PRECONCENTRATION OF TRACE HEAVY METAL IONS IN TEXTILES AND DETERMINATION BY FLAME ATOMIC ABSORPTION SPECTROMETER

TEKSTİL ÜRÜNLERİNDE ESER DÜZEYDEKİ AĞIR METAL İYONLARININ ÖNDERİŞTİRİLMESİ VE ALEVLİ ATOMİK ABSORPSİYON SPEKTROMETRESİ İLE TAYİNİ

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For Whom My Heart Beats with Loving Them

(My Family)

ABSTRACT

PRECONCENTRATION OF TRACE HEAVY METAL IONS IN TEXTILES AND DETERMINATION BY FLAME ATOMIC ABSORPTION SPECTROMETER

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In this thesis, a simple, low-cost, and green effervescence-assisted dispersive liquid-liquid microextraction based on deep eutectic solvent (EA-DES-LPME) method was developed for separation, preconcentration, and determination of lead, copper, nickel, and cobalt in cotton textile products in different colors that collected from various fabric shops in Ankara, Turkey, prior to analysis with flame atomic absorption spectrometry (FAAS). The complexation of metal ions was carried out by using 0.1 % (w/v) 8-hydroxyquinoline (8-HQ) as a complexing agent. The deep eutectic solvent was prepared by mixing choline chloride (ChCl) and phenol (Ph), where used as an extraction solvent. In this method, the dispersion of the extraction solvent is achieved by effervescence reaction, which is performed by carbon dioxide bubbles production. The effervescent agent is environmentally friendly and only produces an increase in the ionic strength and a negligible variation in the pH value of the aqueous sample, which does not interfere with the extraction of the analytes. For this purpose, a special tablet containing ascorbic acid (AA) as a proton donor and sodium carbonate (SC) as a carbon dioxide source in a certain molar ratio was used. THF was utilized as an aprotic solvent and allows the extraction solvent to separate from the aqueous phase by self-aggregation. All parameters related to the effervescence agent and deep eutectic

solvent that affects the extraction efficiency were investigated and optimized in detail, as well as the effect of pH, and the amount of 8-HQ and THF. As a result of the optimization studies, the highest extraction efficiency was achieved when pH is 6.0, effervescence tablet composition is (AA: SC) with (2:1) molar ratio, effervescence amount is 0.457g powder or 2 tablets, effervescence time is 2.5 min, DES volume is 1000 µL and synthesized from ChCl: Ph at 1:3 mole ratio, 8-HQ volume is 750 µL, and THF volume is 1000 µL. Under these optimal conditions, the analytical performance of the proposed method was evaluated based on the enhancement factor (EF), limit of detection (LOD), limit of quantification (LOQ), percent recovery, and accuracy criteria. When applying the EA-DES-DLLME method under optimum conditions for Pb (II), Cu (II), Ni (II), and Co (II) the results are as follows: the EFs are 62.5, 52, 57.5, and 52.5 respectively; the LODs in µg L^{-1} are 2.44, 1.29, 0.58, and 1.55 respectively; the LOQs in µg L^{-1} are 8.13, 4.30, 1.93, and 5.16 respectively; the percent RSDs (n=10) are 1.07, 1.61, 1.35, and 1.97 respectively. In addition, the method was applied to the real samples (different cotton textiles with different colors), and the recovery results were found as follows: 97.20 – 101.70 % for Pb (II), 97.60 – 101.92 % for Cu (II), 95.65 – 104.35 % for Ni (II), and 96,76 – 101.52 % for Co (II). The concentrations of these trace metal ions in cotton textile samples were found to be in the range of 0.26 – 7.52 µg g^{-1} Pb (II), 1.27 – 77.35 µg g^{-1} Cu (II), 0.66 – 5.00 µg g^{-1} Ni (II), and 0.64 – 5.58 µg g^{-1} Co (II). On the other hand, it was revealed that the matrix effect is not important in the method and the method can be applied to real samples with high accuracy and sensitivity.

Keywords: Preconcentration, Enhancement, Lead, Copper, Nickel, Cobalt, Microextraction, Deep eutectic solvents, Effervescence-assisted, EA-DES-DLLME, cotton textile samples, Flame Atomic Absorption Spectroscopy.

ÖZET

TEKSTİL ÜRÜNLERİNDE ESER DÜZEYDEKİ AĞIR METAL İYONLARININ ÖNDERİŞTİRİLMESİ VE ALEVLİ ATOMİK ABSORPSİYON SPEKTROMETRESİ İLE TAYİNİ

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Bu tez çalışmasında, kurşun, bakır, nikel ve kobaltın alevli atomik absorpsiyon spektrometrisi (FAAS) ile tayini öncesinde ayrılması ve zenginleştirilmesi için basit, düşük maliyetli ve yeşil bir yöntem olan derin ötektik çözücü kullanımına dayalı efervesan destekli dispersif sıvı-sıvı mikroekstraksiyonu (EA-DES-LPME) geliştirilmiştir. Yöntem, Ankara, Türkiye' deki çeşitli kumaş mağazalarından sağlanan farklı renklerde pamuklu tekstil ürünlerine uygulanmıştır. Çalışmada, metal iyonlarının kompleksleştirilmesi %0.1 (a/h) 8-hidroksikinolin (8-HQ) kullanılarak gerçekleştirilmiştir. Ekstraksiyon çözücüsü olarak kullanılan derin ötektik çözücü, kolin klorür (ChCl) ve fenol (Ph) kullanılarak hazırlanmış ve ekstraksiyon çözücüsünün dispersiyonu, karbondioksit kabarcıklarının üretimi ile gerçekleştirilen efervesan reaksiyonu ile sağlanmıştır. Kullanılmış olan efervesan madde çevre dostudur ve sadece iyonik kuvvette artışa ve sulu numunenin pH değerinde ihmal edilebilir bir değişime neden olmakta ve bu durum analitlerin ekstraksiyonu üzerinde herhangi bir olumsuz etkiye sebep olmamaktadır. Efervesan madde, proton verici olarak askorbik asit (AA) ve karbondioksit kaynağı olarak sodyum karbonatın (SC) belirli mol oranında karıştırılmasıyla tablet şeklinde hazırlanmıştır. Aprotik bir çözücü olan THF, ekstraksiyon çözücüsünün self-agregasyon ile sulu fazdan ayrılmasını sağlamıştır. pH etkisi,

8-HQ ve THF miktarının yanı sıra efervesan madde ve derin ötektik çözücü ile ilgili olan ve ekstraksiyon verimini etkileyen tüm parametreler, detaylı olarak araştırılmış ve optimize edilmiştir. Optimizasyon çalışmaları sonucunda en yüksek ekstraksiyon verimi pH 6.0, efervesan tablet bileşimi 2:1 mol oranına sahip AA:SC, efervesan miktarı 0.457 g toz veya 2 tablet, efervesan süresi 2.5 dk, DES kompozisyonu 1:3 mol oranında ChCl:Ph, DES hacmi 1000 µL, 8-HQ hacmi 750 µL ve THF hacmi 1000 µL'dir. Bu optimum koşullar altında, önerilen yöntemin analitik performansı, zenginleştirme faktörü (EF), gözlenebilme sınırı (LOD), tayin sınırı (LOQ), geri kazanım yüzdesi ve doğruluk kriterlerine göre değerlendirilmiştir. EA-DES-DLLME yönteminin optimum koşullar altında Pb (II), Cu (II), Ni (II) ve Co (II) tayini için uygulanmasında elde edilen sonuçlar sırasıyla şu şekildedir: EF, 62.5, 52, 57.5 ve 52.5; LOD µg L^{-1} cinsinden, 2.44, 1.29, 0.58 ve 1.55' tir; LOQ µg L^{-1} cinsinden, 8.13, 4.30, 1.93 ve 5.16; %RSD (n=10) 1.07, 1.61, 1.35 ve 1.97' dir. Ayrıca, yöntem gerçek numunelere (farklı renklere sahip farklı pamuklu tekstil ürünleri) uygulanmış ve geri kazanım sonuçları şu şekilde bulunmuştur: Pb (II) için % 97.20 – 101.70, Cu (II) için % 97.60 – 101.92, Ni (II) için % 95.65 – 104.35 ve Co (II) için % 96.76 – 101.52. Pamuklu tekstil numunelerinde bu eser metal iyonlarının derişimlerinin 0.26 – 7.52 µg g^{-1} Pb (II), 1.27 – 77.35 µg g^{-1} Cu (II), 0.66 – 5.00 µg g^{-1} Ni (II) ve 0.64 – 5.58 µg g^{-1} Co (II) aralığında olduğu bulunmuştur. Öte yandan, yöntemde matriks etkisinin önemli olmadığı ve yöntemin gerçek örneklere yüksek doğruluk ve hassasiyetle uygulanabileceği ortaya konmuştur.

Anahtar Kelimeler: Önderiştirme, Zenginleştirme, Kurşun, Bakır, Nikel, Kobalt, Mikroekstraksiyon, Derin ötektik çözücüler, Efervesans destekli, EA-DES-DLLME, pamuklu tekstil numuneleri, Alev Atomik Absorpsiyon Spektroskopisi.

ACKNOWLEDGMENTS

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SYMBOLES AND ABBREVIATIONS

Symbols

%	Percentage
[M]	Metal Ion
°C	Degree Celsius
m	Slope
min	Minute
n	Numbers of experiment
R^2	Correlation Coefficient
S	Standard deviation
T_m	Melting point temperature
ΔpH	pH Variation
η	Viscosity
ρ	Density

Abbreviations

8-HQ	8-hydroxyquinoline
A	Absorbance
AA	Air-Assisted
AA	Ascorbic Acid
AA: SC	Ascorbic Acid: Sodium Carbonate
AALLME	Air-Assisted Liquid-Liquid Microextraction
AAS	Atomic Absorption Spectrometry
CA	Citric Acid
ChCl	Choline Chloride
ChCl: Ph	Choline Chloride: Phenol
Co-MP-DSPME	Cobalt Magnetic Particles Based Dispersive Solid-Phase Microextraction
CPE	Cloud Point Extraction
CPT	Cloud Point Temperature

CRM	Certified Reference Material
DES-DLLME	Dispersive Liquid-Liquid Microextraction Based on Deep Eutectic Solvents
DES-EM	Deep Eutectic Solvent Extraction Method
DESs	Deep Eutectic Solvents
DES-UALPME	Deep Eutectic Solvent Based Ultrasonic Assisted Liquid Phase Microextraction
DLLME-MSPE	Dispersive Liquid-Liquid Microextraction and Micro-Solid Phase Extraction
DLLME-SQT	Dispersive Liquid-Liquid Microextraction-Slotted Quartz Tube-Flame Atomic Absorption Spectrometry
EA	Effervescence-Assisted
EA-DES-DLLME	Effervescence-Assisted Dispersive Liquid-Liquid Microextraction Based on the Deep Eutectic Solvent
Eco – Label	Ecological Products Certification
EDL	Electrodeless Discharge Lamp
EF	Enhancement Factor
ETAAS	Electrothermal Atomic Absorption Spectroscopy
FAAS	Flame Atomic Absorption Spectroscopy
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HCL	Hollow Cathode Lamp
HF-LPME	Hollow Fiber Liquid Phase Microextraction
HPLC	High Performance Liquid Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IL-DLLME	Ionic Liquid-based dispersive liquid-liquid microextraction
ILs	Ionic Liquids
IL-UA-DLLME-SAP	Ionic Liquid Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction Based on Solidification of the Aqueous Phase
In situ-CO2 DLLME	In Situ-Produced CO2-Assisted Dispersive Liquid-Liquid Microextraction
IP-DLLME	Ion Pair Dispersive Liquid-Liquid Microextraction

LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LPME	Liquid-Phase Microextraction
LR	Liner Range
MADLLME	Microwave-Assisted Dispersive Liquid-Liquid Microextraction
OA	Oxalic Acid Dihydrate
Oeko – Tex	The International Research Community and Testing in the Field of Textile Ecology
PF	Preconcentration Factor
Ph	Phenol
pH-MS-HLPME	pH Modulated Solidified Homogeneous Liquid Phase Microextraction
ppb	Part per billion
ppm	Part per million
PV-IS-DLLME	Pressure Variation in-Syringe Dispersive Liquid-Liquid Microextraction
RP-SHS-LLME	Reversed-Phase Switchable-Hydrophilicity Solvent Liquid-Liquid Microextraction
RSD	Relative Standard Deviation
SA-DLLME	Surfactant Assisted Dispersive Liquid-Liquid Microextraction
SBC	Sodium Bicarbonate
SC	Sodium Carbonate
SDILNDµE	Single Drop Ionic Liquid Based Non-Dispersive Microextraction
SDME	Single Drop Microextraction
SDP	Sodium Dihydrogen Phosphate
SFODME	Solidified Floating Organic Drop Microextraction
SHS-LPME	Switchable Solvent Based Liquid Phase Microextraction
SPE	Solid-Phase Extraction
SPME	Solid-Phase Microextraction
SQT-FAAS	Slotted Quartz Tube-Flame Atomic Absorption Spectrometry
SS-LPME	Switchable Solvent Based Liquid Phase Microextraction Method
SSs	Switchable Solvents
SUPRAs	Supramolecular Solvents

TBAB based ABS	Tetrabutylammonium Bromide Based Aqueous Biphasic System
THF	Tetrahydrofuran
TIL–DμE–ISAVS	Tandem Ionic Liquid-Based Dispersive Microextraction Method Using in-Syringe Air-Assisted Vesicle System
U	Urea
UA	Ultrasound-Assisted
UA-CPE	Ultrasound-Assisted Cloud Point Extraction
UA-IPSE-DLLME	Ultrasound Assisted Ion Pair Based Surfactant-Enhanced Liquid-Liquid Microextraction
Um-CPE	Ultrasonically Modified Cloud Point Extraction
USAEME	Ultrasound Assisted Emulsification Microextraction
USA-FF-DLPME	Ultrasound Assisted Ferrofluid Dispersive Liquid Phase Microextraction
UV-Vis	Ultraviolet Visible
VA	Vortex-Assisted
VA-DES-LPME	Vortex Assisted Deep Eutectic Solvent Based Liquid Phase Microextraction
VA-DLLME	Vortex-Assisted Dispersive Liquid-Liquid Microextraction

1. INTRODUCTION

Environmental pollution has been identified as one of the fundamental issues in the modern world where rapid increase in world population, technological and industrial progress, as well as an increase in irregular urbanization rate, and the excessive consumer demand of people, have caused environmental pollution, especially in emerging countries. As a result, the harmful effects of poor environmental conditions on human health and other living organisms began to appear. The gradual increase in such environmental and health problems makes analytical chemistry even more important. Therefore, it has become necessary to investigate and identify these effects in many different areas such as environmental pollution, electronics, and industry [1].

Heavy trace elements are at the forefront of materials that are widely used in industry, are of vital importance to living organisms when present at the required level, and cause toxic effects, and pollution when used excessively. In general, there are two sources of heavy metals in the environment, they are natural and unnatural. Natural resources are the result of an external influence such as natural and anthropological disasters and hydrothermal processes. As for the unnatural sources, they are largely formed as a result of industrial activities such as smelting and refining activities, mining industry activities, and transportation activities, as well as the manufacture of pesticides and fertilizers used in agriculture.

There are many health risks associated with the toxicity of heavy metal, as it has been shown to be a significant threat to the environment and to humans in particular. Even if these elements do not have any biological role in the human body, they may be present in other forms that are harmful or sometimes act as pseudo-elements while they may interfere with metabolic functions at certain times. These toxic species are not removed from the body, but rather accumulate in the soft tissues and cause mutagenic reactions and fatal diseases such as cancer, damages to organs, heart disease, and disorder to the nervous system [2]. The dosage that has been absorbed, and the method and period length of exposure to these metals determine the degree of toxicity (acute or chronic). This can cause a variety of problems, as well as severe damage from oxidative stress caused by free radical generation. As a result, several public health interventions have been implemented to manage, prevent, and treat metal poisoning at various levels, including occupational exposure, accidents, and environmental influences [3].

The garment and textile industry is one of the important sectors that meet the daily needs of human beings. The chemicals in textile manufacturers can cause allergenic and toxic effects and may pose a health hazard to consumers. Among these chemicals are toxic heavy metals that are often used in various textile processes, such as dyeing, finishing, and printing [4]. Given the direct interaction of textiles with human skin, textile products have received increasing attention for toxicological and ecotoxicological traits. The assessment of metal content in various textile materials is critical not only for the safety of personnel exposed during the manufacturing process but also for the safety of customers.

Furthermore, some of the distinctive colors characteristics required in the textile industry, such as turquoise, bright green, violet, and blue prohibit the cancellation of the use of metals and complex dyes. Based on that, the content of heavy metals in textiles must be regulated through national or international standards, so that these standards are included in all voluntary environmental labels [5].

Oeko – Tex Standard 100 [6] is one of the most important and well-known international textile testing and certification system, which limits the use of certain chemicals. This certification system was adopted in this thesis. Lead, copper, nickel, and cobalt were the heavy metals studied in this work which have an effective presence in the textile and dyeing industry.

Lead is a heavy metal that is present in trace levels in the earth's crust. However, human activities such as burning fossil fuels, mining, and other manufacturing processes are currently the most significant sources of lead in our environment. Lead is a hazardous element that may linger and continue to be harmful for a long time, affecting nearly every organ and system in the human body [7]. The nervous system is the primary target of lead poisoning. It also causes weakness in the fingers, wrists, and ankles, as well as a rise in blood pressure. Furthermore, prolonged exposure to high levels of lead can harm the kidneys and the brain [8].

Copper is found frequently in rocks, soil, water, and air, and enters the environment from other sources represented by human activities such as fossil fuel combustion, wood production, mining, production of phosphate fertilizers, and other industries, as well as natural occurrences like volcanoes and forest fires [9]. Copper is an essential metal for all

living organisms, as it is involved in many biochemical reactions, formation of red blood cells, and bones, in addition to its critical role in the lipid metabolism processes. Despite its importance, exposure to high levels of copper leads to nausea, vomiting, diarrhoea, gastrointestinal bleeding, high blood pressure, and dermatitis [10]. Therefore, the determination of this trace metal is important because of its many benefits and serious effects on the ecosystem and human health.

Nickel is an essential transition element, with beneficial effects on mammals and plants in small doses as it acts as an enzyme cofactor in human physiology [11]. The mining, fuel, sewage industries, and coal combustion are the main sources of nickel entering the environment. Nickel is used in many industrial and commercial applications such as jewellery, cosmetics, textile dyes, printing, electronics, and batteries. Thus, humans are exposed to nickel for long periods, as nickel accumulates mainly in the skin, hair, organs adjacent to the bones, bones, and heart muscle in the human body [12]. Exposure to high levels of nickel leads to some health problems such as dermatitis, kidney and heart diseases, and some nickel compounds may cause cancer [13]. As a result, determining trace amounts of nickel is critical to decreasing the risk of harmful consequences from nickel exposure.

Cobalt is one of the basic and necessary elements for humans and many living organisms, but on condition that it be in trace levels. Cobalt plays an important role in most of the vital processes in the human body because it is an essential part of vitamin B12, so it contributes to the production of blood cells and the formation of hormones, neurotransmitters, and other compounds. Cobalt deficiency leads to some diseases such as digestive disorders and anaemia, in addition to neurological and muscular problems [14]. Cobalt is used in different industries for various purposes such as metal alloys, glass, dyes, and other industries through which humans are more exposed to high quantities of cobalt. Thus, a person is exposed to some diseases such as asthma, heart disease, vasodilation, and dermatitis [15]. For these reasons, it is necessary to detect cobalt levels in environmental and industrial samples.

The precise and accurate determination of toxic species in samples from the environment is difficult and a major challenge for analytical chemists due to their trace levels. The determination of trace heavy metals carries off by several analytical techniques which are commonly used such as inductively coupled plasma-optical emission spectrometry (ICP-OES), graphite furnace atomic absorption spectrometry (GFAAS), and flame atomic

absorption spectrometry (FAAS). In many laboratories, FAAS is one of the comparatively basic analytical equipment that is readily available and provides precise and accurate findings, however, a sensitive and reproducible enrichment method must be developed to identify and quantify trace levels of toxic heavy metals due to low analyte concentrations, matrix effects, and insufficient instrument sensitivity [16].

There are several separation/ preconcentration procedures used for this purpose the most important of which are solid-phase microextraction (SPME) [17] and liquid-phase microextraction (LPME) [18], which were developed to overcome the limitations that are characteristic of traditional methods like long duration, low efficiency, high use of toxic organic solvents known to be toxic for all living things and the environment, and problems of waste disposal [19].

To achieve these goals, dispersive liquid-liquid microextraction (DLLME) was used [20]. DLLME method is one of the LPME methods which has attracted a lot of attention and is still quite a common preconcentration technique with its easy application and being open to modification. Although chlorine-containing extraction solvents in conventional DLLME [21,22] may supply high recoveries, they are flammable, poisonous, and unstable (volatile) [23].

In the last few years, deep eutectic solvents (DES) have gained attention in the DLLME, especially because of their modifiable properties [24] and as alternatives to traditional toxic extractant solvents. DESs are typically synthesized from two compounds which are hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). HBA may be an ammonium halide as choline chloride and HBD as urea, oxalic acid, phenol or sugar, etc, that interact with each other via hydrogen bonds in a special molar ratio, thus forming a eutectic mixture and the most important feature of the eutectic mixture is that it has a much lower melting point than its constituent components [25]. DESs have many distinctive properties that make them desirable extraction solvents such as high thermal stability, high conductivity, low vapor pressure, low toxicity, and high biodegradability. Moreover, their composition is less complicated, inexpensive, and does not require organic solvents, which reduces its environmental impact [26].

The usage of high volumes of water-miscible dispersive solvents in classical DLLME such as acetonitrile or methanol may lead to an increase in the solubility of analytes in the aqueous solution, thus obtaining a low extraction efficiency. In addition, the large volumes of toxic organic solvents used as dispersive solvents are environmentally unfriendly [27]. Very recently, A new method called effervescence-assisted microextraction (dispersive solvent-free) was developed in 2014 [28]. This method is based on the generation of CO_2 gas caused by the effervescence reaction. Effervescence reaction resulting from mixing of two components as a proton donor source and a carbon dioxide source in a certain molar ratio and making it in a form of tablet or powder according to the terms of the procedure. Thanks to the generated CO_2 bubbles, the extraction solvent is completely and efficiently dispersed in its aqueous solution and the analyte species are transferred to the extraction phase. [29].

In this thesis, Pb (II), Cu (II), Ni (II), and Co (II) were determined in various 100% cotton textile samples using the DLLME method that supported by DES as an extraction solvent, effervescence agent as emulsification strategy, and THF as an aprotic solvent. In this proposed method several parameters that affect the extraction efficiency were optimized. Among them are variables related to DESs like composition, the mole ratio, and volume; the variables related to the effervescent agent like the ratio and type of CO_2 source and H^+ donor, effervescence time, effervescence amount, and adding style and formula. In addition, volume of 8-Hydroxyquinoline as a chelating agent, the volume of THF, and various matrix effects have been also optimized. The accuracy of the method was verified in three different ways: using certified reference material (NCS ZC 73013, spinach leaves), spiking recovery tests, and matrix effect study.

2. GENERAL INFORMATION

2.1. Trace Heavy Metals and Textiles

Metal is a term used to classify elements that have silvery cross-sections and are good conductors of electricity and heat. Since ancient times, metals have been processed by humans and have been used for centuries in the manufacture of jewellery, hunting tools, or many materials used in daily life, without knowing the effects on the environment or themselves. The increased use of metal with the industrial revolution caused an increase in metal pollution, especially in industrial areas, and poisoning began to be seen due to metal pollution [30].

The definition of a heavy metal refers to metals having density higher than 5.0 g cm^{-3}. More precisely, it is the name given to metals with a density 5 times higher than the density of water, which is accepted as 1.0 g cm^{-3}. More than 60 metals are included in this group, such as lead (Pb), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn). Although heavy metals are classified according to their density values, it is not sufficient to determine their biological effects. The biological effect of heavy metal and the severity of this effect depend on the concentration of that heavy metal in the biological system, its chemical structure, its ability of oxidation-reduction and form complexes, and its solubility value. in addition the way it is taken into the body, and the local pH value are other important parameters [30].

Heavy metals are naturally found in the earth crust due to natural and anthropological disasters such as weathering and volcanic eruptions [31,32], and they are in the form of carbonates, silicates, oxides, and sulphur, or as stable or trapped compounds in silicates. However, industrial activities are among the most important factors affecting the spread of heavy metals in nature such as refineries, power plants, Industries specialized in petroleum and its derivatives, plastics, textiles, and etc. [33,34]. Heavy metals naturally mix with water resources by industrial wastes and acid rains, which dissolve the soil and therefore the heavy metal it contains, and these metals contaminate all the water resources (seas, lakes, rivers, etc.). Heavy metals reach humans and animals through the food chain inhalation of the air as aerosols and dust or, by direct contact or absorption through the skin. In addition, the mixing of industrial wastes with water resources causes heavy metals to reach people and other living things.

Nowadays, due to the increase in human daily requirements, heavy metals have been widely used in the environment through several fields such as agriculture, industry, medicine, technology, etc. Accordingly, it raised concerns about its potential repercussions on health and the environment. Some metals such as arsenic, cadmium, chromium, lead, and mercury are the most important metals affecting public health, even at low levels, as they are toxic substances with the potential to damage one or more organs in the human body. The degree of toxicity of these metals depends on several factors such as the dose and method of exposure, in addition to the age, sex, genetics, and dietary habits of the exposed person.

The dyes used in the textile industry are considered one of the main pollutants that have an impact on the environment and humans because they contain toxic heavy metals as metal complexes (reactive dyes) and carcinogenic amines, as well as pentachlorophenol and other harmful chemicals. Heavy metals in textiles are often used as oxidizing agents, dye stripping agents, fastness improvers, or used in finishing processes (odor-protective agents, flame retardants, and antifungals), so they are considered as a source of contamination throughout textile processing [35,36]. Therefore, heavy metals represent a potential danger to customers and workers in the textile sector [37]. The toxic effects of heavy metals on human health are innumerable when present in/on textile materials and have been subject to many scientific studies, so some of the health risks caused by these metals have been briefly listed in Table 2.1.

Table 2.1. Metals from textiles cause health hazards on different biological systems [38].

Metals	Nervous System	Cardiovascular	Gastroenteric	Endocrine	Immune System	Kidney	Liver	Lungs	Blood	Skin
Al	+							+		
As	+		+	+			+	+	+	+
Cd	+	+	+			+		+		
Co	+	+	+	+	+			+		+
Cr			+		+	+		+		+
Cu	+		+						+	
Fe	+		+				+	+		
Hg	+		+			+		+		
Mn	+			+	+			+		
Ni					+			+		+
Pb	+	+	+	+	+	+			+	+
Tl	+		+	+		+		+		
Zn			+						+	

In addition to tumours or cancers, which are one of the most prevalent diseases in our world, one of its causes is the piling up of heavy metals in the body's tissues and their association

with enzymes, which in turn disrupts the work of cells [39]. Therefore, it is necessary to use certain standards for the use of these metals in textile materials, in addition to determining the maximum permissible values.

The most common labels in the textile industry are the Eco - Label and Oeko - Tex Standard 100. The Eco - Label is a voluntary label that sets specific quality standards for textile products [40] as well as sets limits on permissible levels of pesticides, heavy metals, and other toxic substances. The Eco - Label limit values for textiles are given in Table 2.2.

Table 2.2. Limits for heavy metals suggested by Eco-Label standards for textiles (mg kg^{-1}).

Heavy metals	Limit value
Arsenic (As)	0.010
Cadmium (Cd)	0.005
Chromium (Cr)	0.100
Cobalt (Co)	0.200
Copper (Cu)	3.000
Lead (Pb)	0.040
Mercury (Hg)	0.001
Nickel (Ni)	0.200
Zinc (Zn)	3.000

Oeko - Tex is one of the most popular and widely used labels in the textile industry and is approved in scientific researches specializing in textile analysis. It is well designed to protect consumers, as it is divided into several sections, which are products for general use or decorative materials and products that contact the skin directly and indirectly, in addition to a special section for children's products. Oeko - Tex Standard 100 forbids or restricts the use of certain substances that are dangerous to human health [6]. Oeko - Tex limit values for heavy metals were given in Table 2.3.

Table 2.3. Limit values of extractable heavy metals given by Oeko - Tex (mg kg^{-1} textile).

Heavy metals	Baby wear	With skin contact	Without skin contact	Decoration materials
Antimony (Sb)	30.0	30.0	30.0	-
Arsenic (As)	0.2	1.0	1.0	1.0
Lead (Pb)	0.2	1.0	1.0	1.0
Cadmium (Cd)	0.1	0.1	0.1	0.1
Chromium (Cr)	1.0	2.0	2.0	2.0
Cobalt (Co)	1.0	4.0	4.0	4.0
Copper (Cu)	25.0	50.0	50.0	50.0
Nickel (Ni)	1.0	4.0	4.0	4.0
Mercury (Hg)	0.02	0.02	0.02	0.02

-: No value given.

2.2. Chemical Properties, Health Effects and Environmental Effects of Some Heavy Metals That Involve in The Textile Industry

2.2.1. Lead (Pb)

It is bluish-gray in color. Its frequency in the earth's crust is 12.5 g ton^{-1}. Lead is rarely found in free form in nature but is found in the form of a combination with other chemicals such as (PbS, $PbCO_3$, $PbSO_4$). Lead is characterized by flexibility, softness, relatively poor conduction of electricity, as well as good resistance to corrosion, and it also fades when exposed to air [41] . In addition, there are some chemical properties listed in Table 2.4.

Table 2.4. The chemical and physical properties of lead.

Atomic Number	82
Atomic Mass	207.2 g.mol $^{-1}$
Electronegativity According to Pauling	1.8
Density	11.34 g.cm^{-3} at 20°C
Melting Point	327 $^{\circ}$C
Boiling Point	1755 $^{\circ}$C
Vander Waals Radius	0.154 nm
Ionic Radius	0.132 nm (+2); 0.084 nm (+4)
Isotopes	13
Electronic configuration	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$

2.2.1.1. Health Effects

Lead is known as one of the most environmentally harmful metals in human activities. Lead poisoning is also the first known case of metal-related poisoning in history due to its wide applications since 5000 BC [30]. Although it is the most common type of occupational exposure, there are also various cases of exposure due to its widespread prevalence in the world. Lead can enter the human body by accumulating in drinking water at 20% and various foods at 65%, or by inhaling it from the air at 15%. According to the World Health Organization report, lead poisoning accounts for about 0.6% of the diseases that occur in the world [42]. Lead does not perform a primary function when it enters the human body, but harms organs and causes many undesirable effects as shown in Figure 2.1 [43] .
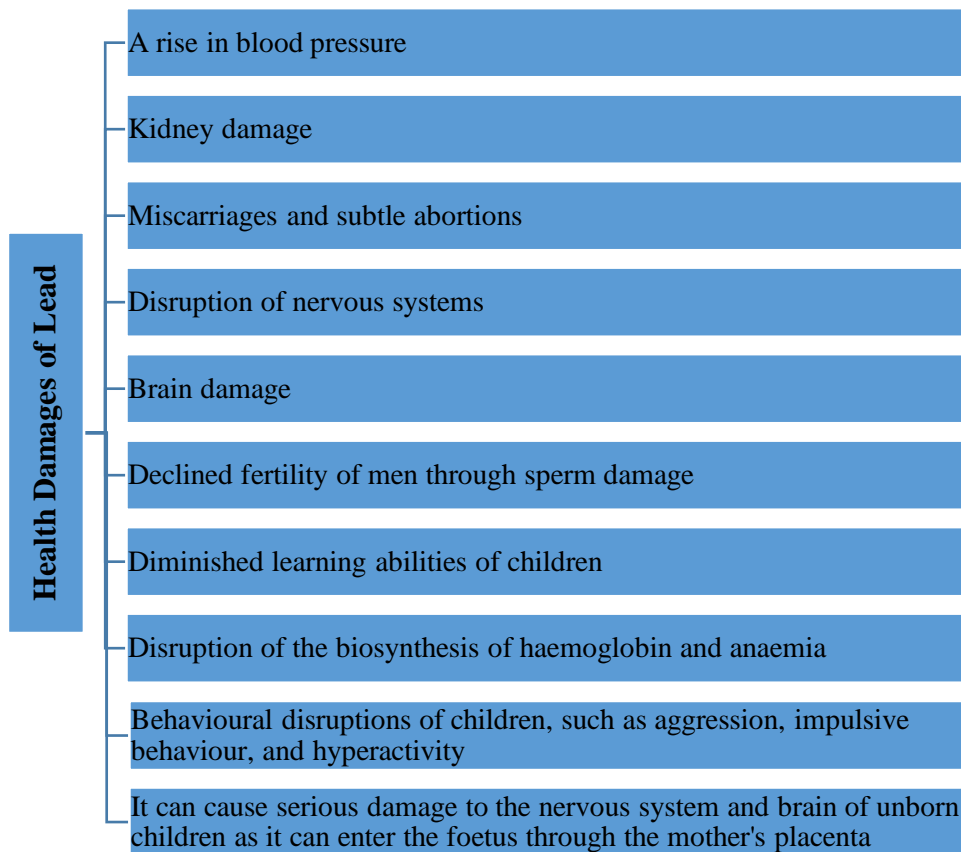
Figure 2.1. The negative effects on human health caused by exposure to lead.

The surest way to prevent its negative effects is to stop the use of lead. However, this situation is difficult due to the use of lead in many areas without alternatives in the developing industry. Therefore, serious precautions should be taken, especially in working conditions [44].

- Necessary engineering measures should be taken to prevent the spread of lead dust and fumes in the working environment,
- Adequate ventilation should be provided,
- Employees should wear special protective clothing and masks,
- Workbenches, shelves, walls, and floors should be washed frequently with water or wiped with a wet cloth,
- Personal hygiene should be given importance,
- The amount of lead in the working environment should not be more than 1.5 mg in 10 m^3 of air.

2.2.1.2. Environmental Effects

As mentioned earlier, human activities are the biggest factor causing heavy metal pollution in the environment in general. It is possible to believe that only gasoline and petroleum derivatives containing lead are the sole cause of high concentrations in the environment, but today industrial waste is the largest cause of lead accumulation in the environment. Lead is used in several industrial fields, including [30,45]:

- As a raw material in paint,
- In food storage containers,
- In the battery industry,
- In pesticides,
- In the fertilizer industry,
- In water pipes,
- Dyes and other main substances in cosmetics,
- In recovering gold in jewellery,
- In the ammunition industry,
- Radiation insulation and cable insulation,
- In children's toys,
- In the manufacture of ceramics and porcelain,
- In the rubber industry

And because the residues of these industries contain lead, it leads to its accumulation in the bodies of all living organisms (human, aquatic and soil organisms). For aquatic organisms, they will suffer from health effects due to lead poisoning, even if it is in low concentrations. When lead interferes with the biological activities of phytoplankton, it can impair their action and thus reduce the source of oxygen generation in the seas. Phytoplankton is the main source of oxygen and a food source for many marine organisms. As well as concerning soil, where soil organisms can suffer from lead poisoning as well, especially in areas near highways due to the combustion of vehicle fuels, as well as soils of agricultural land due to the heavy use of industrial fertilizers and pesticides. Lead is a particularly hazardous chemical since it may build up in individual organisms as well as entire food systems [46].

2.2.2. Copper (Cu)

It is located in the eleventh group of the periodic table conjointly with silver and gold. It has a nice reddish colour with a face-centred cubic crystal structure. Copper is the second most widely used element since the beginning of the industry to the present day, and its use is increasing day by day with the development of the industry. The fact that copper is spread over a wide geographical area on Earth also increases the demand for copper. Although copper is an easily available element, its bioavailability is low. At this point, copper is at the fore in mining exploration programs. It is ranged between 0.005% and 0.015% on the earth and 0.01% on the structure of the earth. At 99.95%, it is the second-best conductor of electricity after silver. Table 2.5 shows some fundamental features of copper [47].

Table 2.5. The chemical and physical properties of copper.

Atomic Number	29
Atomic Mass	63.546 g.mol $^{-1}$
Electronegativity According to Pauling	1.9
Density	8.9 g.cm^{-3} at 20°C
Melting Point	1083 $^{\circ}$C
Boiling Point	2595 $^{\circ}$C
Vander Waals Radius	0.128 nm
Ionic Radius	0.096 nm (+1); 0.69 nm (+3)
Isotopes	6
Electronic configuration	[Ar] 3d^{10} 4s^{1}

It is known that humans used copper to produce various materials about 10,000 years ago. The fact that copper can be found easily and with high purity on earth, and is a strong and corrosion-resistant conductor makes it attractive for use in many industrial fields. The main areas of use are [47];

- Electro-electronic industry
- Coins and guns
- Transportation industry
- Chemicals
- Jewellery
- Paint industry
- Construction industry

2.2.2.1. Health Effects

The effects of copper on the living body have been known for many years. Trace amounts of copper are known as an essential nutrient required for functions of the human body such as carbohydrate metabolism, haemoglobin formation, drug metabolism, catecholamine biosynthesis, and antioxidant defence mechanism [48]. The daily requirement of copper for humans is 2.5 mg. This element may be found in a variety of foods, as well as drinking water and the air. As a result, we absorb enormous amounts of copper every day through food, drink, and breathing. Despite the important role of copper in metabolic functions, when it exceeds an adequate concentration, it causes functional problems in the tissue, organ, or system in which it is involved. According to the report prepared by the World Health Organization, the lethal dose for an adult human being has been determined as 4.0 to 400 mg of copper (II) per body weight [49].

Human exposure to copper through breathing is almost negligible because the air contains low concentrations of it, while people who live near copper ore processing plants or who work in them are more exposed to this type of exposure. In addition, people who live in houses with old water pipe systems may be exposed to high levels of copper through their drinking water by eroding the pipes.

Excess amount exposure of copper leads to its accumulation in various parts of the body such as the brain, liver, and eyes. As a result, the genetic disease known as Wilson's disease appears. With the development of the disease, serious problems with the liver, hepatitis B virus, and diseases such as cirrhosis of the liver were observed. Copper accumulated in the brain directly affects the central nervous system, exerts a mutagenic effect, and causes Alzheimer's disease and Parkinson's disease. Cases of acute copper poisoning are rare but show symptoms such as increased salivation, stomach pain, nausea, and diarrhoea due to irritation of the gastrointestinal mucosa. The copper concentration determined as the lethal dose in acute poisoning is 100 mg kg^{-1}. Up to 600 mg kg^{-1} can be treated, but in larger amounts, coma and death occur. Occupational exposure is common. The presence of copper in the workplace leads to an influenza-like illness called metallic fever [50].

2.2.2.2. Environmental Effects

As was mentioned, copper is included in a wide range of different industries, so industrial waste is considered one of the main sources of copper in the environment. Copper can travel

long distances through running water after its casting. But when it enters the soil, it binds to other materials and remains fixed in its place, meaning that it does not move far after its release, and even rarely moves to groundwater.

The lack of plant diversity is observed in the lands near factories whose waste contains copper because copper does not decompose in the environment, as it accumulates in plants when it is found in the soil rich in it. Therefore, copper is considered a serious threat to the production of agricultural lands. Additionally, when the soil of farmland is contaminated with copper, animals and livestock absorb levels that may be harmful to their health. [51].

On the other hand, copper is toxic to small creatures as it is used against insects and agricultural slugs as a fungicide, antibacterial agent, pesticide, and biocide. Despite this, copper-containing pesticides and fertilizers are still used [9].

2.2.3. Nickel (Ni)

It is a metal with a shiny silvery-white color, and belongs to the transition metals group, meaning that it has valence electrons in two orbits instead of one, which enables it to form different oxidation states. It is one of the four elements that are considered to have a high magnetic permeability (it magnetizes easily) at room temperature and normal pressures. Nickel is hard and ductile, and it is an acceptable conductor of electricity and heat [52]. Table 2.6 shows the chemical and physical properties of nickel.

Table 2.6. The chemical and physical properties of nickel.

Atomic Number	28
Atomic Mass	58.71 g.mol $^{-1}$
Electronegativity According to Pauling	1.8
Density	8.9 g.cm^{-3} at 20°C
Melting Point	1453 $^{\circ}$C
Boiling Point	2913 $^{\circ}$C
Vander Waals Radius	0.124 nm
Ionic Radius	0.069 nm (+2); 0.06 nm (+3)
Isotopes	10
Electronic configuration	[Ar] 3d^8 4s^2

It is naturally present in the Earth's crust at a level of about 0.0075%, and is an element of moderate toxicity compared to other transition metals. Although the metal nickel is rarely found in nature, it is often found with sulphur and iron in the form of complex compounds. Most nickel compounds are blue or green. It is usually found in two main locations: red

sandstone rocks formed as a result of intense erosion of the surface of nickel-rich rocks, and the second location is molten sulphate deposits. Nickel is resistant to external influences due to its magnetic properties. For this reason, it is used in the electrolytic coating of goods, to obtain alloys with high corrosion resistance even at high temperatures, in the production of steel and special coins. It is also used as a catalyst in hydrogenation reactions in batteries, accumulators, paints, glass and ceramics to give glass a green color [52].

2.2.3.1. Health Effects

Nickel is generally found in industrial wastewater where it is used. Although the effect of nickel on humans has not been fully determined, it has negative effects on human life. It is transmitted to humans by inhaling air, drinking water, eating food, or smoking cigarettes. Absorption of very large amounts of nickel has some undesirable health effects as shown in Figure 2.2 [53].
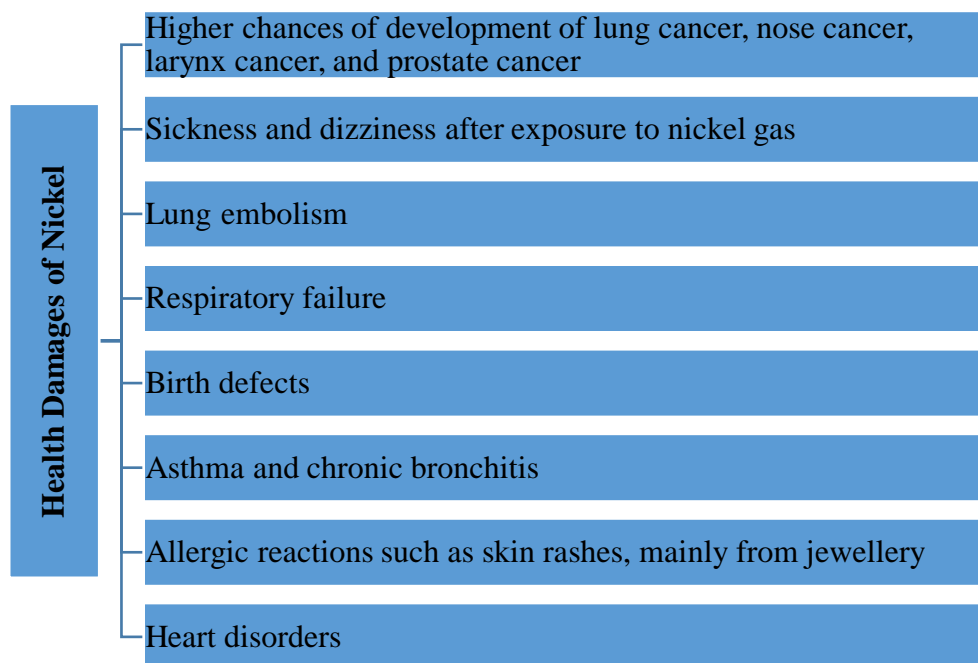
Figure 2.2. The negative effects on human health caused by exposure to nickel.

The International Agency for Research on Cancer has designated all nickel compounds, with the exception of the metallic nickel, in the category of carcinogens. People working in jobs such as nickel plating, nickel electroplating, and welding are exposed to the most serious health problems. Workers who inhale small amounts of nickel dust are at increased risk for lung cancer, fibrosis, and other diseases [54].

2.2.3.2. Environmental Effects

Nickel is a pollutant that can be released into the atmosphere as a heavy metal, widely used in everyday equipment and many areas as industrial activities progress. Therefore, it has caused a great deal of environmental pollution through direct pollution of air, soil, and bodies of water by power plants, garbage incinerators, and other sources, where it settles on the ground or falls after reactions with raindrops. The bulk of all nickel compounds released into the environment are absorbed into sediments or soil particles and become immobile as a result.

Given the ever-increasing consumption, pollution with heavy metals affects not only agricultural products, but also the quality of the atmosphere in the food cycle, environmental pollution from nickel-containing products, and thus human and animal health.

While the main source of nickel in drinking water originates from metal pipes and fittings in contact with drinking water, the concentrations of nickel in groundwater vary with soil type and pH. In areas where industrial activities are intense, it has been determined that there are higher concentrations of nickel in drinking water. On the other hand, high nickel concentrations in sandy soils may cause obvious damage to plants, and algae growth rates may be reduced in surface waters containing high levels of nickel [55].

2.2.4. Cobalt (Co)

It is a member of the ninth group of the periodic table. It is a hard, brittle, bright, and bluish substance. It is a ferromagnetic metal, similar to iron and nickel in their physical properties. Cobalt ranks 33rd among all the elements on Earth. While the cobalt on Earth is about 20 mg kg^{-1}. It is obtained as a by-product from ores of copper, nickel, silver, gold, lead, and zinc. Cobalt exists in different valence states (0, 2$^+$, and 3$^+$). Cobalt is a powerful oxidizing agent and because cobalt salts are highly soluble in water, it is attacked by air oxygen and water vapor, where it oxidizes to cobalt (II). It is a non-fusible metal, chemically reactive. Although dilute acids can destroy it slowly, Cobalt is stable in air and unaffected by water [56]. Table 2.7 shows some of the chemical and physical properties of this element.

Table 2.7. The chemical and physical properties of cobalt.

Atomic Number	27
Atomic Mass	58.9332 g.mol $^{-1}$
Electronegativity According to Pauling	1.8
Density	8.9 g.cm^{-3} at 20°C
Melting Point	1495 $^{\circ}$C
Boiling Point	2927 $^{\circ}$C
Vander Waals Radius	0.125 nm
Ionic Radius	0.078 nm (+2); 0.063 nm (+3)
Isotopes	8
Electronic Configuration	[Ar] 3d^7 4s^2

Cobalt minerals are found in concentrations high enough to support economic extraction in a variety of environments, each showing very different patterns of mineralization. The most common metallic groups are sulphides, sulphides, arsenic, and oxides. There are many uses for cobalt in people's lives; Among the most important are the following [57]:

- It used to make powerful magnets,
- Because of the resistance of its alloy to high temperatures, it is used in the production of jet turbines and gas turbine generators,
- It is used to make dye pigments,
- Because of its corrosion resistance, it is used in electroplating
- It is used in paint, porcelain, glass, pottery, and enamel,
- Cobalt 60 is used to treat cancer,
- It is also used to preserve some types of food, and is also used to sterilize medical and food supplies.

2.2.4.1. Health Effects of Cobalt

Cobalt is considered one of the beneficial elements for the health of the human body, its deficiency causes anaemia, fatigue, indigestion, and muscle problems. These symptoms can be eliminated by taking enough vitamin B12 (cyanocobalamin) [58].

Due to the widespread of cobalt through its wide applications in the environment (natural and industrial), humans may be exposed to high levels of cobalt that exceed the specified limits by inhaling air, drinking water, and eating foods containing cobalt. Occupational exposure is considered one of the most dangerous types of cobalt exposure because it lasts for a long period of time, which causes major health problems.

When exposed to cobalt for occupational reasons, the respiratory system is primarily affected. In addition, exposure through the skin is also important and can cause contact dermatitis. Figure 2.3 illustrates the health effects of absorbing high concentrations of cobalt for a long period of time.
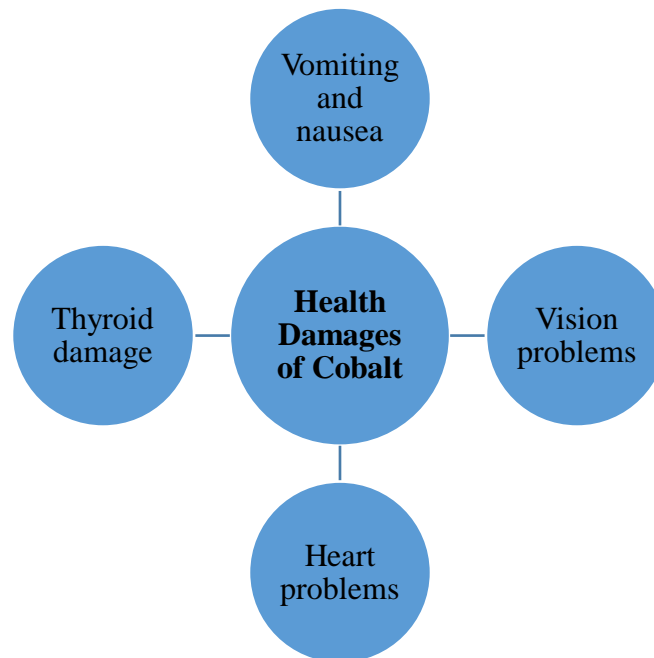
Figure 2.3. The negative effects on human health caused by exposure to cobalt.

Pulmonary fibrosis is one of the most important diseases closely related to chronic exposure to inhalation of cobalt dust and leads to death as it is known as solid metal disease. In addition, there are also other symptoms such as weight loss, dermatitis, and hypersensitivity in the respiratory tract that occur due to overexposure to cobalt and its compounds [59].

2.2.4.2. Environmental Effects of Cobalt

It is also known that environmental pollution depends mainly on human activities represented in the industrial field to a large extent. Cobalt is the same as the rest of the heavy metals that are present in the residues of these industries, such as mining, sewage waste, coal combustion, and ores processing, in addition to the production and use of chemical compounds that contain cobalt.

The concentration of cobalt is high in places near and adjacent to industrial areas. Where water, air, and soil are polluted, and thus the danger of pollution is transmitted to humans, plants, and animals, directly or indirectly [60].

2.3. Preconcentration Methods

The preconcentration process (enrichment) is a set of methods, generally, separation-based methods that are applied when the analyte in the sample is at levels too low to be determined by analytical methods. The preconcentration process contributes to:

* Removal of the main components by keeping the trace components in the solution,
* The trace components are removed from the solution and the main components left in the solution,
* Separation of trace components from other trace components.
* The preconcentration aimed to ensure that the analyte contained in the sample reaches detectable levels [61]. Preconcentration processes are often used in the determination of trace elements due to their advantages and can be listed as follows:
* By increasing the trace element concentration in the medium, the detection limit of the applied method is reduced and the sensitivity of the method is increased.
* By removing the trace element from the matrix, interference from the environment is prevented.

The incorporation of a trace element into a known matrix leads to facilitates the simulation of matrices in real sample analysis.

There are numerous preconcentration methods, some of which are selective for only one or a few elements or compounds, while others have a wide range of applications. These methods are consisting of ion exchange, co-precipitation, evaporation, adsorption, electrodeposition, extraction methods.

2.3.1. Ion Exchange

It is based on the principle that the electrons of the solid material (a resin) are replaced by an equivalent number of other ions present in the surrounding solution according to the law of preservation of mass (physicochemical process). There are two types of solid materials through which ion exchange is achieved, which are natural and artificial. Factors including reusability, ion exchanger selectivity, and the functional group types associated with the ion exchanger should be considered while using this method. The method is frequently used in water purification, wastewater treatment, energy conversion and storage, redox reactions, and various electrochemical methods [62].

2.3.2. Co-Precipitation

It is the separation process based on the solubility differences of the compounds in the aqueous solution. Precipitation takes place in the form of entrapment, mixed crystal formation, or adsorption considering the physical and chemical properties of the trace element to be analyzed and precipitator together. It should be noted that the percent purity obtained from this process will not be very high, but is sufficient for most analyses. The most important point to be considered when choosing a precipitator is that the precipitator is not similar to the target analyte in terms of chemical properties. Otherwise, mixed crystal formations may precipitate.

While applying the co-precipitation method, some negativities may be encountered. These are;

- the method is very time-consuming because many processing steps are required so, the element is lost during operations to considerable amounts,
- the use of many reagents leads to the occurrence of contamination,
- some chemical interference occurs if the precipitate does not dissolve [63].

2.3.3. Volatilization

The principle of this separation process is based on removing the more volatile main component or trace element from the medium by evaporation. Water, an organic solvent, and volatile acid, or an ammonia solution are the major ingredients.

In order for the method to be applicable, the volatility difference between the components should be quite large. Although the most important advantage of the method is that there is no need for high volumes of reagents for the enrichment process, the application area is very limited due to the high probability of analyte loss. However, in some cases, the process can be carried out by taking measures to reduce the volatility of the trace element.

2.3.4. Electrodeposition

The use of this method is appropriate in the analysis of samples with very low concentrations. When using this procedure, the electrode is selected primarily based on the element to be enriched, this electrode is immersed in the solution to be analyzed and it has waited for a

while until the elements are collected on this electrode. Thus, the enrichment process is performed.

2.3.5. Adsorption

Separation process that is based on the bonding of molecules or ions from a gas, liquid, or any solution with a solid surface depending on the contact forces of the molecules or ions on this surface. In other words; It is the increase in the concentration of one substance in another at the interaction between two phases. It occurs in three different ways: physical, chemical and change. It has an important role, especially in liquid-liquid interactions in biological organisms. Therefore, it is often preferred for the determination of bio-organic substances [64].

2.3.6. Extraction Methods

Extraction is a separation and purification process, which is the separation of one analyte or more from the dense matrix (solid, liquid, or gas) in the same sample. Employing extraction, the determination of the analyte can be performed away from the interference effects of other components in the sample. Extraction methods; It is divided into two conventional main groups as solid-phase extraction (SPE) and liquid-liquid extraction (LLE) [65].

- **SPE:** SPE is carried out with sorbents composed of silica or organic resins that have convenient physical and chemical characteristics. The parent material's nature, as well as additional functional groups, is influenced the way solids are used. In any case, solid materials are three-dimensional materials produced under conditions designed to provide a very porous yet rigid material with a high surface area. The working principle of this method is based on passing the liquid sample through the solid materials which are in the form of columns or discs. After the entire sample is kept on the solid phase, the analytes are recovered by elution solution with a suitable solvent where separation is done. This method is very easy and simple to implement. It also has great advantages such as very low final volume and very high enrichment. For these reasons, its use is widespread.
- **LLE:** The basis of the method is the separation of elements or compounds in two immiscible liquid phases according to their relevance to these two phases, depending on their solubility differences. In general, its phases consist of water and an organic solvent immiscible with water. It is a frequently preferred method not only for separation but

also for the purification of many organic substances. Due to its very simple use, it has widespread use, especially in metal determinations. With the help of a complexing agent, the ions in the aqueous phase are converted to less soluble species in water, and their transfer to the organic phase is ensured. As a result of this; The separation process and the enrichment process are carried out simultaneously.

2.4. Microextraction Techniques

The sample preparation stage is of great importance, especially for quantitative determinations. The primary purpose here is to eliminate the interference effect as much as possible and to increase the signal of the sample by increasing the concentration of the target analyte. LLE and SPE methods are the most common traditional preconcentration processes and the most preferred. However, conventional preconcentration methods involve expensive, lengthy, and laborious procedures that requiring huge amount of toxic organic solvents. Miniaturized extraction techniques used in recent years have been a way out to reduce/eliminate the handicaps mentioned. The developed methods are semi or fully automated procedures that are faster, require fewer volumes from samples and extractants compared to conventional methods. Microextraction methods consist of two main groups as liquid and solid phase [66,67].

2.4.1. Solid-Phase Microextraction

SPME, the most popular way of sample preparation, was developed by Pawliszyn as a solvent-free method (1990) [67]. This method provides simultaneous extraction and enrichment of analytes from aqueous samples. SPME in chemical analysis is widely used by working on a broad spectrum of samples that are present in different physical environments, characterized by a complex matrix composition, for example environmental, biological, and food samples. Despite the advantages of this method such as simple application and the absence of organic solvents, most commercial adsorbents used in SPME are relatively expensive and fragile, and their lifetimes are limited. Also, it has poor reproducibility and sample transfer is a possible shortcoming.

SPME is a valuable advance in sample preparation and has several advantages over conventional techniques. One of its most important features is: It does not require organic solvents, which are often expensive and harmful to health and the environment. The technique is simple and fast, the entire extraction and analyte transfer process usually takes

only a few minutes and is easily automated, portable. The shortcomings have led to the development of liquid-phase microextraction (LPME) [68].

2.4.2. Liquid-Phase Microextraction

The basis of LPME is the transfer of analytes in the aqueous sample into a water-immiscible solvent with a volume as small as a few microliters. LPME methods are basically miniaturization of LLE by using a few microliters of solvent to separate the analytes from the complex matrix medium, resulting in a reduction of the potentially toxic exposure of the analyst. Because of the minimal consumption of organic solvents and the high enrichment factor (EF), LPME may be regarded as a more popular approach than SPME. in addition to being easy to implement and use, generally fast, and characterized by its availability and reliance on affordable devices or materials. It is also versatile and has high automation potential [69].

Many LPME methods have been developed which are illustrated in Figure 2.4. These methods overcome many of the disadvantages of traditional methods, which are both fast and inexpensive. On the other hand, new modalities are continually being developed in addition to the main methods. Figure 2.5 depicts the main temporal stages in the development of LPME approaches.

Figure 2.4. A schematic presentation of the existing LPME methods.

Year	Development
1995	• First drop-based system
1996	• Solvent microextraction into a single drop
1997	• Single drop microextraction • Microsyring as a holder • Dynamic LPME
1998	• LLLME
1999	• Use of fiber in LPME (HF-LPME)
2001	• Headspace (HS-SDME)
2003	• IL base as the extracting agent
2004	• solvent-bar microextraction (SBME) • Deep eutectic solvent (DES)
2005	• Water used as the solvent in LPME
2006	• Ultrasound as a factor supporting LPME • DSDME • Dispersive liquid–liquid microextraction (DLLME)
2007	• Microwave radiation as a factor supporting LPME • Automation of SDME • Liquid-phase microextraction method based on solidification of floating organic drop (SFODME)
2008	• Combining LPME with FAAS
2009	• Using IL in LPME and dispersion of analytes by thermal desorption device
2010	• DLLME-SFOD • Solvent-terminated DLLME (ST-DLLME) • Supramolecular solvents
2013	• Sensitive analytical technique for arsenic compounds based on SDME coupled in-line with CE was developed.
2014	• Effervescence assisted dispersive liquid-liquid microextraction (EA-DLLME) • Magnetic ionic liquid-based dispersive liquid-liquid microextraction (MILs)
2015	• Dispersive liquid-liquid mcroextraction based on deep eutectic solvent (DES-DLLME) • Switchable-hydrophilicity solvent liquid-liquid microextraction (SHS-LLME)
2017	• First study on UA-DLLME for the analysis of human whole blood • Magnetic deep eutectic solvents for ultrasound-assisted liquid-liquid microextraction (UALLME-MDES)
2018	• Magnetic ionic liquid-based stir bar dispersive liquid microextraction (SBDLME) • Effervescence-assisted switchable fatty acid-based microextraction with solidification of floating organic droplet • Deep eutectic solvents as a new kind of dispersive solvent for dispersive liquid-liquid microextraction
2019	• CO2-controlled switchable hydrophilicity solvent and solidification of the aqueous solution (CSHS-SAP) • Magnetic effervescent tablet-assisted ionic liquid-based dispersive liquid-liquid microextraction (META-IL-DLLME)
2020	• In-syringe dispersive liquid-liquid microextraction using deep eutectic solvent as disperser • Homogenous liquid-liquid extraction followed by dispersive liquid-liquid microextraction

Figure 2.5. Most relevant developments in LPME [70].

2.4.2.1. Single Drop Microextraction (SDME)

The first development of this method in 1996, where uses a single immiscible drop of organic solvent that can be suspended at the tip of a micro syringe needle immersed in the aqueous solution by continuing to stir throughout the extraction process. After the extraction is complete, the micro drop is withdrawn into the micro syringe and analyzed by injecting it into analytical instruments such as capillary electrophoresis, GC or HPLC, ETAAS, ICP-MS for direct analysis. This approach is regarded as the fundamental LPME technique because it provides an appropriate strategy for preconcentration and matrix separation prior to detection [71]. One of the most important reasons for the widespread of this technique is that the system is non-complicated, cheap, and easy to implement, a very little solvent is used, and derivatization can be performed. The disadvantages are, the small amount of extraction solvent used does not have enough surface area to collect the analytes, the extraction kinetics is low, and therefore the extraction process is slow. It is not preferred in devices that require large volumes of samples [72].

Various analytical applications have been developed by the researchers as depicted in Figure 2.6 to increase the steadiness of the micro drop, enlarge the method's applicability range, supply better automation, and compatibility with additional analytical techniques. Figure 2.7 shows the schematic diagram for each mode of SDME.
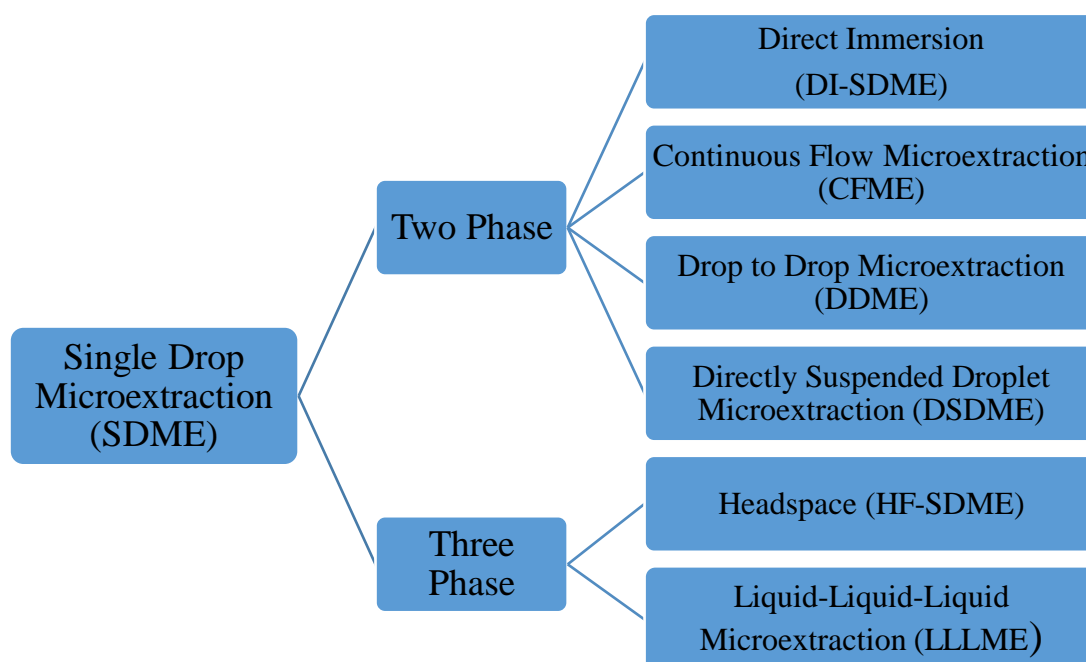
Figure 2.6. Single drop microextraction classification.
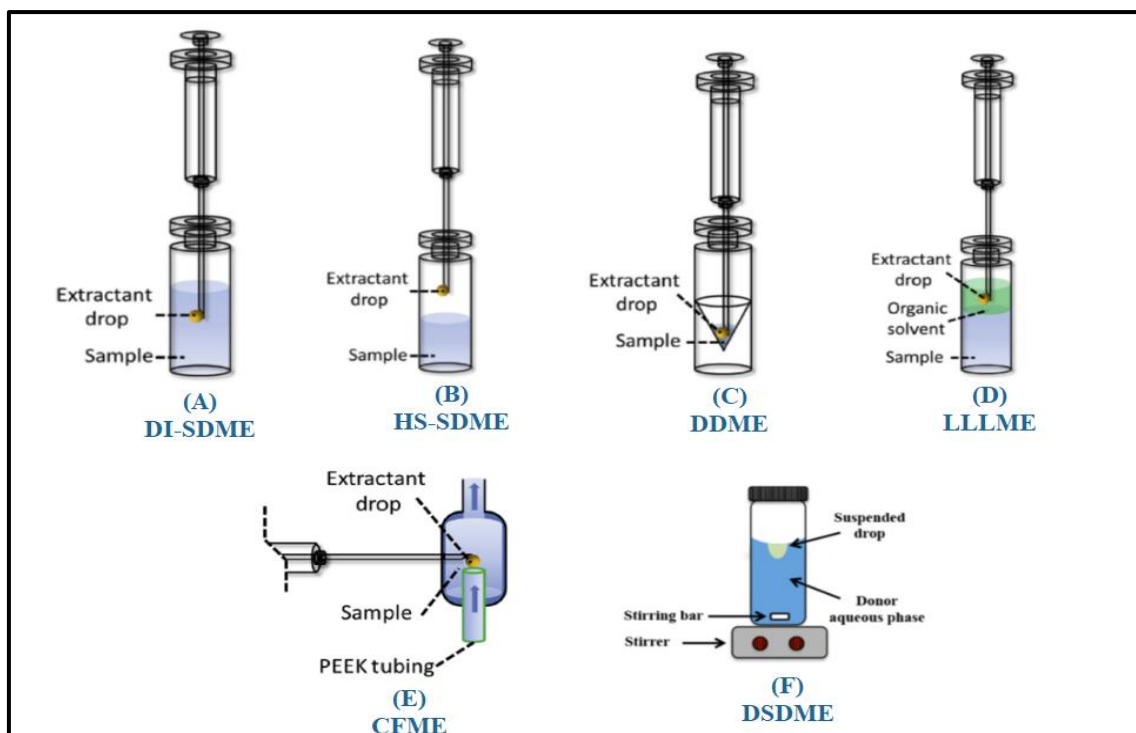
Figure 2.7. Different modes of SDME. (A) Direct immersion SDME, (B) Headspace SDME, (C) Drop-to-drop microextraction, (D) Liquid-Liquid-Liquid microextraction, (E) Continuous-flow microextraction and (F) Directly suspended droplet microextraction [73].

2.4.2.2. Cloud Point Extraction (CPE)

It is a process generally used for the pre-concentration of an analyte solution (metal, organic, or inorganic) from an aqueous phase sample and determined by using spectroscopic or UV-Vis techniques. This method involves reacting an analyte present in an aqueous sample solution with an organic ligand in a surfactant-containing medium to form hydrophobic species. Surfactants replace the toxic organic solvents used in traditional extraction methods as they are characterized by being low toxicity, low volatility, as well as non-flammable and thus reduce the risks of the extraction process. Then the temperature of the solution is increased to reach the cloud point temperature (CPT), where the solution becomes cloudy as a result of the formation of micelles. Micelles are organized structures that contain the analyte that is trapped by the surfactant. As a result of the different densities of micelles and aqueous solutions, the solution separates into two phases, the first being the weaker phase with the surfactant, and the second being the stronger phase with the surfactant (containing the micelles). Finally, the extraction process is carried out similar to the steps of traditional liquid extraction. The general method of cloud point extraction is presented schematically in

Figure 2.8. As an enrichment method; The CPE method has several advantages over conventional liquid (or solid-liquid) extraction techniques in terms of experimental convenience, relatively low-cost, negligible environmental pollution, use of relatively low concentrations of low-toxic surfactant instead of organic solvents, with a high enrichment factor of analytes with very different structures and high recovery rates [74,75].
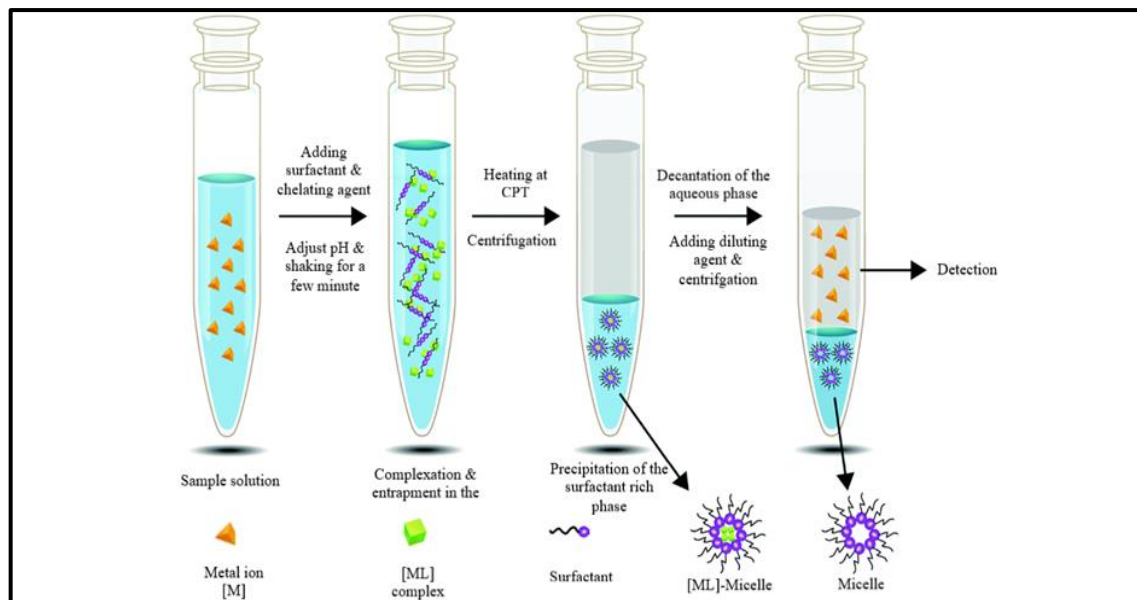
Figure 2.8. schematic presentation of CPE [76].

2.4.2.3. Hollow Fiber Liquid Phase Microextraction (HF-LPME)

In 1999, Pedersen-Bjergaard and Rasmussen applied the HF-LPME method for the first time in the methamphetamine study for solving the problem of the single drop instability. When the used method is considered in general terms; Firstly, the porous hollow fiber (polypropylene) is kept in a low polarity organic solution for a short time. This organic solvent is trapped in the pores of the immersed hollow fiber structure and a thin film layer is formed. The extraction solvent forms the acceptor phase in the fiber and is attached to the tip of the injector. After this stage, the fiber filled with the acceptor phase is kept in the aqueous solution for a short time and thus the analytes are extracted in the aqueous solution by transiting to the acceptor phase in the fibers. The presence of the extraction solvent in the hollow fiber increases its stability during extraction. The extraction solution is then removed with a micro syringe and transferred to the final instrument for analysis [77].

The disadvantages of the method are the long extraction process, and the negative effects of air bubbles formed on the surface of the fibers on the extraction efficiency and reproducibility. Fiber exposure to the sample can be done in three different forms that illustrated in Figure 2.9.
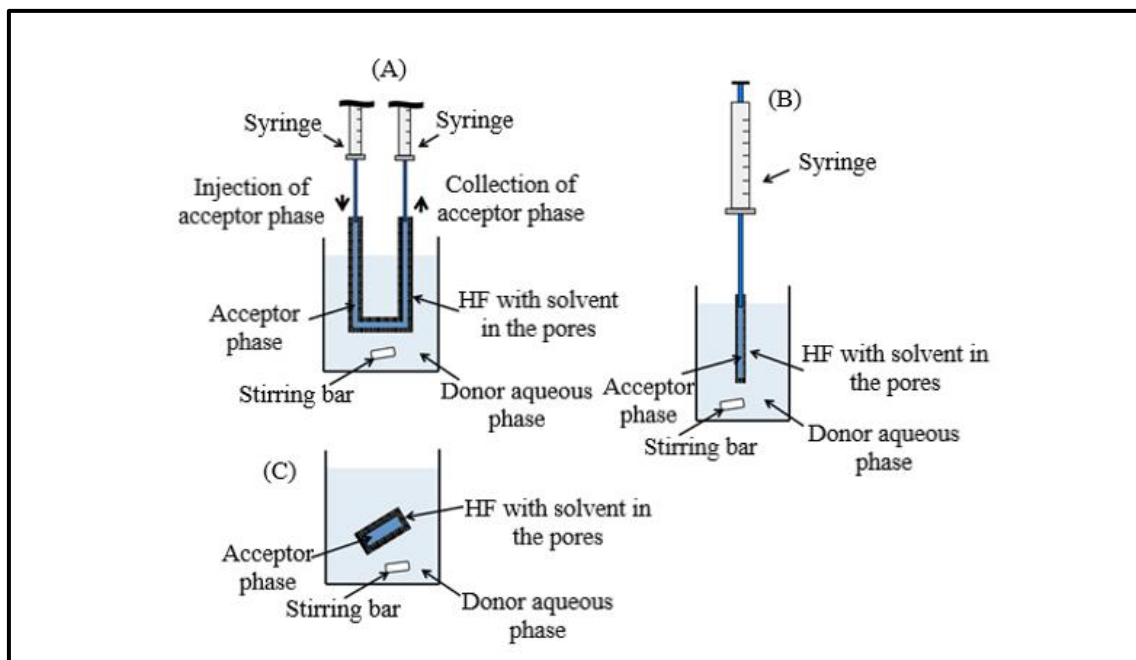
Figure 2.9. Different arrangement of HF-LPME: (A) U-shaped; (B) rod-like; and (C) solvent bar microextraction [78].

2.4.2.4 Solidified Floating Organic Drop Microextraction (SFODME)

One of the objectives of this method is to use the lowest possible volume of the organic solvent, as well as to eliminate the defects of some techniques such as SDME represented by the difficulty of taking the micro-drop from the injector, and its easily separating off from the injector tip by the force of gravity. Therefore, this method was developed based on the ease of collecting the organic phase from the aqueous solution [79].

This method is summarized by adding a small volume of an organic extraction solvent that has a melting point close to room temperature and is also immiscible with water, to the solution containing the analyte to be determined. By applying continuous stirring to ensure that the analyte is transferred to the extraction solvent as much as possible, the organic phase separates and floats in drop form on the surface of the solution. Then the entire solution is immersed in an ice bath to freeze the organic phase, which is removed with the help of a

spatula and sending it to the system where the analyte will be determined at room temperature.

Although the method can be used in the determination of metal complexes, it is not preferred much because of the need for preliminary cleaning and limitations in solvent selection [80]. The application of the method is shown schematically in Figure 2.10.
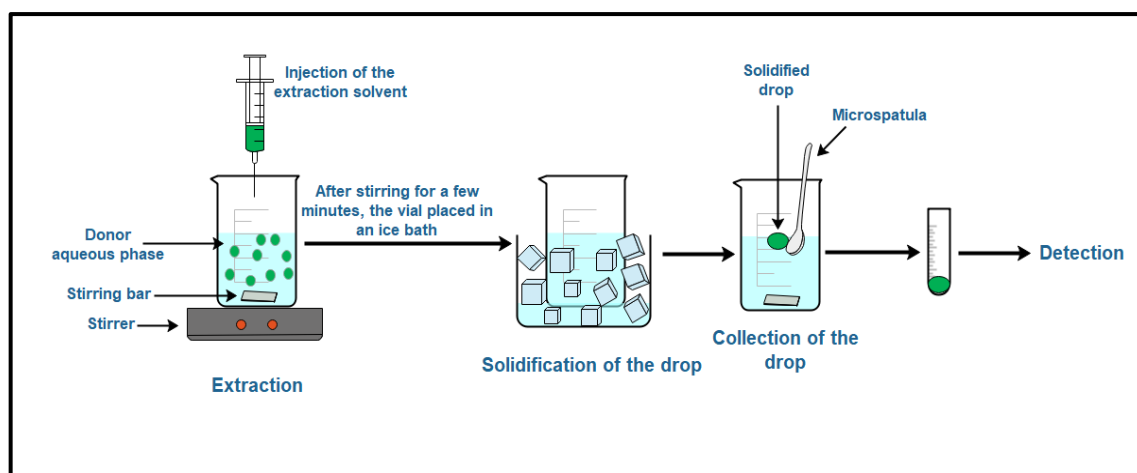
Figure 2.10. SFODME procedure.

2.4.2.5. Dispersive Liquid-Liquid Microextraction (DLLME)

Compared with the old classical methods, the DLLME method is a method that allows it to be used at miniature levels, where the consumption of the extraction solvent is much lower and the enrichment factor obtained is higher. The amazing aspect of this method is that it is very fast, low cost, and easy to apply [81,82] . Because of these advantages, it is a modern extraction method used by researchers in many fields such as pharmacology, toxicology, engineering, agriculture, and modern analytical chemistry [83].

The conventional method conception is based on three constituents solvent system that includes a disperse solvent, an extraction solvent, and an aqueous phase sample containing the analyte. The deciding factor of this method is the selection of a suitable dispersion solvent as it must be miscible with both water and the extraction solvent. In addition, the extraction solvent must be immiscible with water. A mixture of the dispersion solvent and the extraction solvent is prepared and rapidly injected into the aqueous sample. By means of the dispersion solvent, a cloudy solution is formed in which the extractant is broken down into small droplets allowing to obtain a high surface area (the equilibrium state is achieved

quickly), and therefore a high reaction with the sample and high extraction efficiency [20] [84] . As mentioned above, the extraction solvent is not miscible with water, therefore, after the formation of a cloudy solution, it can be separated with the help of a centrifuge then, it can be analysed using a suitable instrument. Figure 2.11 show the described procedure as a scheme.
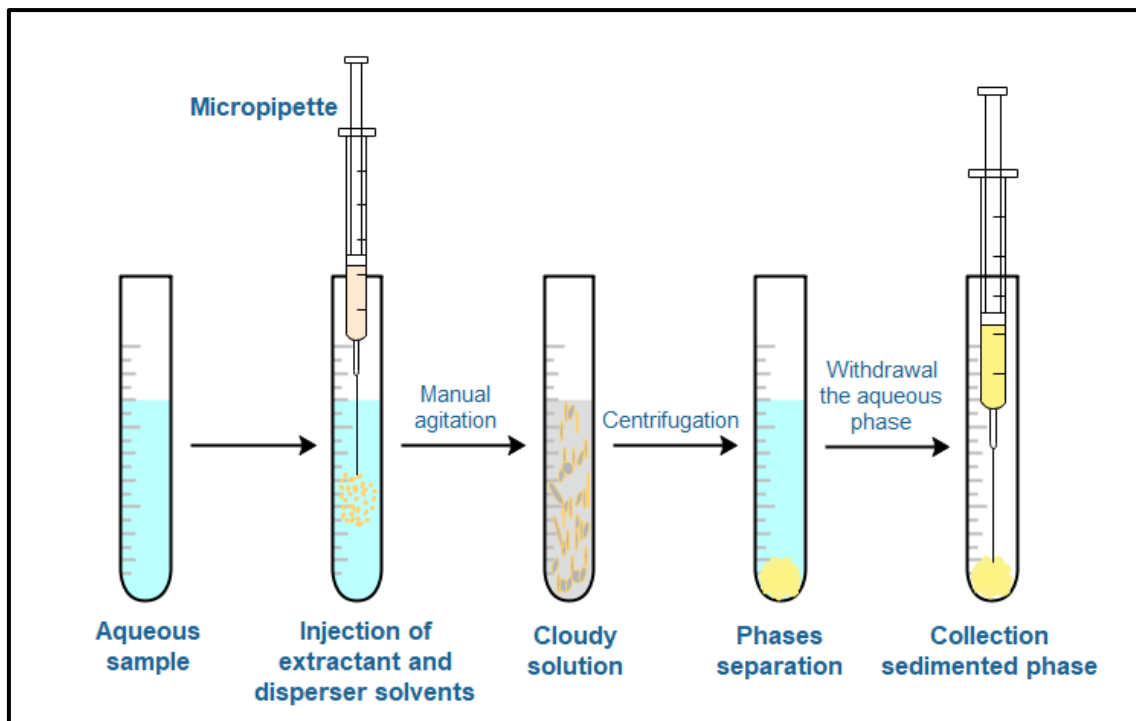
Figure 2.11. General procedure of classical-DLLME.

2.5. Advancement in The DLLME Method

Although quick and simple analyses can be performed with DLLME, better environmentally-friendly behavior can be achieved by adding an extractant solvent with different properties as an alternative to the chlorinated solvents adopted in this method. However, a relatively large volume of disperser is required, which not only affects the consumption of organic solvents but also reduces the extraction efficiency to some extent by lowering the partition coefficient to the extraction solvent. Therefore, to solve the aforementioned technological limitations and offer efficient, simpler, and more ecologically friendly alternatives, several improvements to the traditional DLLME have been proposed. Figure 2.12 gives a summary of recent advances.
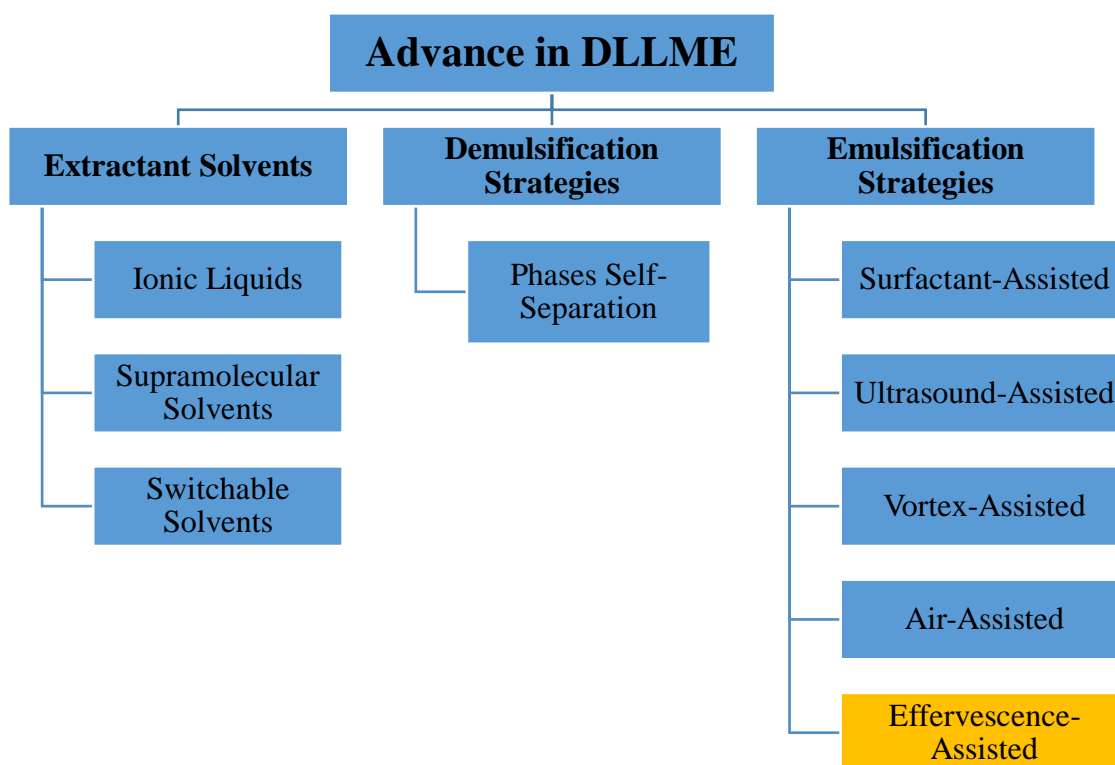
Figure 2.12. Recent advances in DLLME.

2.5.1. Extractant Solvents

According to the principles of green chemistry, new strains of solvents with different physical and chemical properties have been used instead of the classical solvents (chlorinated) that were using for liquid-phase extraction. The Ionic liquids (IL), supramolecular solvents (SUPRAs), and switchable solvents (SSs) are the most common of these solvents. One of the most important features of these solvents is that they are non-toxic and thus do not cause any harm to the environment and do not generate waste. Although used in small amounts (at microliter levels), they can achieve impressive quantitative results. In addition, they have a good ability to extract the analyte without needing a long time for application.

2.5.1.1. Ionic Liquids (IL)

In traditional extraction methods; Many extraction methods have been developed within the scope of green chemistry to minimize the consumption of high rates of toxic organic solvents that produce secondary wastes during the extraction. In this context, the use of highly toxic solvents is avoided or at least minimized. This offers the development of new solvents and different application areas in terms of environmental and economic aspects. One of these

solvents is the IL. IL consists of a group of organic ionic salts present in a liquid state at a low temperature (room temperature or less) [85] . The advantages of these solvents that distinguish them from traditional organic solvents are [86] :

- Its high thermal stability,
- Its vapor pressure is immeasurably low,
- Its viscosity is immiscible with both aqueous and organic solvents,
- Its flammability is almost non-existent,
- Its high ionic conductivity.

Because of these properties they have been widely examined as solvents and have been increasingly used in extraction studies [87] . Deep eutectic solvents are a relatively new class of ionic liquids analogs that have many similar characteristics that will be discussed in section 2.6.

2.5.1.2. Supramolecular Solvents (SUPRAs)

They are among the new generation environmentally friendly solvents, emerging as a substitutional to conventional extraction studies in terms of organic solvents. These solvent systems are water-immiscible solvents in a continuous phase of dispersion, consisting of colloidal solutions of surfactants with effects such as electrolyte effect, temperature effect, and acidity effect (pH). Supramolecular solvents are molecular and nano-sized and consist of polar and nonpolar parts. Different polarity parts of these solvents can interact with analytes, and the interactions can be controlled by altering the polarity of the amphiphilic groups [88] .

The adding of SUPRAs to the medium in extraction experiments leads to an increase the efficiency even it is in very small volumes. It is seen that the extraction studies with SUPRAs are similar to the cloud point extraction studies in terms of the solvents used. SUPRAs are obtained by combining long-chain molecules such as hydroxyl or carboxylic acid with species such as tetrabutyl ammonium and tetrahydrofuran. Supramolecular solvents are formed by three separate mechanisms: aqueous micelle formation, reverse micelle formation, and vesicle formation.

Extraction studies with SUPRAs have advantages such as higher surface area and higher recovery efficiency, thanks to the formation of nano-sized micelles when compared to cloud

point extraction. Once more, this microextraction method stands out with its features such as minimizing the amount of organic solvent used and the most important advantage of the method is the preparation of supramolecular solvents at room temperature using cheap and harmless chemicals [89].

2.5.1.3. Switchable Solvents (SSs)

Switchable solvents, also called as "smart" solvents. The solvents used in the replaceable extraction strategy offer extractions that are economical, non-toxic, safe, and compatible with most detection systems. Therefore, SSs can be considered eco-friendly compounds. The main advantage of using SSs is the ease of the analyte extraction into a homogeneous phase without the need for a dispersant solvent.

SSs have attracted attention as new generation solvents because they can change their physical properties reversibly. SSs are an intelligent solvent system that converts from a water-miscible form to another form by adding or removing CO_2. Amidines and tertiary amines are a group of interchangeable solvents with specific behavior. Amidines and tertiary amines have been identified as switchable solvents that can be exchanged between the two forms by the addition of CO_2 and then returned to their non-ionic form by the addition of nitrogen, inert gas, and/or sodium hydroxide [90].

CO_2 is used as a stimulating in the switching process due to its easy removal, low cost, and low toxicity. Because of these properties of CO_2, it is an ideal stimulating for phase transition used for protonating amines into water-soluble carbonate salts, and as a suitable stimulant for the non-ionic conversion to ionic form. After the addition of CO_2, a hydrophilic carbonate or bicarbonate of the protonated amine is formed, which becomes miscible with water. This protonated amine can be reconstituted by removing CO_2 with nitrogen, inert gas, or sodium hydroxide. For this reason, they are called switchable solvents [91].

2.5.2. Demulsification Strategies

2.5.2.1. Phases Self-Separation

New strategies have been developed to be an alternative to the centrifugal step that is needed to separate the organic phase from the aqueous phase. The centrifugal step is one of the main weaknesses in DLLME, where it is a time-consuming step. Some of the alternative strategies

were to use a conventional plastic syringe used as an extraction unit [92] or by implementing an automated online sequential injection system [93] and also to use a narrow-bore tube containing the aqueous sample [94]. These strategies do not require a centrifugation procedure. Furthermore, the extraction time was extremely short, and the sample throughput was very high.

2.5.3. Emulsification Strategies

The quantity and type of dispersion solvent in DLLME have an important role in the efficiency of extraction as it is responsible for the emergence of the cloudy state of the solution. As the use of low volumes of these solvents is not sufficient for the generation of the cloudy state, while large volumes reduce the division of the analyte to the extractant phase. Therefore, researchers have developed mechanical or chemical assistant techniques to limit the use of these solvents or use them in small volumes at the microliter level.

2.5.3.1. Surfactant-Assisted

Low volumes of surfactants were used to generate effective dispersion as they are amphibious compounds that lower the surface tension between the organic and aqueous phases, thus facilitating the process of dispersing the extract in the sample. In addition, it is considered more benign from the environmental point of view compared to the traditional dispersion solvents [95].

2.5.3.2. Ultrasound-Assisted

The UA technique is one of the applications that can be used as an aid to extraction in the sample preparation step, which affects the sensitivity, accuracy, and speed of the analysis. Ultrasonic energy is used in many analytical applications. The contribution of ultrasound energy in the experimental is based on the emulsification process. This term means that one of the two immiscible liquids is separated into micro-sized droplets by the effect of ultrasonic energy and dispersed in the other in a homogeneous structure, creating the maximum surface area for extraction. As a result, UA permits the disperser solvent to be removed, increasing extraction efficiency. In other investigations, the dispersant is still used, albeit at lower quantities than in traditional DLLME, to guarantee that the cloudy state is formed effectively [96].

2.5.3.3. Vortex-Assisted

It is the mechanical energy applied to the sample solution to achieve the aim of dispersion. Because the dispersion in vortex mixing is unstable, the phases can be readily separated. When employing the VA, the formation of free radicals that always appear in ultrasonic energy was eliminated. In addition, this methodology is more cost-effective than UA baths [97]. More recently, the vortex methodology has been combined with the ultrasonic methodology in which the solution (analyte, extractant, disperser) is vortexed prior to the application of the ultrasonic bath [98].

2.5.3.4. Air-Assisted

AA is a new version of the DLLME method that is applied with the absence of disperser solvent or even supplementary devices such as vortex or ultrasonic, which simplifies the procedure of the method. the basis of this methodology is to repeatedly suck a solution of the sample and extraction solvent into a glass syringe and then inject it into the conical test tube. By this procedure, the turbidity of the solution increased more and more. thence, the mixture was centrifuged after a specific number of cycles were completed [99].

2.5.3.5. Effervescence-Assisted

The importance of green chemistry is gradually increasing in laboratories in order to be able to perform accurate and sensitive analyses by reducing the consumption of organic solvents including dispersion solvents during sample preparation before detection analysis. Therefore, improving sample use using alternatives is a problem discussed by several researchers. As is well known, the dispersion process is carried out using either an organic solvent or by using additional energy (ultrasound and vortex). Based on this context, effervescence-assisted (EA) methods appeared in 2014 in order to replace the dispersion methods that require external energy [28,100]. The EA procedures are based on the simple effervescent mixture reaction consisting of an effervescent agent (a source of carbon dioxide) and a proton donor agent (a source of a proton). A large amount of fine carbon dioxide bubbles are produced from the bottom of the tube, where the effervescent powder or tablet is dissolved in the aqueous solution, thereby the extraction solvent is dispersed homogeneously and effectively throughout the sample solution [101]. This method is characterized by reducing the experimental procedure steps and shortening the extraction time as it provides extraction and dispersal operations in one step [29]. This emulsification strategy has been used for facilitating dispersion throughout the thesis.

2.6. Deep Eutectic Solvents

Since the emergence of the definition of green chemistry in the nineties of the last century until this time, it has been met with ideal importance by many chemists in order to develop it further. Because one of the most important purposes of this strategy is to reduce the generation and use of hazardous materials and reduce environmental pollution, it has developed rapidly in all branches of chemistry in general. So initially the researchers focused on developing green solvents [102].

Although ionic liquids (IL) are commonly used as a "green solvent" in extraction methods due to their special chemical and physical properties, IL has drawbacks such as toxic effects and biodegradation. In addition, further development and application of ILs have been restricted due to their high cost and the necessity of their pure forms. In order to solve these problems, a new group of solvents called deep eutectic solvents (DES) with similar physical and chemical properties to ILs has emerged [103]. Nevertheless, it should be noted that these two types of solvents have are different [104,105] as shown in Figure 2.13.

Ionic Liquids	Similarities	Deep Eutectic Solvents
• Composed primarily of an anion and a cation • Interaction of ions through ionic bonds • Expensive • Hazardous • Difficult to synthesize on a large scale • Non biodegradable	• Wide liquid range • Low volatility • High tunable • The ability to dissolve both organic and inorganic compound	• Formed with HBA and HBD • interactions of its components through hydrogen bonds • Less expensive • Less hazardous • Simple to synthesize • More biodegradable • Stable

Figure 2.13. Similarities and differences of ionic liquids and deep eutectic solvents.

In general, DES is a mixture system consisting of at least two substances, a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). These substances have the ability to form self-linking to form a eutectic phase. This phase is characterized by a lower melting point (less than 100°C) compared to the main individual components [106]. As an example,

the first-mentioned DES that was first introduced in 2004 is a mixture of choline chloride (ChCl) as a HBA and urea (U) as a HBD. Although the melting point of ChCl is 302 °C and the melting point of U is 133 °C, the melting point of the formed DES was 12 °C. This feature is considered one of the most important features of DESs, which is that they are melted at room temperature [106,107]. A schematic representation of the first DES formation is given in Figure 2.14.
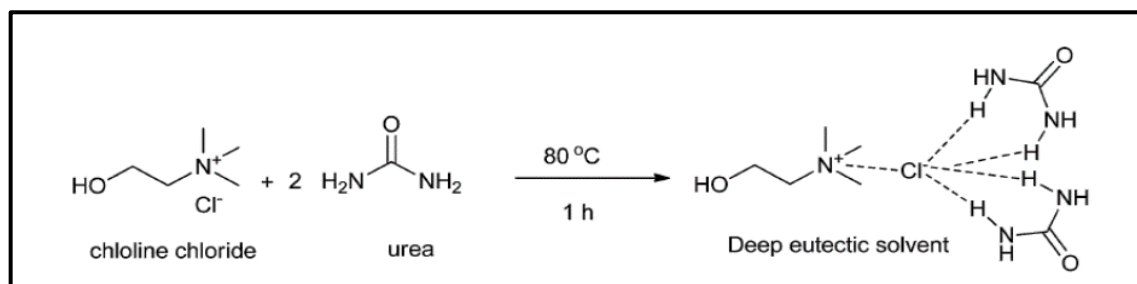
Figure 2.14. Schematic representation of the formation of a DES, illustrated with ChCl and U [106].

This binary combination of a eutectic mixture can be illustrated in a diagram as shown in Figure 2.15. Because of the interactions that occur between the components of the mixture, which is a large network of hydrogen bonds formed between them, a eutectic liquid obtained with lower lattice energy, which is characterized by a large deviation from the ideal (melted at room temperature) [108].

Figure 2.15's analysis also allows for a comparison of the different compositions and temperatures for which a homogenous liquid phase is obtainable. That means the compositions of eutectic mixtures and operating temperature ranges can be chosen to match DES characteristics to the intended use, and this makes DES be as designed solvents with confidence.
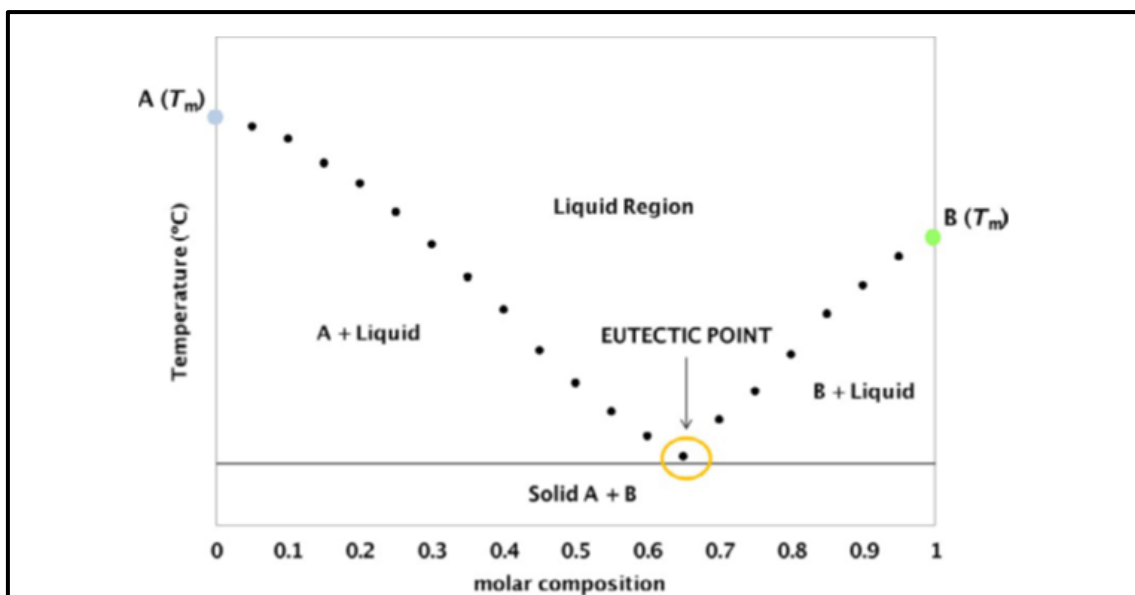
Figure 2.15. Simple binary eutectic behavior [109].

2.6.1. Type of Deep Eutectic Solvents

Because there are so many salts and hydrogen bond donors that may be utilized to make these compounds, there is no limit to the number of DESs that can be made with readily accessible chemicals. Therefore, DESs have been classified into four basic types according to the properties and behavior of these solvents as shown in Figure 2.16 [109,110].
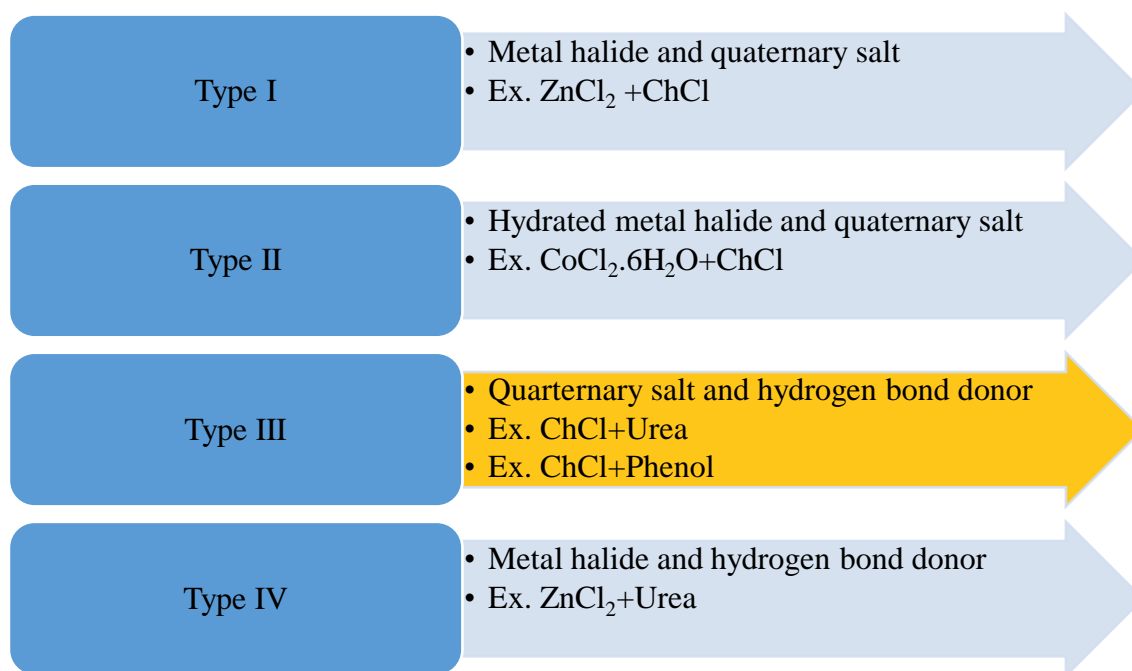
Figure 2.16. Different types of DESs depending on HBDs and HBAs.

Hydrophilic DES is obtained according to the first and second types of DES. This type is characterized by several disadvantages, the most important of which is its high solubility in water [111]. On this basis, researchers focused intensely on the formation of DES with high stability in the aquatic environment, as it is in the third and fourth types of classification [112].

The most common HBA are tetraalkyl quaternary ammonium/phosphorous salts. Furthermore, phenols, carboxylic acids, alcohols, and glycols are the most common HBD. Some of them, however, may act as both donors and acceptors of hydrogen bonds [26]. The most common structures of HBDs and HBAs are presented in Figure 2.17.
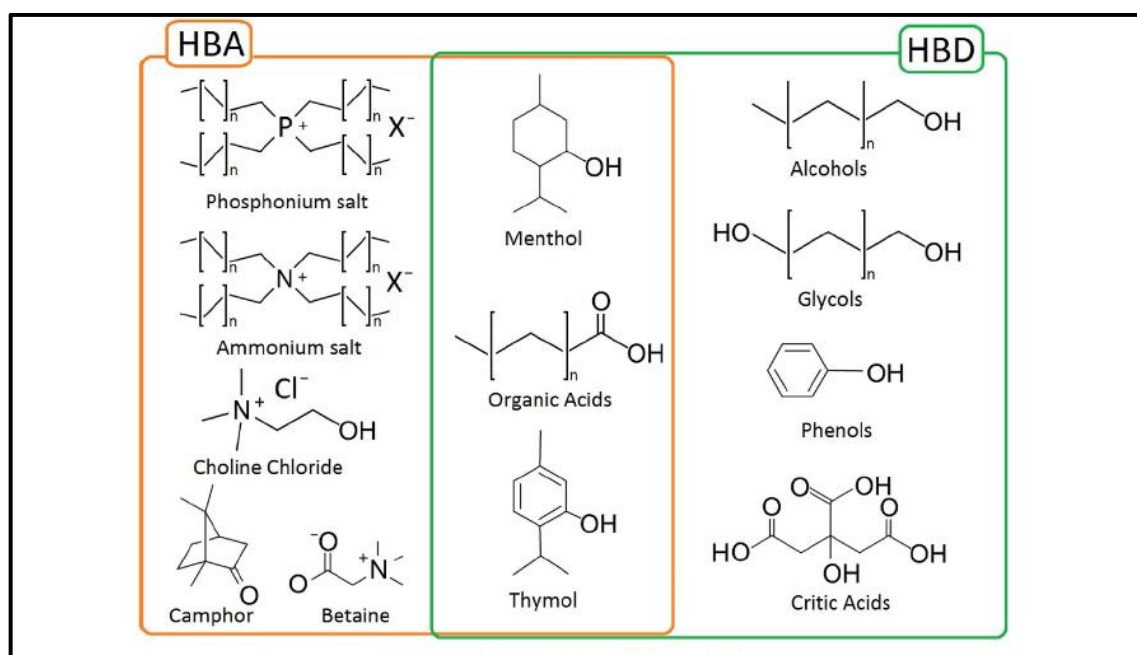
Figure 2.17. Structures of typical HBAs and HBDs [26].

2.6.2. Preparation of DES

In terms of application and experimentation, the preparation procedure for DES is simple (Figure 2.18), which depends on the melting point of its main components (HBA and HBD) and it is carried out in one of the two ways at a certain molar ratio for each component:

1. The component with the lower melting point is melted at first, then the component with the higher melting point is added to the liquid, and the mixtures melt together.
2. When the two components have high melting points or close, the two components are mixed and melted together.

In these ways, many of the DES were prepared from a variety of starting materials such as ChCl/carboxylic acid, ChCl/fructose, ChCl/phenol, ZnCl2/urea, etc. [113,114].

Figure 2.18. The main synthesis processes of DESs.

2.6.3. Application of DESs

By the excellence of interesting properties of DES and widely accepted have been allowed to prevalence its application in many different fields in general and the analytical chemistry fields in particular as shown in Figure 2.19.
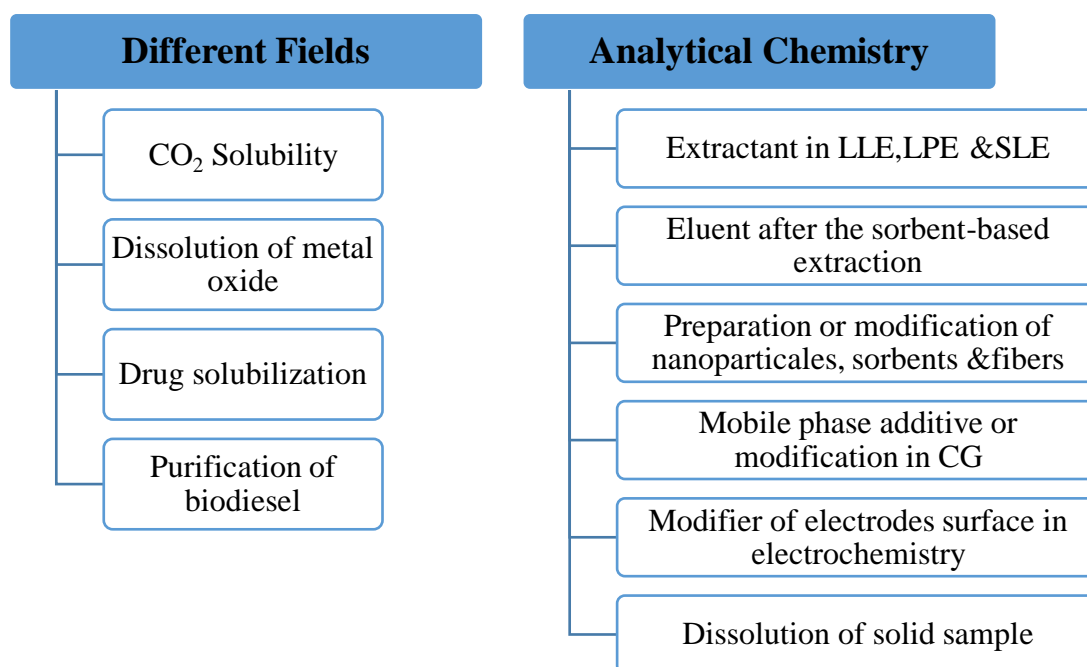
Figure 2.19. Application of DESs in the different fields and in analytical chemistry fields.

2.6.4. Properties of DES Affecting on Extraction Processes

As a common that the development of green solvents aims to find new solvents with chemical and physical properties and an extraction efficiency similar to the traditional solvents used in different extraction methods [115]. DESs are acceptable chemical solvents because they can be designed by combining different HBA with different HBD. Therefore,

the physical and chemical properties of DES depend on the properties of the different components that lead to unique reactions during the extraction process. In addition, DES application possibilities are further influenced by some features such as thermal stability, toxicity, and biodegradability [116]. As a result, the essential physicochemical features of DES will be described in the following subsections.

2.6.4.1. Melting Point

As is customary, the melting point (T_m) of DESs is substantially lower than that of their constituent components according to the definition. There is a relationship between the size of the interactions that occur between HBA and HBD, and the melting point, as these interactions increased, the melting point decreasing will be increased [103,117]. In another word, the melting of DES increases with the increase of the HBA chain (tetra-alkyl ammonium/quaternary phosphonium) as well as the increase of the HBD chain (alkyl).

2.6.4.2. Density

The density (ρ) of DES must be higher or lower than that of water when used as a solvent in extraction processes [118]. The density of the DES is affected by several factors, the most important of which are:

1. Structures of both HBA and HBD: the density decreases with the increase in the chain of each.
2. The solubility of DES: the density decreases with the increase in the solubility of DES in the aqueous phase and vice versa.
3. Temperature: the density decreases with the increase in the temperature and vice versa
4. Molar ratios of HBA and HBD.

2.6.4.3. Viscosity

Viscosity (η) is one of the most important physical properties that affect extraction processes. It is preferable to use DES with low viscosity as it is an extraction solvent. The existence of a huge network of hydrogen bonds between each component leads to less movement of the free species in the DES, therefore the high viscosity of DES was achieved.

There is a relationship linking viscosity to the solubility of DES, where the higher solubility of DES in the aqueous phase leads to lowering its viscosity due to the decrease in the

viscosity of water. In addition, the viscosity increases with the increase of the HBA chain. Even so, a decrease in viscosity appears when using long, branched chains, meaning that the viscosity depends on the chemical characteristics of HBA and HBD. On the other hand, the viscosity of DES differs considerably depending on the temperature. and also increases with the increase of the ratio of HBA to HBD in the mole fraction [119].

2.6.4.4. Hydrophobicity

Mostly, the formed DES is hydrophilic due to the existence of hydrogen bonds between HBA and HBD. The hydrophobic character of DES depends largely on the structure of the basic components of each of HBA and HBD. As the long chains of HBA and HBD lead to a decrease in the solubility of them and DES in the aqueous phase.

Furthermore, DES made up entirely of hydrophobic components is more stable in water than DES made up of both hydrophobic and hydrophilic substances. According to their solubility, the hydrophilic substances in DES can dissolve in the aqueous phase, reducing DES stability [120].

2.6.4.5. Surface Tensions

The interactions that occur between HBA and HBD have a significant impact on the surface tension value, when the interactions that occur are strong, the high surface tension values are obtained and thus leads to an increase in the extraction efficiency. There is little research that includes the effect of surface tension on extraction processes using DES as an extraction solvent. From these studies, the effect of temperature on the surface tension was investigated, it was observed that an increase in the temperature leads to a decrease in the value of the surface tension due to the breaking of hydrogen bonds in the DES, thence low extraction efficiency was obtained [121,122]. The influence of HBA and HBD chain length on the surface tension value was investigated in another research. Studies have shown the highest extraction efficiency with DES synthesizing of the shortest chain of both HBA and HBD. It was found that the extraction efficiency increases by decreasing the length of the alkyl chain [123,124].

2.7. Atomic Absorption Spectrometry

Atomic absorption spectroscopy (AAS) is one of the most widely used instrumental techniques in analytical chemistry. This is because of the low cost of usage, is easily

accessible, the technique offers sufficient sensitivity for many applications, and it is relatively free from interference [125]. Atomic absorption occurs when an atom in the ground state absorbs light of a certain wavelength.

Since the method is based on energy absorption by free atoms, it can be used without prior separation or isolation from other elements in the sample. In addition, through this technique, concentrations ranging from one part per million (ppm) to one part per billion (ppb) can be detected despite the small sample size required.

There are some basic principles of AAS that can be expressed simply as follows: (i) all atoms can absorb light; (ii) There is a specific wavelength for each element at which it absorbs light. For example, if there is a sample that contains lead with other metals such as copper and nickel when exposed to light at the specific wavelength of lead, the lead atoms only will absorb this light. (iii) to determine unknown samples, the relationship between the amount of light absorbed and the concentration of the analyte present in the known standard samples can be used by measuring the amount of light they absorb [126,127].

In order to quantitative determination for a specific element through AAS technique, it is necessary to know the stages that the sample goes through, which are as follows:

- Converting the analyte solution that contained an element into aerosol (Nebulization).
- Evaporation of the analyte, i.e., converting the vapor of the sample into gas.
- Converting the sample into free atoms by providing sufficient thermal energy (atomization).
- The interaction of the atomized sample with the rays coming from the light source
- Stable atoms at the ground state energy level absorb light and become unstable at the excited energy level (excitation)
- Analyte concentration obtained by measuring the absorption signal at the detector.

2.7.1. Quantitative Analysis

Using an optical detection system, quantitative analysis is implemented by calculating the ratio between the intensity of the light source before entering the atomic cell (the incident beam, I0) and the intensity of light outgoing from the atomic cell after absorption (the transmitted beam, I). The absorbance (A) represents the logarithm of this ratio, which is

directly proportional to the concentration of the analyte (C) in the atomic cell. In other words, the amount of absorbance increases with the increase of the atomic number of the element. The amount of absorbed light can be expressed by the Lambert-Beer law [128].

$$A = \log (I_0/I) = abC$$

Where:

a: is a constant called the absorptivity (L/g cm);

b: is the pathlength of the cell (cm);

C: the concentration of the analyte absorbent (mol L^{-1}).

2.7.2. Instrumentation

An atomic absorption spectrometer contains three basic required components: a light source, a sample cell, and a method for measuring a specific light illustrated in Figure 2.20 and Figure 2.21 [129,130].

Light Source	•A light source used to generate light at the wavelength which is characteristic of the analyte element. This is most often a hollow cathode lamp, which is an intense narrow line source.
Sample Cell	•An atomizer to create a population of free analyte atoms from the sample. The source of energy for free atom production is usually heating. •The sample is introduced as an aerosol into the heating source and the burner is aligned in the optical path so that the light beam passes through the heating source, where the light is absorbed. •An optical system to direct light from the source through the atom population and into the monochromator.
Specific Light Measurment	•A monochromator to isolate the specific analytical wavelength of light emitted by the light source lamp from the non-analytical lines including those of the fill gas. •A light-sensitive detector (usually a photomultiplier tube) to measure the light accurately. •Suitable electronic devices measure the response of the detector and translate this response into useful analytical measurements (readout).

Figure 2.20. The basic components for spectrometer.
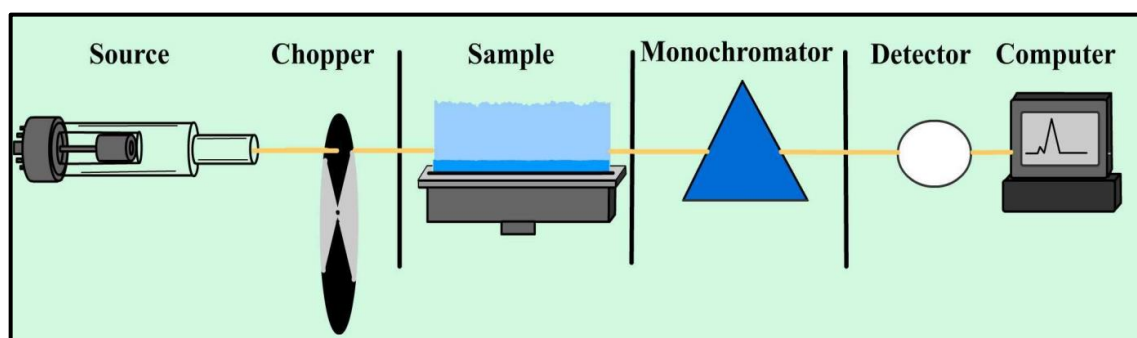
Figure 2.21. Basic atomic absorption spectrometer instrument [131].

2.7.2.1. Unit of Light Source

The function of light sources is to emit light at wavelengths that can be absorbed by the atoms in the sample. There are two types of light sources (line source): continuous and intermittent. Although continuous light sources such as hydrogen and tungsten lamps emit light of all wavelengths in a certain range, very little of it is absorbed by atoms with low line absorption, and as a result, the measured absorption signal is small. One of the disadvantages of the narrow lines sources is to constraint the atomic absorption technique as an analytical method through spectral interferences, and thus to obtain a low sensitivity. The hollow cathode lamp (HCL) and the electrodeless discharge lamp (EDL) are represented as the two most prevalent line sources employed in atomic absorption techniques [132].

The HCLs are cylindrical lamps filled with an inert gas (1.0 - 5.0 Torr pressure) such as neon or argon. Figure 2.22 shows the cross-section of HCL. The lamp is made of glass and/or quartz. Inside the lamp, there are a cathode and anode. The cathode consists of the metal to be analysed or another metal surface coated with this metal, in the form of a hollow cylinder. The anode is a wire made of tungsten or nickel [133]. The cathode and the anode are very close together. The individual metal atoms are obtained by applying a different electrical potential between the cathode and the anode (300 – 500 Volt), where the inert gas atoms are ionized at this stage (Ar^+ or Ne^+). Gas ions (Ar^+ or Ne^+) pass quickly through the electric field to collide with the cathode (negatively charged), as a result of this collision, the individual metal atoms are dislodged. Subsequently, through kinetic energy transferred by impact with inert gas ions, the sputtered metal atoms are excited to an emission state and then return to their ground state immediately. When the metal atoms return to their ground

state, they emit the absorbed energy in the form of radiation [126]. The emission process is illustrated in Figure 2.23.
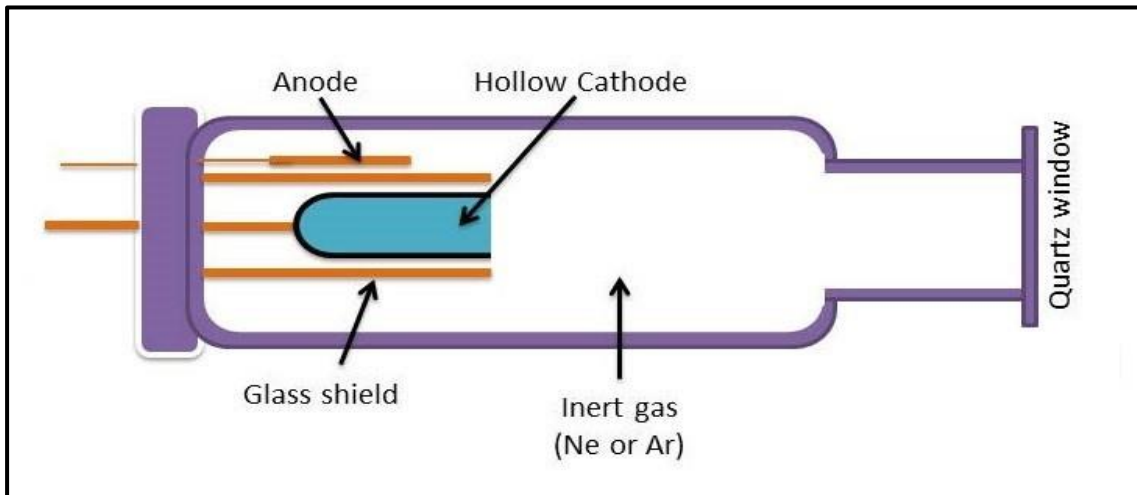
Figure 2.22. Hollow cathode lamp [134].
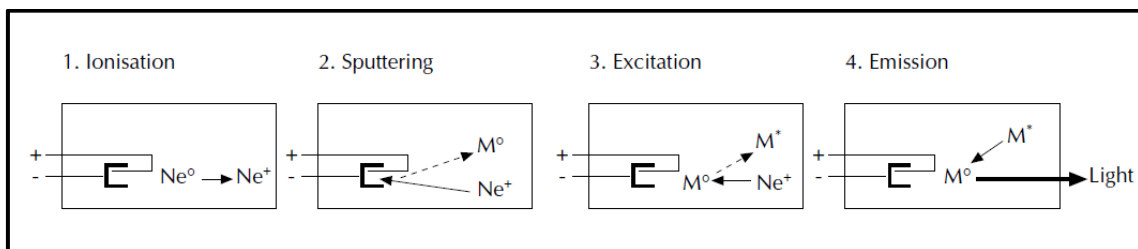
Figure 2.23. Hollow cathode lamp process [135].

HCLs have some limitations that are summarized as follows [126]:

- When using this lamp repeatedly, the deposition of a black layer on the inner surface of the lamp, which is the element atoms released from the cathode layer. Therefore, each lamp has a finite lifetime for work.

- One of the main causes of lamp failure is the decreasing fill gas pressure that occurs as a result of adsorption of the filling gas atoms (Ar^+, Ne^+) on the internal surfaces of the lamp. This, lead to decreases in the sputtering efficiency and in the excitation process of the sputtered metal atoms, which reduces the intensity of the lamp emission.

- The presence of hollow cathode lamps specific to each element (single element HCL) is a disadvantage for AAS. For this reason, multielement HCLs are designed to determine more than one element in succession.

EDLs are glass or quartz bulb that contains a salt of the element or a small amount of the metal which the source is to be used, and it is filled with an inert gas under low pressure (Ne, Ar). Figure 2.24 shows the design of the (EDL). Since these lamps do not contain electrodes, a strong radiofrequency field is required for the ionization of the inert gas inside the bulb. For this reason, the glass bulb is surrounded by a ceramic cylinder surrounded by a copper coil (radiofrequency coil), which is connected to a radiofrequency or microwave electric source. As a result of the passage of the electric field, the temperature of the bulb rises, releasing the atoms of the element in the form of an atomic cloud. Then the metal atoms are excited, and a beam is emitted from the characteristic element of this metal element [136]. This type of lamp is characterized by:

- The light beam emitted from it has a relatively high intensity.
- Its work is limited to a few elements.
- More expensive than HCL
- It needs a separate unit for the radiofrequency field.
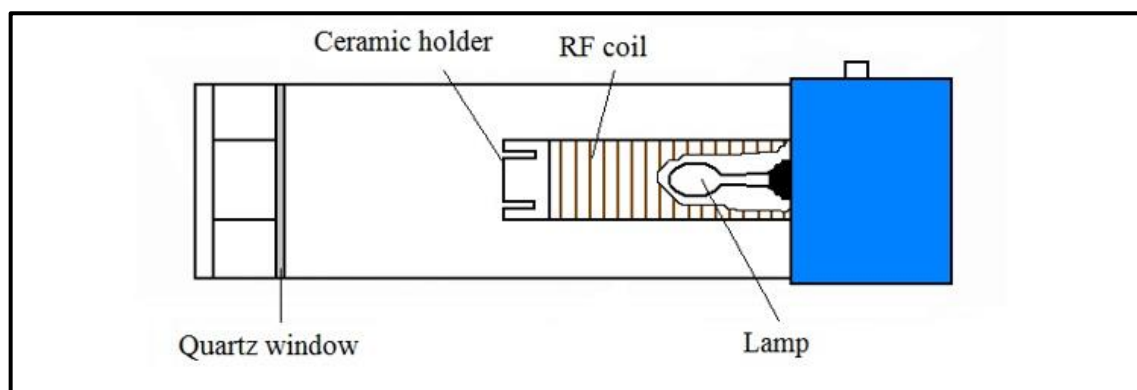
Figure 2.24. Electrodeless discharge lamp [137].

2.7.2.2. Unit of Nebulizer

Nebulizers are used as sampling units in AAS. The oxidizing gas passed through the tubes in the nebulizer creates a negative pressure effect on the liquid solution in the inner tube, resulting in the formation of an aerosol that disperses as fine droplets. Large-sized droplets are thrown by separating the aerosol according to their size in the spray chamber. A spray chamber is a significant part of the nebulizer for ensuring the nebulizer aerosol and the fuel are mixed properly. Homogeneous combustion and atomization are achieved with the small-sized droplets carried by the gas. Nebulizers are especially used in continuous flame

atomizers with their short response times and high repeatability. On the other hand, a very small portion (1.0-10%) of the solution sent to the system reaches the flame, and this loss of solution reduces the overall sensitivity of the system [138].

2.7.2.3. Unit of Atomization

The quality of the results obtained from atomic absorption spectroscopy depends on the efficiency of the atomization of the analyte and thus the sensitivity of the system, so the atomizer is the most important part of the AAS instruments. There are several atomizer techniques that developed, the most important of which are: flames, electrothermal, and special atomization techniques [127].

2.7.2.3.1. Flame Atomizer Technique

The homogeneous mixture between small droplets of aerosol (containing the analyte) and the fuel, that obtained from the nebulizer unit, passes into the burner where combustion and sample atomization take place. Figure 2.25 shows an atomic absorption burning system called a "premix" design. The Premix burner has some characteristics as follows:

- The beam path through the flame is long, which makes the sensitivity high.
- The mixing of fuel, oxidant, and sample drops (aerosol) is highly homogeneous, making the signal stable.
- The aerosols are homogeneous and approximately equal in size, which results in the stability of the flame, and leads to the stability of the signal.
- It is safe in the case of lack of oxidant or fuel, it is not possible for the flame to enter the burner body, because the mixture outside and inside the burner body is not different, so there is no risk (flashback) of an explosion.
- Expensive.
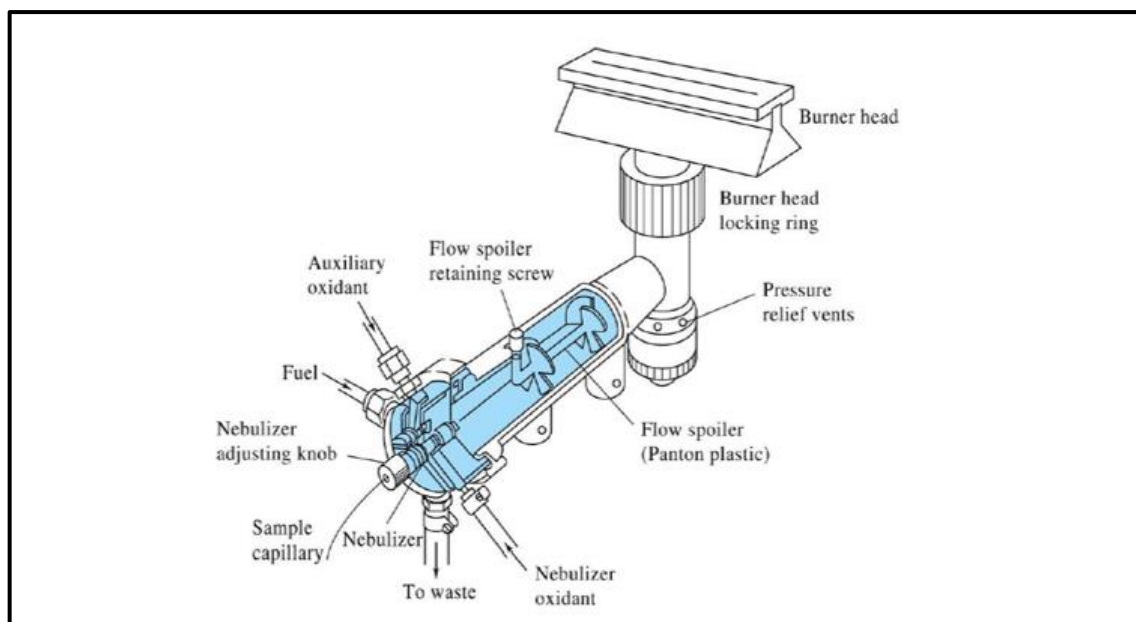
Figure 2.25. Premix or laminar flow burner used in flame atomic absorption spectroscopy [129].

In order to obtain maximum absorbance, the following must be observed:

- Passing as much as possible of the light from the light source lamp via the flame.
- For each individual analysis, the burner position must be adjusted so that the highest population area of free atoms corresponds with the optical path.

As soon as the solution's reached the flame's base, the solvents evaporated, turning the aerosol droplets into very tiny solid particles. These particles undergo changes (fusion or melting and vaporization) to form molecules [139].

Flame atomization uses flammable gas as a fuel source that is mixed with an oxidizing gas to form a high-temperature flame. These gases must be mixed in equal ratios for optimum efficiency and stable combustion. the thermal energy from the flame is responsible for producing the absorbing species. Therefore, the flame temperature is an important parameter governing the flame process. There is a linear relationship between the amount of energy supplied by a flame and the temperature of the flame. Thus, different mixtures of flammable gases can be used in AAS depending on the desired temperature. The gas mixtures used in AAS and their characteristics are shown in Table 2.8 [126,129,136].

Table 2.8. Properties of spectroscopic flames for AAS.

Fuel	Oxidant	Temperature °C	Maximum Burning Velocity (cm s^{-1})
Natural gas	Air	1700-1900	39-43
Natural gas	Oxygen	2700-2800	370-390
Hydrogen	Air	2000-2100	300-440
Hydrogen	Oxygen	2550-2700	900-1400
Acetylene	Air	2100-2400	158-266
Acetylene	Oxygen	3050-3150	1100-2480
Acetylene	Nitrous oxide	2600-2800	285

The air-acetylene and the nitrous oxide-acetylene are the two types of flames that are most used for the FAAS technique. Although the air-acetylene flame is adequate for the greater number of elements determined by atomic absorption, the hotter nitrous oxide-acetylene flame is required for numerous refractory elements, in addition, that is also effective in the control of some types of interference [140].

The efficiency of combustion also depends on the rate of combustion velocity and on the rate of gas flow, which are inversely related. If the gas flow rate is slower than combustion velocity, the flame propagates itself back into the burner, giving a flashback. As if the flow rate is too fast, the combustion rate will be low and the flame will rise until it reaches a point above the burner. When the gas flow rate and the combustion velocity are equal, at this region the flame is stable [141].

The temperature inside the flame is not completely homogeneous, but varies according to the distance from the top of the metal body of the burner, as well as how near or far the point at which we measure the heat from the tip of the flame. Figure 2.26 shows a cross-section through the flame, looking down the source radiation's optical path. It is possible to distinguish three regions within the flame, the characteristic for each one are shown in Figure 2.27 [141,142]:
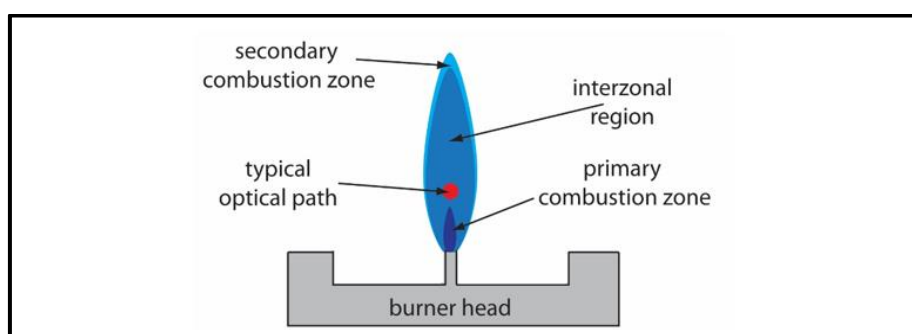
Figure 2.26. Regions in the flame of AAS [137].

The primary combustion zone	• Is the area near the burner head top and distinguished from others by its blue colour, where the amount of oxygen is little, and the temperature in this region is usually low. This region has the most of the combustion reactions take place and is rich in radicals. Due to collisions with high-energy gas molecules, the atomizing of the analyte takes place.
The interzonal region	• Has a more stable temperature, a more homogeneous composition, and is the richest region in terms of analyte atoms and therefore high sensitivity. It contains fewer radicals, therefore, fewer interferences take place when compared with other regions. This region is the most appropriate in the analysis process, as most elements are analysed.
The secondary combustion zone	• It is the area farthest from the burner head top, where the flames come into contact with the atmospheric air surrounding it. This region is characterized by an abundance of oxygen (mostly oxidation reactions occur), which results in the region creating CO_2 and stable metal-oxide compounds. Its temperature is not high, as it is located on the outer ends of the flame.

Figure 2.27. The characteristics of regions within the flame.

2.7.2.3.2. Electrothermal Atomizer Technique

The electrothermal atomization, also known as flameless atomization, was developed for low-concentration materials, the most famous of which is the graphite furnace. The graphite furnace technology is the most preferred, which appeared in the 70s (that is, after a long time of use of the flame). Although electrothermal atomizers have advantages such as requiring small sample volumes, working with viscous samples that are difficult to be in the form of an aerosol, low detection limit, high evaporation and atomization efficiency, and analysis of solid samples; but It is not preferred much because of its high cost, large power supply requirement and covering large areas [143].

A typical graphite furnace consists of a cylindrical graphite tube approximately 1–3 cm in length and 3–8 mm in diameter. As shown in Figure 2.28, 1-100 µL of sample is injected into the furnace through the hole in the middle of the furnace. Light from the light source lamp passes through the windows at each end of the graphite tube. A continuous stream of inert gas is passed through the furnace, protecting the graphite tube from oxidation and

removing the gaseous products produced during atomization, and the highest recommended temperature is 2550 °C in less than 7 s.

In FAAS the analyte stays in the optical path is less than one second while in graphite furnace traps the atomized sample for a few seconds in the optical path. Thus, it achieves greater sensitivity [143,144]. To achieve adequately atomization of the sample, the furnace is heated in three stages, which are shown in Figure 2.29 [145].
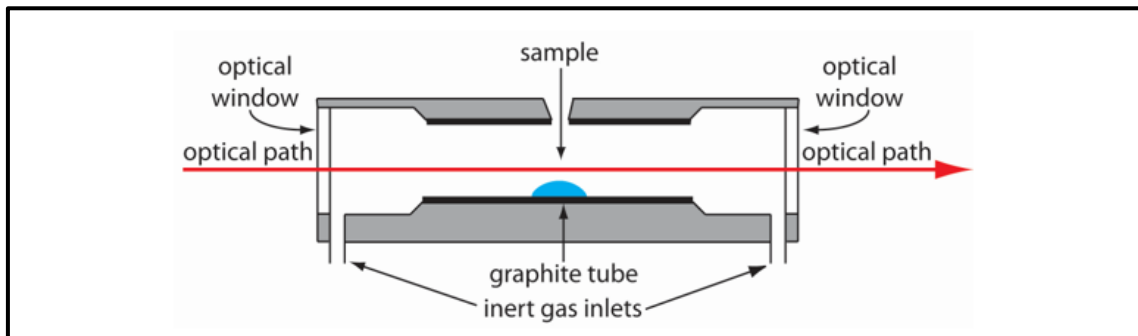
Figure 2.28. Cross-section of an electrothermal analyser [142].

Drying Step

- The sample is dried to a solid residue, and the solvent is removed by using a current that raises the temperature of the graphite tube to about 125 °C and kept for 20 s.

Ashing Step

- The sample is heated between 350 – 1200°C to remove the organic substances by converting them to CO_2 and H_2O, and the volatile inorganic substances are vaporized. These gases are removed by the inert gas flow. This step is especially important in the analysis of elements with high volatility, because with the increase in temperature, the analyte element may be removed prior to the atomization step. Therefore, in this step, the signals are not recorded.

Atomization Step

- The temperature of the graphite furnace is rapidly increased to between 2000 – 3000 °C, at which gaseous atoms of the element to be analysed. This temperature is kept for 5 seconds according to the analysis element. The light absorbed by the analysis element is measured in the atomization step. Signals are received using a printer and evaluation is made by measuring the peak areas or heights.

Figure 2.29. The heating program steps of graphite furnace.

2.7.2.3.3. Special Atomization Techniques

There are some other atomizers that can be used with the same spectrometer to produce ground-state atoms. The basic principle of different atomization processes uses was to eliminate the limitations of classical techniques for some specific elements.

One of these is the hydride generation technique (Figure 2.30), which is designed for volatile elements such as (Arsenic, Tin, Bismuth, Antimony, Tellurium, Germanium, and Selenium). These elements have the ability to react with reducing agents (sodium borohydride) and converted into gaseous hydride form in an acidic medium and move to the vaporizer using the gas flow (argon). During the atomization process, hydride compounds exposed to temperatures of 800-900 °C are broken down and analyte atoms are formed. The analytical signal is the highest absorption reading, peak height, or integrated peak area. In this method, while the matrix effect is eliminated by the transition of analyte atoms to gas form, on the other hand, the sensitivity of the method is increased to a very high extent by sending only the target analyte to the system [131].
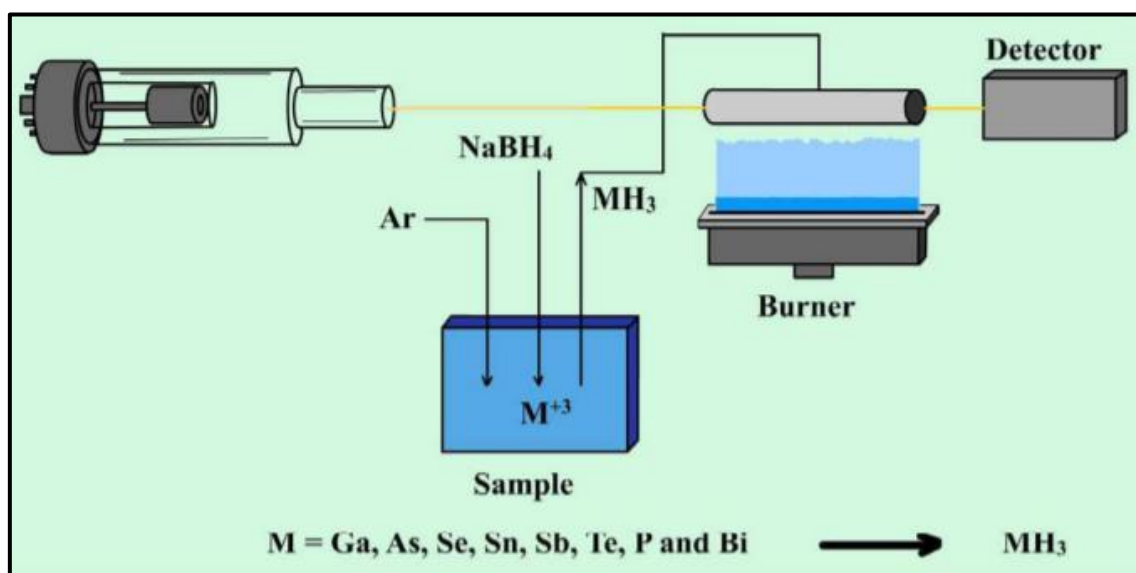
Figure 2.30. Scheme of the hydride generation technique [131].

The cold vapor technique is another specific atomization technique developed for mercury since mercury is the only metal that can evaporate even at room temperature. the process occurs by a mercury sample reaction with a strong reducing agent in an acidic medium and in a closed container. In the same way as the prior system, the formed elemental mercury atoms are sent to the quartz absorption cell with a carrier gas (air or argon bubbling). By

adding an extra amount of the sample, the sensitivity can be increased, and this is the advantage of the technique [131].

2.7.2.4. Monochromator Unit

Since the element to be determined gives absorbance at a certain wavelength, the wavelength at which the element gives maximum absorbance must be separated from the other light beams, where monochrome is responsible for this task (Figure 2.31). It should be preferred considering the wavelength ranges of the materials used in the construction of monochromator components such as slits, lenses, windows, and prisms. After that, the chosen wavelength light is sent to the sample. The separation power of the monochromator is directly related to the excellence of the instrument in almost all spectroscopic methods. In the FAAS system, there is no need to use monochromators with high separation power except for a few elements and a bandwidth of 0.2 nm is enough for the measurements for most elements [146].
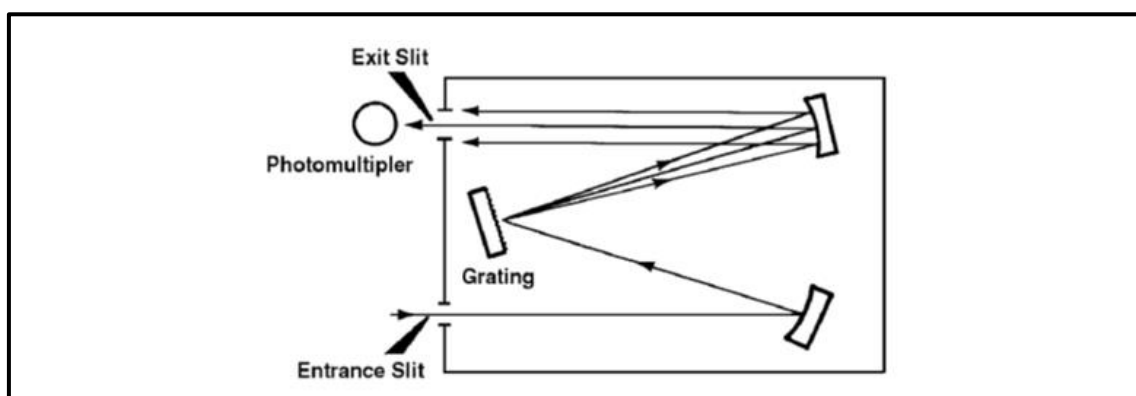
Figure 2.31. A monochromator [126].

2.7.2.5. Detection Unit

The main principal objective of the detectors is to convert the monochromator's beams into electrical signals using a photomultiplier device. A photomultiplier tube (Figure 2.32) is a highly sensitive device. This device consists of an anode and nine dynodes, where each one is more positive than the previous one. The accelerated electrons collide between the nine surfaces of the dynodes with more energy than their initial kinetic energy. This process is repeated several times, and finally, with each photon striking the initial surface, many additional electrons are collected. In this way, extremely low light intensities are converted into measurable electrical signals [147].
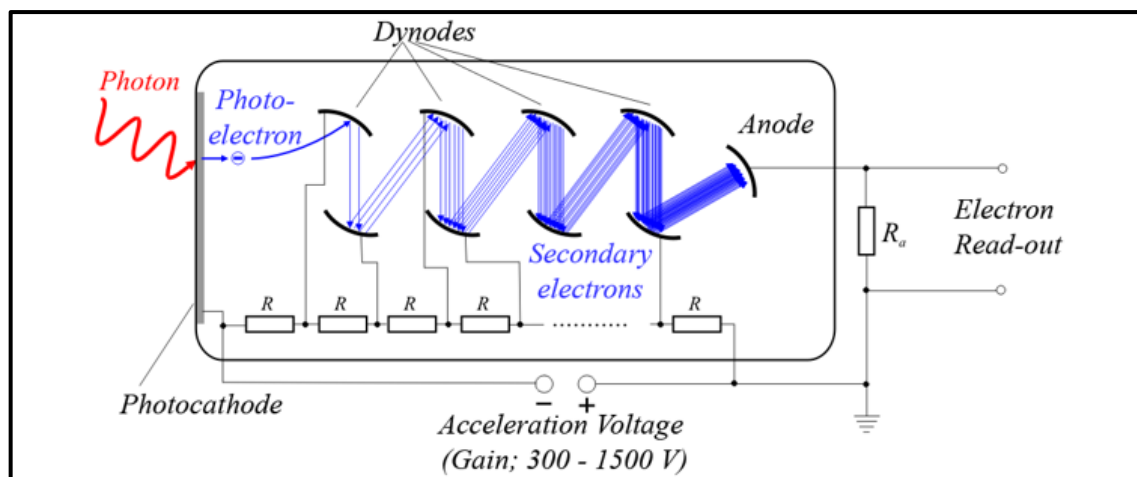
Figure 2.32. Photomultiplier tube [147].

2.7.3. Interferences

Atomic absorption is a specialized technique with a few interferences. It can be classified into two basic categories as spectral and non-spectral interferences. Non-spectral interferences are matrix interferences, chemical interferences, and ionization interferences. Spectral interferences consist of emission interference, spectral interference, and background interference. If the existing interference can be determined very well, it is possible to prevent it.

2.7.3.1. Chemical

This type of interference can often be minimized by choosing appropriate working conditions. In chemical interference, the sample analyte forms thermally stable compounds (for example, refractory phosphates, oxides, silicates, or carbides), which do not completely decompose at the flame temperature, thus the population of analyte atoms that can absorb will be reduced and this will decrease the analysis sensitivity.

There are two methods applied to overcome this interference normally;

- The first method; is to do away with the interference by inserting another element or compound (a sequestering agent) into the medium that will form a thermally stable compound with the interfering species.
- The second method; since interference occurs due to inadequate energy to atomize the thermally stable analyte compound, it can be got rid of by increasing the amount of

energy; that is means, chemical interference can be overcome by using a hotter flame. For this, it is most appropriate to use a nitrous oxide-acetylene flame.

2.7.3.2. Ionization

The nature of this Interferences can be a problem in the analysis of alkali metals at relatively low temperatures and in the analysis of other elements at high temperatures. In these two cases, the elements are ionized and the total number of atoms at the ground state decreases. Since ions have different energy levels than neutral atoms, the expected atomic signal decreases, which leads to a negative error.

This interference can be avoided in two ways.

- A lower temperature flame may be preferred for atomization. For example, if an air/propane flame is used instead of an air/acetylene flame in the analysis of alkali metals, atomization without ionization may be achieved. Nevertheless, the use of low temperature is not a definitive solution as it will reduce the atomization efficiency of many elements.
- Another way in which ionization interference can be eliminated is to use an ionization suppressor that reduces the degree of ionization of the analyte. In this method, by adding another element with low ionization energy such as K, Na, Rb, and Cs to standard and sample solutions. When adding these metals, the number of electrons in the flame will be increased and reduce the formation of cations.

2.7.3.3. Matrix

This type of interference occurs because the physical and chemical properties of the sample matrix differ from the parameters of the calibration curve standards. These properties may be are viscosity, surface tension, and combustion rate. The difference in these characteristics is due to several reasons, including the use of different solvents; the difference in sample temperatures; and the difference in the concentrations of dissolved salts, acids, and bases. Therefore, the resulting signal may be increase or decrease due to this type of interference. They are controlled by using the matrix spike test or dilution of the sample.

2.7.3.4. Emission

The intensity of the light emission of the analyte has an effect on sensitivity, If the light emission intensity is great, the analyte absorption amount may decrease due to the reduction of the ground state atoms numbers. Emission interference can be removed by increasing the source current, reducing the slit width, or diluting the sample. Increasing the source current or decreasing the slit width will result in an increase in the signal to noise ratio.

2.7.3.4. Spectral

Spectral interferences occur in the presence of an element or multiple atoms that absorb light of the same wavelength as the element analyzed in the absorption cell. As a result, less light reaches the detector than it should, and absorbance values are greater than expected. The most straightforward approach to avoid this is to use a line of the analyzed element that does not overlap with the interfering element or to use an alternative wavelength of the studied element. In another hand, it must be ensured that the analyte sample is free of elements that may interfere in the line and try to obtain previous information about it to confirm and solve.

2.7.3.5. Ground Interferences

Serious problems can be getting up in the analysis if the multiple atomic species (molecules or radicals) in the sample solution absorb light. In addition, reactive oxides formed during combustion also affect ground interferences. The presence of such substances in the flame causes light scattering, causing a positive error in absorbance. Ground interferences, also called ground absorption, are the most important source of error in atomic absorption spectroscopy.

To solve this problem, the analyte absorbance must be found by measuring the total absorbance value (analyte absorbance + ground absorbance) and subtracting the absorbance from the interference.

It is not possible to prevent this type of interference from occurring, but various methods have been developed to correct ground interferences such as continuum source, Zeeman effect, the double line effect, and pulsed HCL.

Continuum Source: The ground correction is achieved by using a continuous light source, a light source that can radiate in a wide wavelength range (deuterium/halogen) other than

the HCL. The wide emission of the deuterium lamp and the emission of the HCL are sequentially passed through the atomizer. The light of the HCL is absorbed by the analyte and the ground, while the light from the deuterium lamp is only absorbed by the species that cause ground interferences. The difference between the two absorbance values is the absorbance of the analyte.

Zeeman Effect: Is based on the principle of splitting the electronic energy levels of the atom when a magnetic field exists. In order to avoid ground interference, the magnetic field is applied to the atomizer using the Zeeman effect. In this technique, the two components of the analyte atoms behave differently when interacting with polarized light. The absorption of the analyte and the molecules causing the ground interference can be separated from each other because their magnetic and polarization properties are different.

The Double Line Effect: The double line method is based on the principle of measuring the absorbance twice at two different wavelengths. The first measurement is made at the characteristic wavelength of the analyte. The resulting absorbance is the sum of the absorbance of the analyte and the absorbance of the species causing the ground interference. The second measurement is made at a wavelength close to the first wavelength where the analyte does not absorb. The absorbance obtained from this measurement belongs only to the ground absorbance and the difference between the results of the first and second measurements gives the true absorbance value.

Pulsed HCL: This ground correction method is based on operating the hollow cathode lamp at low and high current, respectively. The total absorbance is measured with the source beam obtained at a low current. In the high current pulse, the emission line of the lamp expands and the center of this line is absorbed by the atomic vapor in the CHL, causing a decrease in the emission intensity of the lamp (self-absorption). In this case, the measured absorbance value belongs to the ground absorption. Analyte absorbance is calculated from the difference between absorbances.

3. EXPERIMENTAL

3.1. Chemicals and Solutions

Each of the chemicals utilized was an analytical grade certification. All the information related to the chemicals used and preparation procedure were listed in detail as follows:

3.1.1. Heavy Metals Solutions

Standard solutions (1000 mg L^{-1}) of lead, copper, nickel, and cobalt ions were prepared, as shown in Table 3.1.

Table 3.1. The details of heavy metals solutions.

Metal ions	Added as	Company	Preparation
Pb (II)	Pb $(NO_3)_2$	Merck, Germany	0.4 g in 250 mL
Cu (II)	Cu $(NO_3)_2.3H_2O$	Merck, Germany	0.95 g in 250 mL
Ni (II)	Ni $(NO_3)_2.6H_2O$	Merck, Germany	1.23 g in 250 mL
Co (II)	Co $(NO_3)_2.6H_2O$	Merck, Germany	1.23 g in 250 mL

3.1.2. Complexing Agent Solution

The heavy metal ions were chelated using 8-hydroxyquinoline (8-HQ) in this study. The chemical properties and the preparation procedure of 0.1 % (w/v) 8-HQ was shown in Table 3.2.

Table 3.2. The chemical properties of 8-hydroxyquinoline.

Chemical formula	C_9H_7NO
Company	BDH, UK
Color	White
Solubility	Alcohol-soluble
Molecular mass	145.16 g mol^{-1}
Melting point	76 ° C
Preparation	0.05 g in 50 mL ethanol
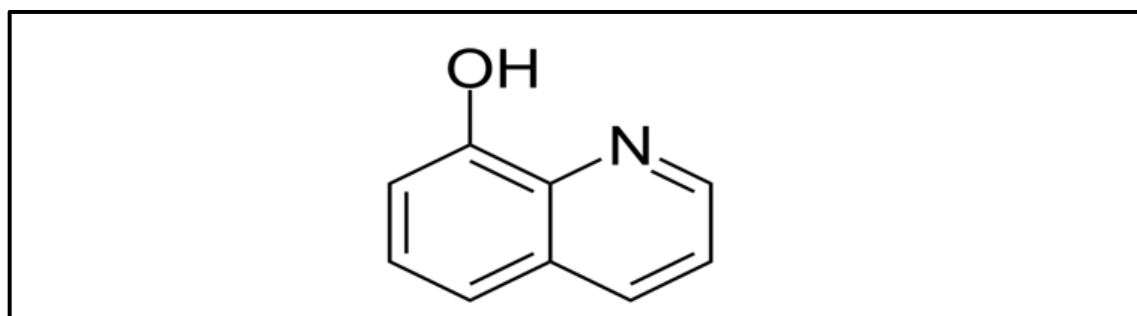
Figure 3.1. Chemical structure of 8-Hydroxyquinoline.

The quinoline molecule contains a portion of phenol (Figure 3.1), which in turn contains an acidic proton. If this proton is deprotonated, the anion formed is fixed by resonance. The formed anion is alongside the nitrogen pyridine, as the two have unbonded electron pairs that create covalent bonds with highly polarized ions. The metal ions displace the acidic protons in the phenol part of the quinoline molecule to form these complexes according to

$$M^{Z+} + ZHQ \rightarrow MQ_Z + ZH^+$$
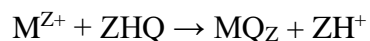
Where z is the metal ion charge and HQ is hydroxyquinoline. Thus, the ion charge of the metal determines the amount of quinoline molecules existent in the complexes [148] as shown in Figure 3.2.
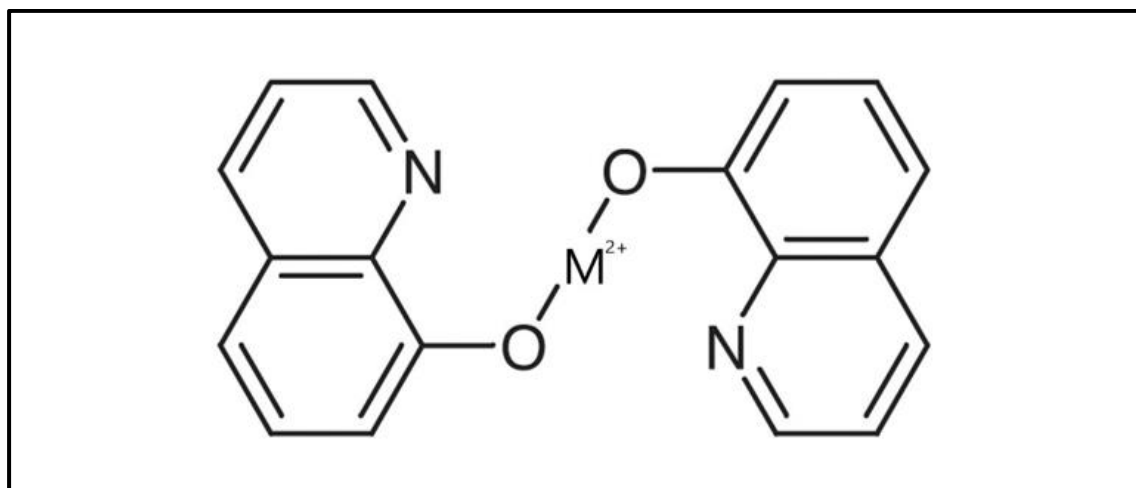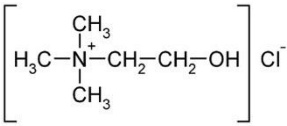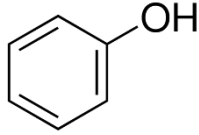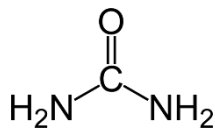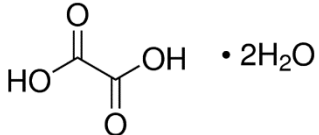
Figure 3.2. Structure of 8-HQ-metal complexes, where M = Pb^{2+}, Cu^{2+}, Ni^{2+}, and Co^{2+} [149,150].

3.1.3. Extraction Solvent Materials

In this thesis, deep eutectic solvents (DESs) were used as an extraction solvent. The chemicals used for deep eutectic solvents preparation were listed in Table 3.3. All chemicals in this table were purchased from Sigma Aldrich, Germany.

Table 3.3. The chemicals used for the preparation of extraction solvent.

Chemical	Formula	Used as	Structure
Choline chloride (ChCl)	$C_5H_{14}ClNO$	HBA	
Phenol (Ph)	C_6H_6O	HBD	
Urea (U)	CH_4N_2O	HBD	
Oxalic acid dihydrate (OA)	$C_2H_2O_4.2H_2O$	HBD	

3.1.4. Effervescence Agent for Emulsification

Effervescence agent was used as an emulsification method in this study. To choose the suitable effervescence agent for the extraction process, different effervescent powders were prepared from different substances which purchased from Merck, Germany as illustrated in Table 3.4.

Table 3.4. The chemical substances used for the preparation of effervescence agent.

Chemical	Formula	Used as
sodium dihydrogen phosphate (SDP)	NaH_2PO_4	
citric acid (CA)	$C_6H_8O_7$	proton donor
ascorbic acid (AA)	$C_6H_8O_6$	
sodium carbonate (SC)	Na_2CO_3	carbon dioxide source
sodium bicarbonate (SBC)	$NaHCO_3$	

3.1.5. Buffer Solutions

The buffer systems CH_3COOH/CH_3COONa, KH_2PO_4/Na_2HPO_4, Na_2HPO_4/HCl, $Na_2CO_3/NaHCO_3$ were used to select the appropriate pH during pH optimization, which is a very effective parameter on the performance of extraction method. The CH_3COONa was purchased form Sigma Aldrich, USA and others from Merck, Germany. The preparation procedures of buffer solutions were given in Table 3.5.

Table 3.5. The preparation procedures of buffer solutions.

Buffer solution	Preparation procedure
pH 2.0-3.0	0.10 M (17.799 g L^{-1}) Sodium hydrogen phosphate + 0.10 M Hydrochloric acid
pH 4.0-6.0	0.10 M (8.204 g L^{-1}) Sodium acetate + 0.10 M Acetic acid
pH 7.0-8.0	0.10 M (13.608 g L^{-1}) Potassium dihydrogen phosphate + 0.10 M (17.420 g L^{-1}) Sodium hydrogen phosphate
pH 9.0-10.0	0.10 M (10.598 g L^{-1}) Sodium carbonate + 0.10 M (8.401 g L^{-1}) Sodium bicarbonate

3.1.6. Other Chemicals

Tetrahydrofuran (THF) (Merck, Germany) was used as an aprotic solvent. Ethanol (Merck, Germany) and HCl 37 % (w/v) with 1.19 g mL^{-1} (Merck, Germany) were used to prepare 1 % (v/v) acidic ethanol that used to dissolve and dilute the extracting phase. HNO_3 65 % (w/v) with density 1.39 g mL^{-1} and H_2O_2 30 % (w/v) with density 1.11 g mL^{-1} (Merck, Germany) were used in the microwave digestion of real samples. Besides, NH_3 30 % (w/v) with density 0.90 g mL^{-1} (Merck, Germany) was used to adjust the pH of real samples after performing the microwave digestion process.

All of the solutions and samples were prepared with deionized water with a conductivity of 18.2 M cm^{-1}. To avoid contamination, all glassware was soaked in 10 % (v/v) hydrochloric acid for at least 24 hours before being rinsed with deionized water.

3.2. Apparatus

3.2.1. Detection Instrument

In this procedure, flame atomic absorption spectrophotometer was used as an instrument for lead, copper, nickel, and cobalt determination. The specifications of this instrument were listed in Table 3.6, and the measurement conditions recommended by the manufacturer for each metal were illustrated in Table 3.7.

Table 3.6. The specifications of flame atomic spectrometry instrument.

Company	Perkin Elmer, USA
Model	AAnalyst 800 Atomic Absorption Spectrophotometer
Atomizer	Flame atomizer
Background correction	Deuterium lamp
Flame type	Air/acetylene
Flow rate	17.0 / 2.0 L min^{-1}
Radiation source	Hollow cathode lamps for lead, copper, nickel, and cobalt

Table 3.7. Measurement conditions for flame atomic absorption spectrometry.

Parameter	Pb (II)	Cu (II)	Ni (II)	Co (II)
Lamp current (mA)	28	15	30	30
Wave length (nm)	283.3	324.8	232.0	240.7
Slit width (nm)	0.7	0.7	0.2	0.2

3.2.2. Other Instruments

There are many instruments needed during laboratory work to apply and complete the advanced method mentioned in Table 3.8 with details.

Table 3.8. The complementary laboratory instruments.

Instrument	Company	Purpose of use
Millipore Simplicity deionized water device	Merck, Germany	Preparation and dilution of all solutions.
Digital pH meter	Isolab Laborgeräte GmbH, Germany	pH amendment of sample solutions
UNE 500 Universal oven	Memmert, Germany	Drying the textile products and some chemicals
Manual hydraulic press	GRASEBY SPECAC, UK	Preparation the effervescent tablets
MARS 6-One Touch Microwave Digestion System	CEM Corporation, USA	Real sample digestion
Analytical balance	Mettler Toledo, Switzerland	Weighing all solid chemicals

3.3. Preparation of Deep Eutectic Mixture

In general, the preparation process for DESs simply boils down to mixing specific weights of HBA material with HBD material according to the molar ratio to be prepared. The DESs mixtures were magnetically stirred in glass beakers until clear solutions were obtained. Some solutions require slight heating, while others require heating to a higher temperature. Based on that, three different types of DESs with different molar ratios were prepared (ChCl: Ph, ChCl: U, and ChCl: OA) as shown in Table 3.9

Table 3.9. The preparation Procedure of different DESs.

DES type	Amount for given molar ratios				
	1:1	1:2	1:3	1:4	1:5
ChCl: U	1.39 g:0.60 g	1.39 g:1.20 g	1.39 g:1.80 g	1.39 g:2.40 g	1.39 g:3.00 g
ChCl: OA	1.39 g:0.90 g	1.39 g:1.80 g	1.39 g:2.70 g	1.39 g:3.60 g	1.39 g:4.50 g
ChCl: Ph	13.96 g:9.41 g	13.96 g:18.82 g	13.96 g:28.20 g	13.96 g:37.60 g	13.96 g:47.05g

Clear solutions of ChCl: U and ChCl: OA were obtained after continuous stirring for 45 minutes at 80°C for ChCl: OA and 90°C for ChCl: U, while ChCl: Ph solution was achieved after 5 minutes at 50°C as shown in Figure 3.3. On the other hand, Figure 3.4 shows the synthesis reaction of ChCl: Ph DES at a 1:3 mole ratio.
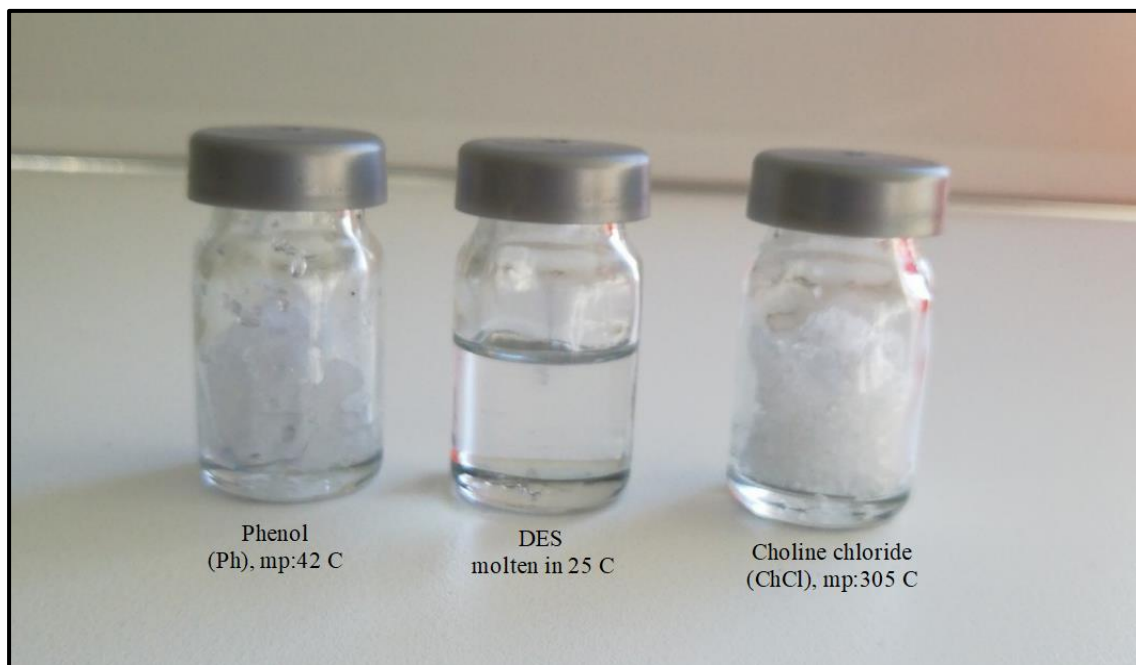
Figure 3.3. Image of the DES that composed from ChCl and Ph.
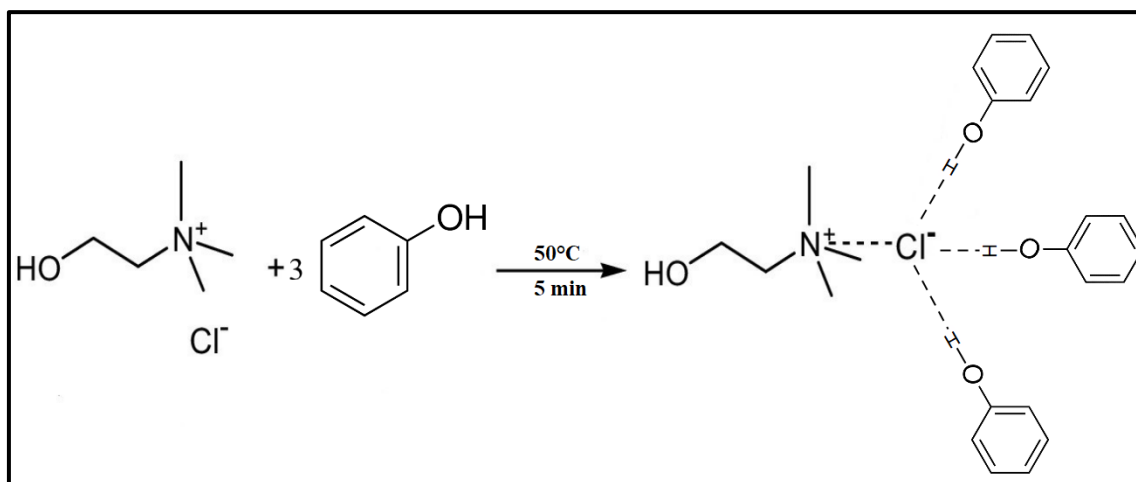
Figure 3.4. Schematic representation for ChCl: Ph DES formation at a 1:3 mole ratio.

3.4. Effervescent Agent Preparation

3.4.1. Effervescent Powder

The requirements for effervescent powder preparation are a proton donor and a source of carbon dioxide. For this purpose, SDP, CA, and AA were used as proton donors and SBC and SC as carbon dioxide sources (see Table 3.4 for abbreviations). Before using, all these chemicals were dehydrated in an oven at 90°C for 2 hours and then the powders were manually integrated in a ceramic mortar and pestle until a fine and homogeneous powder was obtained. The procedure of effervescent powders preparation was illustrated in Table 3.10.

Table 3.10. The procedure of effervescent agents' preparation.

Effervescent agent compositions	Mole ratio	Preparation	Effervescent agent amount
SDP: SBC	0.001: 0.001	0.156 g: 0.084 g	0.240 g
SDP: SC	0.002: 0.001	0.312 g: 0.105 g	0.417 g
CA: SBC	0.001: 0.001	0.192 g: 0.084 g	0.276 g
CA: SC	0.002: 0.001	0.384 g: 0.105 g	0.489 g
AA: SBC	0.001: 0.001	0.176 g: 0.084 g	0.260 g
AA: SC	0.002: 0.001	0.352 g: 0.105 g	0.457 g

3.4.2. Effervescent Tablet

The adequate amount of effervescent powder for the proposed method was weighed accurately and compressed in a manual hydraulic tablet press apparatus at 12 tons for 1 min as shown in Figure 3.5. The obtained tablets were stocked in an inert atmosphere like a desiccator.
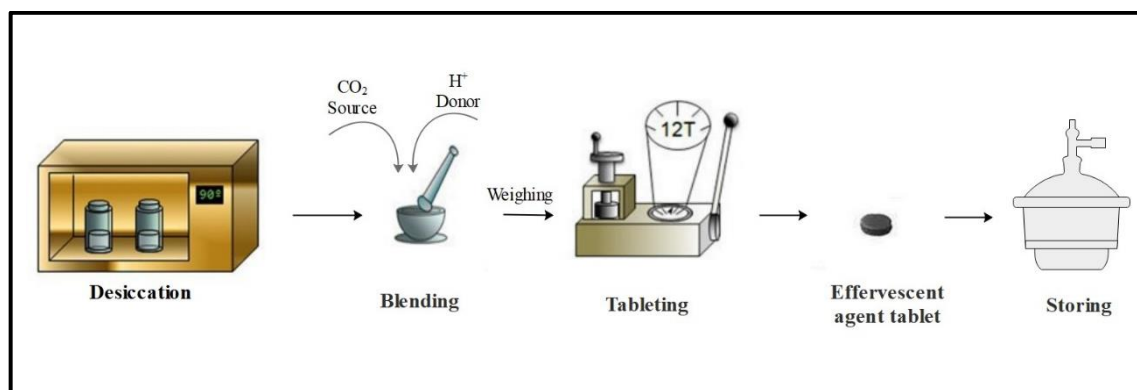
Figure 3.5. Schematic representation of making effervescent tablets.

3.5. The Procedure of Effervescence-Assisted Dispersive Liquid-Liquid Microextraction Based on the Deep Eutectic Solvent (EA-DES-DLLME)

3.5.1 Using Effervescent Powder

The EA-DES-DLLME method consists of several stages, the first stage involves preparing two conical bottom tubes with a capacity of 50 mL, the first is for the analyte and the other is for the effervescent agent. The analyte tubes containing the extraction solutions for Pb (II), Cu (II), Ni (II), and Co (II) which contains 25 mL of standard or sample solutions, pH 6.0 buffer (CH_3COOH/CH_3COONa), 0.1 % (w/v) of 8-HQ as a complexing agent, (ChCl: Ph) DES as an extraction solvent and THF as an aprotic solvent. The contents of analyte and effervescent agent tubes for each ion were clearly mentioned in table 3.11.

Table 3.11. The contents of analyte and effervescent agent tubes.

Metal ions	Analyte tube					Effervescent agent tube	
	Standard solution (μgL^{-1}) in 25 mL	pH 6.0 buffer solution (mL)	8-HQ (μL)	DES (μL)-mole ratio	THF (μL)	Effervescent powder composition	Effervescent powder amount (g)
Pb (II)	500	2	750	1000-1:3	1000	AA+SC	0.457
Cu (II)	50	2	750	1000-1:3	1000	AA+SC	0.457
Ni (II)	100	2	750	1000-1:3	1000	AA+SC	0.457
Co (II)	100	2	750	1000-1:3	1000	AA+SC	0.457

The second stage involves pouring the analyte tube contents into the tube that containing the effervescent powder. Thereafter, effervescence occurred instantaneously from the bottom to the top of the tube. Thus, the extraction solvent in the aqueous sample solution was homogeneously dispersed as a result of the effervescent reaction which produced small bubbles by the CO_2 released, which effectively accelerated the extraction process. The effervescent reaction is completed within 2.5 minutes.

In the third stage (phase separation), the ChCl: Ph phase at the top of the tube was collected automatically once the effervescent reaction was completed, and the aqueous phase was carefully discarded with a pipette.

In the last stage, the volume of the ChCl: Ph phase that remaining in the tube was diluted to 500 µL with 1% (v/v) acidic ethanol and finally introduced into FAAS by using the micro-sampling technique [151,152]. The micro-sampling technique was applied with a short capillary tube specially designed (homemade) for this system to ensure that the sample

transfers directly to the nebulizer, where the obtained absorption peak height is recorded for each target metal, so, it is possible to determine these metals in microliter volumes.

The blank solutions without any metal ions were also treated in the same way as the procedure. The schema of the EA-DES-DLLME procedure was represented in Figure 3.6.
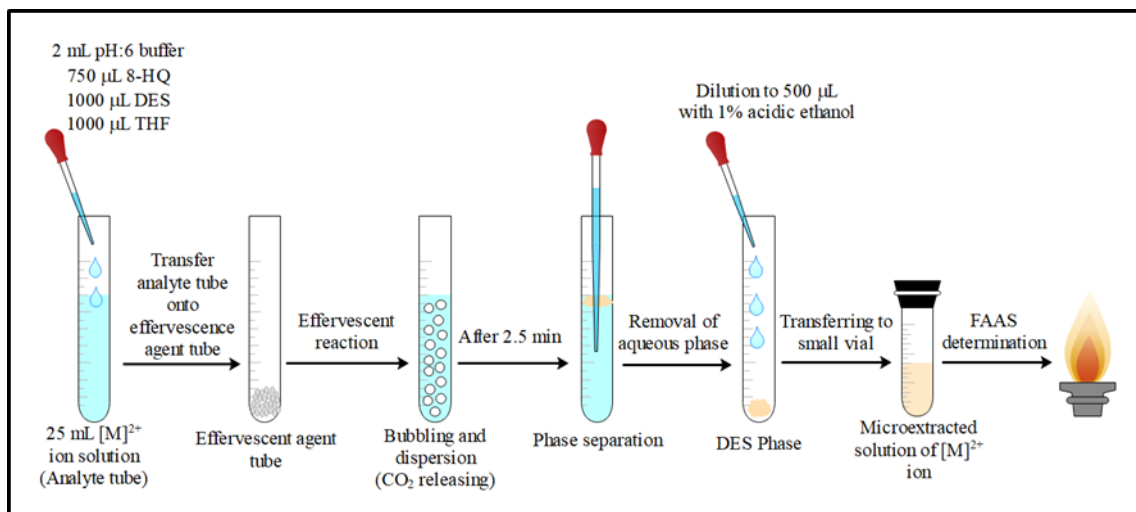
Figure 3.6. The schematic of the EA-DES-DLLME procedure with effervescent powder.

3.5.2. Using Effervescent Tablet

Figure 3.7 illustrates the EA-DES-DLLME procedure using the effervescent tablet. The effervescent tablet was added directly to the analyte tube which containing the extraction solutions of metal ions that were described in the previous section. The tablet settled down at the bottom of the tube and began to effervesce from the bottom to the top and then it was followed by the stages outlined in the preceding section.
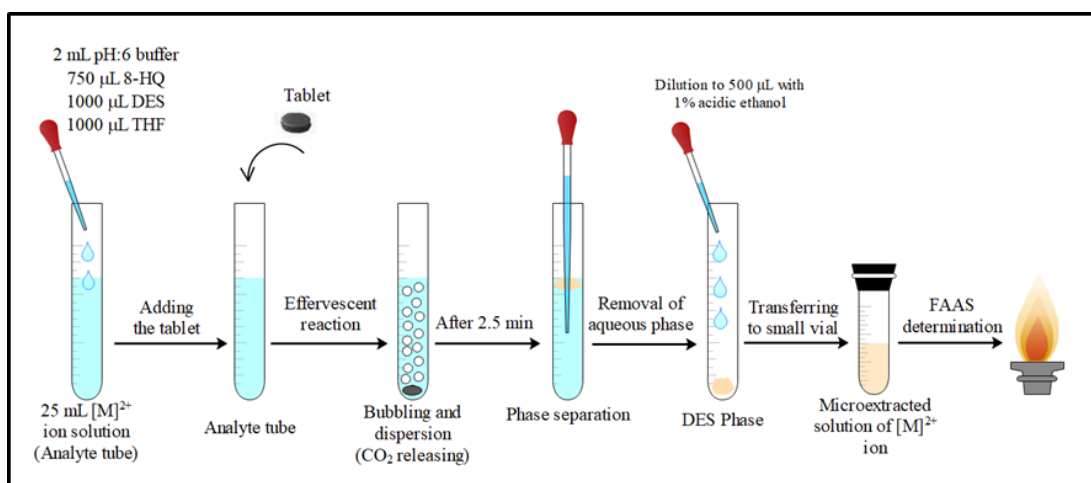
Figure 3.7. The diagram of the EA-DLLME procedure with effervescent tablet.

3.6. Sample Preparation

The digestion process with a closed vessel system has several advantages comparing with open container digestion methods. Teflon containers release fewer metal contaminants compared to glass or ceramic beakers. Hermetically sealed vessels prevent the pollution of airborne dust and help to reduce evaporation and volatile metal losses. Moreover, the procedure with microwave digestion is less time-consuming than open container dissolution methods [153].

To determine the total metal content in the textile products, cotton fabric samples were collected from various fabric shops in Ankara, Turkey. The samples were cut and dried for 48 h at 60 °C then weighed. In the pre-digestion step, 0.5 g of dried samples were put into the vessels of the microwave oven (Figure 3.8 A) and digested with 8 mL of HNO_3 (65 %, w/v) and adding 2 mL of H_2O_2 (30 %, w/v) to increase the oxidizing power of the mixture, so that leads to a clear digest solution. Before sealing the vessels, they should be kept open for a minimum of 15 min at room temperature to allow samples to be digested. Blank digestion was performed in the same manner. Afterward, the vessels were closed by inserting them in the frame support module (Figure 3.8 B) and assembled in the turntable (Figure 3.8 C) so that they are ready to be placed in the microwave digestion oven (Figure 3.8 D).

Digestion conditions of microwave reaction system were consisting of three main steps as follows: (ramping the temperature to 210 ˚C for 20 min with 1600 W and 800 psi), (holding at 210 ˚C for 15 min) and (cooling for 10 min) respectively. The resulting solutions were filled up to 25 mL with deionized water and used as a stock solution of the textile samples. 12.5 mL of textile sample stock solution was adjusted to pH (6.5-7.5) with NH_3 solution, thus, the final volume was 25 mL and the EA-DES-DLLME procedure was applied with a flame atomic absorption spectrometer determination.
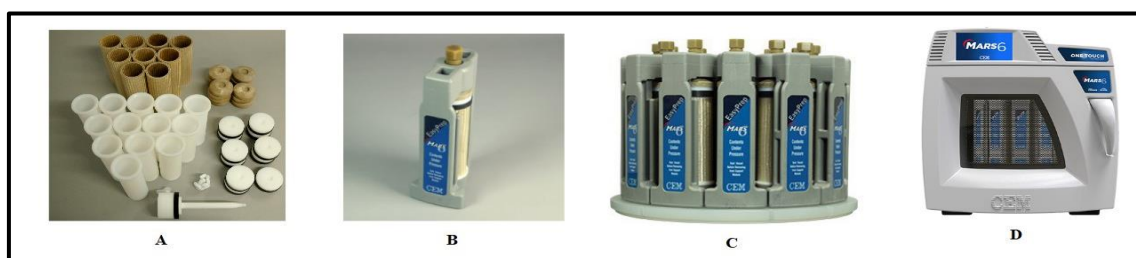
Figure 3.8. (A) microwave vessels, (B) frame support module, (C) assembled the vessels in the turntable and (D) microwave digestion oven.

4. RESULT AND DISCUSSION

EA-DES-DLLME method with FAAS was used for preconcentration and determination of lead, copper, nickel, and cobalt ions. To improve the efficiency of the extraction process, several optimization studies were performed. These are the effect of pH, the effect of effervescent agent properties, the effect of DESs properties, the effect of 8-HQ and THF amount, and the influence of interfering ions.

In this work, the single parameter variable method was applied while keeping the rest of the variables constant. All the optimization parameters were carried out three times and average results were used.

4.1. Effect of pH

The effect of the pH is one of the most important factors that affects the efficiency of the extraction process for the metal ions to be analysed., whereas it has a direct effect on the complex formation between 8-HQ and these metal ions. The complexing agent, 8-HQ is an amphoteric compound that has transferable hydrogen and an atom with lone electron pairs. So, in alkaline solutions, it dissolves as an oxinate, and in acidic solutions dissolves as oxinium [154]. Therefore, the effect of pH on the absorbance value of target metal ions was examined between 3.0 and 10.0 while the other parameters remained constant. The buffer solutions mentioned in Section 3.1.5 were used to adjust the pH in the range of 3.0 to 10.0. The preconcentration process was carried out by applying the conditions in Table 4.1 and the results obtained from the absorption value of each metal are shown in Figure 4.1.

It can be seen from Figure 4.1 that the highest absorbance values for lead, nickel, and cobalt were at pH 6.0, as the results showed low absorbance values for these metal ions at pH below or above 6.0. On the other hand, the highest absorbance value of copper was observed in the range of 6.0-7.0. Therefore pH 6 was chosen as the optimal pH for the subsequent experiments for EA-DES-DLLME.

Table 4.1. Experimental parameters used in pH optimization for lead, copper, nickel, and cobalt determination by EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution	2 mL of pH 3.0 – 10.0
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g
Dispersion time	2.5 min

Figure 4.1. The effect of pH on the EA-DES-DLLME of Pb (II), Cu (II), Ni (II), and Co (II).

4.2. The Effect of Effervescent Agent Properties

The properties of the effervescent agent have an important function in the process of dispersing the target analyte and transferring it from the aqueous phase to the organic phase (ChCl: Ph phase) and thus affect the efficiency of extraction [155]. To obtain the highest EA-DES-DLLME performance, some parameters that have a relation with the effervescent agent should be optimized such as, type and ratio of carbon dioxide (CO_2) source and proton (H^+) donor. In addition to the amount, adding style, and formulation of the effervescent agent was also optimized.

4.2.1. Type of CO_2 Source and H^+ Donor

To carry out the effervescence reaction, it is necessary to have a source of proton donor and a carbon dioxide compound for producing enough bubbles that assist in the dispersion of the extraction solvent. For that reason, the composition of the effervescent agents has great importance in the EA-DES-DLLME method.

In order to prepare six various effervescent powders with all possible combinations and considering the stoichiometry of the reaction between the sources of proton donor and CO_2, for this purpose, SDP, CA, and AA were examined as proton donors, and SBC, SC were examined as a source of CO_2. In this section, the quantity of the effervescent agent was selected on the basis of the production of 0.001 mol of carbon dioxide. Effervescent capacity can be affected by the humidity of effervescent powders. Therefore, initial thermal treatment for drying is requisite.

Some characteristics related to the effervescent agent performance were also determined. For this purpose, the effervescence times for each combination were noted and the pH changes of the aqueous sample at the end of the effervescent reaction were calculated. Obtained results were tabulated in Table 4.2. This process was carried out by applying the conditions that are given in Table 4.3 and the effect of effervescent agents having different compositions on the absorbance value was shown in Figure 4.2.

Table 4.2. Some properties of six types of effervescent agent by using various sources of components.

Effervescence sources		Main characteristics	
Proton donor	Carbon dioxide	Effervescence time (min)	ΔpH^a
SDP	SBC	1.2	+ 1.10
SDF	SC	3.3	+ 1.30
CA	SBC	0.5	- 2.00
CA	SC	1.3	- 2.20
AA	SBC	2.0	- 0.15
AA	SC	2.5	- 0.13

^{a}pH change value of aqueous sample after addition of effervescent agent.

Table 4.3. Experimental parameters used in the effervescent agent composition study for lead, copper, nickel, and cobalt determination by EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF

Figure 4.2. Effect of effervescent agents having different compositions.

From the results that listed in Table 4.2, it can be noticed that the pH variation (ΔpH) of the analyte solution after adding the effervescent agent was unimportant.

Effervescence time has two antithetical characteristics that affect extraction efficiency. The first is a rapid effervescence that includes strong dispersion of the extraction solvent, which may increase the extraction efficiency, but the shorter extraction time may lead to a lower extraction efficiency. The second one is slower effervescence, means weak dispersion of extraction solvent, which may reduce the extraction efficiency, but also has longer extraction time, which may lead to increased extraction efficiency [29,100]. SBC combinations have a shorter effervescence duration concerning the effervescent time (Table 4.2), which can minimize the extraction efficiency due to the lower interaction time between the extraction

solvent and the analytes as shown in Figure 4.2. Therefore, SBC was excluded and SC was chosen to be the source of carbon dioxide.

According to the results shown in Figure 4.2, AA and SC combination has high absorbance values for each lead, copper, nickel and cobalt due to the fact that the extraction process took place in a suitable time (2.5 min) with a strong dispersion was occurred. Despite the combination of SDP and SC it also looks a bit good which has a longer effervescence time (3.3 min) and a rather weak dispersion process.

Therefore, the composition of AA and SC was selected as a suitable effervescent powder for further improvement and applied for the preparation of tablets.

4.2.2. The Ratio of CO_2 Source and H^+ Donor

The ratio between the CO_2 source and the H^+ donor is important in the effervescent reaction, and to obtain the uttermost possible extraction efficiency, the ratio of CO_2 and the H^+ donor must be determined. Usually, more effective effervescence can be obtained by adding an excessive CO_2 source or an excess of H^+ donor.

In this study, the AA and SC molar ratios were listed to be 0.001:0.001 (depending on the chemical reaction's stoichiometry), 0.001:0.002, 0.001:0.003 (CO_2 source excess), and 0.002:0.001, 0.003:0.001 (H^+ donor excess) as illustrated in Figure 4.3. This process was applied under conditions which listed in Table 4.4.

Table 4.4. Experimental parameters used in the optimization of ratio of AA and SC for lead, copper, nickel, and cobalt determination by EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 1:1, 1:2, 1:3, 2:1, 3:1 mole ratio
Dispersion time	2.5 min
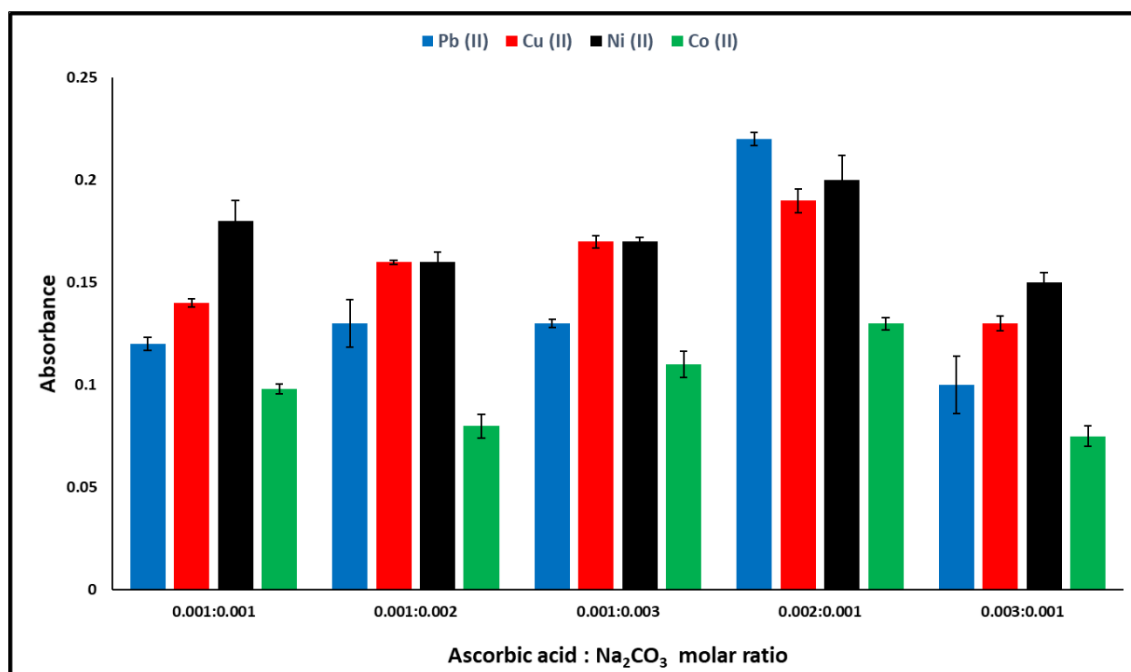
Figure 4.3. The mole ratio between ascorbic acid and sodium carbonate.

The maximal absorbance values obtained when there was an excessive proton donor with a molar ratio of 0.002:0.001 as seen in Figure 4.3, so the AA and SC molar ratio was set to be 0.002:0.001 in subsequent optimizations.

4.2.3. Effervescent Agent Amount

Increasing the amount of effervescent agent in the aqueous sample leads to the production of more CO_2, thus increasing the bubbles that responsible for accelerating the dispersion process and enhance the transport of target analytes, and thus increasing the extraction efficiency, but sometimes with the increase in the amount of the effervescent agent the ionic strength and viscosity of the solution increase, which contributes to reducing the extraction efficiency [101]. For this reason, the amount of the effervescent agent (AA and SC 2:1) was examined in the range of 0.228 - 0.914 g (to generate CO_2 in the range of 0.0005 - 0.002 mole). The results in Figure 4.4 showed that the absorbance values for studied metal ions increased by increasing the amount of the effervescent agent to 0.457 g and then remained constant until 0.914 g, as the absorbance values began to decrease. Therefore, 0.457 g of AA and SC combination was chosen for further experiments. This process was carried out under conditions which listed in Table 4.5.

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Table 4.5. Experimental parameters used in the optimization of effervescent agent amount for lead, copper, nickel, and cobalt determination by EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.228 – 0.914 g
Dispersion time	2.5 min
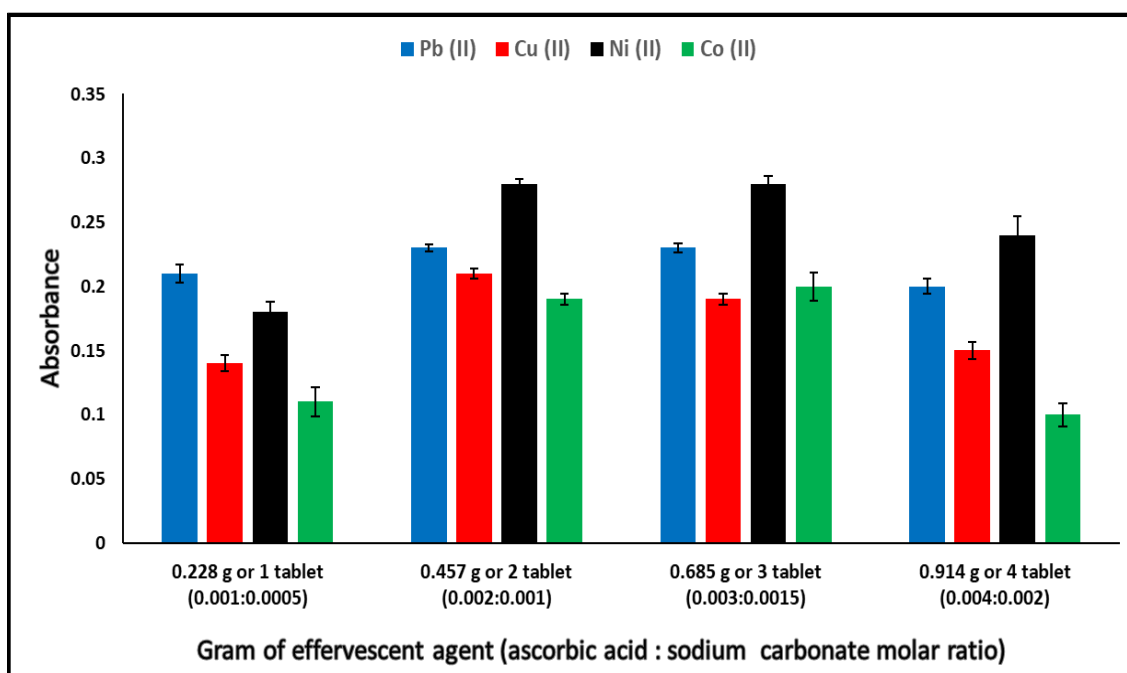
Figure 4.4. The effect of effervescent agent amount.

4.2.4. Addition Style of Effervescent Powder

Effervescence must occur from the bottom to the top of the test tube to achieve good dispersion efficiency. So, two different styles of addition were used in order to get maximized dispersion as shown in Figure 4.5. Style A; at the beginning, the effervescent powder was put in the bottom of the test tube and then the analyte solution which containing the metal ions, complexing agent, DES, and THF was added into the tube of effervescent powder. In style B; the analyte solution containing metal ions, complexing agent, DES, and

THF was initially introduced to the test tube and then the effervescent powder was added into the analyte solution.
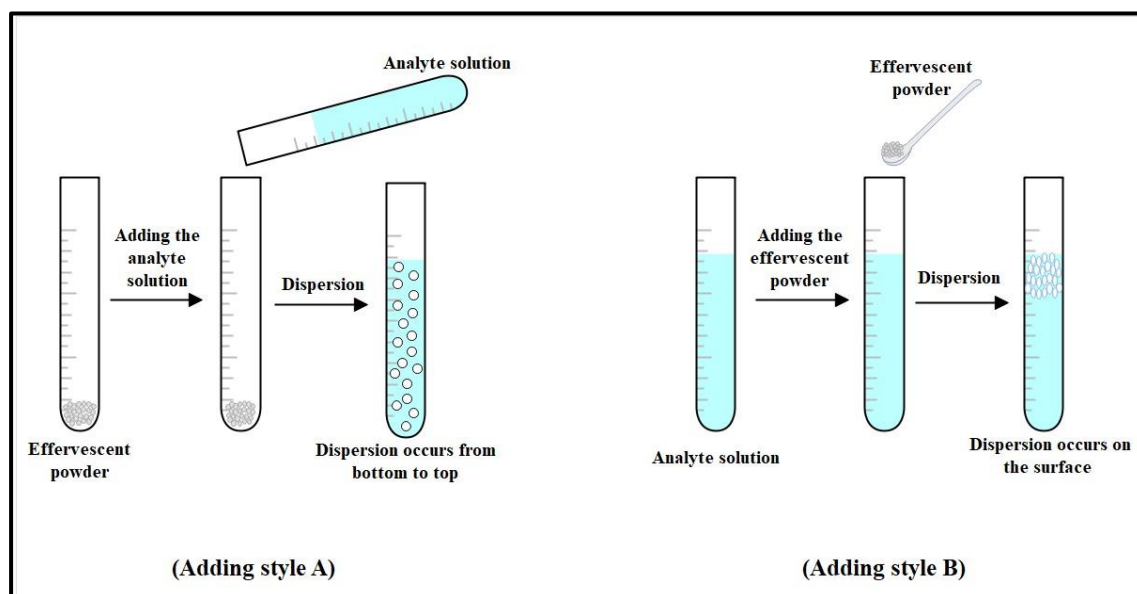
Figure 4.5. Two different adding styles of effervescent agent.

Figure 4.5 shows that style A is better than style B, because in style A, effervescence began immediately from the bottom to the top of the tube and homogeneous dispersion of the extraction solvent into the sample solution occurred, but in style B the dispersion was not homogeneous because the effervescence occurred on the surface of the solution, resulting in a decrease in the extraction efficiency. This process was carried out under conditions which listed in Table 4.6.

Table 4.6. Experimental parameters used in the optimization of effervescent agent addition style for lead, copper, nickel, and cobalt determination by EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g
Dispersion time	2.5 min

Figure 4.6. Effect of different addition style of effervescent powder.

4.2.5. Effect of Physical Form of Effervescent Agent

The extraction performance was compared on two physical form of the effervescent agent, the powder, and the tablet. It became clear from the results shown in Figure 4.7 that the powder and the tablet have the same good results, so the choice remains according to the ease of use and convenience. Therefore, the tablet form was selected for the following experiments. As for the powder, it is only used at the beginning of the experiments until the composition, molar ratio, and amount of the effervescent agent are determined. This experiment was carried out under the conditions listed in Table 4.7.

Table 4.7. Experimental parameters were used to examine effervescent agent formulas for lead, copper, nickel, and cobalt determination by the EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g
Dispersion time	2.5 min

Figure 4.7. Formulation effect of the effervescent agent.

4.3. Evaluation of the Effervescence-Assistance (EA) Performance

In this section, the efficiency of EA was compared with various other dispersion methods, such as ultrasound-assistance, vortex-assistance, and manual shaking-assistance. In addition, one of the samples was kept without any dispersion method. These assistance methods were applied for 2.5 min as effervescence time and all other parameters were kept at the optimal values. Experimental conditions were listed in Table 4.8. The results showed that the extraction efficiency of EA and ultrasound-assistance was almost similar and greater than the vortex-assistance, and manual shaking-assistance. In addition, the extraction efficiency for the sample without any dispersion assistance was negligible as shown in Figure 4.8. Therefore, it can be inferred that the performance of EA dispersion was equal to that of ultrasound-assistance and higher than that of manual shaking-assistance and vortex-assistance. It can be concluded that among all these dispersion methods examined, effervescence is the superior dispersion assistance as it does not need external energy.

Table 4.8. Experimental parameters that are used in efficiency evaluation of the EA as a dispersion for lead, copper, nickel, and cobalt determination by the EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g in the form of 2 tablets
Dispersion time	2.5 min

Figure 4.8. Comparison of EA with other dispersion processes.

4.4. The Effect of Extraction Solvent Properties

The extraction solvent is one of the most important parameters of the DLLME method generally, and in EA-DES-DLLME especially. As is known, good extraction solvent criteria are as follows: higher or lower density than that of water, low aqueous phase solubility, ability to extract the target analyte, and it has the characteristic of easy dispersion in the aqueous solution during the dispersion process. The kind of DES has a significant effect on

the extraction efficiency and to achieve the above conditions, the effect of type, molar ratio, and volume of DES must be studied.

4.4.1. Selection of the DES Type and Mole Ratio

There are a large number of salts used as HBA (hydrogen bond acceptor) as well as HBD (hydrogen bond donor) that can be used to prepare DESs, so there is no limit to the number or types of DESs that can be prepared [107]. To choose the suitable DES as an extraction solvent for the EA-DES-DLLME method, three different types of DES according to availability, abundance and price of the material were prepared, where ChCl was used as an HBA, and Ph, U, and OA were used as an HBD with different mole ratio for each type ranged from 1:1 to 1:5.

It is observed from the results that shown in Figure 4.9 that the optimum molar ratios for ChCl: U and ChCl: OA compositions were 1:2 and 1:1, respectively and this corresponds with the information mentioned in the literature reviewed [156,157]. Despite this, the extraction efficiency when using these two formulations was very poor, in other words, the extraction process not occurred. According to the expectations, these two formulations have the ability to be soluble in aqueous samples, for this reason, they were excluded from this study. As for the composition of the ChCl: Ph, high extraction efficiency was obtained when it was used in the EA-DES-DLLME method as shown in Figure 4.8. So, the ChCl: Ph was chosen as an extraction solvent to carry out the remainder of the experiments. This experiment was completed under the conditions listed in Table 4.9.

Table 4.9. Experimental parameters were used to examine the suitable DES type for the EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph, ChCl: U, ChCl: OA with mole ratio ranged from 1:1 - 1:5
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g in the form of 2 tablets
Dispersion time	2.5 min
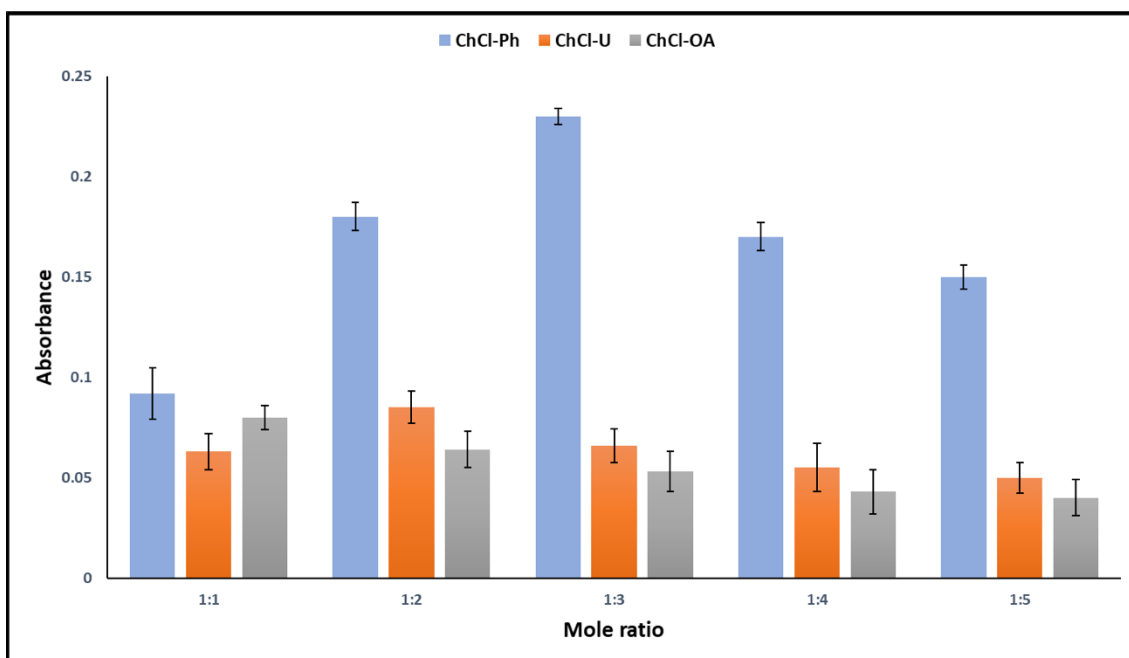
Figure 4.9. Effect of DES type on the extraction efficiency.

On the other hand, the viscosity of the ChCl-Ph influences the extraction efficiency, where it is preferable to use DES in general with lower viscosity. According to this fact, the ChCl: Ph mole ratio was examined as 1:1, 1:2, 1:3, 1:4, 1:5, as viscosity decrease with decreasing of HBA: HBD mole fraction, or in other words, the viscosity decreases with the increase in the mol of HBD over HBA.

Figure 4.10 shows the effect of different molar ratios of ChCl: Ph on the extraction efficiency, as it can be seen that the absorbance values increase with increasing molar ratio of phenol from 1:1 to 1:3 and then decrease at 1:4 and 1:5 for metal ions studied, and therefore the 1:3 is considered the best mole ratio of the ChCl: Ph combination. The reason is that ChCl: Ph at a ratio of 1:1 and 1:2 is somewhat viscous due to the presence of extensive hydrogen bonds between ChCl and Ph, which impede the mobility of the metal complex within the DES. Besides, in the ratio of 1: 4 and 1: 5, the viscosity of ChCl: Ph is very low, almost close to the viscosity of water, and thus the solubility of ChCl: Ph increases in the aqueous phase. for this reason, ChCl: Ph with a 1:3 mole ratio was selected as the optimum composition for the experiments. This investigation was carried out under the conditions listed in Table 4.10.

Table 4.10. Experimental parameters were used to investigate the ChCl: Ph mole ratio for lead, copper, nickel, and cobalt determination by the EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:1, 1:2, 1:3, 1:4, 1:5 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g in the form of 2 tablets
Dispersion time	2.5 min
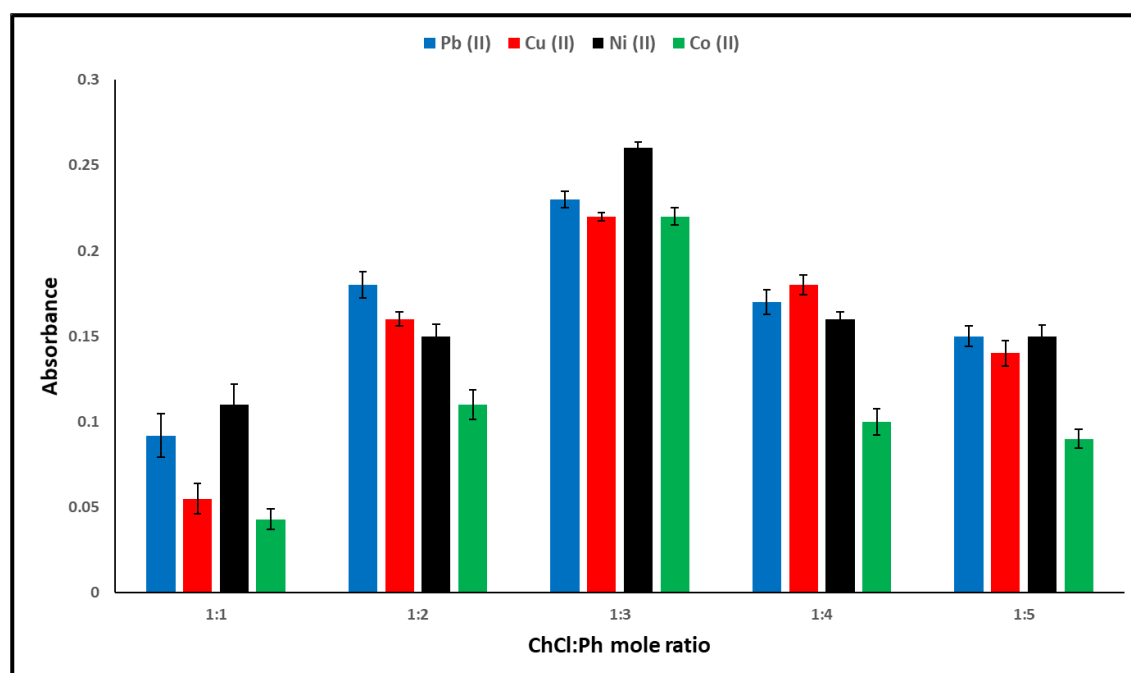
Figure 4.10. Effect of ChCl-Ph mole ratio.

4.4.2. Volume of the DES

After choosing the appropriate DES components, as well as the appropriate molar ratio, now should investigate the optimal volume of DES and its effect on the extraction efficiency of the EA-DES-DLLME method. The volume of DES was checked by adding different volumes ranging from 100 to1500 µL and the results showed that the maximum extraction efficiency was achieved at 1000 µL for lead, copper, nickel, and cobalt, and then decreased when DES volumes were greater than 1000 µL, as shown in Figure 4.11. Extraction

efficiency decreases when the volume of DES is less or greater than 1000 µL. Because, when the volume is less than 1000 µL, the DES is not enough to extract the metal ions from the analyte solution. In addition, when the volume is greater than 1000 µL, an increase in the final volume of the DES phase is obtained, and therefore a larger volume of acidic ethanol is needed to dilute it. Therefore 1000 µL of DES was selected to be the suitable volume for the following experiments. This optimization was carried out under the conditions listed in Table 4.11.

Table 4.11. Experimental parameters were used to check the volume of ChCl-Ph for lead, copper, nickel, and cobalt determination by the EA-DES-DLLME method.

Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	100 – 1500 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g in the form of 2 tablets
Dispersion time	2.5 min

Figure 4.11. Effect of DES volume.

4.5. Effect of the Complexing Agent

For the extraction of metal ions into the organic phase, a non-polar complex must be formed before the extraction process. For this purpose, a complexing agent should be used. Different complexing agents that can form complexes with the studied metal ions with the highest selectivity were tested. Quinalizarin Red, dithizone, diphenyl carbazide, and 8-HQ were tested complexing agents. As a result of the preliminary experiments, 8HQ was chosen as the complexing agent, since the highest efficiency was obtained with 8HQ for all metal ions studied.

8-HQ is widely used for the separation and analysis of metal ions due to its excellent coordination ability and as well as good metal identification properties, well it can form complexes with divalent metal ions by the process of metal chelation [150].

The extraction efficiency depends on the amount of analyte that transfers to the DES phase by complexing with 8-HQ. Therefore, there is a necessity to find a sufficient volume of the 8-HQ as a complexing agent that will collect all the metal ions targeted for the determination in the analyte solution. The effect of the volume of 8-HQ on EA-DES-DLLME for target metal ions was investigated in the range of 100 -1500 µL and the results showed that the signals of these metals were increased with the increase in the volume of 8-HQ up to 750 µL and then stayed constant, as shown in Figure 4.12. Therefore, 750 µL of 8-HQ was selected as an ideal volume and applied in subsequent works. These results were achieved under the conditions listed in Table 4.12.

Table 4.12. Experimental parameters were used to optimize the 8-HQ volume for lead, copper, nickel, and cobalt determination by the EA-DES-DLLME method.

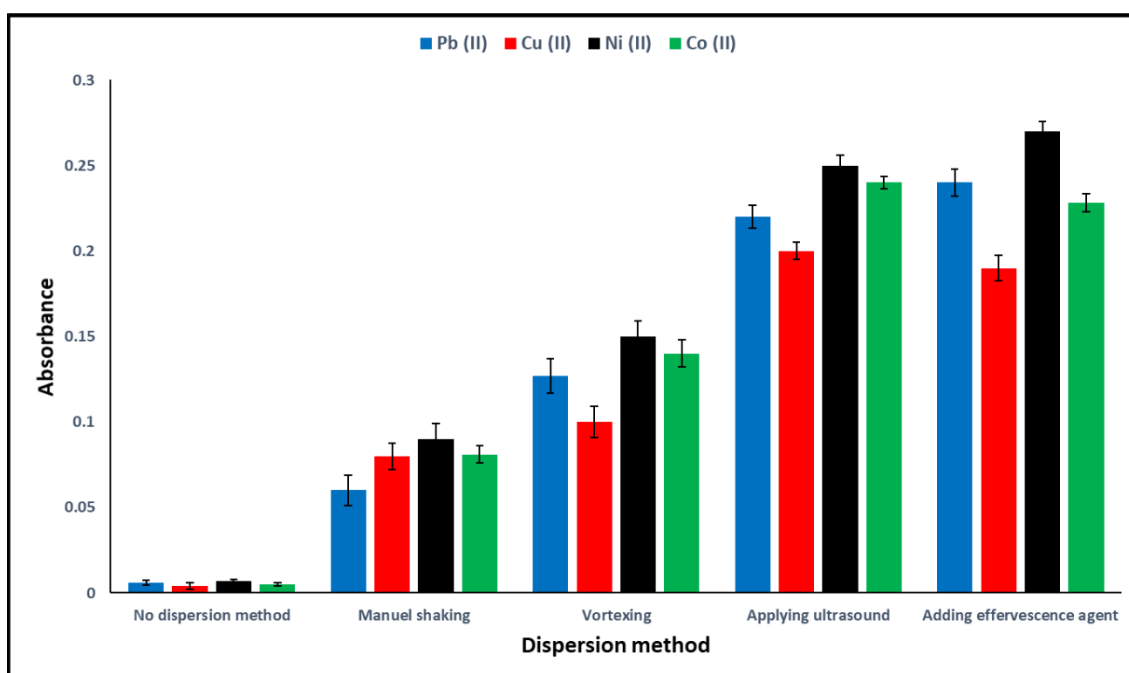|	
Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	100 – 1500 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	1000 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g in the form of 2 tablets
Dispersion time	2.5 min

Figure 4.12. Effect of complexing agent 8-HQ volume.

4.6. Effect of THF Volume

In this study, THF was used as an aprotic solvent that had an ability to react with water molecules more quickly than DES. In other words, there is an inverse relationship between the THF-H_2O and the DES-H_2O interactions, whereby an increase in the THF-H_2O interaction leads to a decrease in the DES-H_2O interaction, thus DES is separated from the aqueous phase and self-aggregation will occur [158].

The final volume of the extracted DES phase varies with the volume of THF that was added to the analyte solution, so the effect of the THF volume was examined in the range of 0 - 1500 µL. The results illustrated in Figure 4.13 showed that the extraction efficiency was almost zero when THF was not added and then began to increase with the increasing of THF volume until 1000 µL and then decreased with THF volume greater than 1000 µL for each target metal ions. The decrease in the extraction efficiency at lower volumes of THF is due to, the DES-H_2O interaction is predominant and therefore the process of DES separation from the aqueous phase did not occur completely. In addition, the large volume of THF leads to an increase in the solubility of the analyte and DES in the aqueous phase. Hence, 1000 µL of THF volume was selected as the optimal value for the next experiments. These experiments were performed under the conditions listed in Table 4.13.

Table 4.13. Experimental parameters were used to select the THF volume for lead, copper, nickel, and cobalt determination by the EA-DES-DLLME method.

|	
Concentration of metal ions	500 (µg/L) of Pb, 50 (µg/L) of Cu, 100 (µg/L) of Ni, 100 (µg/L) of Co
Sample volume	25 mL
Buffer solution volume	2 mL of pH 6
Complexing agent concentration	0.1 % (w/v) 8-HQ
Complexing agent volume	750 µL
DES composition	ChCl: Ph with 1:3 mole ratio
DES volume	1000 µL
Aprotic solvent volume	0 – 1500 µL of THF
Effervescent agent composition	AA:SC with 2:1 mole ratio
Effervescent agent amount	0.457 g in the form of 2 tablets
Dispersion time	2.5 min

Figure 4.13. Effect of THF volume.

4.7. Effect of Interfering Ions

Real samples include certain possible ions, some of which cannot be complexed with ligands such as alkali, alkaline earth elements, and anions, and some have the capacity to associate with complexing agents at high concentrations such as transition metal ions, resulting in incorrect measurements at FAAS and lower extraction performance for studied metal ions. In order to decide the specific selectivity of the EA-DES-DLLME method for the target ions in the presence of other ions, the influence of the various ions was examined. For this reason, the EA-DES-DLLME procedure has been applied under optimized conditions (previously

achieved) by introducing foreign ions at varying concentrations to solutions each containing 250, 50, 100,100 μgL^{-1} for Pb (II), Cu (II), Ni (II), and Co (II) respectively. Throughout the EA-DES-DLLME process, the foreign ions concentration was tested up to 2000 times that of the target analytes. If the foreign ion causes a difference of ± 5 % in the recovery value, that means the ion has an interfering effect. If the added ion did not have an interference effect when added 2000 times of analyte concentration, it is expressed as > 1:2000 in the table. The results in the Table 4.14 and 4.15 showed that the EA-DES-DLLME method has powerful selectivity and that the generally prevalent foreign ions in real samples have no interference effect on the extraction and determination of examined metal ions. Moreover, the effect of the matrix on the extraction efficiency was studied using the matrix matched calibration, details of which will be given in the Section 4.9.3. The obtained matrix effects in this study were 0.70 % for Pb (II), -3.76 % for Cu (II), -2.94 % for Ni (II), and -0.50 % for Co (II). So, it was confirmed that there was no prominent effect of the sample matrix on the extraction efficiency of the EA-DES-DLLME method.

Table 4.14. Effect of foreign ions for Pb (II) and Cu (II) on the EA-DES-DLLME method.

Interfering ion, [Int.]	Added as	[Pb^{2+}]		[Cu^{2+}]	
		[Pb]: [Int.]	Recovery (%)	[Cu]: [Int.]	Recovery (%)
Na$^+$	NaCl	> 1: 2000	96.8 ± 1.2	> 1: 2000	98.3 ± 1.3
K$^+$	KCl	> 1: 2000	98.1 ± 1.1	> 1: 2000	96.4 ± 2.2
NH$_4$$^+$	NH$_4$Cl	> 1: 2000	97.6 ± 1.3	> 1: 2000	99.1 ± 1.3
Cl$^-$	NaCl	> 1: 2000	99.2 ± 2.0	> 1: 2000	97.6 ± 1.8
NO$_3$$^-$	KNO$_3$	> 1: 2000	97.7 ± 1.8	> 1: 2000	95.8 ± 1.7
SCN$^-$	KSCN	> 1: 2000	98.3 ± 1.5	> 1: 2000	99.0 ± 2.1
Cd^{2+}	CdCl$_2$	1:1000	96.9 ± 1.9	1:500	97.2 ± 1.7
Cu^{2+}	Cu (NO$_3$)$_2$	1:1000	95.8 ± 2.1	-	-
Mn^{2+}	MnCl$_2$	1:1000	97.8 ± 1.8	> 1: 2000	97.5 ± 1.6
Zn^{2+}	Zn (NO$_3$)$_2$	1: 2000	96.3 ± 1.3	1:500	97.1 ± 1.8
Pb^{2+}	Pb (NO$_3$)$_2$	-	-	1:1000	98.7 ± 1.5
Co^{2+}	Co (NO$_3$)$_2$	1:1000	98.1 ± 1.7	1:2000	96.5 ± 1.3
Ni^{2+}	Ni (NO$_3$)$_2$	1:1000	97.8 ± 1.7	1:500	95.9 ± 1.4
Mg^{2+}	Mg (NO$_3$)$_2$	1: 2000	98.8 ± 1.7	> 1: 2000	96.2 ± 1.6
Ca^{2+}	Ca (NO$_3$)$_2$	1:1000	98.7 ± 1.7	1:500	98.5 ± 1.5
SO$_4$$^{2-}$	Na$_2$SO$_4$	1:2000	96.4 ± 1.2	1:1000	97.7 ± 2.0
CrO$_4$$^{2-}$	K$_2$CrO$_4$	> 1: 2000	99.2 ± 1.8	> 1: 2000	96.9 ± 1.5
Fe^{3+}	Fe (NO$_3$)$_3$	1:500	97.5 ± 1.4	1:1000	96.8 ± 2.2
Cr^{3+}	Cr (NO$_3$)$_3$	1:1000	98.8 ± 1.3	> 1: 2000	98.6 ± 1.9
Al^{3+}	Al (NO$_3$)$_3$	> 1: 2000	98.2 ± 1.7	1:1000	99.1 ± 1.9
PO$_4$$^{3-}$	(NH$_4$)$_3$PO$_4$	1:2000	97.4 ± 1.6	1:2000	97.2 ± 1.4

Table 4.15. Effect of foreign ions for Ni (II) and Co (II) on the EA-DES-DLLME method.

Interfering ion, [Int.]	Added as	[Ni²⁺]		[Co²⁺]	
		[Ni]: [Int.]	Recovery (%)	[Co]: [Int.]	Recovery (%)
Na^+	NaCl	> 1: 2000	97.7 ± 1.8	> 1: 2000	96.8 ± 1.8
K^+	KCl	> 1: 2000	98.8 ± 1.7	> 1: 2000	97.8 ± 1.7
NH_4^+	NH_4Cl	> 1: 2000	95.8 ± 2.1	> 1: 2000	96.3 ± 1.3
Cl^-	NaCl	> 1: 2000	97.6 ± 1.3	> 1: 2000	96.2 ± 1.6
NO_3^-	KNO_3	> 1: 2000	97.4 ± 1.6	> 1: 2000	99.2 ± 1.8
SCN^-	KSCN	> 1: 2000	98.8 ± 1.3	> 1: 2000	98.6 ± 2.1
Cd^{2+}	$CdCl_2$	> 1: 2000	98.7 ± 1.7	1:1000	96.4 ± 2.2
Cu^{2+}	Cu $(NO_3)_2$	1:500	96.5 ± 1.2	1:500	96.9 ± 1.9
Mn^{2+}	$MnCl_2$	> 1: 2000	97.5 ± 1.4	1: 2000	97.6 ± 1.3
Zn^{2+}	Zn $(NO_3)_2$	1:1000	96.4 ± 1.2	1:500	98.1 ± 1.1
Pb^{2+}	Pb $(NO_3)_2$	1:500	98.1 ± 1.1	1:1000	97.7 ± 1.8
Co^{2+}	Co $(NO_3)_2$	> 1: 2000	97.8 ± 1.7	-	-
Ni^{2+}	Ni $(NO_3)_2$	-	-	1:1000	99.2 ± 2.0
Mg^{2+}	Mg $(NO_3)_2$	> 1: 2000	96.2 ± 1.6	1: 2000	96.5 ± 1.5
Ca^{2+}	Ca $(NO_3)_2$	1:500	98.1 ± 1.7	1:1000	97.2 ± 1.7
SO_4^{2-}	Na_2SO_4	1:1000	99.2 ± 2.0	1:2000	97.5 ± 1.6
CrO_4^{2-}	K_2CrO_4	> 1: 2000	99.2 ± 1.8	> 1: 2000	97.6 ± 1.8
Fe^{3+}	Fe $(NO_3)_3$	1:500	97.8 ± 1.8	1:1000	98.3 ± 1.3
Cr^{3+}	Cr $(NO_3)_3$	1:1000	98.2 ± 1.7	1:1000	99.0 ± 2.1
Al^{3+}	Al $(NO_3)_3$	1:1000	96.3 ± 1.3	1:500	99.1 ± 1.3
PO_4^{3-}	$(NH_4)_3PO_4$	1:2000	96.9 ± 1.9	1:2000	98.3 ± 1.5

4.8. Analytical Performance Characteristics of the EA-DES-DLLME Method

The data of calibration curves, which are generated with standard aqueous solutions were presented in Table 4.16. The data include the linear concentration ranges, the linear equations obtained by direct analysis in FAAS, and the correlation coefficient for each lead, copper, nickel, and cobalt ions.

Table 4.16. Aqueous calibration curves data for Pb (II), Cu (II), Ni (II), and Co (II).

Ion	Linear concentration range ($\mu g\ L^{-1}$)	Linear equation	Correlation coefficient, R^2
Pb (II)	2500 – 50000	A= 8 x10^{-6} C + 0.0073	0.9984
Cu (II)	500 – 8000	A= 5 x10^{-5} C + 0.0035	0.9986
Ni (II)	500 – 8000	A= 4 x10^{-5} C + 0.0046	0.9990
Co (II)	500 – 8000	A= 4 x10^{-5} C + 0.0053	0.9992

The data of calibration curves, which are obtained after applying the EA-DES-DLLME method (after preconcentration) in the optimum conditions for investigated ions were illustrated in Table 4.17.

Table 4.17. Calibration curves data after EA-DES-DLLME for Pb (II), Cu (II), Ni (II), and
Co (II).

Ion	Linear concentration range ($\mu g\ L^{-1}$)	Linear equation	Correlation coefficient, R^2
Pb (II)	10 – 750	A= 0.0005 C + 0.0131	0.9992
Cu (II)	2.5 – 200	A= 0.0026 C + 0.0387	0.9989
Ni (II)	5 – 150	A= 0.0023 C + 0.0076	0.9994
Co (II)	5 – 150	A= 0.0021 C + 0.008	0.9994

The definitions and the calculation methods for the various analytical parameters used to evaluate the analytical performance of the developed EA-DES-DLLME technique were illustrated in Table 4.18 and the results of these calculations were presented in detail in Table 4.19.

Table 4.18. Analytical performance criteria and calculation methods for the EA-DES-DLLME.

Analytical parameters	Definition	Calculations
Enhancement Factor (EF)	It is described as the ratio between the slope (m_1) of the calibration curve obtained after applying the preconcentration method (EA-DES-DLLME) and the slope (m_2) of the calibration curve getting from the aqueous standards (without preconcentration) method [159].	EF= m_1/m_2
Preconcentration Factor (PF)	It is defined as the volume ratio of the analyte in the final diluted DES phase and in the initial solution [160].	PF=$V_{sample}/V_{DESphase}$
Limit of Detection (LOD)	It is the lowest concentration level that can be determined separately from the ground interference in the introduced method but cannot be quantitatively determined. It calculates by the formula 3 S/m where S is the standard deviation of blank (n=10) and m is the slope of the calibration curve prepared with the developed EA-DES-DLLME in optimum conditions [159].	LOD = 3 S/m
Limit of Quantification (LOQ)	It is the lowest concentration that can be determined by the introduced method. It calculates by the formula 10 S/m where S is the standard deviation of blank (n=10) and m is the slope of the calibration curve prepared with the developed EA-DES-DLLME in optimum conditions [159].	LOQ= 10 S/m
Correlation Coefficient (R^2)	It is the characteristic of linearity among the standards used in plotting the calibration curve. A more linear calibration curve can be achieved when the R^2 is closer to 1.	It is obtained from the calibration curve

Table 4.18. (Continued)

Relative Standard Deviation (RSD%)	Reproducibility Relative standard deviation is the scale of accuracy and the convergence of the data obtained as a result of repeated extraction for a certain number of consecutive days (n) of the analyte with a known concentration. Relative standard deviation is calculated by dividing the standard deviation of the analysis made at a certain concentration and the number of samples by the average of the analysis results and multiplying it by 100.	RSD= $S/X_{(Average)} \times 100\%$
	Repeatability The convergence of the data obtained as a result of a certain number of extractions (n) applied by the same person on the same day under the same conditions.	

Table 4.19. Analytical performance characteristics achieved from the EA-DES-DLLME method advanced for Pb (II), Cu (II), Ni (II), and Co (II) ions.

Analytical parameters	Obtained value for metal ions			
	Pb (II)	Cu (II)	Ni (II)	Co (II)
Linear concentration range ($\mu g\ L^{-1}$)	10 – 750	2.5 – 200	5 – 150	5 – 150
Initial sample volume (mL)	25	25	25	25
Extracted sample volume (μL)	500	500	500	500
The enhancement factor (EF)	62.5	52.0	57.5	52.5
Preconcentration factor (PF)	50	50	50	50
Limit of detection (LOD) ($\mu g\ L^{-1}$) (n = 10)	2.44	1.29	0.58	1.55
Limit of quantitation (LOQ) ($\mu g\ L^{-1}$) (n = 10)	8.13	4.30	1.93	5.16
RSD (%) Reproducibility ($n=10$), 500 $\mu g\ L^{-1}$ Pb (II), 50 $\mu g\ L^{-1}$ Cu (II), 100 $\mu g\ L^{-1}$ Ni (II), 100 $\mu g\ L^{-1}$ Co (II)	1.07	1.61	1.35	1.97
RSD (%) Repeatability ($n=10$), 500 $\mu g\ L^{-1}$ Pb (II), 50 $\mu g\ L^{-1}$ Cu (II), 100 $\mu g\ L^{-1}$ Ni (II), 100 $\mu g\ L^{-1}$ Co (II)	0.94	1.36	1.24	1.75

4.9. Validation of the EA-DES-DLLME

In order to validate the proposed method, three different validation methods were performed:

- Using the certified reference materials (CRM),
- Spiked sample analysis,
- Matrix matched calibration study

4.9.1. Validation of the EA-DES-DLLME by CRM

In the beginning, the accuracy of the EA-DES-DLLME method was verified by analysis of CRM, namely NCS ZC73013 for spinach. This CRM was containing $11.10 \pm 0.90\ \mu g\ g^{-1}$ for lead, $8.90 \pm 0.40\ \mu g\ g^{-1}$ for copper, $0.92 \pm 0.12\ \mu g\ g^{-1}$ for nickel, and $0.22 \pm 0.03\ \mu g\ g^{-1}$ for cobalt. This experimental procedure was applied using the microwave digestion method which was described in section 3.6. The results of this study are given in Table 4.20. The percent recovery values for the CRM experiment are in the range of 97.2 – 98.6 %, so the recovery values show that the separation/enrichment process of the presented EA-DES-DLLME method has been applied successfully. Although the CRM used does not have the same matrix as the textile sample, it is important in proving the applicability of the proposed method to samples with a complex matrix.

Table 4.20. Analysis results of certified material NCS ZC 73013 (Spinach) for Pb (II), Cu (II), Ni (II), and Co (II) ions ($n = 4$).

Ion	Certificated value ($\mu g\ g^{-1}$)	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)	Recovery (%)
Pb (II)	11.10 ± 0.90	-	10.95 ± 0.14	98.6 ± 1.3
Cu (II)	8.90 ± 0.40	-	8.74 ± 0.25	98.2 ± 2.9
Ni (II)	0.92 ± 0.12	-	0.89 ± 0.01	97.2 ± 1.4
Co (I)	0.22 ± 0.03	2.5	2.67 ± 0.07	98.3 ± 2.5

After obtaining the results, it must be compared with the certificate values of the CRM to understand whether there is a significant difference between them. Therefore, the t-test (Equation 4.1) was applied to verify this purpose.

$$t_{Cal.} = \frac{\mu - X}{S/\sqrt{n}} \tag{4.1}$$

X: Average of analysis results

μ: Value of certified reference material

S: Standard deviation of the analysis results

n: Number of analyses performed.

The value of the t_{table} was calculated according to the n-1 degree of freedom at the $\alpha = 0.05$ significance level (95% confidence level). When comparing the results of the $t_{cal.}$ values with the t_{table} values, that shown in Table 4.21, it is observed that the $t_{cal.}$ values smaller than the t_{table} values and that means there is no significant difference in the results. Thus, the results of the developed EA-DES-DLLME method can be accredited.

Table 4.21. The results of *t*-test calculated for the CRM sample for Pb (II), Cu (II), Ni (II), and Co (II) ions ($n = 4$).

CRM type	Ion	$t_{cal.}$	t_{table}	Evaluation
NCS ZC73013 (Spinach)	Pb (II)	0.53	3.18	$t_{cal.} \leq t_{table}$
	Cu (II)	0.31	3.18	
	Ni (II)	0.99	3.18	
	Co (II)	0.34	3.18	

4.9.2. Determination of the Target Heavy Metals in Real 100% Cotton Textile Samples (Spiking studies)

Humans are subjected to various chemical compounds that they get from textile materials as a result of their daily contact with textiles such as garments, bed linen, and related goods. Also, many of these chemicals including heavy metals used in textiles may pose a risk to customers' health, so the amount of these materials should be reduced to a minimum as possible. Therefore, to determine and calculate the Pb (II), Cu (II), Ni (II), and Co (II) content in some textile samples and in order to demonstrate the capability of EA-DES-DLLME, the spiking method was used. For this purpose, 100% cotton textile samples were gathered and dissolved by the microwave digestion method as described in Section 3.6. The standard solutions for target metal ions were spiked into the samples at three different concentrations, and then the EA-DES-DLLME method was applied and repeated three times for each sample. The recovery values obtained by spiking for the cotton textile sample by the proposed method were calculated according to Equation 4.2.

$$Recovery \% = \frac{C_{measured} - C_{initial}}{C_{added}} \times 100\% \qquad (4.2)$$

$C_{measured}$: Total concentration value of target obtained after adding ($\mu g\ L^{-1}$)

$C_{initial}$: Concentration of the target in the sample before adding ($\mu g\ L^{-1}$)

C_{added}: Concentration of added to the sample ($\mu g\ L^{-1}$)

The obtained results were illustrated in detail in Table 4.22, which includes the spiking level, measured concentration, recoveries, and calculated metal content for each metal in each cotton textile sample.

Table 4.22. Analysis results for Pb (II), Cu (II), Ni (II), and Co (II) metal ions, which spiked by application of the EA-DES-DLLME method for various real cotton textile samples ($n = 3$).

Sample No. & color	Sample picture	Metal ion	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery %	Calculated metal content ($\mu g\ g^{-1}$)
T₁ (Red for Adults)		Pb	0	9.40	-	0.94 ± 0.09
			100	107.20	97.80 ± 0.81	
			250	260.60	100.48 ± 1.31	
			500	507.40	99.60 ± 1.82	
		Cu	Direct aspiration with FAAS			1.27 ± 0.07
		Ni	0	10.96	-	1.09 ± 0.06
			25	35.04	96.35 ± 1.63	
			50	59.65	97.39 ± 1.74	
			100	109.22	98.26 ± 1.28	
		Co	0	42.14	-	4.21 ± 0.10
			25	66.43	97.14 ± 1.91	
			50	91.19	98.10 ± 2.40	
			100	140.71	98.57 ± 1.74	
T₂ (Plaid Green for Adults)		Pb	0	23.60	-	2.36 ± 0.11
			100	120.80	97.20 ± 0.93	
			250	270.00	98.56 ± 1.26	
			500	520.82	99.44 ± 1.79	
		Cu	Direct aspiration with FAAS			22.58 ± 0.19
		Ni	0	45.74	-	4.56 ± 0.15
			25	70.09	97.39 ± 1.10	
			50	94.87	98.26 ± 1.24	
			100	141.39	95.65 ± 1.55	
		Co	0	32.62	-	3.26 ± 0.13
			25	56.90	97.14 ± 2.04	
			50	81.43	97.62 ± 0.99	
			100	131.19	98.57 ± 2.69	

Table 4.22. (continued)

Sample No. & color	Sample picture	Metal ion	Added (µg L^{-1})	Found (µg L^{-1})	Recovery %	Calculated metal content (µg g^{-1})
T$_3$ (Khaki for Adults)		Pb	0	18.78	-	1.88 ± 0.14
			100	117.40	98.62 ± 1.04	
			250	266.80	99.21 ± 2.15	
			500	520.20	100.28 ± 0.82	
		Cu	Direct aspiration with FAAS			15.49 ± 0.14
		Ni	0	19.65	-	1.96 ± 0.08
			25	44.04	97.57 ± 1.13	
			50	68.78	98.26 ± 2.34	
			100	117.48	97.83 ± 1.51	
		Co	0	15.95	-	1.59 ± 0.09
			25	41.19	100.95 ± 1.89	
			50	65.48	99.05 ± 1.98	
			100	113.57	97.62 ± 3.06	
T$_4$ (Dark Blue for Adults)		Pb	0	7.20	-	0.72 ± 0.15
			100	104.80	97.60 ± 1.39	
			250	253.40	98.48 ± 1.21	
			500	513.60	101.28 ± 2.10	
		Cu	Direct aspiration with FAAS			2.64 ± 0.06
		Ni	0	32.70	-	3.26 ± 0.11
			25	58.78	104.35 ± 2.11	
			50	81.39	97.39 ± 1.19	
			100	129.65	96.96 ± 1.56	
		Co	0	18.81	-	1.88 ± 0.09
			25	43.10	97.14 ± 1.90	
			50	67.86	98.10 ± 1.45	
			100	120.24	101.43 ± 1.12	

Table 4.22. (continued)

Sample No. & color	Sample picture	Metal ion	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery %	Calculated metal content ($\mu g\ g^{-1}$)
T₅ (Dark Brown for Adults)		Pb	0	75.20	-	7.52 ± 0.24
			100	176.00	100.80 ± 2.63	
			250	329.40	101.68 ± 1.05	
			500	581.20	101.20 ± 1.19	
		Cu	Direct aspiration with FAAS			77.35 ± 0.33
		Ni	0	15.30	-	1.52 ± 0.04
			25	40.96	102.61 ± 1.00	
			50	67.48	104.35 ± 2.07	
			100	113.13	97.83 ± 1.34	
		Co	0	23.57	-	2.34 ± 0.12
			25	47.76	96.76 ± 1.19	
			50	72.14	97.14 ± 1.78	
			100	121.67	98.10 ± 1.04	
T₆ (Beige for Bed Linen)		Pb	0	45.40	-	4.52 ± 0.20
			100	147.10	101.70 ± 1.51	
			250	297.80	100.96 ± 1.43	
			500	547.40	100.40 ± 2.00	
		Cu	0	36.20	-	3.60 ± 0.10
			25	61.00	99.20 ± 2.78	
			50	86.76	101.12 ± 1.32	
			100	138.12	101.92 ± 0.62	
		Ni	0	41.39	-	4.14 ± 0.15
			25	65.78	97.57 ± 2.08	
			50	90.09	97.39 ± 1.49	
			100	138.35	96.97 ± 1.18	
		Co	0	55.95	-	5.58 ± 0.21
			25	81.33	101.52 ± 2.99	
			50	105.48	99.05 ± 1.83	
			100	153.57	97.62 ± 1.53	

Table 4.22. (continued)

Sample No. & color	Sample picture	Metal ion	Added (µg L⁻¹)	Found (µg L⁻¹)	Recovery %	Calculated metal content (µg g⁻¹)
T₇ (Light Blue with Stars for Kids)		Pb	0	31.60	-	3.16 ± 0.15
			100	132.80	101.20 ± 2.08	
			250	278.00	98.56 ± 1.48	
			500	521.40	97.96 ± 1.24	
		Cu	0	92.20	-	9.21 ± 0.17
			25	117.68	101.92 ± 1.75	
			50	141.80	99.20 ± 2.00	
			100	193.00	100.80 ± 3.12	
		Ni	0	41.39	-	4.12 ± 0.24
			25	67.04	102.61 ± 1.74	
			50	90.52	98.26 ± 1.91	
			100	137.48	96.09 ± 1.44	
		Co	0	17.86	-	1.78 ± 0.1
			25	42.48	98.48 ± 1.67	
			50	68.33	100.95 ± 2.52	
			100	119.29	101.43 ± 2.17	
T₈ (White for Babies)		Pb	0	BDL*	-	nd*
			100	101.40	99.60 ± 1.84	
			250	248.20	98.56 ± 1.67	
			500	500.94	99.83 ± 1.48	
		Cu	0	15.40	-	1.54 ± 0.05
			25	39.96	98.24 ± 1.63	
			50	64.80	98.80 ± 1.83	
			100	116.44	101.04 ± 2.36	
		Ni	0	6.61	-	0.66 ± 0.02
			25	30.97	97.43 ± 1.71	
			50	55.74	98.27 ± 1.29	
			100	104.43	97.83 ± 1.54	
		Co	0	6.43	-	0.64 ± 0.04
			25	30.95	98.10 ± 1.27	
			50	55.95	99.05 ± 2.83	
			100	106.90	100.48 ± 2.42	

*BDL: Below the detection limit; nd: Not detected

Table 4.22. (continued)

Sample No. & color	Sample picture	Metal ion	Added (μg L⁻¹)	Found (μg L⁻¹)	Recovery %	Calculated metal content (μg g⁻¹)
T₉ (Light Yellow for Kids)		Pb	0	11.20	-	1.12 ± 0.11
			100	110.80	99.60 ± 1.54	
			250	257.60	98.56 ± 1.90	
			500	503.00	98.36 ± 1.84	
		Cu	0	115.80	-	11.56 ± 0.15
			25	141.00	100.80 ± 1.97	
			50	165.00	98.40 ± 1.53	
			100	213.60	97.80 ± 1.61	
		Ni	0	50.09	-	5.00 ± 0.11
			25	75.48	101.57 ± 1.07	
			50	99.04	97.91 ± 2.16	
			100	146.96	96.87 ± 1.22	
		Co	0	26.90	-	2.69 ± 0.12
			25	51.19	97.14 ± 1.46	
			50	76.19	98.57 ± 2.37	
			100	125.95	99.05 ± 1.24	
T₁₀ (Light Pink for Babies)		Pb	0	2.60	-	0.26 ± 0.10
			100	102.40	99.80 ± 1.34	
			250	250.00	98.96 ± 1.73	
			500	503.60	100.20 ± 2.43	
		Cu	0	76.60	-	7.66 ± 0.11
			25	101.00	97.60 ± 1.69	
			50	127.00	100.80 ± 2.06	
			100	175.40	98.80 ± 1.25	
		Ni	0	9.22	-	0.92 ± 0.12
			25	34.65	101.74 ± 1.61	
			50	58.78	99.13 ± 1.39	
			100	107.04	97.83 ± 1.54	
		Co	0	11.19	-	1.12 ± 0.14
			25	35.71	98.10 ± 2.32	
			50	61.67	100.95 ± 1.44	
			100	112.62	101.43 ± 1.30	

According to the results obtained after microwave digestion and EA-DES-DLLME application for cotton textile samples which presented in Table 4.22, copper has the highest concentration values, followed by lead, nickel, and cobalt.

The concentrations of Pb (II), Cu (II), Ni (II), and Co (II) in cotton textile samples were found to be in the range of $0.26 - 7.52$, $1.27 - 77.35$, $0.66 - 5.00$, and $0.64 - 5.58$ µg g^{-1}, respectively. Comparing the results of the metal content values in the samples with the limit values provided by Oeko - Tex (Table 4.23), it was found that the lead has a high content in most samples. The highest lead values were found to be 2.36, 1.88, 7.52, 4.52, and 3.16 µg g^{-1} for T_2, T_3, T_5, T_6, and T_7 samples respectively. The highest copper value was found in T_5 as 77.35 µg g^{-1}. In addition, since the amount of copper in the solutions prepared by microwave digestion of 0.5 g of textile sample was at a level that does not require the preconcentration step, the amount of copper in T_1, T_2, T_3, T_4, and T_5 samples was determined directly with FAAS. As for nickel and cobalt, there was a slight difference in the metal content compared with the limit values for T_9 which were 5.00 µg g^{-1} for nickel, and T_6 was 5.58 µg g^{-1} for cobalt.

Table 4.23. Limit values for Pb (II), Cu (II), Ni (II), and Co (II) metals given by Oeko-Tex (mg kg^{-1} textile).

Heavy metals	Baby wear	With skin contact	Without skin contact	Decoration materials
Lead (Pb)	0.2	1.0	1.0	1.0
Copper (Cu)	25.0	50.0	50.0	50.0
Nickel (Ni)	1.0	4.0	4.0	4.0
Cobalt (Co)	1.0	4.0	4.0	4.0

The results of T_8 and T_{10} samples (as cotton flannel for neonate) were found to be within the permissible limits of Oeko - Tex criteria that revealed minimum values for babies. On the other hand, the results of the target trace metal concentrations obtained by EA-DES-DLLME were compared to the values found in other literature, it was discovered to be slightly higher as shown in Table 4.24. Therefore, samples of textile products in stores and local markets must be evaluated regularly for trace metal followability.

Table 4.24. Literature values of Pb (II), Cu (II), Ni (II), and Co (II) metal content (as µg g^{-1}) in cotton textile samples.

Ion	Study [161]	Study [162]	Study [35]	Study [36]	Study [37]	This study
Pb (II)	0.18 – 6.00	0.28 – 0.30	-	1.23 – 1.83	-	0.26 – 7.52
Cu (II)	0.28 – 0.84	0.26 – 0.78	0.76 – 341	1.96 – 4.40	0.008–193.9	1.27 –77.35
Ni (II)	0.24 – 1.52	0.20 – 0.70	1.20 – 4.69	-	1.15 – 2.55	0.66 – 5.00
Co (II)	0.04 – 0.96	-	-	-	0.16 – 1.81	0.64 – 5.58

-: not examined

The quantitative recovery values after implementing the spiking process by the EA-DES-DLLME method were in the range of 97.20 - 101.70 % for Pb (II), 97.60 - 101.92 % for Cu (II), 95.65 - 104.35 % for Ni (II), and 96.76 - 101.52 % for Co (II), and their standard deviation values ranged of 0.81 - 2.63 for Pb (II), 0.62 - 3.12 for Cu (II), 1.00 - 2.34 for Ni (II), and 0.99 - 3.06 for Co (II), as shown above in Table 4.22. These results show that the proposed EA-DES-DLLME method can be applied accurately and reliably commute for trace heavy metals determination in textile samples.

4.9.3. Matrix Effect

Another way to obtain more reliable results for the EA-DES-DLLME method is to calculate the matrix effect factor (ME), which is used to evaluate the effect of other compounds present with the target metal ions in real sample solutions. The matrix effect was examined by comparing the calibration slopes in the matrix (matrix-match calibration, MC) and in the solvent (solvent-based calibration, SC). The MC can be obtained by adding known quantities of the standard analyte solution to the real samples and then extracting by the recommended method (EA-DES-DLLME) as in the spiking process. The SC is the calibration based on standard solutions using the EA-DES-DLLME method. The ME can be calculated according to Equation 4.3.

$$ME \% = \frac{M_{MC} - M_{SC}}{M_{SC}} \times 100\% \qquad (4.3)$$

ME: Matrix effect factor
M_{MC}: The slope of matrix-matched calibration
M_{SC}: The slope of solvent-based calibration

When the slope of the MC is greater than the slope of the SC (ME > 0) it can be concluded that the matrix has a signal enhancement, while signal suppression occurs when the slope of the MC is smaller than that of the SC (ME < 0). There is no matrix effect if the slope of MC equals the slope of SC (ME = 0).

The MCs have been established for targeted metal ions with 10 cotton textile samples, and then compared with SC for each metal ion. The results obtained were shown in Table 4.25.

Table 4.25. Comparison of matrix-matched calibration data with solvent-based calibration data for Pb (II), Cu (II), Ni (II), and Co (II).

Ion	Parameters		ME %
	SC Equation	MC Equation*	
Pb (II)	$A = 5x10^{-4}\,C + 0.01314$ $R^2 = 0.9992$	$A = 5.035x10^{-4}\,C + 0.02335$ $R^2 = 0.9996$	0.70
Cu (II)	$A = 2.60x10^{-3}\,C + 0.03872$ $R^2 = 0.9989$	$A = 2.502x10^{-3}\,C + 0.17217$ $R^2 = 0.9999$	-3.76
Ni (II)	$A = 2.30x10^{-3}\,C + 0.00756$ $R^2 = 0.9994$	$A = 2.232x10^{-3}\,C + 0.12855$ $R^2 = 0.9997$	-2.94
Co (II)	$A = 2.10x10^{-3}\,C + 0.00796$ $R^2 = 0.9994$	$A = 2.09x10^{-3}\,C + 0.06403$ $R^2 = 0.9999$	-0.5

*($n = 10$)

As a result, the obtained matrix effects were 0.70 % for Pb (II), -3.76 % for Cu (II), -2.94 % for Ni (II), and -0.50 % for Co (II), and these results are considered acceptable when compared with the interference limit value ±5 %. So, it was confirmed that there was no significant effect of the sample matrix on the extraction efficiency of the EA-DES-DLLME method.

4.10. Comparison of the EA-DES-DLLME Method with Other Literature Methods

Performance characteristics of the proposed EA-DES-DLLME method for Pb (II), Cu (II), Ni (II), and Co (II) preconcentration were compared with other microextraction methods by various detection techniques. Several parameters were used to compare the EA-DES-DLLME results, including overall extraction time, LOD, EF or PF, LR, and RSD%. Furthermore, other comparative data such as the extraction solvent type, disperser type, and the extraction instruments were also compared as shown in Tables 4.26-4.29.

Table 4.26. Comparison of the proposed EA-DES-DLLME method for Pb (II) preconcentration with other techniques in literatures.

Matrix	Analytical technique	Detection	Extraction solvent	Disperser type	Extraction tool	Total extraction time (min)	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	EF	PF	RSD %	Ref.
Oilfield-produced water samples	VA-DLLME	ICP-OES	Carbon tetrachloride (CCl_4)	Methanol	Vortex & centrifugation	14	0.5-250	0.15	20	-	1.85	[163]
Spring and well water	TBAB based ABS	ICP-OES	Tetrabutylammoniums bromide, TBAB	-	-	>16	1-160	0.2	-	150	1.8	[164]
Water, tea and human hair samples	SHS-LPME	GFAAS	protonated triethylamine carbonate (P-TEA-C)	-	Vortex & centrifugation	5	0.04-2	0.016	49	-	4.2	[165]
Lipsticks and hair dyes	MADLLME	GFAAS	1-undecanol	acetone	Centrifugation & ice bath	10	0.3-50	0.1	96	-	8.3	[166]
Real water samples	DLLME	HPLC	Carbon tetrachloride (CCl4)	Methanol	Centrifugation	5	0.15-100	0.054	311	-	5.2	[167]
Urine samples	DLLME	UV-Vis	Carbon tetrachloride (CCl4)	Methanol	Centrifugation	<5	0.01–100	0.0036	-	50	9.1	[168]
Eye makeup products	SDILNDµE	ETAAS	1-butyl-3-methylimidazolium hexafluorophosphate [C4mim] [PF6]	-	-	<5	1.0-20	0.126	50	-	<4.2	[169]
Artificial sweat	TIL–DµE–ISAVS	FAAS	1-hexyl-3-methylimidazolium hexafluorophosphate [HMIM] [PF6]	syringe air-assisted vesicle system	Centrifugation for two times	~15	10-50	0.09	78.6	-	3.4	[170]
Milk samples	VA-DES-LPME	SQT-FAAS	DES	-	Vortex	~5	50-1000	8.7	48	-	3.1	[171]
Serum samples	Um-CPE	FAAS	polyethyleneglycol tertoctylphenylethe (Triton X-114)	Ultrasound	Ultrasound & Centrifugation	~20	1.5-200	0.450	30	-	4.12	[172]
Black tea samples	Co-MP-DSPME	SQT-FAAS	-	Manual shaking	-	-	25.0-500	7.78	64.3	-	4.2	[173]
100 % cotton textile	EA-DES-DLLME	FAAS	DES	Effervescence	No extraction instrument	5	10-750	2.44	62.5	50	1.07	This study

Table 4.27. Comparison of the proposed EA-DES-DLLME method for Cu (II) preconcentration with other techniques in literatures.

Matrix	Analytical technique	Detection	Extraction solvent	Disperser type	Extraction tool	Total extraction time (min)	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	EF	PF	RSD %	Ref.
Food &environmental water	DLLME-MSPE	ICP-MS	Carbon tetrachloride CCl_4	-	Ultrasound	6	0.02-20	0.017	81	-	2.6	[174]
Sediment Samples	DES-EM	ICP-OES	DES	-	Vortex & centrifugation	4 h	-	1.2	-	-	7.5	[175]
Real water samples	DLLME	HPLC	Carbon tetrachloride CCl_4	Methanol	Centrifugation	3	0.2-100	0.0483	327	-	4.9	[167]
Real water samples	UA-CPE	UV-Vis	Triton X-114	-	Ultrasound	20	0.0-500	0.7	17	-	2.9	[176]
Tap water and seawater	SDME	GFAAS	Decane	-	Stirrer	10	0.1-1.3	0.025	45.2	-	6.77	[177]
Water & fruit juice samples	In situ-CO2 DLLME	GFAAS	1,1,2,2-tetrachloroethane $C_2H_2Cl_4$	Produced CO_2	centrifugation	5	0.015–0.25	0.006	150	-	2.3	[178]
Water samples	DLLME-SQT	FAAS	Chloroform $CHCl_3$	2-propanol	Ultrasound & Centrifugation	2.5	2.0-100	0.7	79	-	3.7	[9]
Oil samples	RP-SHS-LLME	FAAS	Triethylamine (TEA)	-	Vortex, ultrasound & centrifugation	6	23-1000	6.9	-	22.7	4.7	[179]
Water & food samples	DLLME	FAAS	Chloroform $CHCl_3$	Acetone	Centrifugation	5	3.0-120	0.6	49	50	1.5	[16]
soil, multivitamin tablet, tea &water samples	IP-DLLME	FAAS	Chloroform $CHCl_3$	Dimethylformamide (DMF)	Centrifugation	15	6.0-100	3.7	10	-	1.9	[180]
Rice, lettuce & water samples	AALLME	Image colorimetric analysis	Carbon tetrachloride CCl_4	Air bubbles by glass syringe	Centrifugation	7	5.0–500	1.5	-	-	4.53	[181]
100 % cotton textile	EA-DES-DLLME	FAAS	DES	Effervescence	No extraction instrument	5	2.5-200	1.29	52	50	1.61	This study

Table 4.28. Comparison of the proposed EA-DES-DLLME method for Ni (II) preconcentration with other techniques in literatures.

Matrix	Analytical technique	Detection	Extraction solvent	Disperser type	Extraction tool	Total extraction time (min)	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	EF	PF	RSD %	Ref.
Rice samples	USAEME	ICP-OES	Carbon tetrachloride (CCl4)	-	Ultrasound	8	0.2-250	0.03	85	-	3.30	[182]
Seafood	DLLME	ICP-OES	Trichlorethylene C_2HCl_3	Ethanol	Centrifugation	3	0.12-100	0.12	20	-	≤3.6	[183]
Water & fruit juice samples	In situ-CO2 DLLME	GFAAS	1,1,2,2-tetrachloroethane $C_2H_2Cl_4$	Produced CO_2	Centrifugation	5	0.02–0.2	0.012	139	-	4.50	[178]
Marine brown algae	SA-DLLME	UV-Vis	Dichloromethane CH_2Cl_2	-	Ultrasound & centrifugation	12	0.1-100	0.031	119	-	-	[184]
Water samples & synthetic samples	USAEME	UV-Vis	Carbon tetrachloride CCl4 & chloroform CHCl3 (1:2 V/V%)	-	Ultrasound & centrifugation	22	2.0-120	0.13	-	200	1.70	[185]
Water & fruit juice samples	DLLME	FAAS	DES	Methanol	Centrifugation	8	0.8-50	0.30	24	-	4.10	[25]
Vegetable oil samples	IL-DLLME	FAAS	Tetrabutyl phosphonium phosphate [TBP] [PO4]	Chloroform CHCl3	Centrifugation	20	1.0-200	0.77	63	-	3.20	[186]
Chamomile tea, & coffee samples	DLLME	SQT-FAAS	Chloroform CHCl3	Isopropyl alcohol	Vortex & centrifugation	4	0.025-0.2	0.0049	66.4	-	8.10	[12]
Water samples	pH-MS-HLPME	FAAS	Caprylic acid	-	Vortex, centrifugation & refrigerator	4	10.0-450	3.2	45	-	2.00	[187]
Chocolate samples	PV-IS-DLLME	FAAS	1-butyl-3-methylimidazolium hexafluorophosphate [C4MIM][PF6]	The pressure generated by the movement of the plunger	Centrifugation	11	6.7-100	2.0	17	-	4.80	[13]
Milk-based samples	UA-CPE	FAAS	Triton X-114	-	Ultrasound, centrifugation & refrigerator	30	3.0-180	0.78	48.6	50	3.60	[188]
100 % cotton textile	EA-DES-DLLME	FAAS	DES	Effervescence	No extraction instrument	5	5.0-150	0.58	57.5	50	1.35	This study

Table 4.29. Comparison of the proposed EA-DES-DLLME method for Co (II) preconcentration with other techniques in literatures.

Matrix	Analytical technique	Detection	Extraction solvent	Disperser type	Extraction tool	Total extraction time (min)	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	EF	PF	RSD %	Ref.
Rice samples	USAEME	ICP-OES	Carbon tetrachloride (CCl4)	-	Ultrasound	8	0.3-300	0.04	86	-	3.80	[182]
Real water samples	USAEME	ICP-OES	Carbon tetrachloride (CCl4)	Sonication	Ultrasound & centrifugation	11	1.0-1000	0.28	19	-	5.08	[189]
Water & fruit juice samples	In situ-CO2 DLLME	GFAAS	1,1,2,2-tetrachloroethane $C_2H_2Cl_4$	Produced CO_2	Centrifugation	5	0.02-0.3	0.008	148	-	4.60	[178]
Natural water samples	IL-UA-DLLME-SAP	LC-UV	trihexyl(tetradecyl) phosphonium bis[(2,4,4-trimethyl) pentyl] phosphinate (Cyphos IL 104)	-	Ultrasound, centrifugation & freezer	66	0.2-75.0	0.03	-	210	5.00	[190]
Food samples	PV-IS-DLLME	Digital image colorimetry	Trichloroethylene C_2HCl_3	The pressure generated by the movement of the plunger	Centrifugation	6	3.27-100	0.08	65	-	4.60	[191]
Tobacco & food samples	SS-LPME	FAAS	N,N dimethyl-n-octylamine bicarbonate	-	Vortex & centrifugation	11	-	3.2	40	-	-	[192]
Environmental Samples	USA-FF-DLPME	FAAS	DES	Sonication	Ultrasound	4	2.0-100	0.7	-	50	2.60	[193]
Vegetable & herb samples	UA-IPSE-DLLME	FAAS	1-dodecanol	Sonication	Ultrasound, centrifugation & ice bath	6	10.0-400	2.4	48	-	3.80	[194]
Water samples	DES-DLLME	FAAS	DES	Methanol	Centrifugation	5	5.0-100	1.7	-	40	2.00	[195]
Milk-based samples	UA-CPE	FAAS	Triton X-114	-	Ultrasound, centrifugation & refrigerator	30	2.0-160	0.56	53.9	50	3.80	[188]
Tea and pharmaceutical supplement	DES-UALPME	MS-FAAS	DES	-	Vortex, ultrasound &centrifugation	17	0.25-2.0	1.1	-	15	7.10	[196]
100 % cotton textile	EA-DES-DLLME	FAAS	DES	Effervescence	No extraction instrument	5	5.0-150	1.55	52.5	50	1.97	This study

As shown in Table 4.26, it was noticed that the proposed method for preconcentration of lead was better or similar in terms of results to EF, LOD, LR, and repeatability when compared with other methods using FAAS [170–173], except for the TIL-DµE-ISAVS method [170] which looks better as it has low LOD and high EF but it has some disadvantages, including narrow LR, the use of an external extraction tool, and a longer extraction time than the proposed method. The other detection procedures using ICP [163,164], GFFAS [165,166], HPLC [167], UV-Vis [168], and ETAAS [169] for lead determination have lower LOD values than the proposed method. Nevertheless, the proposed method was better in term of EF than the methods [163,165,169] and it was better than some methods in one term or more together such as, using a toxic extraction solvent [163,164,167,168], using disperser solvent [163,166–168], external extraction equipment [163,165–168], and long time to extraction [163,164,166].

The comparison of the proposed method for copper preconcentration was presented in table 4.27. The proposed method looks better than most other methods in different aspects of analytical properties. Some of these methods use a chlorinated organic solvent [167,174,178,181], some have a long extraction time [175,176], and some of them have a low enhancement factor value [176,177]. As for the FAAS methods, the EA-DES-DLLME method was better than [16,179] in terms of enhancement or preconcentration factor values and better than [179,180] in terms of LOD. In addition, several methods in the table were used for toxic extraction solvents [9,16,179,180].

On the other hand, Table 4.28 illustrated the comparison of analytical characteristics for nickel preconcentration by the proposed method with other methods. The other methods that used FAAS as a detection instrument have several disadvantages, for example, the methods [13,187,188] have higher LOD and lower EF values than the proposed method, and [12,187] were used disperser solvents or chlorinated solvent as [186]. Although the DLLME method [25] uses DES as an extraction solvent it has a lower EF value and uses a dispersed solvent compared to the proposed method. Also, Table 4.28 includes the detection methods other than FAAS such as ICP, GFAAS, and UV-Vis which have a lower LOD than the provided method however all of them are used for chlorinated solvents [178,182–185] and have lower EF value and used for disperser solvent [183] or some of them used extraction instruments and required a long time to end the extraction processes [184,185].

The proposed study for cobalt determination was similar or even better than the other methods that used FAAS as detection in some terms such as, EF or PF values [188,192–196], LOD values [192,194,195], extraction time [188,196], external extraction instruments [188,192–194,196] , and using disperser solvent [195] as shown in table 4.29. The methods that use different detection tools (ICP, GFAAS, LC-UV, digital image colorimetry) compared to the proposed method have some advantages and disadvantages in analytical features. For example, the methods [178,182,191] have good features such as low LOD and high EF values but also have disadvantages as used toxic extraction solvents and needed to an extraction tool. As well, the method LC-UV required a long extraction time [190] and ICP-OES have a lower EF value [189] than the proposed method.

As a result, in this study, DES was used as a green extraction solvent with an environmentally friendly effervescent tablet as a dispersion without the need for any extraction device such as vortex, ultrasound, or even a centrifuge to separate the DES phase from the aqueous phase. For this reason, the proposed EA-DES-DLLME technique is superior and preferable to most of the reported techniques presented in Tables 4.26, 4.27, 4.28, and 4.29.

5. CONCLUSIONS

In the present dissertation, the EA-DES-DLLME technique (effervescence-assisted dispersive liquid-liquid microextraction) with the aiding of deep eutectic solvents was evolved for preconcentration and determination of four heavy metals is lead, copper, nickel, and cobalt in cotton textile samples by employing micro-sampling flame atomic absorption spectrometry. The EA-DES-DLLME procedure offered the following merits:

- Dispersive liquid-liquid microextraction is a technique that is well known and widely used in many fields because of its major characteristics such as simplicity, low cost, ease of process development, and most significantly of this, its need for very limited amounts of extraction solvent (at the microliter level), which makes it harmless to human health and the surrounding environment.

- Since the final extracted sample volumes are at the microliter level, measuring them directly using flame atomic absorption instrument is difficult. As a result, the microsampling technique allowed the simple and rapid introduction of small quantities of samples into the flame atomic absorption instrument, resulting in high extraction performance.

- Deep eutectic solvents are one of the greener alternatives that used as an extraction solvent. A clear solution of DES which used in the proposed method (EA-DES-DLLME) was synthesized by simply mixing of choline chloride and phenol within 5 minutes. When comparing DESs with the conventional organic solvents used in the extraction processes, the DES is distinguished by: easy storage, long expiration, low cost, easy to obtain, non-volatility, low toxicity so it is considered safer on the environment.

- The effervescence agent played an important role in the proposed method because it provided a green force (environmentally friendly) that contributed to dispersing the extraction solvent in the aqueous sample and transferring the target analytes homogeneously. This process is done by producing carbon dioxide as bubbles in the sample solution as a result of the reaction of a mixture of ascorbic acid (as a proton donor) with sodium carbonate (as a carbon dioxide source) that has been added to the sample solution as a tablet or powder. Furthermore, after the effervescence reaction is finished which takes just 2.5 minutes, the DES phase is separated from the aqueous phase spontaneously, so there is no need to use a centrifuge. Finally, effervescence

assistance is a simple, efficient, and energy-free alternative to other well-known assistance approaches such as ultrasound or vortex.

- The EA-DLLME method was distinguished by good selectivity to determine each of lead, copper, nickel, and cobalt in cotton textile samples through the absence of matrix effect or foreign ions interference, which is attributed to the high sensitivity of the complexing agent (8-HQ) for the target metal ions.

- The use of THF as aprotic solvent contributed to increasing the efficiency of this method, as it has the capacity to separate DESs from the aqueous phase through its interaction with water molecules, which is faster than the interaction of DES.

To achieve the best performance of the proposed method, we attempted to examine all of the factors that could influence the extraction efficiency of lead, copper, nickel, and cobalt, considering the selection of the most effective and suitable one.

Initially, the pH effect was improved because it is considered the basic parameter that has an influence on the complex formation between the metal ion and the complexing agent. The pH of metal ions solutions was examined in the range of 3-10 and the pH 6 was chosen to be the suitable value.

The effervescence formation is an important parameter in EA-DES-DLLME whose properties should be investigated, and among these properties is to select the appropriate type and ratio of the effervescent agent that achieves high extraction efficiency. For this reason, six different types of effervescent agents have been prepared by mixing different proton donors and carbon dioxide sources and as a result, the ascorbic acid and sodium carbonate combination with a 0.002:0.001 mole ratio that has a convenient effervescence time (2.5 min) was selected as the best effervescent agent for the studied metal ions determination. Also, the amount of the selected effervescent agent was examined in the range of 0.228 - 0.914 g, and it was noticed that the extraction efficiency reaches a pinnacle when 0.457 g of the effervescent agent is added. As for the formulation of the effervescent agent, there is no difference in the extraction efficiency results when it is used as a powder or as a tablet, so the selection occurred on the tablet for ease of use in experiments.

The type, molar ratio and volume of DES as an extraction solvent were also optimized to obtain a good performance for proposed method. Three kind of DESs ChCl: Ph, ChCl: U,

and ChCl: OA were examined and the result showed that the ChCl: Ph was the best in terms of the efficiency of extraction. After completing the selection of the DES composition that will be applied in EA-DES-DLLME, a molar ratio test was performed for it, and the results indicated that 1: 3 was the optimal ratio among five different molar ratios of ChCl: Ph. Finally, to verify the sufficient volume of ChCl: Ph for the extraction process, various volumes of ChCl: Ph ranging from 100 to 1500 µL were added, and the maximum extraction efficiency was observed at 1000 µL for each of lead, copper, nickel, and cobalt.

Moreover, the volume of complexing agent (8-HQ) and THF was improved by adding different volumes of them ranging from (100-1500 µL) for 8-HQ and (0-1500 µL) for THF. Therefore, 750 µL of 8-HQ and 1000 µL of THF were selected as appropriate volumes that contributed to the amelioration of extraction efficiency.

The optimal conditions investigated to develop the EA-DES-DLLME method which applied for determination and preconcentration of targeted metal ions are listed in Table 5.1.

Table 5.1. The optimum condition values of the developed EA-DES-DLLME method for Pb (II), Cu (II), Ni (II), and Co (II) determination.

Parameters	Optimum values for Pb (II), Cu (II), Ni (II), and Co (II)
Sample volume (mL)	25
Extracted phase volume (mL)	0.5
pH	6
Buffer solution volume (mL)	2
Effervescent agent composition	AA:SC
Effervescent agent molar ratio	2:1
Effervescence time (min)	2.5
Effervescent agent amount (g)	0.457
Effervescent agent form	tablet
DES composition	ChCl: Ph
DES molar ratio	1:3
DES volume (µL)	1000
8-HQ volume (µL)	750
THF volume (µL)	1000

The EF, LOD, and LOQ that achieved from applying the EA-DES-DLLME method at optimum conditions were listed in Table 5.2.

Table 5.2. Some analytical performance of the EA-DES-DLLME method at optimum conditions for Pb (II), Cu (II), Ni (II), and Co (II).

Metal ions	Analytical performance			
	EF	LOD (μg L^{-1})	LOQ (μg L^{-1})	RSD %
Pb (II)	62.5	2.44	8.13	1.07
Cu (II)	52	1.29	4.30	1.61
Ni (II)	57.5	0.58	1.93	1.35
Co (II)	52.5	1.55	5.16	1.97

The validation of the proposed method was checked in three ways: (i) applying the developed method on certified reference material (NCS ZC73013 spinach), (ii) applying the spiking procedure on the various 100 % cotton textile samples, and (iii) studying the sample matrix effect by matrix matched calibration.

As a result of the 4 replicates analysis for certified reference material, the recovery values were calculated as 98.6% ± 1.3 for Pb (II), 98.2% ± 2.9 for Cu (II), 97.2% ± 1.4 for Ni (II), and 98.3% ± 2.5 for Co (II). The spiking procedure was applied for determination of target heavy metal ions in different 100 % cotton textile samples. The recoveries that obtained were in the range of 97.20 - 101.70 % for Pb (II), 97.60 - 101.92 % for Cu (II), 95.65 - 104.35 % for Ni (II), and 96,76 - 101.52 % for Co (II). On the other hand, the sample matrix effect was studied and the results were as follows: 0.70 % for Pb (II), -3.76 % for Cu (II), -2.94 % for Ni (II), and -0.50 % for Co (II). The recoveries and matrix effect results show that the EA-DES-DLLME method can be applied accurately and reliably commute for trace heavy metals determination in textile samples.

Finally, the analytical characteristics of the proposed method were compared with several different reported methods. As a result, it can be concluded that the proposed EA-DES-DLLME method has several distinct characteristics that make it preferable than others, such as environmentally friendly, don't have any need to use a dispersion solvent or even an external tool and energy to complete the extraction, has a short time to the extraction process, and have a good LOD, EF values.

Therefore, in general terms, it can be said that the EA-DES-DLLME method was sensitive, repeatable, inexpensive, rapid, and easy to apply for preconcentration and determination of lead, copper, nickel, and cobalt traces in cotton textile samples.

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