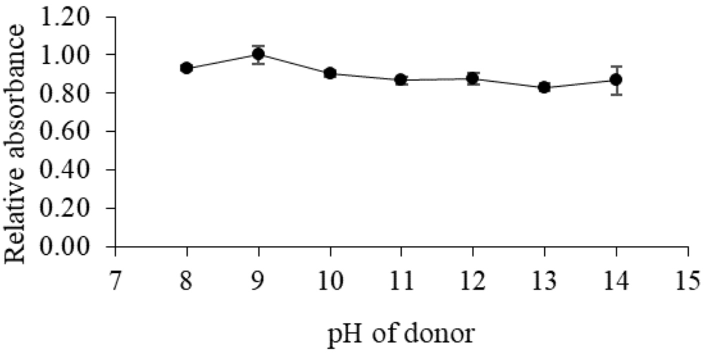


Figure 78 *Effect of pH in donor phase*

Composition of DES as accepter media Since, the method was developed as semi-three-phase-HFLPME mode, mixtures of choline chloride (ChCl) and phenol (Ph) were used as DES composition. In this work, DES can maintain the stability in the lumen of HF during the extraction process. Especially, the mixing of DES in pH buffer as acceptor gave high affinity for Pb-oxoanions. Choline chloride was polar ionic liquid that was easy to interact to polar analyte. Phenol can cause the intermolecular force with analyte. Therefore, the ratio of ChCl-Ph mixtures was investigated from 1:1 to 1:5 by mole ratio (ChCl:Ph). As can be seen in Fig. 79, the maximum relative absorbance was observed at 1:2 mole ratio. DES mixtures over 1:2 had the high densities that affected the viscosity of acceptor. Too viscous acceptor solution can decrease the extraction efficiency. Therefore, mixture of ChCl:Ph at 1:2 was selected for the further experiments.

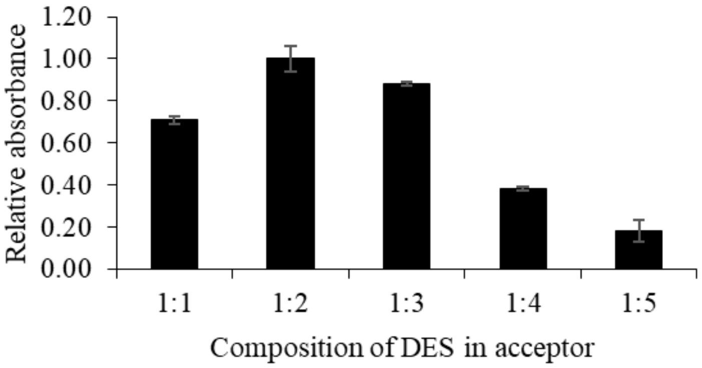


Figure 79 *Composition of DES as media in acceptor phase*

pH of acceptor phase Ionic concentration of the donor and acceptor phases was controlled by the pH adjustment. In this study, pH of the acceptor solution was also investigated in the range 7-14, whereas the pH of the donor phase was kept at 9.0. pH value of acceptor was a key role for ion balance ($Ions_{donor}$/ $Ions_{acceptor}$) and controlled the ionic forms of Pb. As the result in Fig. 80 indicated that if the pH of the acceptor phase was enhanced, the extraction performance was improved. Total anionic forms of Pb in donor phase were effectively transferred and reached maximum pH 8. In more alkali condition, relative absorbance was slightly decreased and constant until 14. Therefore, the pH of acceptor phase at 8 was used for further experiment.

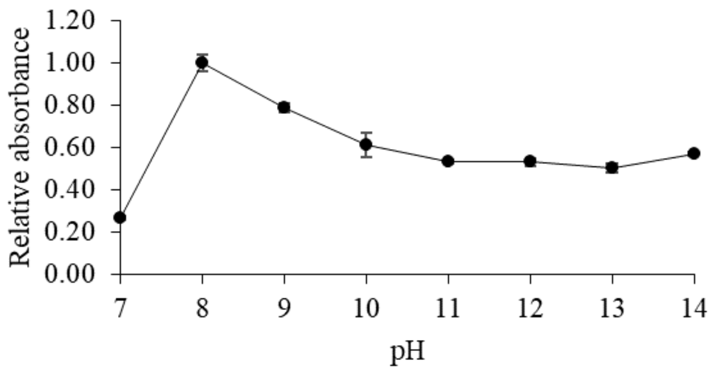


Figure 80 *Effect of pH in acceptor phase*

Ratio of DES and buffer in acceptor phase EM-DES-HFLPME was a semi-three-phase-HFLPME coupled with electromembrane extraction method. DES mixed with buffer solution can affect to the extraction efficiency. Therefore, percentage of acceptor mixtures was optimized with various DES volume from 0:100, 25:75, 50:50, 75:25, and 100:0 by volume (DES : pH 8). DES was mixed with pH 8 buffer solution and vortexed for few seconds. The result was indicated in Fig. 81. Volume of DES increased, the relative absorbance also increased until maximum point at 75:25 by volume. Presence of DES in buffer solution as acceptor can promote the mass transfer via passive diffusion to maintain the acceptor volume in lumen and increased the viscosity of the mixtures of acceptor. Higher volume of DES caused the acceptor phase too viscous that can influence and reduce the ionic transportation and relative absorbance. Thus, mixture of 75:25 by volume (DES : pH 8 buffer) was an optimum acceptor in this method.

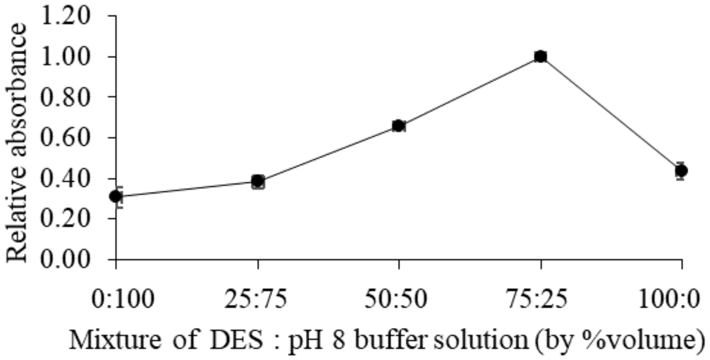


Figure 81 *Mixture of DES and pH 8 buffer solution in acceptor phase*

Effect of applied voltage An important parameter in EM-DES-HFLPME influencing the extraction efficiency and mass transfer across the membrane (Donor to SLM to acceptor) was the electric potential. Therefore, to obtain maximum extraction efficiency, the effect of the applied voltage ranging from 0 to 50 V was evaluated. The results in Fig. 82 showed that at 0 V, low relative absorbance of Pb-oxyanions was obtained because only passive diffusion through DES carrier-mediated transport was occurred. At low voltages relatively high mobility until the maximum point at 30 V was observed. At high voltages can cause the production of a faradic current causing heat and electrolysis in solution which was unwanted for EM-DES-HFLPME. It was observed that the highest extraction efficiency in EM-DES-HF LPME method reached maximum at voltages of 30 V. For these reasons, an applied voltage of 30 V was chosen for further experiment.

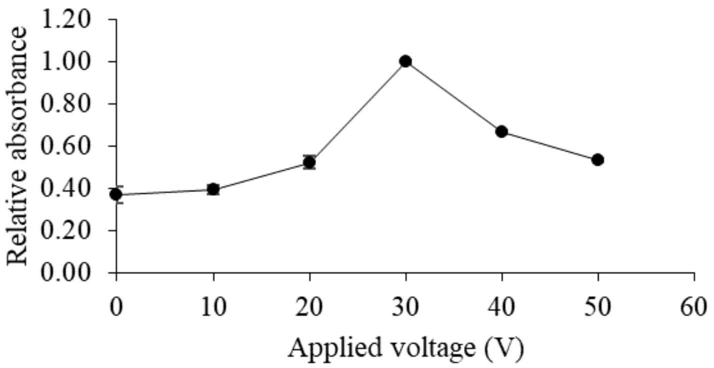


Figure 82 *Effect of applied voltage for EM-DES-HFLPME*

Effect of stirring rate Ionic transportation of Pb-oxyanions could be enhanced by stirring rate in the donor phase. This agitation also encouraged to reduce the boundary layer at the donor/SLM interface. In this work, the agitation speed was investigated in the range of 0-100 rpm. The result (Fig. 83) showed that the relative absorbance was increased when the stirring rate increased up to 500 rpm. After 500 rpm, the signal was decreased due to the possible reduce of acceptor volume. Therefore, stirring rate at 500 rpm in donor phase was used for the next experiments.

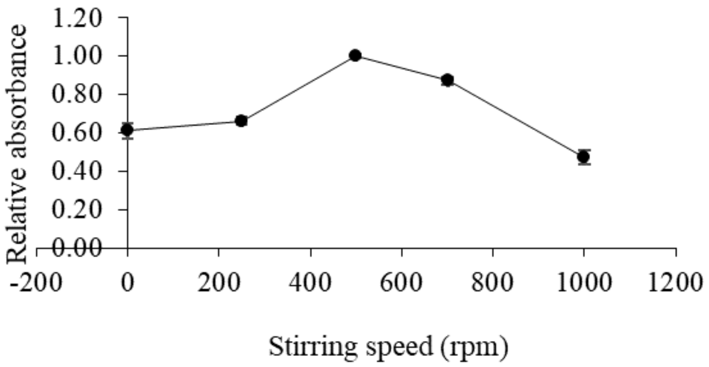


Figure 83 *Effect of stirring rate for EM-DES-HFLPME*

Effect of extraction time The mass transfer in EM-DES-HFLPME should be completely occurred for the effective extraction. In this experiment, extraction time was optimized. As shown in Fig.84, the various extraction times from 10 to 40 min were carried out. The result indicated that relative absorbance of analyte was increased until 25 min and slightly decreased after that. Less extraction time was not sufficient for complete extraction. While the extraction reached the maximum point, Pb-oxyanions was greatly transferred to acceptor. At long extraction times (>25 min), decreasing in relative absorbance were observed, probably due to back extraction to aqueous phase by ionic balance process, solvent loss of DES media in acceptor, dissolution of the SLM into the donor phase, or the occurrence of undesired electrolytic reactions on the electrodes. Therefore, 25 min was taken as the best extraction time.

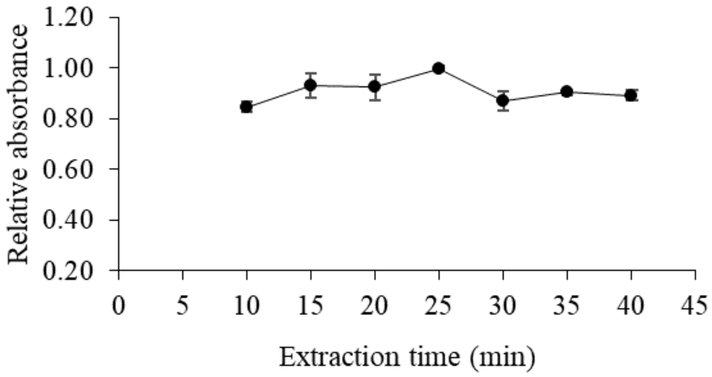


Figure 84 *Effect of extraction time*

Sample volume Sample volume directly affected to preconcentration factor and extraction efficiency. In this work, EM-DES-HFLPME was carried out at different initial sample volume in the range 10-50 mL. The results (Fig. 85) indicated that the good relative absorbance can be obtained by using of sample volume at 30 to 50 mL. For effective preconcentration, the large initial sample volume with small final volume was desired. Therefore, cost effective extraction and low waste generation were considered under this condition. Hence, EM-DES-HFLPME was achieved by using of sample volume at 30 mL with the preconcentration factor for 750.

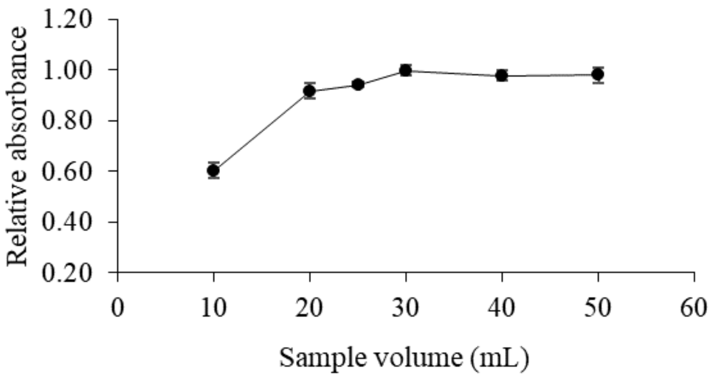


Figure 85 *Sample volume*

7.2.2 Analytical performance

Under optimized condition of EM-DES-HFLPME-GFAAS, figures of performance of the method including linearity, limit of detection (LOD), limit of quantitative (LOQ), relative standard deviation (%RSD), extraction recovery (%Recovery), enrichment factor (EF), and preconcentration factor (PF) were investigated. The equations of the calibration plots were established by linear regression of absorbance versus the concentration of Pb. The results in Fig. 86 represented the ranges of linearity that were in the range 0.036-1.00 ng/mL ($Y = 0.111X + 0.0064$, R^2 0.9834). By the linear equation of normal-GFAAS and EM-DES-HFLPME-GFAAS, the EF calculated from the slope of calibration curves was 111. LOD and LOQ were calculated by eq. 8 and eq. 9, respectively. The proposed procedure obtained with LOD and LOQ at 0.011 and 0.036 ng/mL, respectively. Precision was defined as the %RSD at concentration 0.3 and 0.5 ng/mL of Pb solution were in the range 1.49-2.43%. The results showed that Pb contents can be efficiency extracted with preconcentration factors of 750 with corresponding %recoveries, of Pb at 87.5-110.9% in drinking water samples and 95.5-110.5% in tap water samples. Figures of merit of the proposed method was summarized in Table 29. Characteristic mass (M_0) calculated from eq. 14 represented the improving sensitivity which was better than 117 times ($M_{0\,(GFAAS)}$ was 70.0 pg and $M_{0\,(EM\text{-}DES\text{-}HFLPME\text{-}GFAAS)}$ was 0.6 pg).

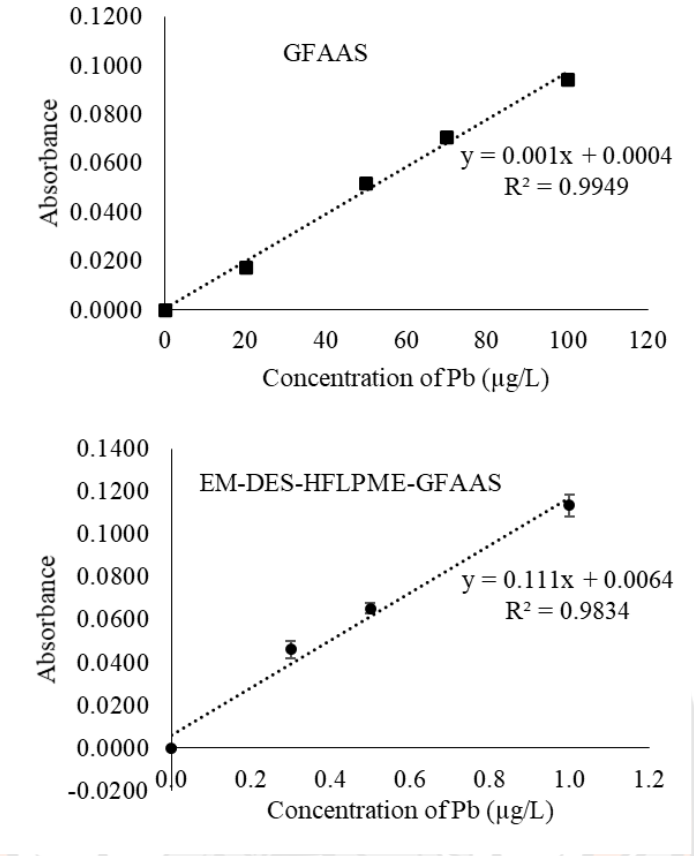


Figure 86 *Working ranges for Pb analysis using GFAAS and proposed method*

Table 29 *Figures of merit from EM-DES-HFLPME method*

Figures of merit	**Value**
Linearity (ng/mL)	0.036-1.00
LOD (ng/mL)	0.011
LOQ (ng/mL)	0.036
%RSD	1.03-3.49
%Recovery	83.90-110.9
EF	111
PF	750

7.2.3 Method validation

To investigate the accuracy of the developed method, river water certified reference material for trace metals and other constituents (SLRS-6) as standard reference material was used for method validation. The reference material contained Pb at a certified concentration value of 0.170±0.026 µg/L. The concentration of Pb in reference material measured by the EM-DES-HFLPME method was found to be 0.166±0.007 µg/L. These values were comparable and were not significantly different at the 95% confidence level.

7.2.4 Application of EM-DES-HFLPME for Pb analysis

The application of the developed method was tested by applying for the quantification of Pb in drinking water and tap water samples. The extraction procedure was carried out after adjusting the pH of sample to 9.0. The concentrations of Pb standard solution at 0.30 and 0.50 ng/mL were spiked in all samples before EM-DES-HFLPME extraction. The result was obtained in Table 30. Pb contamination in all drinking water and tap water samples did not exceed MRL guideline. Drinking water-(1), (2), and (3) containing Pb at concentration 0.11±0.06, 0.34±0.09, and 0.31±0.01 ng/mL, respectively. Pb in tap water-(1), (2), and (3) were for 1.20±0.01, 1.06±0.01, and 1.86±0.02 ng/mL, respectively. The analytical performances of the proposed EM-DES-HFLPME method for quantification of trace levels of Pb in ppb level were compared with other previous works and presented in Table 31. It can be summarized from the result that the proposed method not only gave an excellent enrichment and preconcentration factors but also a low LOD and LOQ (higher sensitivity) as compared to the reported methods [142, 171, 172, 190]. In addition, this method provided precis extraction and satisfied accuracy.

Table 30 *Results for the determination of Pb in water samples by the EM-DES-HFLPME-GFAAS method (n=3)*

Sample	**Pb concentration (ng/mL)**		**%Recovery±SD**
	Added	**Found**	
Drinking water (1)	-	0.11±0.06	-
	0.30	0.41±0.01	99.8±3.5
	0.50	0.55±0.01	87.5±2.5
Drinking water (2)	-	0.34±0.09	-
	0.30	0.63±0.00	95.7±1.2
	0.50	0.86±0.02	104.0±3.5
Drinking water (3)	-	0.31±0.01	-
	0.30	0.62±0.01	105.62±4.7
	0.50	0.86±0.01	110.9±1.4
Tap water (1)	-	1.20±0.01	-
	0.30	1.53±0.00	110.5±0.5
	0.50	1.67±0.00	95.5±0.4
Tap water (2)	-	1.06±0.01	-
	0.30	1.36±0.00	99.5±1.2
	0.50	1.59±0.01	105.0±1.3
Tap water (3)	-	1.86±0.02	-
	0.30	2.18±0.00	105.2±5.9
	0.50	2.41±0.00	108.4±2.0

Table 31 *Comparison of proposed EM-DES-HFLPME method with previous works for quantification of Pb*

Method	**Detection**	**Sample**	**Linearity (ng/mL)**	**LOD (ng/mL)**	**LOQ (ng/mL)**	**%RSD**	**%Recovery**	**EF**	**PF**	**Ref.**
CM-HFLPME	GFAAS	blood	1-200	0.1	0.4	2.3-4.4	-	-	33	[142]
MIL-DLLME	GFAAS	bee products	0.01-3.00	0.003	-	3.6	-	107	-	[189]
HFLLSME	GFAAS	soft drinks	0.00-2.00	0.007	0.024	6	85-100	22-66	-	[191]
VA-SHS-LPME	GFAAS	water, tea, human hair	0.04-2.00	0.016	0.045	4.2	94.9-110	49	-	[171]
HLILM-SAP	GFAAS	urine and blood	0.10-50	0.014-0.018	0.046-0.060	3.77-6.79	95-103	43-45	-	[190]
SFODME	GFAAS	tap water	0.2-10	0.058	0.20	8.8	92-95	-	113	[172]
EM-DES-HFLPME	GFAAS	water	0.036-1.00	0.011	0.036	1.03-3.49	83.90-110.9	111	750	This method

Remarks;

CM-HFLPME	Carrier-mediated hollow fiber liquid phase microextraction
MIL-DLLME	Magnetic ionic liquid- dispersive liquid-liquid microextraction
HFLLSME	Hollow-fiber liquid–liquid–solid micro-extraction
VA-SHS-LPME	Vortex assisted switchable hydrophilicity solvent based liquid phase microextraction
HLILM-SAP	Hydrophobic, lighter-than-water ionic liquid microextraction- solidification of the aqueous phase
SFODME	Solidified floating organic drop microextraction

7.2.5 Robustness

Tolerance limit represented the robustness of the developed method. EM-DES-HFLPME-GFAAS was applied for evaluation of existing Pb signal under the contamination of interfering ions. Solution of interfering ions namely Na^+, Ca^{2+}, K^+, Cd^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, chloride (Cl^-), and sulfate (SO_4^{2-}) probably presented in real water sample were investigated. These solutions contained 0.3 ng/mL of Pb solution prior to extraction. The evaluation was carried out under optimum condition. The result. It was observed in the Table 32 that the Na^+, Ca^{2+}, and K^+ ions did not have any impact on the method performance at the ratio of 1500 This proved that when the concentration of these coexisting anions was <450 ng/mL, there was no substantial impact on extraction efficiency and the suggested EM-HF-LPME method had no interferences from these elements. However, some heavy metals can form metal-oxyanions (Generic formula $A_xO_z^{-y}$ or hydrate form as $A_x(OH)_y^{-z}$) in alkali condition. The results revealed no interference up to 150 ng/mL (ratio of 500) of Cd^{2+}, Cu^{2+}, and Zn^{2+} ions and 90 ng/mL of Fe^{3+} (ratio of 300). Owing to the common anion interferences in sample matrices, Cl^- and SO_4^{2-} were used as representative samples. The effect of these interfering ions was quite low at concentration up to 300 times or 150 ng/mL.

Table 32 *The study of interfering effect for the determination of Pb using EM-DES-HFLPME-GFAAS (Using 0.30 µg/L of Pb solution)*

Interfering ions	**Tolerance limit**	**%Recovery ± SD**
Na^+	1500	89.0±3.9
Ca^{2+}	1500	101.4±2.5
K^+	1500	82.6±2.2
Cd^{2+}	500	106.2±4.7
Cu^{2+}	500	104.9±1.2
Zn^{2+}	500	89.4±3.9
Fe^{3+}	300	97.6±0.8
Cl^-	300	82.9±1.9
SO_4^{2-}	300	84.6±2.9

7.3 Conclusions

The simple EA-DES-HFLPME method has been successfully employed for the determination of Pb in different types of water samples by GFAAS. The influencing parameters were optimized by univariate optimization method. Under the optimum condition, the analytical performances of the proposed method were elucidated as shown in Table 29. Furthermore, effect of interfering ions was investigated as demonstrated in Table 32. The found concentration of contaminated-Pb in drinking water samples were 0.11-0.34 µg/L and tap water samples were 1.06-1.86 µg/L. Meanwhile, the accuracy of the proposed method was validated by SRM.

CHAPTER VIII

CONCLUSIONS AND SUGGESSION

This chapter presented the summary of the contribution and the concluding remarks of this dissertation. In addition, some suggestions of the work were explored for the future.

8.1 Conclusions

First, air-assisted solvent terminated dispersive liquid-liquid microextraction (AA-ST-DLLME) was utilized for the extraction and preconcentration of Pb in water, beverage, and fruit juice samples using GFAAS detection. Pb-PAN complexes were extracted with chloroform according to the dispersion on the solution using surfactant and the use of a second surfactant for demulsification in the centrifuge-less step. The method gave high extraction efficiency with the low detection limit. Applying of home fish-tank pump for bubble generation in extraction system for improving of extraction efficiency for the first time and reducing the extraction time, as well as to increase the efficiency, and to make the method cost effective. Nonetheless, the home fish-tank pump in AA-ST-DLLME method was an alternative approach for air-assisted techniques. The method could be applied for trace metal determination in environmental, biological, medical, and other samples in future works.

Second, a fast, simple and time saving MdSPE method was developed for the determination of Pb using magnetic GO-Fe_3O_4-DTZ composite. Normally, Fe_3O_4 nanoparticles had drawbacks of severe aggregation between particles which restricted their application on adsorption. The modified-Fe_3O_4 to GO surfaces can improve the selectivity and extraction capacity. Preferably, GO based material from used-graphite tube consisting of high porous surfaces and high adsorption capacity can enhance the preconcentration factor. Therefore, the modified GO was a new alternative source of graphene powder for cost-effective GO synthesized method. Meanwhile, the synthesized GO-Fe_3O_4-DTZ was characterized by Zeta potential, ATR-FTIR, and SEM before extraction. This method had a good preconcentration factor (13.33) and good adsorption capacity (30.53 mg/g). Percentage recoveries for Pb in the sample matrices and in interference indicated that the method had good accuracy and selectivity. Magnetic GO-Fe_3O_4-DTZ sorbent can be simply used with fast analysis time. The GO-Fe_3O_4-DTZ-MdSPE-FAAS can be successfully applied for the drinking and environmental samples.

Third, a fast-sequential EA-SS-LPME-HR-CS-FAAS analysis was successfully developed for the determination of Pb and Cd in drinking water, canned tuna, and canned fruit samples. The proposed method involved the use of the effervescent tablet assisted technique for enhancement the preconcentration with good precision. The synthesized switchable solvent was simply synthesized by mixing dry ices with TEA . Time saving method was achieved under 8 min (6-8 min for extraction) for preconcentration and separation of both analytes. HR-CS-FAAS facilitated multi-element sequential analysis. Pb and Cd were co-extracted and analyzed in the same run with the use of 3.0 mL final volume. Hence, the consumption of reagents in extraction and fuel gas for instrumental detection could be cost effective. Besides, the wastes from extraction were easily managed.

Fourth, sequential multi-element analysis of Pb and Cd using AA-DES-DLLME for preconcentration and extraction coupled with HR-CS-FAAS was successfully developed. In the proposed method, alternative to conventional toxic organic extracting solvents, small volume of DES (0.5 mL) was used as an extracting solvent, implying a reduction in the risk for human health and environment, and the time consuming of routine analysis (11-15 min per extraction). In addition, the obtained results provided that its advantages were good repeatability, low LOD and LOQ (lower than MRL from Thailand standard), and simple operation. The application of proposed method indicated that the determination of trace level of Pb and Cd in drinking water and canned food samples was fast and applicable for multi-element analysis using small final volume (3.0 mL) by HR-CS-FAAS.

Novelty of the proposed EM-DES-HFLPME method facilitated to determine the trace level of Pb in water samples together with high sensitivity GFAAS. Analysis of Pb-oxyanions were successfully performed. Meanwhile, the use of DES carrier in buffer solution (in acceptor phase) provided the remaining of aqueous phase in hollow fiber, increasing of viscosity of acceptor, and passive transportation. The existence of solvent on SLM (1-octanol) and acceptor (DES) avoided the leaking of aqueous in acceptor and losing of analyte by the back diffusion. The use of electro-enhancement was effective method which can increase the extraction efficiency with high enrichment factor of 111. Under the extraction by EM-DES-HFLPME for 30 min per sample, the method consumed low reagents and minimize waste generation. Finally, all water sample matrices were not interfered with Pb over the MRL or standard levels.

The present dissertation consisted of five techniques for Pb and Cd preconcentration. The analytical performances were compared and summarized in Table 33. The advantages of each technique were described in Table 34.

Table 33 *Summarization of dissertation*

Topic	**Dissertation-I**	**Dissertation-II**	**Dissertation-III**	**Dissertation-IV**	**Dissertation-V**
Technique	AA-ST-DLLME	MdSPE	EA-SS-LPME	AA-DES-DLLME	EM-DES-HFLPME
Spectrometry	GFAAS	FAAS	HR-CS-FAAS	HR-CS-FAAS	GFAAS
Analyte	Pb	Pb	Pb and Cd	Pb and Cd	Pb
Sample	Water, beverage, and fruit juice	Drinking and surface water	Drinking, canned tuna and fruit	Drinking and canned vegetable	Water samples
Linearity (ng/mL)	0.08-8.0	70.00-3000	(Pb) 64.90-1500 (Cd) 22.80-10000	(Pb) 16.79-4500 (Cd) 9.43-2000	0.036-1.00
LOD (ng/mL)	0.025	70.0	(Pb) 19.50 (Cd) 6.80	(Pb) 5.07 (Cd) 2.83	0.011
LOQ (ng/mL)	0.084	230	(Pb) 64.90 (Cd) 22.80	(Pb) 16.79 (Cd) 9.43	0.036
%RSD	1.02-2.62	3.41	(Pb) 1.25-1.69 (Cd) 1.07-1.64	(Pb) 1.49-2.43 (Cd) 1.39-4.19	1.03-3.49
%Recovery	82.70-117.3	88.60-118.8	(Pb) 82.30-119.0 (Cd) 81.67-120.0	(Pb) 84.20-114.6% (Cd) 84.70-118.0%	83.90-110.9
EF	58.9	7.7	(Pb) 1.4 (Cd) 2.6	(Pb) 2.60 (Cd) 3.08	111
PF	60.0	13.3	3.3	6.67	750
Analysis time (min)	< 7	< 10	< 8	< 15	<30

Table 34 *Advantages and disadvantages of proposed microextraction methods in this dissertation*

Method	**Advantages**	**Disadvantages**
AA-ST-DLLME-GFAAS	- Using low sample volume - Green disperser and demulsifier - Centrifuge-less method - Fastest extraction - Low LOD and LOQ	- Limitation of demulsifier reagent - Using toxic and volatile solvent (Chloroform)
MdSPE-FAAS	- High adsorption efficiency - Using external magnet for separation - Centrifuge-less method - Rapid separation	- Using high volume for detection - Precaution for oxidation procedure of sorbent synthesis
EA-SS-LPME-HR-CS-FAAS	- Low LOD and LOQ - Simplicity of synthesis for the switchable solvent and extraction operation - Green alternative solvent - Fast sequential analysis - Multi-element analysis by HR-CS-FAAS	- Long time consumption for solvent synthesis - Unpleasant smell of synthesized solvent

Table 34 *(Cont)*…

Method	**Advantages**	**Disadvantages**
AA-DES-DLLME-HR-CS-FAAS	- Low LOD and LOQ - Simplicity of synthesis for the DES and extraction operation - Simple air-assisted technique - Fast sequential analysis - Multi-element analysis - Environmentally friendly DES	- Unpleasant smell of DES - Viscous solution for HR-CS-FAAS detection and dilution needed
EM-HF-LPME-GFAAS	- High preconcentration factor - High enrichment factor - Low final volume for detection (50 µL) - No need for large volume of solvent - Easy to improve the LOD/LOQ via increasing of the length of hollow fiber	- Instability of SLM - Long extraction - Avoiding of volatile solvent in SLM - Avoiding the use of high voltage

8.2 Suggestion

As discussed in the dissertation chapter III (AA-ST-DLLME-GFAAS), the direct air bubble insertion into extraction tube enhances the extraction efficiency. However, the caution of this technique is that the extraction tube needs to have a free space above solution for bubble spreading. Large sample volume in the extraction tube should be avoided.

As presented in the dissertation chapter IV (MdSPE-FAAS), the used-graphite tube from GFAAS is a novel modification of GO source. Therefore, the future work, graphite from pencil lead or mechanical pencil leads for which in-expensive materials and simply purchase from local stores can use. Furthermore, the limit of detection and quantitation can improve for the ultra-trace determination of Pb. Selection of new type of complexing agent such as L-cysteine, thiourea, 1-(2-Pyridylazo)-2-naphthol, and ammonium pyrrolidinedithiocarbamate may be useful for the better of LOD and LOQ with high extraction efficiency.

In the dissertation chapter V (EA-SS-LPME-HR-CS-FAAS), effervescent tablets are the important materials enhancing the extraction efficiency. The special tip for the future woks is the tablet making. The tablet of effervescence should be freshly prepared for each extraction. Preparation of tablets in advance may affect for decomposition by the humidity. The prepared tablet should always keep in the desiccator.

As mentioned in the dissertation chapter VI (AA-DES-DLLME-HR-CS-FAAS), the digested sample solutions are quite in acidic solution. In the extraction procedure, solution should be neutralized by basidic solution such as NaOH or buffer solution before extraction. Besides, the synthesized DES should be thoroughly mixed before introduction to AA-DES-DLLME procedure.

Finally, for the dissertation chapter VII (EM-DES-HFLPME-GFAAS), since high voltage is applied for the extraction, care must be taken. Because the use of high power might be inducing heat and electrolytic reaction in the system. Importantly, distance of two electrodes should be controlled. In the future works, improving of LOD and LOQ are interesting subjects that could be made by extending of length of hollow fiber.

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