A NEW APPROACH FOR THE DETERMINATION OF TRACE HEAVY METALS IN HAIR DYEING COSMETICS

SAÇ BOYAMA KOZMETİKLERİNDEKİ ESER DÜZEY AĞIR METALLERİN TAYİNİ İÇİN YENİ BİR YAKLAŞIM

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ABSTRACT

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Although hair does not have a vital function in humans, it is an essential body component that affects appearance and self-confidence for both men and women. Cosmetic application using chemicals to change hair color is called "hair coloring". There are many different natural and synthetic products used for hair coloring. Many different heavy metal salts are added to increase the color performances of hair dyes or as impurities. Since the harmful effects of heavy metals on human health are known, it is vital to determine the amount of heavy metals in hair dyeing products. The flame atomic absorption spectroscopy (FAAS) method is a fast, practical, and economical method that is widely used to determine heavy metals. However, it is insufficient to determine the ppb and sub-ppb concentrations directly. In addition, the complex matrices of the analyzed samples also cause difficulties in the analysis. For these reasons, separation and/or preconcentration step is needed to increase sensitivity before analysis by FAAS.

In this thesis, ultrasound assisted-deep eutectic solvent based-dispersive liquid-phase microextraction (DES-UA-LPME) was developed, and the determination of

trace elements was carried out by flame atomic absorption spectrometry (FAAS). For this purpose, deep eutectic solvent (DES) prepared from choline chloride (ChCl), and phenol (Ph) as extraction solvent, dithizone as a complexing agent, and THF as aprotic solvent were used. All parameters affecting the extraction efficiency such as pH, DES volume and composition, extraction time, dithizone amount were optimized. As a result of the optimization studies, when pH is 6.0, dithizone ($3x10^{-3}$ M) volume is 500 µL, DES (ChCl: Ph) mole ratio is 1:3, DES volume is 1 mL, ultrasound application time is 3 minutes, and centrifugation time is 4 minutes, the extraction takes place with the highest efficiency. Under optimum conditions, for Cd(II), Cu(II), Pb(II), and Ni(II), the enhancement factor (EF) of 57, 50, 92, and 58, the limit of detection (LOD) of 0.7, 1.4, 2.3 and 0.8 µg L^{-1}, the limit of quantitation (LOQ) of 2.3, 4.7, 7.6 and 2.5 µg L^{-1}, the relative standard deviation (n= 10) of 2.3, 1.8, 1.7 and 2.7, were calculated, respectively.

After optimizing the experimental conditions and evaluating the analytical features, the method was applied to hair dyes and henna samples from different brands and origins. As a result of the experiments, high recovery values were obtained with high sensitivity and accuracy, away from the matrix effect. The amounts in the selected samples are between 0.12 and 2.66 µg g^{-1} for Cd, 0.73 and 7.07 µg g^{-1} for Cu, 3.58 and 15.60 µg g^{-1} for Pb and 1.16 and 7.36 µg g^{-1} for Ni.

Keywords: Preconcentration, Cadmium, Copper, Lead, Nickel, DES-UA-LPME, Microextraction, Hair Dye, Henna, Flame Atomic Absorption Spectroscopy

ÖZET

SAÇ BOYAMA KOZMETİKLERİNDEKİ ESER DÜZEY AĞIR METALLERİN TAYİNİ İÇİN YENİ BİR YAKLAŞIM

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Saçın insan vücudunda hayati bir fonksiyonu olmamasına rağmen hem erkekler hem de kadınlar için görünüşü ve özgüveni etkileyen çok önemli bir vücut bileşenidir. Saç rengini değiştirmek için kimyasallar kullanılarak yapılan kozmetik uygulamaya “saç boyama” denir. Saç boyama için kullanılan birçok farklı doğal ve sentetik ürün bulunmaktadır. Saç boyalarının renk etkinliklerini artırmak amacıyla ya da safsızlık olarak birçok farklı ağır metal tuzu eklenmektedir. Ağır metallerin insan sağlığı üzerine olumsuz etkileri bilindiğinden saç boyama ürünlerindeki ağır metal miktarlarının tayini son derece önemlidir. Alevli atomik absorpsiyon spektroskopisi (FAAS) yöntemi ağır metallerin tayin için yaygın olarak kullanılan hızlı, pratik ve ekonomik bir yöntemdir. Ancak ppb ve sub-ppb derişimleri doğrudan belirlemede yetersiz kalmakta, ayrıca analiz edilen örneklerin sahip olduğu karmaşık matrisler de analizi zorlaştırmaktadır. Bu nedenlerle, FAAS ile analizden önce hassasiyeti artırmak için ayırma ve/veya önderiştirme basamağına gereksinim duyulmaktadır.

Bu tez çalışmasında, saç boyamada kullanılan kozmetik ürünlerindeki (saç boyası ve kına) eser düzeydeki Cd(II), Cu(II), Pb(II) ve Ni(II) iyonlarının önderiştirilmesi için ultrason destekli derin ötektik çözücü temelli dispersif sıvı faz mikroekstraksiyonu (DES-UA-LPME) kullanılmış ve eser elementlerin tayini alevli atomik absorpsiyon spektrometresi (FAAS) ile gerçekleştirilmiştir. Bu amaçla, ekstraksiyon çözücüsü olarak kolin klorür (ChCl) ve fenolden (Ph) hazırlanan derin ötektik çözücü, kompleksleştirici olarak ditizon ve aprotik çözücü olarak THF kullanılmıştır. pH, DES hacmi ve bileşimi, ekstraksiyon süresi, ditizon miktarı gibi ekstraksiyon verimini etkileyen tüm parametreler optimize edilmiştir. Bu optimizasyon çalışmaları sonucunda pH 6.0, $3x10^{-3}$ M ditizon hacmi 500 µL, DES (ChCl: Ph) mol oranı 1:3, DES hacmi 1 mL, ultrason uygulama süresi 3 dakika ve santrifüj süresi 4 dakika olarak seçildiğinde ekstraksiyonun en yüksek verimle gerçekleştiği saptanmıştır. Optimum koşullar altında, Cd (II), Cu (II), Pb (II) ve Ni (II) metal iyonları için zenginleştirme faktörü (EF) sırasıyla 57, 50, 92 ve 58; gözlenebilme sınırı (LOD) sırasıyla 0.7, 1.4, 2.3 ve 0.8 µg L^{-1}, tayin sınırı LOQ sırasıyla 2.3, 4.7, 7.6 ile 2.5 µg L^{-1}; bağıl standart sapma (n= 10) ise sırasıyla 2.3, 1.8, 1.7 ve 2.7 olarak hesaplanmıştır.

Deney koşullarının optimizasyonu ve analitik özelliklerin değerlendirilmesinden sonra, yöntem gerçek numune olarak seçilmiş farklı marka ve menşeili saç boyaları ve kına örneklerine uygulanmış ve yüksek geri kazanım değerleri ile matriks etkisinin gözlenmediği, yüksek duyarlığa ve doğruluğa sahip sonuçlar elde edilmiştir. Seçilmiş olan numunelerde bulunan miktarlar Cd için 0,12 ile 2,66 µg g^{-1} arasında, Cu için 0,73 ile 7,07 µg g^{-1} arasında, Pb için 3,58 ile 15,60 µg g^{-1} arasında ve Ni için 1.16 ile 7.36 µg g^{-1} arasında bulunmuştur.

Anahtar Kelimeler: Önderiştirme, Kadmiyum, Bakır, Kurşun, Nikel**,** DES-UA-LPME, Mikroekstraksiyon, Saç Boyası, Kına, Alevli Atomik Absorpsiyon Spektrometresi

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SYMBOLS AND ABBREVIATIONS

Symbols

T_f	Freezing point temperature (ºC)
ρ	Density
η	Viscosity
S	Standard deviation
R^2	Correlation Coefficient
n	Numbers of experiment

Abbreviations

A.N.	Atomic number
A.W.	Atomic weight
CFME	Continuous flow microextraction method
ChCl- OA	Choline chloride- oxalic acid
ChCl- Ph	Choline chloride- phenol
ChCl- U	Choline chloride- urea
CRM	Certified reference material
DDSME	Drop-to-drop solvent microextraction
DES-DLPME	Deep eutectic solvent-based dispersive liquid phase microextraction
DESs	Deep eutectic solvents
DES-UA-LPME	Deep eutectic solvent-based ultrasound assisted liquid phase microextraction

DI-SDME	Direct immersion single drop microextraction
DLPME	Dispersive liquid phase microextraction
DSDME	Directly suspended droplet microextraction
DTZ	Dithizone ($C_{13}H_{12}N_4S$)
EDL	Electrodeless discharge lamp
EF	Enhancement factor
FAAS	Flame atomic absorption spectrometer
GC	Gas Chromatography
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HCL	Hollow cathode lamp
HF-LPME	Hollow fiber-based Liquide phase microextraction
HPLC	High Performance Liquid Chromatography
HS- SDME	Headspace- Single drop microextraction
ICP-AES	Inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
LLL-SDME	Liquid-liquid-liquid - Single drop microextraction
LOD	Limit of detection
LOQ	Limit of quantitation
LPE	Liquid phase extraction
LPME	Liquid phase microextraction
MALDI-MS	Matrix-assisted laser desorption ionization mass spectrometry
MEPS	Microextraction by packed sorbent
NIOSH	The National Institute for Occupational Safety and Health
RSD	Relative standard deviation

SBSE	Stir-bar sorbtive extraction
SFODME	Solidified floating organic drop microextraction
SPE	Solid phase extraction
SPME	Solid phase microextraction
Ss	Switchable solvents
SUPRAs	Supramolecular solvents
THF	Tetrahydrofuran
USA-LLE	Ultrasound-assisted liquid-liquid extraction

1. INTRODUCTION

In the last few decades, due to the rapid increase in the world population and the advancement in technology, the rate of irregular urbanization, excessive consumption of people, energy, and nutritional deficiency have increased gradually, bringing environmental pollution. Due to human activities, the environment has been heavily polluted by toxic pollutants in the recent past. Exposure to this toxicity puts human health as well as plants, animals, and microorganisms at risk. Heavy metals, called micro-pollutants, are among the most important causes of environmental pollution as they can create toxic effects even at very low concentrations due to their ability to accumulate. The term "heavy metal" generally describes elements whose density is five times greater than that of water [1]. Heavy metals have a wide variety of sources. The main ones are natural events such as volcanic activities, industrial activities, mining operations, exhaust gases of vehicles, pesticides, and fertilizers used in agriculture. While the sources and uses of heavy metals are so diverse, it is almost impossible to avoid exposure to them and their effects. In the last quarter of the twentieth century, heavy metals began to be added to cosmetic products as coloring additives or impurities [2]. These heavy metals, which can pass into the human body through the skin and hair follicles, can cause heavy metal toxicity, leading to various cancers, lung diseases, organ dysfunction, and mental retardation [3, 4]. Therefore, the ingredients of such cosmetic products should be well examined before marketing and use.

According to the regulation on cosmetic products in the European Union and Canada, lead, cadmium, and their compounds, nickel, and some of their compounds are listed as prohibited substances in cosmetics unless they are mandatory in their components [5].

According to European Union regulations tolerable quantities of some heavy metals are decided as 10 ppm, 3 ppm, 3 ppm, 3 ppm and 5 ppm for lead, arsenic, mercury, cadmium, and antimony, respectively [6, 7]. In addition, the FDA completely banned the addition of lead acetate in 2018 [8]. Although the addition of heavy metals as

additives is prohibited, the raw materials used in cosmetic products inevitably contain heavy metals as impurities. For this reason, the heavy metals' determination in cosmetic products is crucial.

For trace heavy metal detection, numerous techniques are used, such as flame atomic absorption spectroscopy (FAAS) [9], inductively coupled plasma-optical emission spectroscopy (ICP-OES) [10], electrothermal atomic absorption spectroscopy (ETAAS), inductively coupled plasma-mass spectroscopy (ICP-MS) [11], and UV- visible spectroscopy (UV-VIS) [12]. Among these techniques, FAAS stands out with its speed, practicality, convenience, and low-cost advantages [13]. However, the sensitivity of FAAS is insufficient because real samples have very low concentrations of heavy metals and a complex matrix. For this reason, detection limits should be improved by applying separation/enrichment to samples before determination by FAAS [14].

Many different methods such as liquid-liquid extraction [15], co-precipitation [16], solid-phase extraction [17], which can be called traditional methods, are being developed and applied for the preconcentration of analytical species in various matrices. However, these methods have several disadvantages: large sample volume, large amounts of toxic organic solvents, high processing steps, and low enrichment factor. In order to eliminate these disadvantages, miniaturized enrichment-separation methods, which adopt the green chemistry approach, have been developed recently [18]. The aforementioned traditional methods have begun to be replaced by their miniaturized versions. For example, conventional liquid-liquid extraction has been miniaturized into liquid-liquid microextraction [19]. In this way, an improved enrichment factor has been achieved with high extraction efficiency by using new generation solvents in much lower volumes. Thus, deep eutectic solvent (DES) selected as an extractant solvent which is environment friendly with a low cost alternative to conventional organic solvent, easy to prepare form available components, too many variety, and a very low volume (in microliter level) sufficiency for high enrichment and separation.

In this thesis, a method based on microextraction of Cd (II), Cu (II), Pb (II), and Ni (II) ions, which are found in cosmetic products using hair dye, are converted into metal chelates with dithizone reagent, and microextraction with deep eutectic solvent (DES) was developed. In this suggested method, various parameters that affect the recovery efficiency are optimized. Among these parameters were pH, amount of choline chloride and phenol forming DES, amount of DES, amount of dithizone providing complexation, amount of aprotic solvent THF, ultrasonic bath time, and centrifugation time investigated. In addition, various matrix effects have been studied. The method's accuracy was investigated using NCS ZC 73013 (spinach leaves) CRM and spiking-recovery tests. The developed DES-based microextraction method was applied to the determination of Cd (II), Cu (II), Pb (II), and Ni (II) ions in various hair dye and henna samples. Heavy metal determinations were performed by FAAS.

2. THEORETICAL

2.1. Definition and History of Hair Dyes

Hair dyes have been one of the most common products in the markets among the different cosmetics products, with a high risk of being contaminated with heavy metals during processing the product [20], which is considered the main source in releasing these elements. So, these kinds of cosmetic products and their ingredients should be safe and well-evaluated before marketing under and usage [4].

The process of altering the hair's color is called as "hair dyeing". Hair dyeing can be applied for different reasons: to camouflage gray hair, to obtain darker or lighter hair's color shades, or to completely change the color of the hair, Today it is a popular method to use hair dyes [21].

From the mummies obtained, it is seen that henna was used to dye hair in the ancient Egyptian civilization about 4000 years ago. In the Roman Empire, it is known that the hair was combed with a lead comb dipped in vinegar to darken gray hair [22]. Recently, hair dyes have become widespread, especially in developing countries; for example, 10% of men over 40 and 35% of women over 18 years of age dye their hair [23].

2.2. Development of Hair Dyes

Henna was one of the first herbs to be used as a beauty product. Henna contains a red-orange pigment and called lawson (2-hydroxy-1,4-naphthoquinone), which causes dyeing of hair and skin. It is also traditionally used as a medication to cure many diseases. When a color change is desired on the skin or hair, powdered henna is mixed with water or oil and turned into a slurry and applied to the surface. After the application, lawsone in henna is absorbed on the outer layer of the skin, causing coloration (Figure 2.1.). In order to make it permanent or more substantial, commercially sold henna is frequently combined with various herbs and various chemical additives (containing high levels of trace elements like lead(II) acetate and

lead(II) sulfate). For this reason, natural henna, which is actually harmless, can cause negative health effects due to the additives added [24, 25].

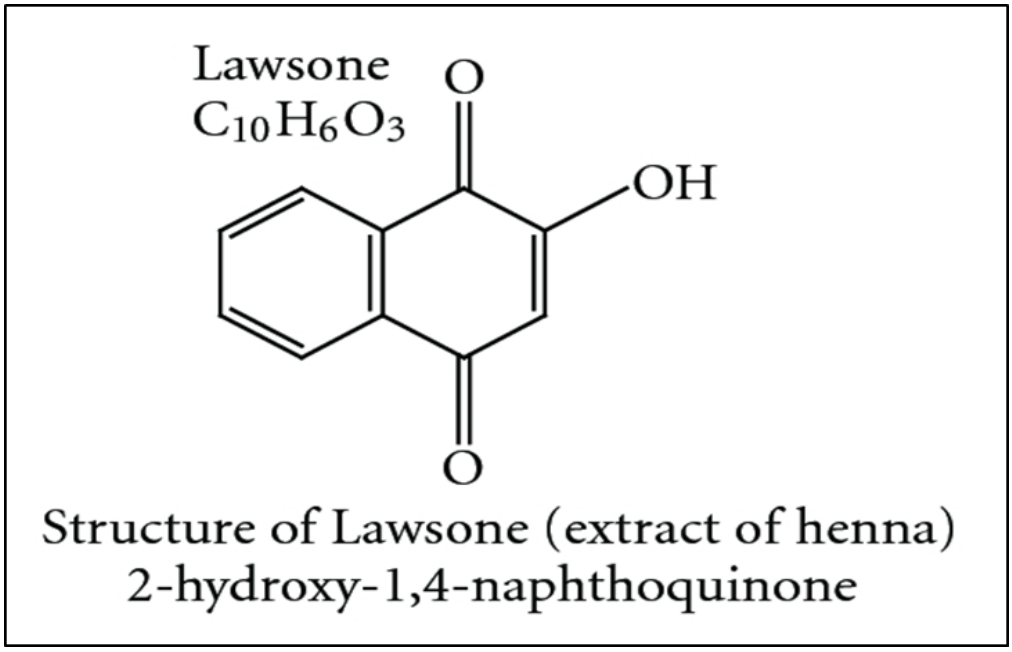

Figure 2.1. Structure of Lawsone in henna [31].

Eugene Schueller, in 1907, for commercial goals, designed the first chemical dye, which was named Aureole—later known "L'Oréal" as a trademark. After that double dyeing process for blonde hair shortly followed. Then, in 1932 the chemist Lawrence Gelb developed a hair-through dye called "Clairol", the first single-step hair dye in 1950 [26].

2.3. Taxonomy of Hair Dyes

The hair dyeing scheme can be divided into two major categories, oxidative and non-oxidative (progressive), with a specific action mechanism and composition [27].

2.3.1. Oxidative Dyes

Oxidative Dyes are characterized by long-lasting effect and considered as the most popular hair dye. These dyes have various substances that react with each other to reach the desired final shade. These essential components are:

2.3.1.1. Primary Intermediates

Typically, all permanent hair dyes have a primary intermediate which is either p-aminophenols or p-phenylenediamines. Oxidation occurs by hydrogen peroxide (H_2O_2) to yield reactive species that interact with couplers, thus gain the required shades.

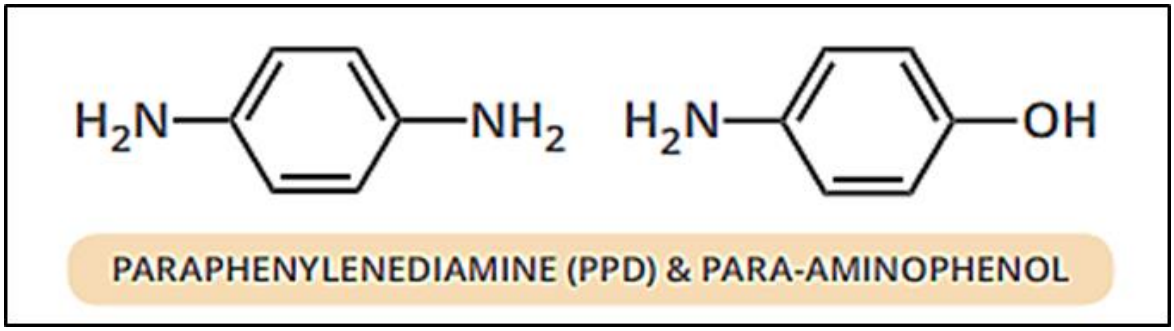

Figure 2.2. Structure of p-phenylenediamines and p-aminophenols.

2.3.1.2. Oxidation Compounds

The oxidation agent of the primary intermediates is hydrogen peroxide. It also works as the lighting of pheomelanin and eumelanin, the natural pigments present in hair. Ammonia has been needed in the dyeing mixture system to make the alkali intermediate that expanded the hair cuticles, aiding dye molecules and peroxide reach the hair's cortex. Ethanolamine has been used sometimes as an alternative to ammonia [28].

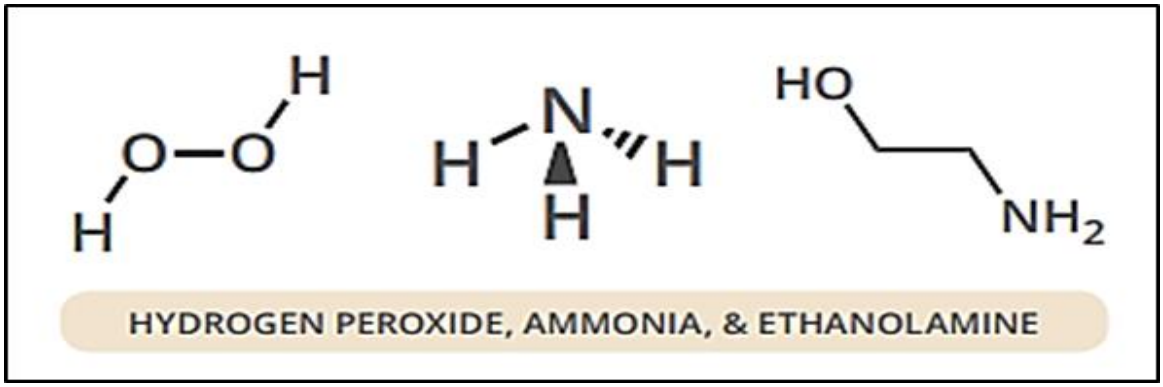

Figure 2.3. Structure of hydrogen peroxide, ammonia and ethanolamine.

2.3.1.3. Joining Agent

It is known as a color coupler, characterized by containing an electron-donating in the aromatic ring systems like OH and NH_2 in the meta position so, can react with the p-aminophenols or p-phenylenediamines in the presence of oxidation mixture to produce the dye molecule with the desired shade but not engaging in an oxidation process and not produce any significant color alone in the presence of oxygen release [29].

Figure 2.4. Structure of colour coupler [29].

2.3.2. Progressive or (Non-Oxidative) Hair Dyes

Progressive or non-oxidative hair dyeing means when the product in use for a while, as each application, the hair had been becoming darkens by combination with the hair proteins [30].

Kind of hair dyes include:

- Gradual hair coloring by using metallic dyes like lead acetate [31].
- Natural dyes (such as henna) [32].
- Temporary dyes are water-soluble, that removed easily with shampoo, it is non-oxidative dyeing, reduces the durability period on the fiber, then released from

hair after the first shampooing due to the great molecular weight of dye that deposits on the hair surface, but unable to penetrate the cortex, that dye formula does not whiten the hair but just adding new shade and not changing the original hair color [33].

- Semipermanent dyes are low molar mass basic or cationic dyes that have better sensitivity to hair keratin, which are resistant to three to six washes. No oxidation reaction has been in the hair dyeing process; the treatment is easy and remains for 10 to 40 minutes, accompanied by rinsing. Many products are present on the market, including lotions, shampoos, and mousses. Low molar mass dyes enter slightly into the scalp, mainly due to the high pH of the product's content, which supports the opening of the cuticles [34].

As mentioned above, the most common hair coloring cosmetics are permanent (oxidative) dyes, and henna which is widely used as a traditional habit despite many hair dyes being present.

In the literature, henna samples and hair dyes had been proved to contain numerous heavy metals by different studies. For example, a study had been surveyed 32 hair dye samples for 10 heavy metals using ICP-MS. Consequently, the amount of Fe, Ba, Al, Pb, Cu in addition to Cd had been founded as 1.19, 0.86, 0.54, 0.185, 0.061, and 0.00045 mg kg^{-1}, respectively. But for Co, Cr, Ag, and Mn, the concentration has been detected as below LOD [35]. In another project performed by Ibrahim et al ICP-MS was established for determination of a series heavy metals (Mn, Pb, Cd, Ba, Al, Bi, Cu, Co, Ni, Mo, Zn, Cr, Ag, and Hg) in 15 types of henna samples. The lower and higher concentrations in mg L^{-1} for each metal ion were found as: 0.70–13.54 to Mn, 1.85–16.42 to Pb, 0.14–0.958 to Cd, 2.50–103.68 to Ba, 10.63–19 681.5 to Al, 21.30–698.1 to Bi, 0.84– 118.9 to Cu, 0.47–1.3 to Co, 3.22–223.11 to Ni, 0.227–2.18 to Mo, 1.676–996.3 to Zn, 1.30–15.75 to Cr, 0.07–1.780 to Ag and 0.1–2.4 for Hg [36].

In a study conducted on two different hair dyes, nine heavy metal ions (Co, As, Hg, Cd, Ni, Cr, Zn, Cu, and Pb) were detected using ICP-OES, the maximum concentration in ppm had been found as follows: 1.458 for Pb, 0.523 for As, 0.461 for Zn, 0.319 for Cr, 0.303 for Cu, 0.139 for Ni and 0.036 for Co. But Hg and Cd cannot be determined because they were under the LOD [37].

In a recent study Cd, Cr, Fe, and Ni were determined in 6 hair dyes samples by using AAS; the maximum and minimum content were founded to be 0.001– 0.169, 0.048–0.130, 0.263–0.416, and 0.08–4.16 mg kg^{-1}, respectively [38].

2.4. The Role of Heavy Metals in Human Health

Heavy metals are regarded as metallic components with a higher density relative to water, which are dangerous to human and environmental health. Substantial metal toxicity is known as a crucial risk, that linked with many health problems, in addition act as pseudo-elements of the body, but sometimes can interact with metabolic processes when activated. Rare metals, like Al, can be removed, but residues accumulate in the food chain and body, displaying a continuous existence. Different public life techniques, like environmental causes and workplace sensitivity, have been implemented to monitor, mitigate and manage metal contamination that occurs at different levels. The toxicity of metals relies mainly on the dosage absorbed, route and length of exposure, which leading to different diseases, thus resulting in excessive harm caused by the formation of free radical [39].

2.4.1. Cadmium (Cd)

(Cd) is a silvery-white, smooth, ductile metal with an atomic number of 48 and lie in the group 12 element in period 5 and d block. Characterized by highly toxic even in low concentrations, causes damage to vital organs including lung, liver and kidneys.

2.4.1.1. Cadmium Exposure Sources

Generally, Cd is restored as a by-product upon sulphide deposits, especially those containing copper, zinc and lead. Also, other primary sources of cadmium are [40, 41]:

1. Fossil fuels,
2. Cement,
3. Waste incineration,
4. Fertilizers as a phosphate fertilizer
5. Nature activities like a volcanic eruption,

6. Mining
7. Pigments,
8. PVC stabilizers.
9. Smoking
10. Iron and steel production
11. Batteries
12. Coating and plating.

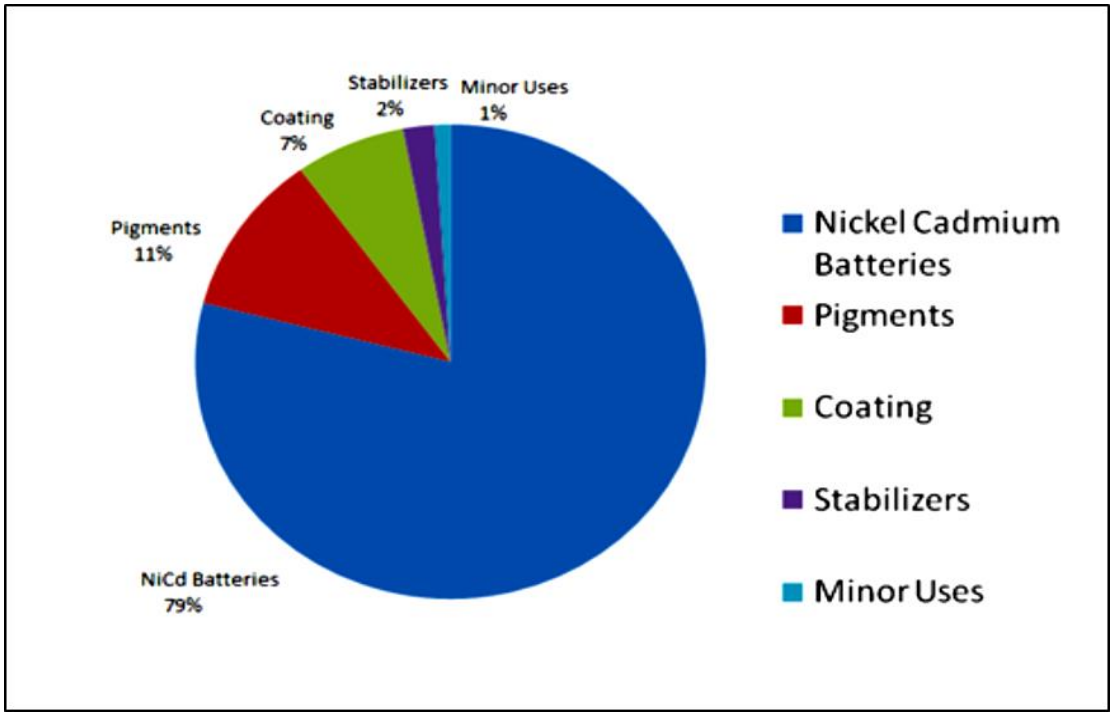

Figure 2.5. Cadmium uses in industries [41].

Cd level in human elevated with age, reaching in mean of nearly 30 mg in the age between 40-50 years then decline slightly [42]. Cd is dangerous to both human beings and the environment. As explained above, Cd exists in the atmosphere, food or water, and use even in cosmetics products because of its color characteristics, so, it has been utilized as a pigment in various industries [43].

2.4.1.2. Effect of Cadmium on Human Health

NIOSH ranked Cd as a human carcinogenic, and if had been absorptive inside body can cause kidney damage; Cd concentrations in urine indicate long term

susceptibility. The quantity of Cd stored in the body, specifically in the liver and kidneys. Also, even that Cd exposure was at a very low level; it can cause lower bone mineral density. Another disease that can cause is bronchitis that are an inflammation of the membrane of bronchial tubes and also other lung diseases such as pulmonary edema or emphysema or, in addition chemical pneumonitis, such as peripheral neuropathy symptoms of numbness, tingling and muscle weakness [44, 45].

2.4.2. Copper (Cu)

Copper (Cu) is a pinkish-orange, malleable, soft metal with a very high electric and thermal conductivity. It has a molecular weight of 63.5 and atomic number of 29. Cu atoms exist more than ten thousand years old have been quarried in northern Iraq from Sumerian civilization and are likely made of indigenous copper, of which fragments can sometimes be found. In the ancient world, Cu was generally used as bronze, alloyed with tin, also, used to make coins, tableware and utensils [46]. Cu was a vital trace element which catalysis processing in iron absorption and heme synthesis. After Fe and Zn, Cu is the third profuse trace element in the human body. Cu contaminant of beverages products and food causes an acute gastrointestinal disease. Origin of intoxication with Cu poisoning includes: Cu, vending machines or beverages from brass vessels and seldom drinking water. The medicinal utilizes of Cu as an emetic had been stopped due to its toxicity. Cu transmission through the skin can give beneficial effects, but still induce generate skin irritation reactions which usually ignored [47]. While Ingesting high levels of Cu can be hurtful, leading to various symptoms, including: vomiting, nausea, diarrhea, kidney or liver damage or even death, so, an exact, accurate and rapid measurement of Cu was very important [48].

2.4.2.1. Copper Exposure Sources

1- The mainly use of Cu is as an alloy (Monel metal, brass, gunmetal and bronze) or a metal. Cu is an essential constituent of newer alloys (palladium and nickel-tin) and white gold used for imitation jewelry [49].

2- A bout 25% of the production of Cu used in electrical applications with transportation, build, machinery and munitions as a metallic copper [50].
3- Industrial purposes involve [51]:

   a) Petroleum refining,
   b) Water treatment,
   c) Dye manufacturing
   d) Wood preservatives
   e) Mineral froth flotation.

4- Agricultural purposes (nutritional supplements, algicides, fungicides) approximately most of the production of copper sulphate [52].
5- Medical purposes:

   a) Copper-based alloys (Al, Co, Mg, Ni, Zn) had been introduced to manufacture dental crowns and bridges [53].
   b) Today, addition of few amounts of palladium or platinum to high-copper amalgams to raise durability and decrease tarnish, which was less exposed to corrosion in comparable to low-copper amalgams [54].
   c) Cu was a component of birth control; which had been used in intrauterine contraceptive devices, releasing of Cu from them essential to their contraceptive impacts, which contain approximately 100–150 mg of Cu, that nearly equal to copper's average total body load [55].
   d) The wearing of Cu bracelets was a traditional treatment for arthritis, in spite of there was poorly reports to confirm the effectiveness of that treatment or remedy [56].
   e) Green or Spiritual water was a purgative applied in African but consuming of that water resulting in renal failure, hemolysis, then death. About 13 g of Cu/L present in samples from solutions of spiritual water [57].

2.4.2.2. Effect of Copper on Human Health

There were three main pathways for the copper to cross the human body and causes many health problems, which were:

1- From Inhalation Exposure:

Hematological, gastrointestinal, hepatic, genetic, ocular and endocrine effects had been noticed in humans.

2- From oral exposure:

a. Various human studies had been examined the possible association between the elevation of serum Cu levels which increased threat of coronary heart disease. However, many studies had been found an elevation serum Cu levels with increased threat of coronary heart disease deaths [58].
b. Numerous reports deal with acute gastrointestinal impacts in humans when ingesting large amounts from Cu in beverages or even drinking water. The main prevalent symptoms vomiting and nausea, which usually happened shortly after ingestion but not persistent. As well as, belly pain and diarrhea have been reported.
c. Limited information about influence of Cu on the hematological system of human, for example a case of acute hemolytic anemia had been recorded in a child (18-month-old) after two days of drinking a solution containing about 3 g of copper sulphate.
d. Hepatic effects, for example, Wilson's disease is a genetically inherited metabolic disorder that develops as a result of excessive copper accumulation in the eye, liver, brain, bone marrow, kidney and other vital organs.
e. Kidney effects from extra copper in the liver overflows and builds up in the kidneys and cause damages.

3- Dermal Exposure effects

a. Optical impacts. Eye irritation had been recorded in factory staff after exposing Cu dust.
b. Allergic contact dermatitis had been observed. In vitro studies proposed that Cu is weakly absorbed a cross intact skin and lower than 6% of Cu precipitated on ex-vivo human skin samples was absorbed as well as absorption of copper chloride was higher extent than copper sulphate [59].

2.4.3. Lead (Pb)

Pb is silvery grey or bluish-white metal, naturally exists in the earth's crust. Its atomic weight is 207.19 and atomic number is 82, with a boiling point of 1740°C under atmospheric pressure and a melting point of 327.5°C. Raw Pb was scarce, that often found in nature with Cu, Ag, Zn and extracted together. Nevertheless, most Pb concentrations exist in nature as a consequence of human actions. Pb is widely spread in the environment due to extensive consumption by recycling old products, consumer products and manufacturing processes. Recent projects and media also recorded the presence of Pb in different cosmetic products, suggesting that exposition to minimal concentrations of Pb causes various problems like reduced learning and hearing, behavioral abnormalities, permanent neurological damage, also could have negative impacts on the hepatic, renal and reproductive systems [60, 61].

2.4.3.1. Lead Exposure Sources

1. Using lead in gasoline.
2. Pb is burnt in vehicle engines; thus, lead salts include: bromines, chlorines, and oxides are formed. Through the exhausts of vehicles, the Pb salts are introduced to the atmosphere. Depending on the size, the heavier particles directly falling to the earth and pollute surface waters or soils, but fine particles fly farther via the air and remain in the atmosphere. During rain, some of those particles drop down into the ground.
3. Using Pb in a painting like a wall paint.
4. Lead can enter drinking water through the corrosion of pipes.
5. In a wide range of products used globally, lead and lead compounds were used, including ceramics, batteries, ammunition and cosmetics.
6. The average amounts of lead in the soil vary from 50 to 400 parts per million. Still, the impact of mining, refinery practices significantly increase the amount of lead in the environment, especially near smelting and refining sites.

2.4.3.2. Effect of Lead on Human Health

Pb inside the human body does not perform any vital purpose but instead causes damage after entry into the body via air, food, or water. Several health effects on

humans, such as interruption of hemoglobin and anemia biosynthesis, blood pressure increases, damages kidneys, nervous system disturbance, damages in the brain that reduce children's learning abilities, and children's behavioral disorders such as impulsive behavior and aggression and hyperactivity. Lead can enter the fetus through the mother's placenta and cause massive damage to the unborn babies' system of nervous and brain [62, 63].

2.4.4. Nickel (Ni)

Ni is a rigid, silvery-white, ductile, and malleable metal. Its atomic mass is 58.71 g/mol, and Its atomic number is 28. There are many industrial and agricultural applications of nickel and nickel compounds, and the progress of the industrial revolution has resulted in increased pollution of the contaminant into the environment. While Ni is necessary for many species to make their functions, the amounts of both anthropogenic release and natural quantities in certain areas can be highly toxic [64]. There are several kinds of exposure to nickel like inhalation exposure, Oral consumption through water and food and daily use of stainless steel and nickel-plated products since nickel is a common sensitizing agent with an increased proportion of skin irritation [65].

2.4.4.1. Nickel Exposure Sources

Naturally, foods contain minimal quantities of nickel, but it's also established that chocolate and fats contain significantly high nickel concentrations [66]. It can also exist in contaminated soils, and then people ingest vast amounts of vegetables so that nickel absorption increases. Another source is smoking cigarettes with a great amount of nickel absorbed in the lung and larynx [67].

One of the most important sources of nickel exposure is metallic uses like in jewelry and tools and staffs for different activities and jobs such as electricians, painters and plumbers and causes serious allergy problems like Occupational contact dermatitis due to nickel allergy [68].

2.4.4.2. Effect of Nickel on Human Health

Several effects of Ni on human health status, like the rising risk of lung and oropharynx cancers and even prostate cancer. Nausea and vertigo can be observed after the exposure to nickel, also headache, Insomnia, Respiratory insufficiency and also possible embryotoxicity or nickel teratogenicity in pregnant women who are exposed to nickel carbonyl and other nickel compounds that then cause a birth deformity, other effects such as chronic bronchitis, pneumonia, heart disturbances and also Allergic effects, primarily from jewels, such as skin irritation [69].

2.5. Preconcentration of Trace Heavy Metals

In case the samples contain analyte in too small concentrations to be analyzed by analytical methods, the "preconcentration" process is applied to give the analytes a suitable level of determination. The preconcentration process is generally based on separation. With applying the preconcentration method on a solution, the main component is removed from the solution, and the trace components remain in the solution in some cases. On the contrary, the main components remain in the solution while trace components are removed. Also, the trace components can be separated from each other in the same sample solution.

The preconcentration method can affect the determination of trace elements by;

- Increasing the determination limit via raising the trace element concentration in the analyte sample.
- Preventing interferences in the analyte sample by removing the trace elements from the matrix.
- Decrease errors that may arise from the inhomogeneity of the sample.
- Increasing the selectivity of the method.
- with the separation and extraction process, the trace element is taken into a known matrix, so it is easy to simulate the standard and sample matrixes.

The fact that the instrumental analytical methods often have not good selectivity, sensitivity, or free from matrix interferences lead to the need for preconcentration of trace elements or ions into an aqueous solution.

Preconcentration methods can be listed as, co-precipitation, solid-phase extraction ‘adsorption’, volatilization, ion exchange, electrodeposition, and liquid-liquid extraction (solvent extraction) [70, 71].

2.5.1. Co-Precipitation

It is the separation process made by taking advantage of the solubility differences of compounds in aqueous solution. It is especially preferred in water analysis. The most important point in the co-precipitation process is that the trace element and the precipitator have different chemical properties. Otherwise, mixed crystal structures may precipitate [17].

2.5.2. Solid-Phase Extraction by Adsorption

The separation process depends on the adherence of ions or molecules from a gas, liquid, or any solution to a solid surface, is called "adsorption". It is widely used because of its important role in the stability of colloids and fluid-fluid interactions especially in biological organisms.

2.5.3. Volatilization

It is a method that can be used when there is a large difference in volatility between matrix and trace element. Whichever the main component or traces element is more volatile, the volatilization process is applied to it. The most significant advantage of the method is that the enrichment process takes place without the need for high volumes of reagents in the evaporation of the main components. Here the main ingredient is a liquid such as water, an organic solvent, a volatile acid or a solution of ammonia.

2.5.4. Ion Exchange

Ion exchange is a physicochemical phenomenon that works according to the law of preservation of mass. It is based on the principle of exchanging the ions of a solid

(a resin) with an equivalent number of ions in the surrounding solution. Ion exchange can be carried out by using two different types of solid materials; natural and artificial. For an excellent chelating resins efficiency, high selectivity, high exchange rate and capacity, chemical and physical stability are usually required.

2.5.5. Electrodeposition

Electrodeposition technique used in preconcentration and separation of very low concentration analyte from the bulk sample by very simple instrument, depending on the variation in the reduction potential of the trace elements. Accumulation of the trace elements affected by the sample and electrolyte composition, electrode types, electrolysis cell and other experimental conditions.

2.5.6. Liquid-Liquid Extraction (Solvent Extraction)

It is the most using preconcentration method known in analytical chemistry. The main characteristic of this separation method is the difference in solubility of compounds and elements in the two immiscible liquid phases. An organic solvent that is immiscible with aqueous solution is generally used for phase separation. The method is widespread because of its simplicity and is preferred, especially in studies with atomic absorption spectrometry. In that method, the analyte ions transfer from the aqueous phase toward the organic solvent phase with a water-soluble complexing agent. Thus, both separation and preconcentration are carried out at the same time.

2.6. Microextraction Technique

Microextraction is similar in process to conventional extraction but differs in that the volume of the extraction phase is very low compared to the sample volume. Due to the tedious sample preparation and the high amount of chemicals required in most of the conventional analysis methods, the use of microextraction methods has been increasing in recent years. In this method, sample preparation is easy, fast and chemical requirements are low [72, 73]. In addition, there is no or as little as possible need for toxic solvents and energy. So, these are methods that support the understanding of green analytical chemistry. It is also known that the enrichment

factors of conventional microextraction methods are much better when compared to extraction methods. Nowadays, many microextraction protocols are accessible. This method is categorized according to type of the extraction phase, as liquid-based and solid-based. Both liquid-based and solid-based microextraction techniques has many different applications for determination of trace elements [18].

2.6.1 Solid-Phase Microextraction (SPME)

In 1990, SPME was proposed as a novel advancing in the scope of sample preparation, which was a solvent-free process [74]. The method is simply implemented using a syringe containing a low volume solid sorbent or high molecular weight polymeric liquid [75]. In this method, polydimethylsiloxane, divinylbenzene, polyacrylate, carboxene, and carbowax are frequently used adsorbent types [76, 77]. Carbon nanomaterials [78], magnetic nanoparticles [79] and polymers are the types of adsorbents developed in recent years [80]. Two features can be highlighted regarding the SPME technique: it is a simple application and it eliminates the use of organic solvents. However, the high cost of the solid phase used, low reproducibility and selectivity are limitations, especially in the analysis of hydrophilic and non-volatile compounds. In addition, its use is limited due to its higher LOD values compared to conventional solid phase extraction (SPE). The efficiency of the method has been increased with nanomaterials, the use of which has become widespread in recent years [81, 82].

2.6.2. Liquid-Phase Microextraction (LPME)

The most important difference of liquid-phase microextraction (LPME) from the classical liquid-liquid extraction method is that the extraction solvent amount is reduced to microliter levels [19]. Besides, it was a quick, simpler, environmentally friendly technique. In LPME extraction process occurs between a small volume of aqueous phase containing target compounds and water-immiscible solvent (acceptor phase). Different types of LPME are available, depending on the method of application. These are single- drop microextraction [83], hollow fiber- LPME [84], cloud point microextraction [85] in addition to dispersive liquid-phase microextraction (DLPME) [86].

Recently, LPME is considered among wide spread commonly used extraction technique to the pretreatment and separation of several samples like environmental [87], food [88, 89], cosmetic [90] and pharmaceutical samples [91]. In LPME, analytes are transferred to the extraction phase and enriched by dispersion between the extraction solvent and the aqueous analyte solution. High enrichment factors can be obtained if the sample volume is high and the extraction solvent volume is low [92].

2.6.3. The Classification of LPME

2.6.3.1. Single-Drop Microextraction (SDME)

The SDME technique, first proposed in 1996, is the oldest, simplest and most widely used among LPME techniques [93]. This method uses a only one tiny drop of organic solvent at the tip of the microsyringe needle, which is immersed in the aqueous solution, which is continued to be stirred throughout the extraction process. Upon completion of the extraction, the microdrop is withdrawn into the microsyringe and directly analyzed by injecting it into analytical instruments such as capillary electrophoresis, GC or HPLC, ETAAS, ICP-MS. Single drop microextraction is a simple method with high recovery and low use of organic solvents [94, 95]. SDME is categorized as two-phase or three-phase methods [96].

1. Two- Phase Modes

a. Direct Immersion- Single Drop Microextraction (DI-SDME)

In this method, a drop from water- immiscible extraction solvent hanging on the head of micro-syringe needle submerged in the sample as seen in Figure 2.6 [97].

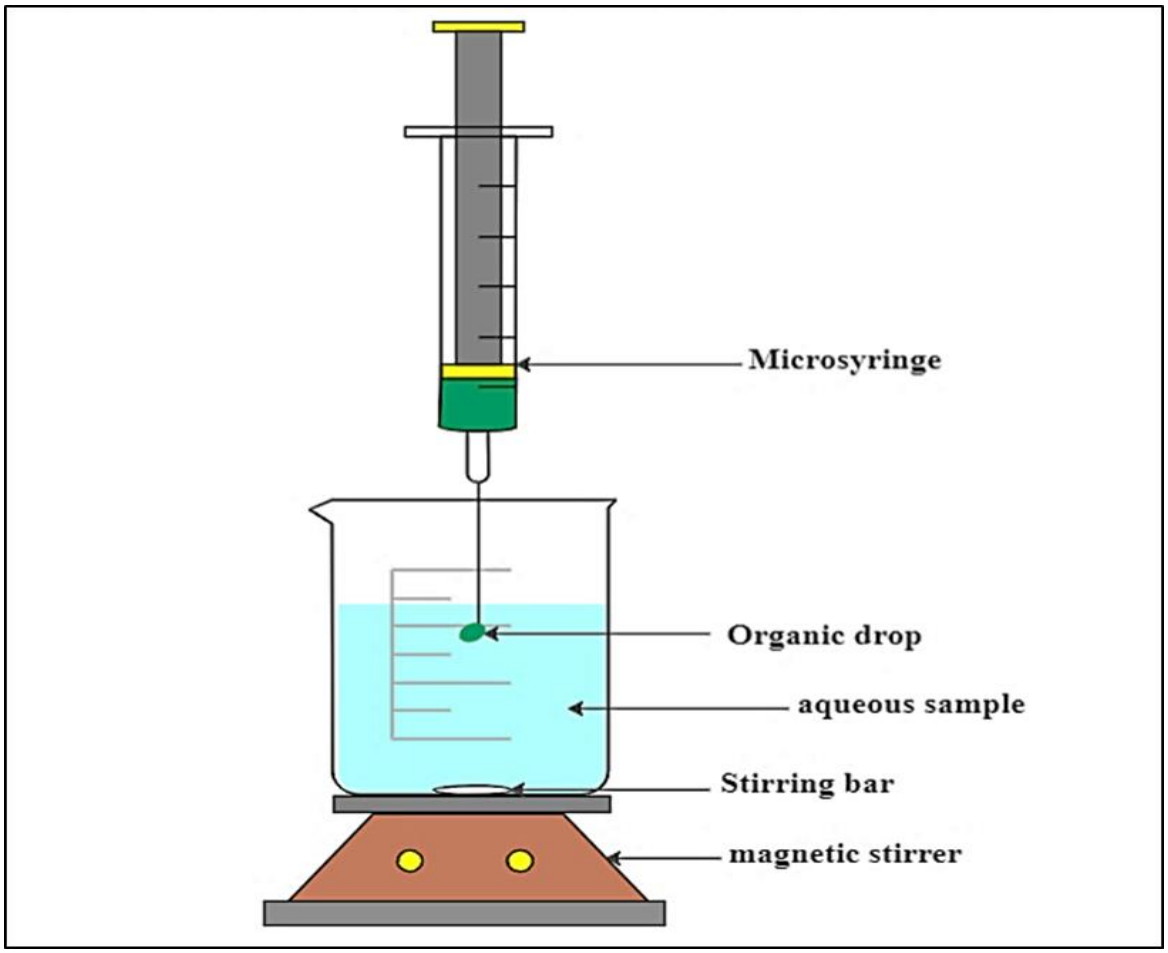

Figure 2.6. Direct immersion single-drop microextraction.

It has an important characteristic that easily and totally automated with chromatographic and spectroscopic determination techniques [98]. This method used has two different application types (dynamic and static modes) to extract and determine various analytes, such as hydrocarbons and heavy metals. As referred before the advantages of DI-SDME made it a very green analytical process. But instability the droplet during rapid stirring rate and complex sample matrices are main disadvantages of the method [95].

b. Drop-to-Drop Solvent Microextraction (DDSME)

DDSME was introduced after modulation of DI-SDME technique that appropriate in conditions just a minimal volume or complex sample was obtainable, like biological specimens (urine, blood and saliva) [99]. Overall, DDSME uses a suspended drop of organic solvent submerged sample drop (often volumes in µl for both). That arrangement secures quick equilibration between two phases so, had not needed stirring as seen in Figure 2.7. The rate of volume of organic to aqueous droplet is

more significant in comparison with DI-SDME, so, preconcentration factor is lower. In 2006 first analysis using DDSME in combination with GC-MS was performed [100].

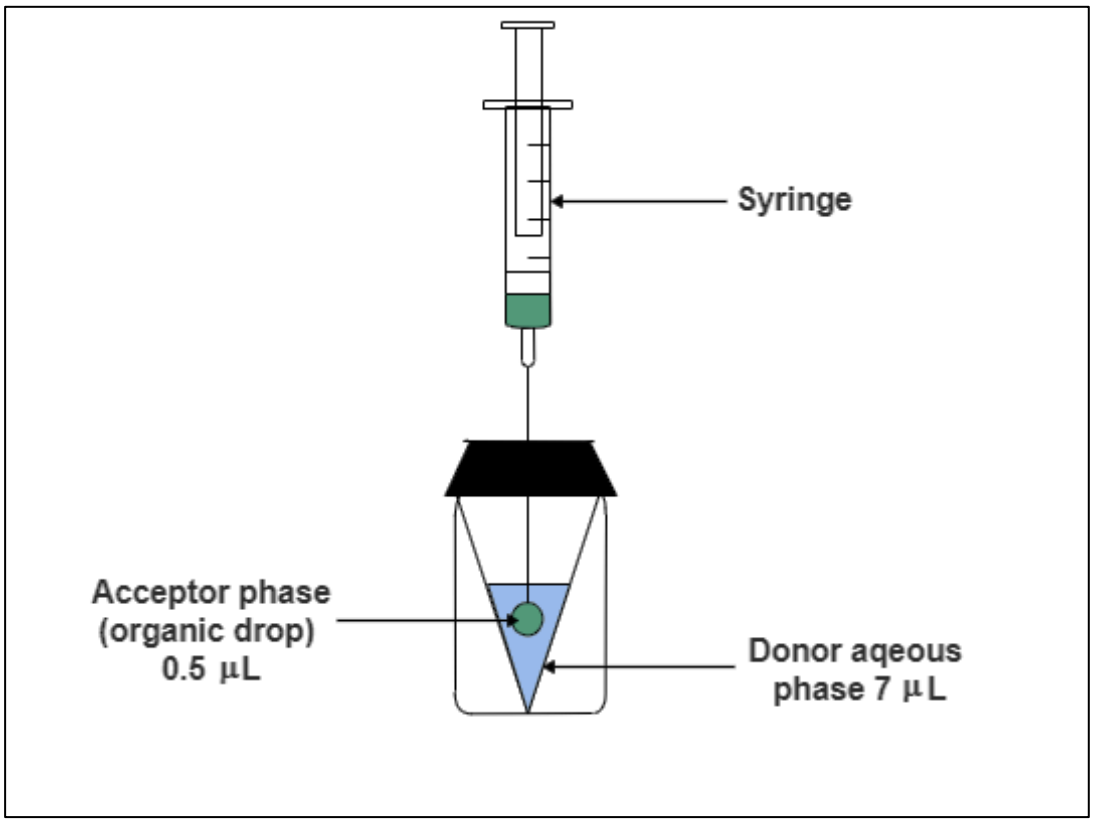

Figure 2.7. Drop-to-drop solvent microextraction.

c. Continuous Flow Microextraction (CFME)

First describing of CFME was in 2000, which enhance the mass transfer between the aqueous and organic phases [101]. CFME, as shown in Figure 2.8, is an extraction method having two-phases and relies on the extraction of analytes from a running sample solution into a single drop of organic solvent. Usually, the drop of solvent is placed on top of the vertically placed PEEK tube through which the sample flows. The microextraction performance is maximized by controlling the volume and type of extraction solvent, sample flow rate, capillary material, and extraction time. Many disadvantages of CFME like using non-standard equipment lead to restriction in the extraction of only slightly polar or non-polar compounds because of sufficient stability non-polar solvents just in the flowing setup. In addition, for avoiding the forming of bubbles in solution, selecting an appropriate pump is necessary [102].

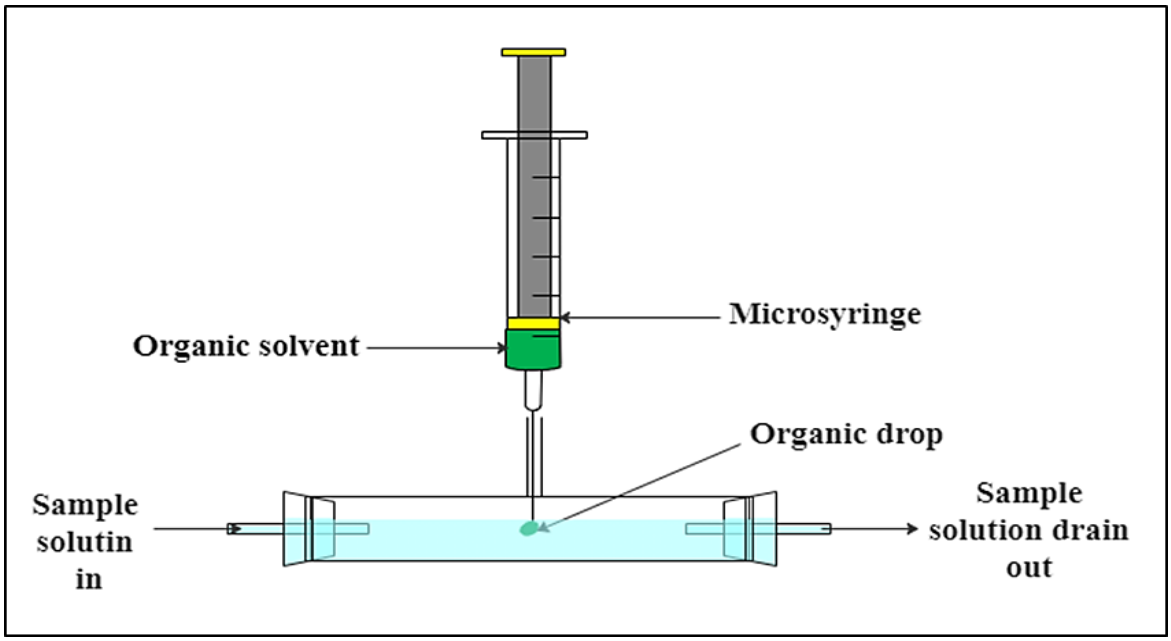

Figure 2.8. Continuous flow microextraction.

d. Directly Suspended Droplet Microextraction (DSDME)

The DSDME technique, developed in 2006, is based on the transfer of the analyte from the stirred aqueous solution to a suspended solvent droplet in a volume of 5–100 µL. By mixing, the contact surface area is increased and the transfer of the analyte to the extraction phase is facilitated. A disadvantage of this method is that the organic solvent is difficult to collect after extraction. The solvent used in this method should have a lower density than the sample solution. For this reason, chlorine containing organic solvents and several ILs are not preferred to be used in this method. In addition, the solvent must have low vapor pressure and low water solubility to prevent losing of solvent during extraction. Solvents that can be used for this purpose are isooctane or toluene [103].

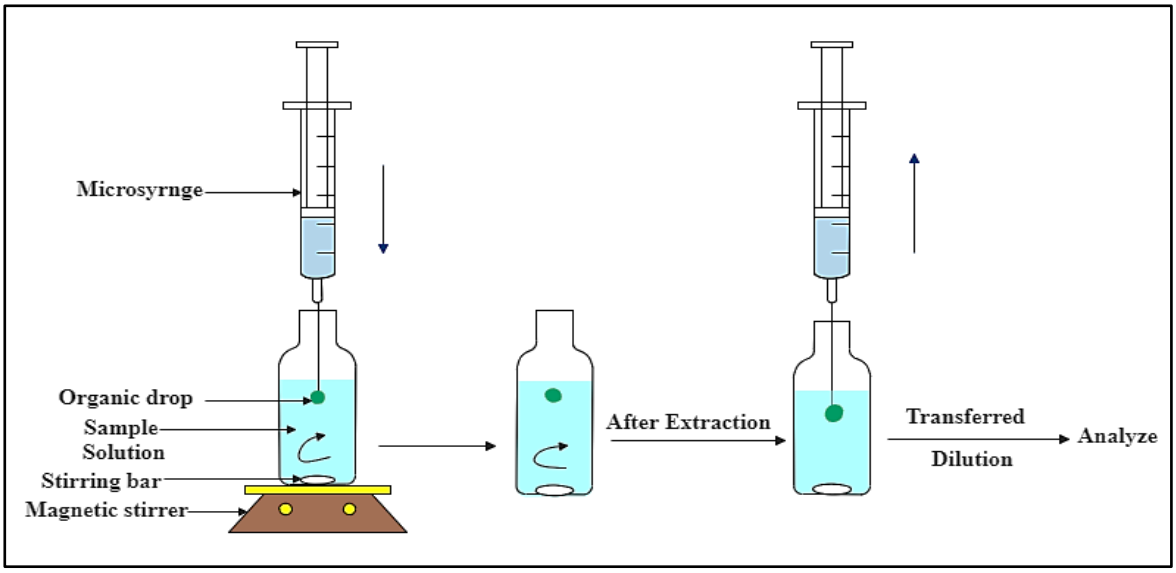

Figure 2.9. Directly suspended droplet microextraction.

2. Three-Phase Modes

a. Headspace (HS-SDME)

In the HS-SDME technique, which is used for the extraction of volatile or semi-volatile analytes and was first published in 2001, the organic extraction droplet is suspended above the aqueous sample solution [104]. In recent years, the advantages of the method have made this method interesting. For example, in this method, the sample solution can be mixed quickly, as the stability of the droplet suspended above the sample solution is not adversely affected. In addition, non-volatile matrix interactions are also reduced. In this mode, the analytes are distributed between the three phases: the aqueous phase, the headspace, and the drop of extraction solvent. Transfer of analytes from the aqueous phase to the organic phase is the step that determines the rate of extraction. High speed mixing of the sample solution simplifies the mass transfer between the three phases [95].

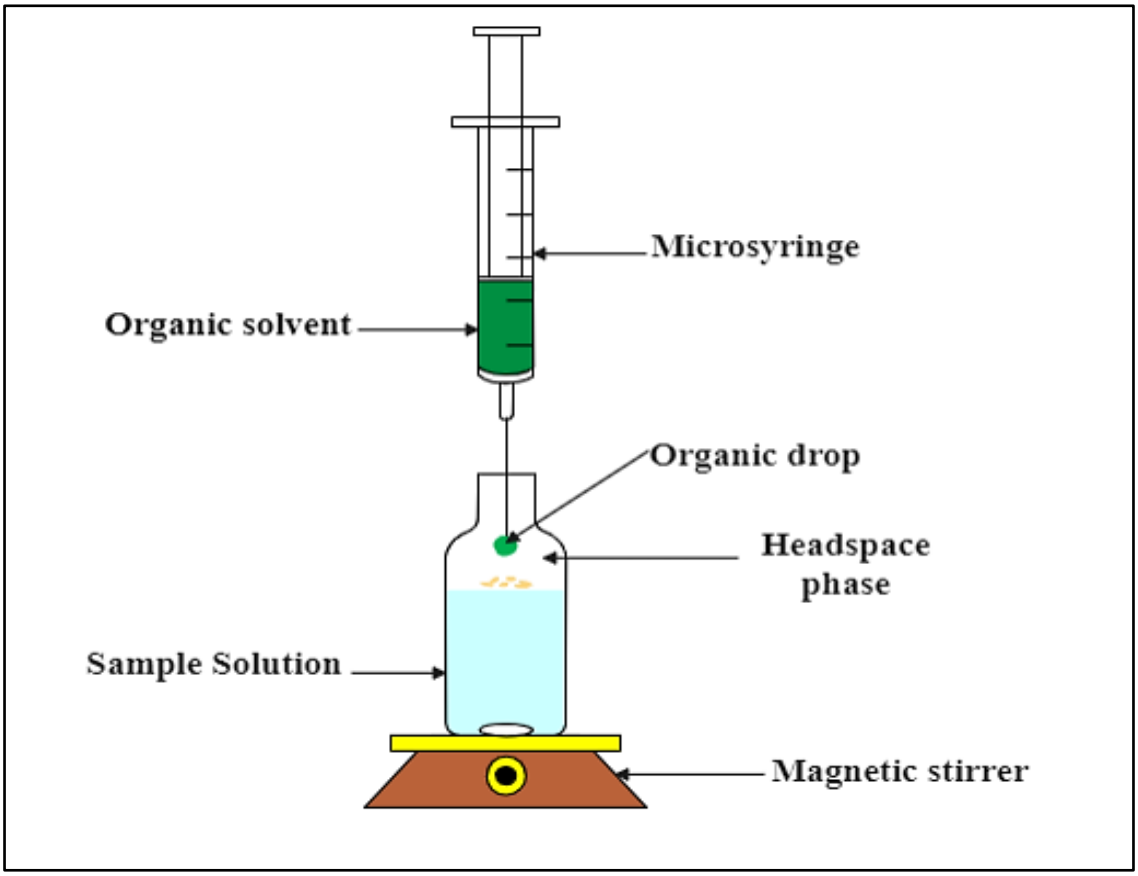

Figure 2.10. Headspace-single drop microextraction (HS- SDME)

b. Liquid-Liquid-Liquid (LLL-SDME)

LLLME is a three-phase method, called solvent microextraction with simultaneous back-extraction, in which DSDME and DI-SDME are usually carried out in two sequential extractions. The organic droplet should be lighter than water for a successful extraction procedure, also must be immiscible. The more commonly used solvents were 1-octanol, toluene and n-octane. Having high extraction efficiency, high selectivity and low LOD are the advantages of this method [96]. To obtain effective extraction, it was essential to study organic phase affinity and protonation therefore it could be easily back-extracted aqueous phase [105]. The extraction depends mainly on the pH adjusting and the two aqueous phases must have inverse polarity [106].

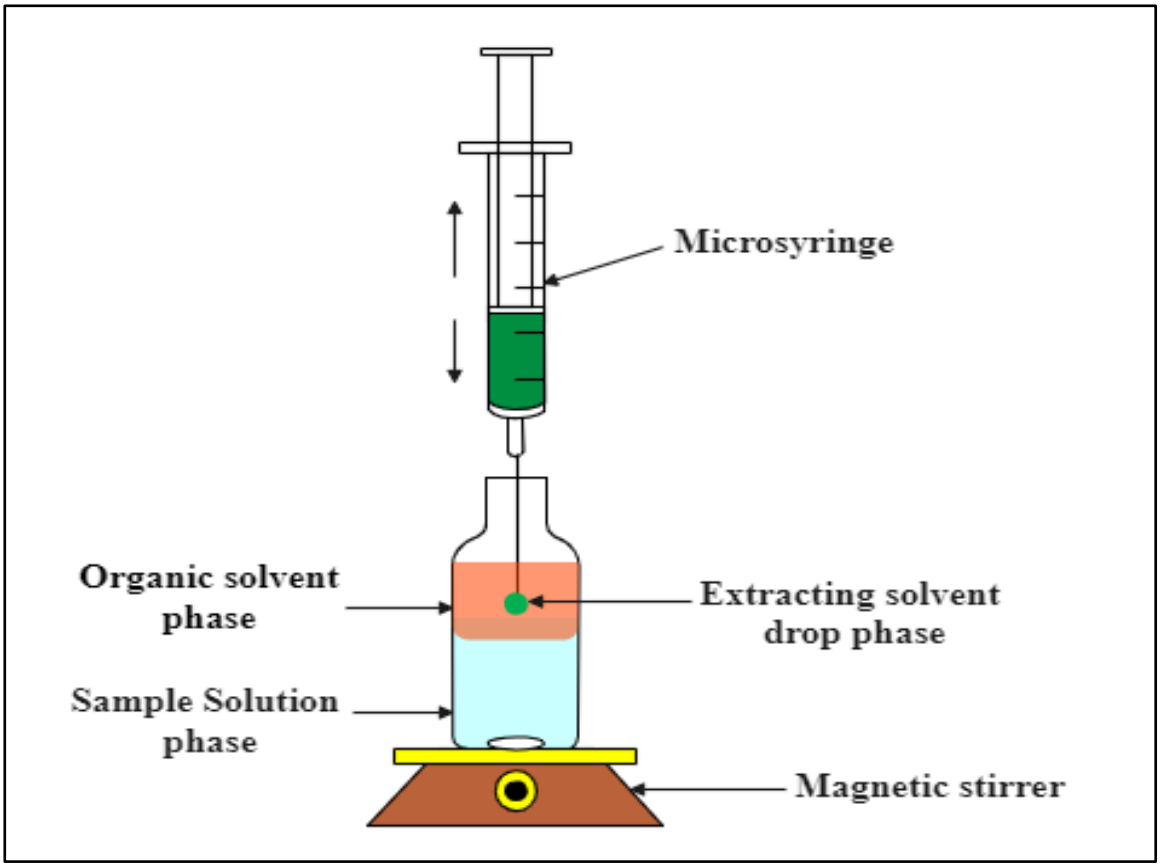

Figure 2.11. Three liquid phase-single drop microextraction.

2.6.3.2. Hollow Fiber-Based Liquide Phase Microextraction (HF-LPME)

For solving problem of the single drop instability, Rasmussen and Pedersen-Bjergaard in 1999, recorded a distinct LPME named hollow fiber based liquid-phase microextraction, HF-LPME [107]. In the method, the target substances in the aqueous solution are extracted into the acceptor phase in the fiber with the help of organic solvent impregnated on the walls of the porous polypropylene hollow fiber. In the experimental setup, hollow fiber is used either in the form of a rod with one end closed and the other end attached to the micro injector or prepared in a "U" shape with both ends connected to the micro injector. First, the pores of the fiber are immersed in a low polarity organic solvent (toluene, octanol, dihexylether, etc.) for a few seconds and filled with capillary action. The organic solvent in the pores forms a thin film on the fiber walls, preventing the acceptor phase in the fiber from mixing with the donor phase. The fiber is attached to the end of the acceptor phase drawn by micro-injector. The acceptor phase is then filled into the fiber from the

micro-injector and dipped in the sample solution for analysis of analytes. The substances are extracted into the acceptor phase trapped inside the fiber Figure 2.12. Then, the substances in the acceptor phase are directly determined by analytical devices such as HPLC, GC. HF–LPME is a simple, fast, inexpensive and highly selective method with a high enrichment factor. The hollow fiber microextraction method can be used in environmental, biological, food samples, acidic and basic drug analysis. Since the fiber cuts off the direct contact of the acceptor phase with the sample solution, it minimizes the loss of extraction solvent at high mixing speeds. Polypropylene fiber has a very cheap cost. For this reason, it is used once in each analysis. Using the fiber once in each analysis prevents contamination from previous analyzes. Since polypropylene hollow fiber has small pores, it makes a good pre-cleaning process by preventing large molecular impurities in the matrix environment from passing to the acceptor phase [108].

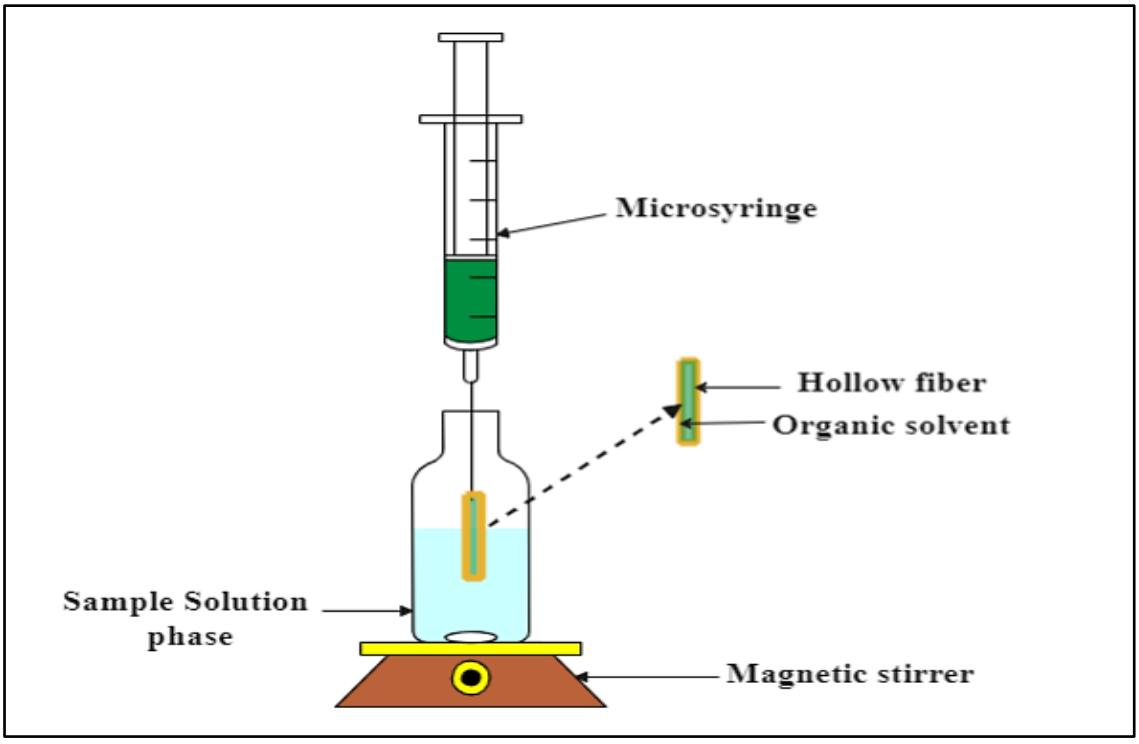

Figure 2.12. Hollow fiber-based Liquide phase microextraction (HF-LPME).

2.6.3.3. Dispersive Liquid Phase Microextraction (DLPME)

In order to get improvement in the extraction efficiency of LPME, Rezaee et al designed the DLPME method in 2006 [109]. As seen in Figure 2.13, the basic

DLPME experiment was performed by adding a few µL of organic solvent that is immiscible with to the sample solution, followed by a rapidly water-miscible solvent (dispersive solvent), thus creating a homogeneous cloudy solution with the help of mechanical or manual agitation. The dispersion resulted in a considerably increased contact surface between the sample and the extraction solvent, which markedly increased the extraction efficiency [110]. After extraction, the aqueous and extraction phase were separated by centrifugation and then the extracted solvent was separated with a microsyringe and injected into the analysis instrument [111].

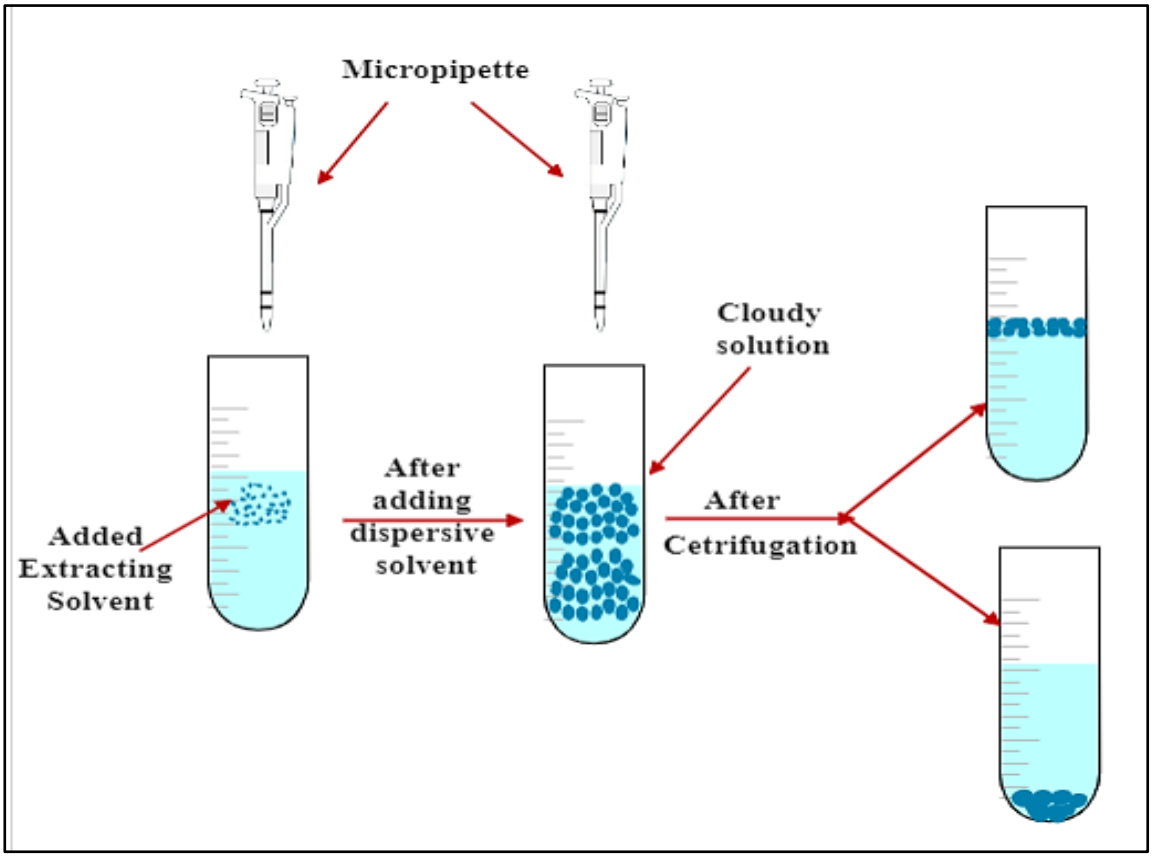

Figure 2.13. Dispersive liquid phase microextraction (DLPME).

DLLME method has attracted the attention of analytical chemists because of its advantages such as simplicity, cheapness, high enrichment efficiency, and short implementation time. Although the first applications of the method were for organic compound extraction from water samples, it was later applied to very different samples: food [112], soil [113], cosmetic products [114] and biological samples

[115]. Moreover, DLLME has been satisfactorily utilized not only for the detection of organic species but also for the determination of inorganic analytes [116].

In order to get highly effective extraction, many requirements should be present in extraction solvent used in DLPME [117]:

- miscibility with dispersive solvent
- having low solubility
- having different density than water hence could be phase-separated.
- having the ability for extracting analytes of interest.

The other most important parameter is the selection of dispersive solvents. An appropriate dispersive solvent should have miscibility properties with both aqueous and extraction phases for generating cloudy solution which higher the interaction between two phases. These interactions increase the extraction efficiency. Acetone, methanol ethanol, acetonitrile, and tetrahydrofuran are usually utilized as dispersing solvents.

In the DLLME technique, solvents such as CCl_4, $CHCl_3$ and CS_2, which have a higher density than water, can be used as extraction solvents. However, due to the fact that most of these solvents are chlorinated toxic organic compounds and their number is limited, a search for a new generation solvent has been started [118, 119].

2.7. New Generation Solvents Used in Microextraction

Recently, solvents that can be applied in a short time have been used in liquid phase microextraction studies, which minimize waste generation, are non-toxic, and provide quantitative results due to their use in µL volumes. These solvents are solvents that are used instead of conventional solvents that are harmful to the environment and that are harmful minimum levels according to the principles of green chemistry [120]. The main ones can be listed as ionic liquids (IL) [121], supramolecular solvents (SUPRAs) [122], switchable solvents (Ss) [123] and deep eutectic solvents (DESs) [124].

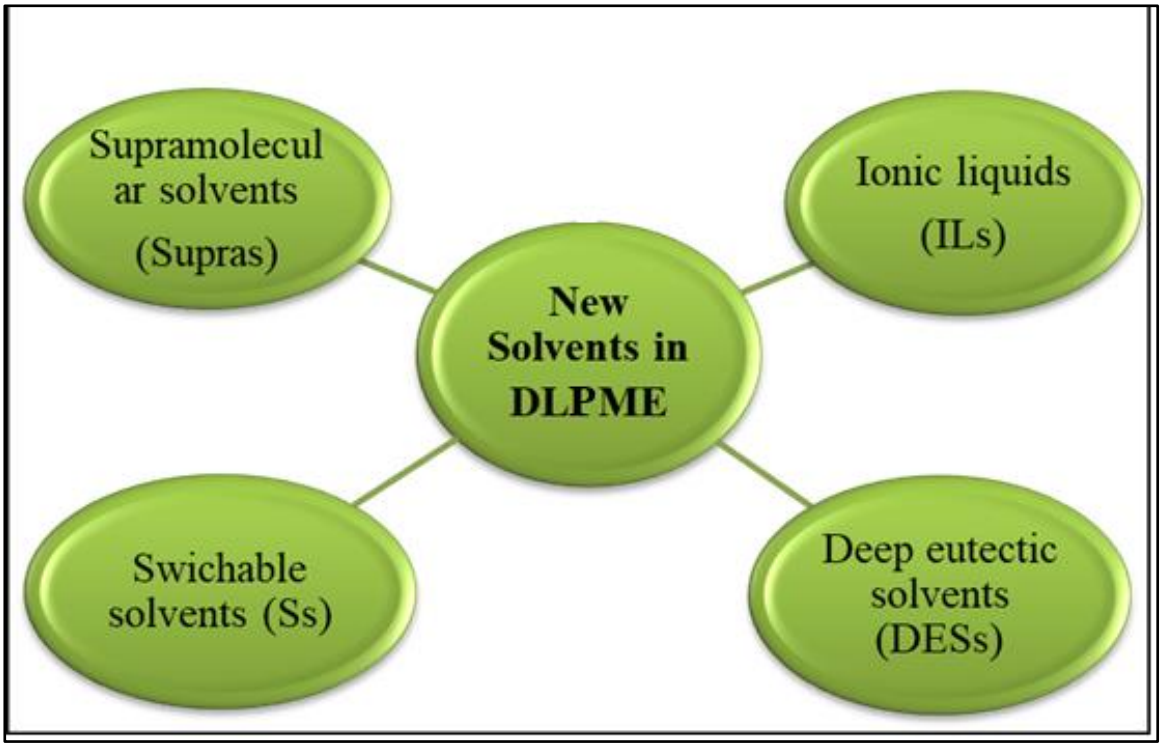

Figure 2.14. Classification of DLPME according to the modifications in the extraction solvent.

2.7.1. Ionic Liquid Solvent (ILs)

Ionic liquids are ionic organic salts that can exist in liquid form at room temperature. The most important feature of these salty organic compounds is liquid at room temperature or below, due to their very low melting points. The crucial property of ionic liquids is that they have negligible vapor pressure. In this way, ionic liquids enter the atmosphere in very small quantities. Because of all these properties, they are defined as green solvents [125]. They can be used safely at high temperatures because of having high chemical and thermal stability and high polarity. Also, compared to normal solvents, ionic liquids have higher thermal and electrical conductivity. This feature increases the use of ionic liquids in extraction studies [126].

2.7.2. Supramolecular Solvent (SUPRA)

SUPRAs based DLPME was developed in 2009 like a simple, rapid, efficient sample treatment process [127]. SUPRASs were water-immiscible solvents formed by

supramolecular gathering and dispersed then, which was nanostructured solvents and occurring into two different scales: (nano and molecular) [128]. The influences like temperature, electrolyte concentration and pH of sample as well as amount and type of solvent were important the global self-assembly process. These techniques, coacervates (a colloid-rich viscous liquid phase) compromised of the reverse micelles sized of long-chain carboxylated acids or alcohols dispersed in tetrahydrofuran that injected to the aqueous sample solution. Finally, hydrophobic phase is discrete from sample using centrifugation. Supramolecular solvents have various interactions (hydrophobic and hydrogen bonding) with the aqueous sample phase to gain efficiency of extraction [129].

2.7.3. Switchable Solvent (SS)

A new homogeneous liquid-liquid microextraction depend on using switchable hydrophilicity like polycyclic aromatic solvents had been introduced for the first time in 2015 [130]. A switchable polarity solvent provides water-miscible hydrophilic form in the presence of CO_2 and at 1 bar pressure, however separated from water and generates hydrophobic form by elimination of CO_2 with a phase transition starter like argon, nitrogen, bubbling air or inert gas under heating as well as adding acids and bases [131].

In a microextraction system using SS, a hydrophilic SS mode as an extractant is totally dissolved in aqueous sample. A phase transition is induced to generate hydrophobic SS mode. Then fine SS micro-droplets is generated and analyte is extracted to hydrophobic SS then analyzed [130].

2.7.4. Deep Eutectic Solvent (DES)

2.7.4.1. General Information and Definition of DESs

Deep eutectic solvents (DESs) are an environmentally or semi-environmentally friendly, low-cost alternative that can be used to replace conventional solvents in recent years [132]. In 2001, Abbot and colleagues obtained a liquid called DES by mixing a mixture of a metal salt (zinc chloride) and choline chloride at temperatures below 100ºC [133, 134]. DES's have emerged as the next generation solvents in various applications since 2004. In the same year, different DESs were developed

by mixing ChCl with various carboxylic acids such as succinic acid, malonic acid, or oxalic acid [135]. In 2015, a DLLME method was studied by using DES prepared from 4-chlorophenol and choline chloride as an extractant and acetonitrile as a disperser was used for the extraction of pesticides from food samples prior to GC determination [136].

Deep eutectic solvents can be defined simply as solvents consisting of two or more components, all solid or sometimes solid and liquid, and having a lower melting point than their constituent components. The reaction of urea and choline chloride to form DES at room temperature is shown in Figure 2.15 [137].

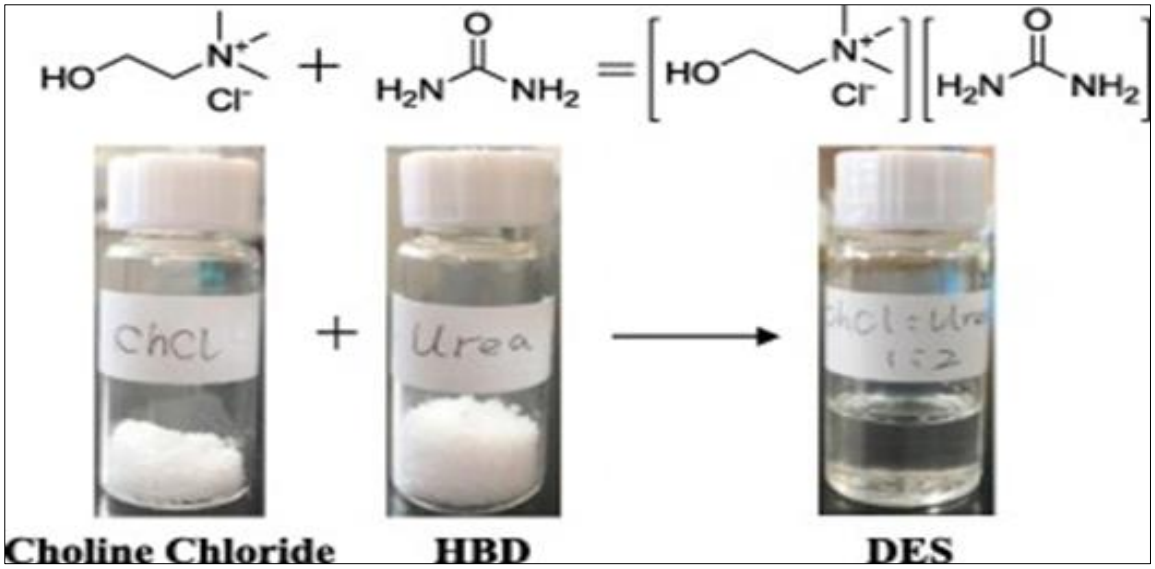

Figure 2.15. Formation of DES from Choline Chloride and urea.

Generally, the formation of these liquid compounds at room temperature because of the formation of hydrogen bonds between a hydrogen bond donor (HBD), like alcohol or carboxylic acid, and a hydrogen bond acceptor (HBA), which often the anion in an ammonium salt like choline chloride for three types of DESs and the fourth type is with metal salt instead of ammonium salt. In 2019, a new type of DESs has been recognized as non-ionic DES for the type V [138]. Five types of DESs are categorized in Table 2.1 [139]:

Table 2.1. Types of DES.

Type I (quaternary salt and metal halide)	Y = MClx, (M = Zn, Sn, Fe, Al, Ga)
Type II (quaternary salt and hydrated metal halide)	Y = MClx. yH_2O, (M = Cr, Co, Ni)
Type III (quaternary salt and hydrogen bond donor)	Y = R_5Z, (Z = $CONH_2$, COOH, OH)
Type IV (metal halides and hydrogen bond donor)	e.g., $ZnCl_2$ + urea, ethylene glycol or acetamide
Type V	(Non-ionic species DES)

Unlike ILs, DESs can be prepared more cheaply with readily available reagents and efficiently by simply complexing the salt with the hydrogen bond donor. In addition, having unique characteristics such as environmental friendliness and high purity. Among DESs, ChCl is commonly used component similar to B vitamins [140]. DESs that prepared from ChCl has many advantages such as non-toxicity, easy preparation by simply mixing components, no need to additional purification, biocompatibilty and low cost DESs prepared from ChCl have several advantages: they are inexpensive, easily prepared by simply mixing the ingredients, are non-toxic, do not require extra purification steps, and are biocompatible. [141, 142]. In order to obtain eutectic, HBD molecules are needed to complex each chloride ion [143]. The choosing of DESs as a suitable extraction solvent is depend on the hydrophobic, electrostatic, and π–π interactions with target analytes [144].

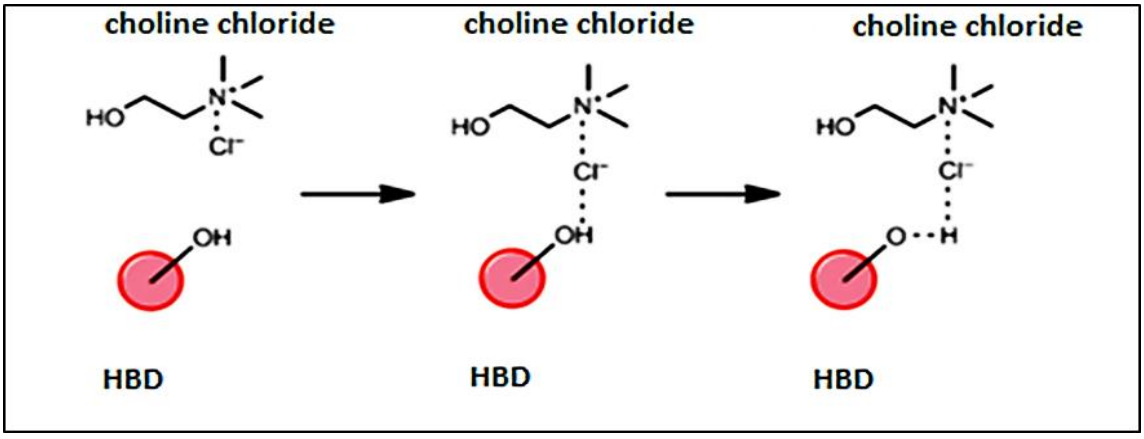

Figure 2.16. Structure composition of DES from choline chloride as ammonium salt with hydrogen bond donor.

2.7.4.2. The Advantages of DESs

There are many advantages to prefer using a deep eutectic solvent in microextraction instead of other solvents [145]:

- Simple preparation procedures
- Environmentally friendly
- Good biocompatibility
- Nonreactive with water
- Non-toxic
- Biodegradable
- Low production cost
- Relatively high polarity
- Can produce several combinations

2.7.4.3. The Disadvantages of DESs

- The attendant high viscosity, that led in a slow mass transfer and inhibits the efficiency; but it can be taken it over by using reagent and/or mechanical tool for dispersion.
- Having poor conductivity because of high viscosity [146].

2.7.4.4. Some Physicochemical Properties of DESs

1. Description of the freezing point (Tf) depression

As we know, DESs are formed by mixing two solids that can form a new liquid phase through self-assembly through hydrogen bonds. The most important feature of this newly formed liquid is that it has a lower freezing point than the two solids that form it. For example, DES obtained by mixing choline chloride and phenol used in this thesis in a 1:3 mole ratio has a freezing point of -110 ºC and this value is well below the freezing point of choline chloride (302 ºC) and phenol (41 ºC) [143]. Generally, DESs with a freezing point below 50 ºC are used in many areas because of their cheapness and safety. The lattice energies of the hydrogen bond acceptor and the hydrogen bond donor and the entropy change in the liquid formation are the factors

affecting the freezing point [147]. As the size of the ion increases and the charge of the ion decreases, the energy required to break the bonds decreases and thus the freezing point decreases [148]. Such DESs can exist as liquids at room temperature due to their low lattice energy [143]. In summary, the freezing point of DESs depends on (1) the lattice energies of HBD and HBA (2) the interaction between HBD and HBA (3) the entropy difference in the formation of the liquid phase [149].

2. Density

Density is among the basic physical characteristics of a solvent. Commonly, densities of DESs can be detected through a specific gravity meter. Most DESs reveal higher densities than water. As in the seen Table 2.2 below [143]:

DES 1 ChCl: phenol (1:2)
DES 2 ChCl: phenol (1:3)
DES 3 ChCl: phenol (1:4)
DES 4 ChCl: phenol (1:5)
DES 5 ChCl: phenol (1:6)

Table 2.2. Densities (ρ) of the DES at different phenol to ChCl mole ratios at pressure p = 0.101 mPa and temperature T = (293.2 to 318.2) K.

	ρ/g·cm^{-3}				
T/K	DES1	DES2	DES3	DES4	DES5
293.2	1.0995	1.0948	1.0918	1.0898	1.0885
298.2	1.0967	1.0921	1.0893	1.0870	1.0852
303.2	1.0930	1.0890	1.0860	1.0838	1.0818
308.2	1.0901	1.0858	1.0819	1.0803	1.0782
313.2	1.0873	1.0829	1.0795	1.0761	1.0745
318.2	1.0843	1.0795	1.0763	1.0736	1.0717

It is expected that the density of DES prepared with a constant molar ratio of Ph and ChCl will decrease as the temperature increases. At a constant temperature, increasing the Ph component in the DES mixture at a constant temperature reduces

the density of DES. This is because the increasing amount of phenol bonded to the anion of ChCl to form more DES and the DES density decreases [150].

3. Viscosity

Phenol-based DES's viscosity decreases with increasing temperature. As the phenol content in DES decreases, the viscosity is more affected by temperature.

Table 2.3. Viscosities (η) of the DES at Different Phenol to ChCl Mole Ratios (as in density table mole ratio) at Pressure p = 0.101 MPa and Temperature T = (293.2 to 318.2) K.

	η/mPa·s				
T/K	DES1	DES2	DES3	DES4	DES5
293.2	120.77	57.84	40.23	31.96	27.03
298.2	90.33	44.64	31.55	25.25	21.43
303.2	68.41	35.17	25.20	19.75	16.82
308.2	53.43	28.22	20.25	16.16	13.76
313.2	42.42	23.08	16.71	13.44	11.45
318.2	34.34	19.14	14.00	11.26	9.46

Table 2.3 indicates that the viscosity of DES decreases with increasing Ph content at a constant temperature. Ph added to ChCl behaves as a HBD to form DES and to reduce the viscosity of DES. Viscosity is an indicator of intermolecular attraction in solution, and Ph added to DES reduces intermolecular attraction in DES [143, 146].

4. Ionic Conductivity

At room temperature the most DESs have ionic conductivity as low as 2 mS cm^{-1} because of their relatively high viscosity. As the viscosity of DES decreases with temperature, increasing temperature markedly increases the conductivity of DES [146].

5. Polarity

Polarity Extensive hydrogen bonding causes DES to be polar. Polar and protic DESs are either acidic or basic. The acidic or basic character of DESs depends on the HBD that creates DES [151].

In summary, a DES

- has a lower freezing point than its constituent components,
- is mostly denser than water and has a high viscosity at room temperature,
- generally has low ionic conductivity and the strength of the hydrogen bond determines its polarity [151].

2.8. Assisting of Extraction: Ultrasonic Energy

Prior to the analysis of trace species, ultrasonic energy is used as an assisting tool for extraction during sample preparation. Ultrasound energy can be used to accelerate the extraction of the analyte without chemically altering the analyte or matrix [152].

The effect of ultrasound energy in the experimental environment is mainly based on emulsification. Emulsification means that two immiscible liquids are divided into micro-sized droplets by the effect of applied ultrasonic energy, and that maximum surface area is provided for extraction by dispersing them homogeneously within each other [153]. During sonication, an acoustic pressure is periodically created in the solution, which triggers mechanisms that accelerate the extraction process [154]. The most important of these mechanisms are cavitation (formation and bursting of air bubbles) and friction at the interface. In addition to these mechanisms, the resulting heat also contributes positively to the extraction process [155]. After the ultrasound application, centrifugation is required in order to separate the two phases [156]. Recently, ultrasonic energy has attracted attention due to some superior features: being clean energy, easy to use, providing high extraction

efficiency and shortening the extraction time. Due to all these features, ultrasound has started to be preferred as an auxiliary tool in liquid phase extraction [157].

There are two types of systems for the application of ultrasonic energy, ultrasonic water bath and ultrasonic probe. The most widely used of these are ultrasonic baths. Although they are widely used, there are two disadvantages that reduce repeatability for ultrasonic baths. The first of these is that the energy applied in the bath does not distribute homogeneously to all the liquid in the bath. The second disadvantage is that the power of the device decreases with the increase of the usage time [158]. However, these disadvantages can be ignored since ultrasonic energy greatly reduces the extraction time.

In the DES based microextraction technique, the high viscosity and low conductivity of DESs compared to the aqueous solution cause a decrease in their ionic mobility and extraction efficiency. Ultrasound energy can be used to overcome this problem. In this way, DES is dispersed homogeneously in the aqueous solution in the form of fine particles in a short time and the extraction efficiency is increased [159].

The first use of ultrasound assistant in LPE was performed in 2007, some compounds having different polarity were extracted from plant samples [160]. In 2008, ultrasound support was used in the miniaturized liquid phase extraction method and the USAE-LLME method was reported for pesticide determination in environmental water samples [161].

2.9. Flame Atomic Absorption Spectroscopy

The FAAS is a quantitative analytical instrument used to find the concentrations of atomic species based on their absorption amounts of radiation selected from the UV-visible region. In atomic absorption spectroscopy, the radiation absorbed by atoms in the gaseous state and in the ground energy level is measured as they rise to the excited energy level. The concentration can be calculated using the linear relationship between absorption and concentration. The most important step of the analysis is the atomization step, and two types of atomizers are used: flame atomization and electrothermal atomization. Atomic absorption spectrometers are

classified according to the type of atomizer they have: flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS). FAAS is widely used due to some features: easy to use, low cost, reproducible results.

2.9.1. Uses of AAS

AAS has wide-uses in wide areas of scientific researches [162]:

1. Environmental analysis. surveillance our environment -detect levels of different elements in air, rivers, water, seawater, petrol and drinks like beer, fruit drinks and wine.
2. Clinical analysis. Analysis of metals in biological fluids like urine and blood.
3. Pharmaceuticals. For many pharmaceutical manufacturing procedure, tiny quantities of a catalyst utilized in process are often existing in final product. These amounts of catalyst exist can be detected by using AAS.
4. Industry. Raw materials are checked for their purity and toxic element content.
5. Mining metals as gold in rocks that detected to check if it is valuable mining the rocks to extract the gold.
6. Cosmetics and Skin Care Products. Different metals are determined in a variety of cosmetics and body care product.

2.9.2. The Constituent of Atomic Absorption Spectrometer

AAS devices consist of 5 basic components. These are

1. Radiation source
2. Wavelength selector
3. Atomizer, also called sample cell
4. Detector
5. Reatout unit

The constituent of atomic absorption spectrometer is given in Figure 2.17 and Figure 2.18.

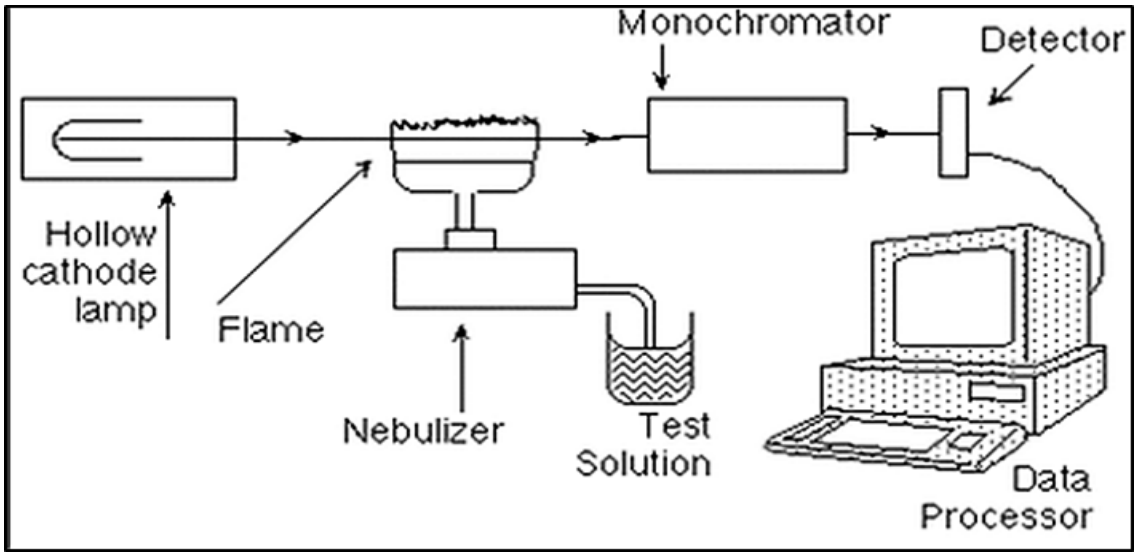

Figure 2.17. The constituent of atomic absorption spectrometer.

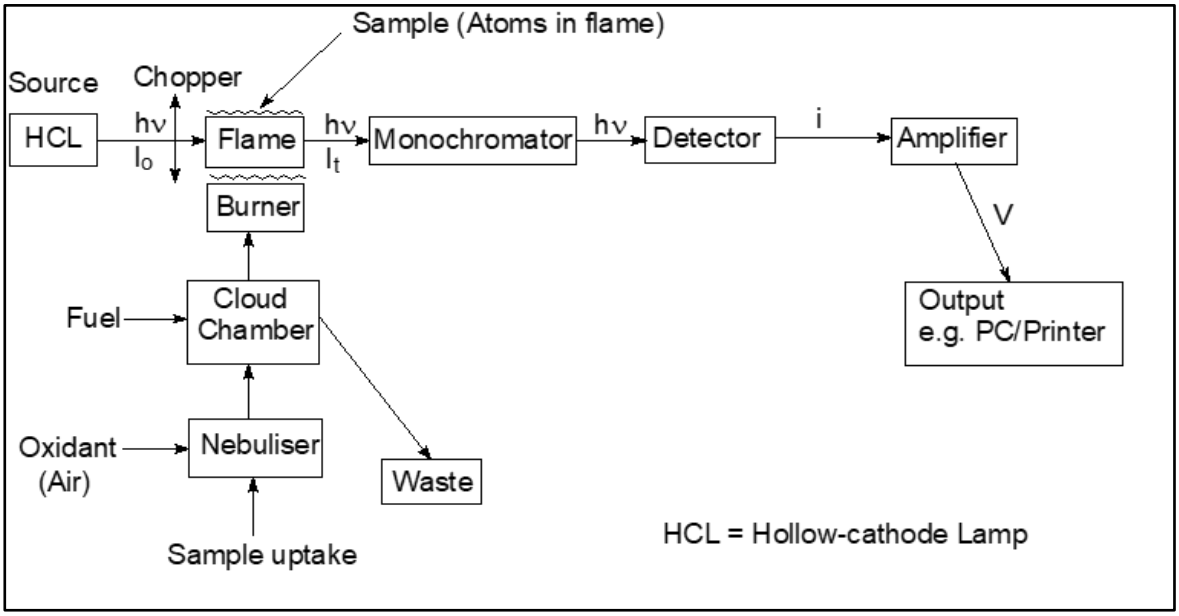

Figure 2.18. Description of the atomic absorption spectrophotometric devices work mechanism. (Absorbance = -log (I_t/I_0), I_0 = incident radiation on sample, I_t= transmitted radiation).

2.9.3. Radiation Source

Since atoms only absorb radiation of specific wavelengths, the radiation source used must be specific to the element to be analyzed. For this reason, light sources

with narrow line spectrum are used. There are two types of light sources used for this purpose. These are the hollow cathode lamp (HCL) and the electrodeless discharge lamp (EDL).

2.9.3.1. The Hollow Cathode Lamp (HCL)

The hollow cathode lamp is a line source that emits stable and bright radiation for more than 60 elements. The cathode inside the lamp is made of a cylindrical metal specific to the element to be analyzed. The cathode is placed in a cylindrical tube containing noble gas together with the anode. When an electrical voltage is applied between the cathode and the anode in the lamp, the noble gas atoms in the lamp are ionized. These ions collide with the cathode, causing it to atomize and then get excited. Excited cathode atoms emit a distinctive radiation as they return to the ground state. The schematic representation of HCL is given in Figure 2.19.

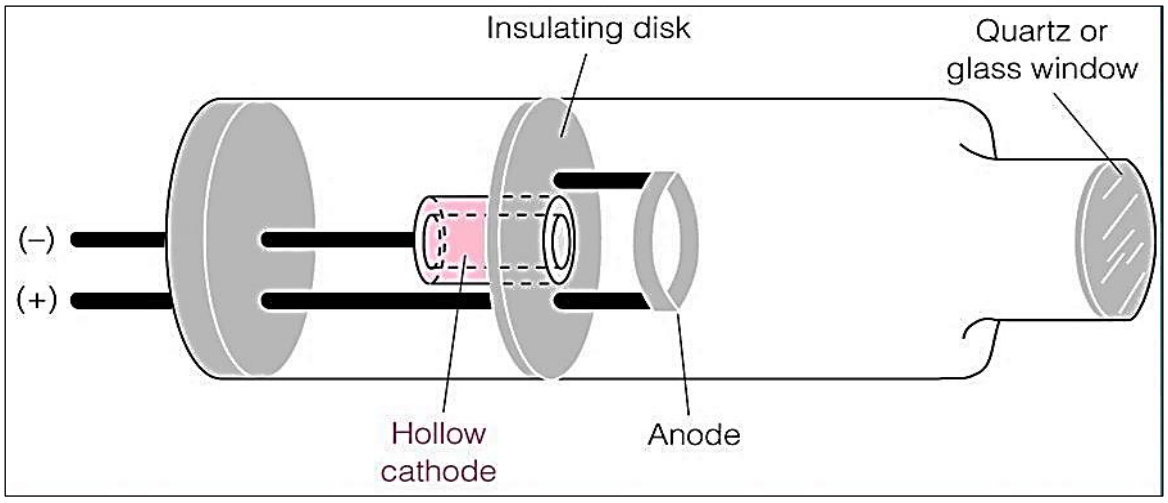

Figure 2.19. The hollow cathode lamps.

2.9.3.2. Electrodeless Discharge Lamp (EDL)

Electrodeless discharge lamps: They are developed for volatile elements such as As, Se, Sb, which can absorb and emit at low wavelengths. In these sources, 1-2 mg of the analysis element was put into a quartz tube with a length of 1-2 cm and a diameter of 5-10 mm with argon gas at low pressure. Excitation is achieved by applying a power of 200 watts between the electrodes in contact with the outer walls of the quartz tube. EDLs provide a higher sensitivity and more intense radiation than

HCL for some elements. The schematic representation of the EDL is given in Figure 2.20. [13, 163].

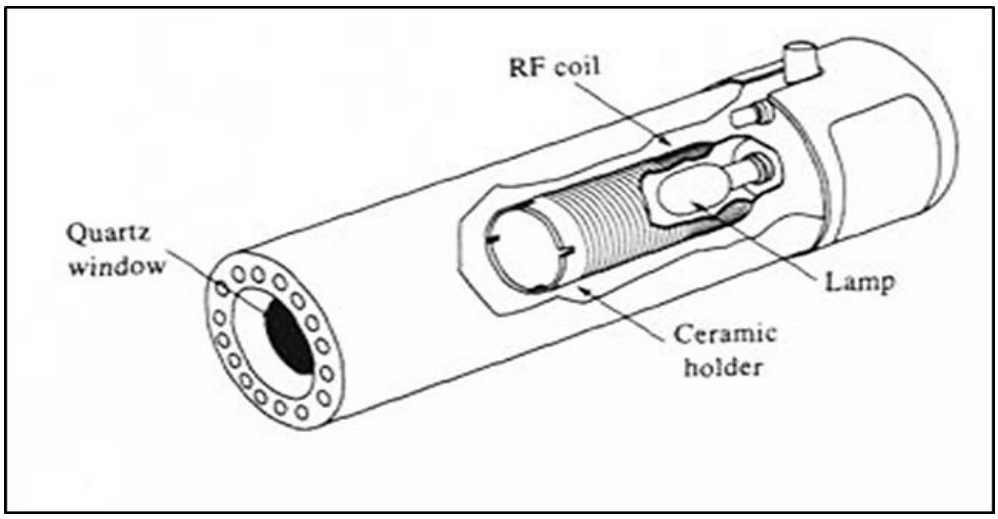

Figure 2.20. Electrodeless discharge lamp.

2.9.4. Atomization System

In atomic absorption spectrophotometers, the atomizer is the part where the ground level atomic vapor of the element to be analyzed is formed from the ions and molecules in the sample. There are two types of atomizers: flame atomizers and electrothermal atomizers.

2.9.4.1. Flame Atomizer

The sample solution is sprayed onto the flame with the help of an air nebulizer. The burners used to create the flame are of two types, non-pre-mixed and pre-mixed. A flexible capillary tube transfers solution to nebulizer. In capillary tip, solution is nebulized, separates into tiny drops. Smaller drops vaporize in the flame, while the ones with larger sizes send out and drain off. A Typical burner scheme of the flame atomizer is given in Figure 2.21.

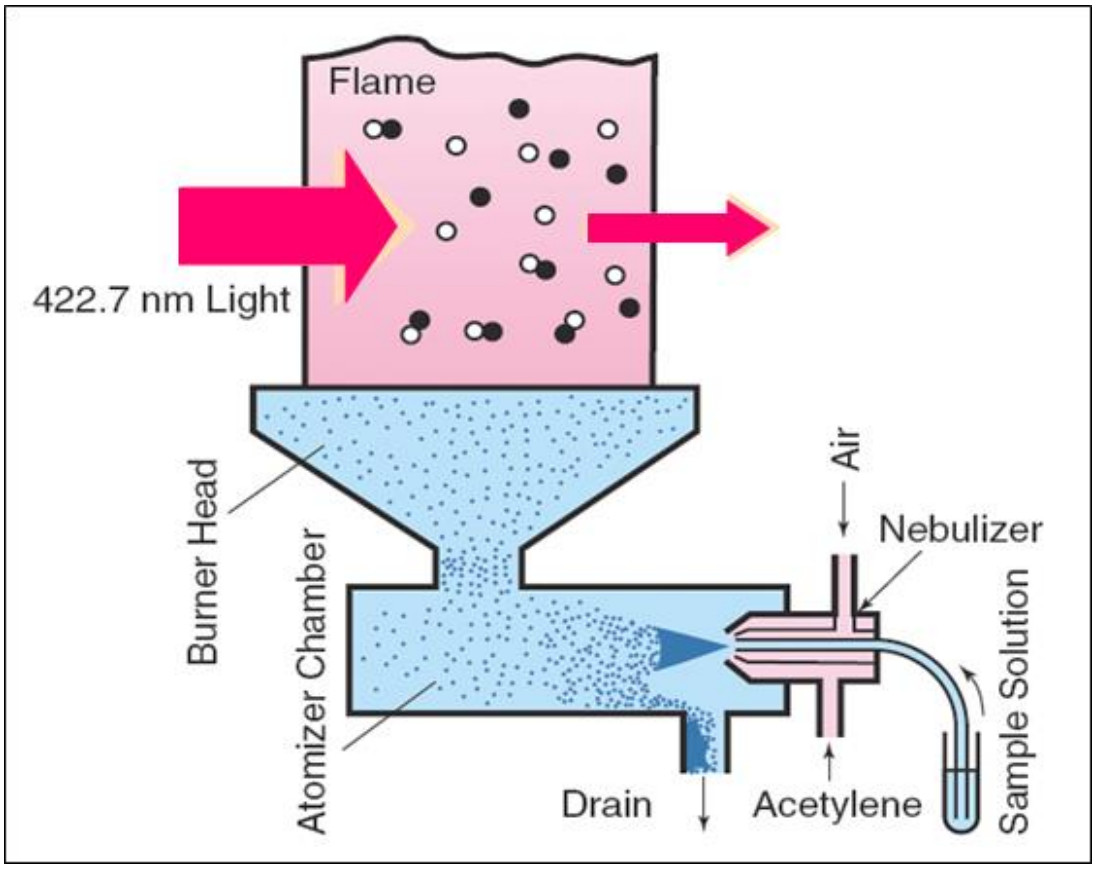

Figure 2.21. Typical burner scheme of the flame atomizer.

2.9.4.2. Electrothermal Atomization

This atomization technique requires a separate power supply to be heated and are more expensive systems. In these, very small sample volumes (5-50 µL) are sufficient and the sensitivity is much higher than flame.

2.9.5. Monochromator

The main task of the monochromator is to separate the resonance line of the target element from the other lines emitted by the light source. For most elements, the use of a 0.2 nm bandwidth monochromator is sufficient. Wavelength ranges must be taken into account when choosing the materials used in the construction of monochromator components (slits, lenses, windows, optical mesh or prisms). The materials used in the construction of the components that make up the

monochromator should be chosen considering the wavelength ranges (Figure 2.22.).

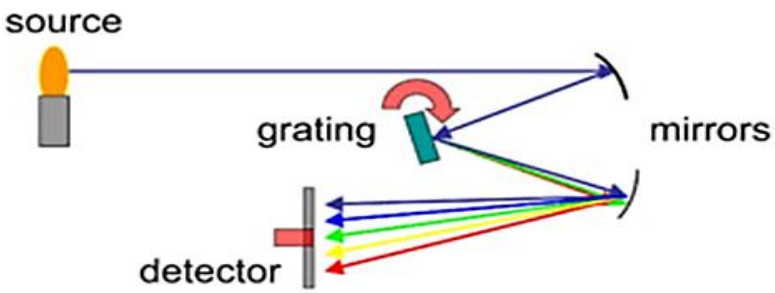

Figure 2.22. Description of the monochromator.

2.9.6. The Detector and Data Processor

The detector is a photomultiplier that converts the beam emanating from the monochromator into an electrical signal. Photomultiplier (PMT; photomultiplier tube) is a vacuum photocell consisting of a photosensitive cathode, a series of dynodes showing a successively more positive potential, and an anode in between. PMTs consist of 9 diodes, each more positively charged than the previous one. With the increasing positive charge, the direction of the electrons to the diodes accelerates, and thus a current is formed to be converted into a signal on the computer screen. It is quite difficult to find a photomultiplier with sufficient sensitivity over the entire spectral range.

2.9.7. Types of Atomic Absorption Instruments

Two basic kinds of atomic absorption instruments are present:

2.9.7.1. Single-Beam Spectrometer

Light source, either electrodeless discharge lamp or hollow cathode lamp emits a specific radiation to element that has made, and focuses via sample cell to monochromator. Light source should mechanically chop or electronically modified in distinguish emission from the sample cell and light from source. The monochromator scatters light, and specific wavelength of radiation isolated passes through detector. An electrical current is generated according to light intensity and processed using electronics of spectrometer. Electronics of instrument will measure amount of light absorbed in sample cell and change these readings to determine the accurate sample concentration. In the version of single-beam systems, a short period of warmup duration usually needed thus allow the source lamp to stabilize a certain light intensity value.

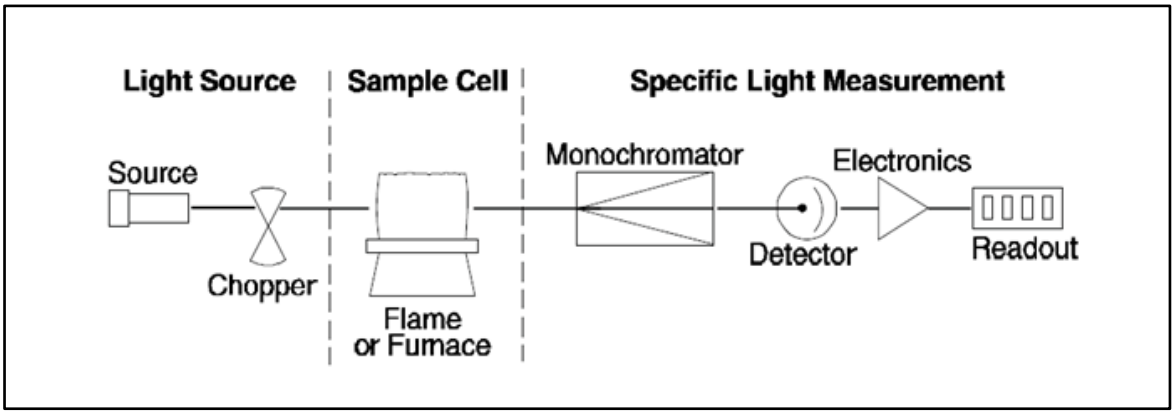

Figure 2.23. Constructs of Single-Beam type of atomic absorption instrument.

2.9.7.2. Double-Beam Spectrometer

The light in source lamp is splited to a reference beam, directed around sample cell and a sample beam, focused via sample cell. With a double-beam system, readout system compares the data obtained from sample and reference beams. So, fluctuations in source intensity had not become fluctuations in instrument readout,

thus constancy can be improved. Commonly, analyses completed directly without lamp warm-up needed.

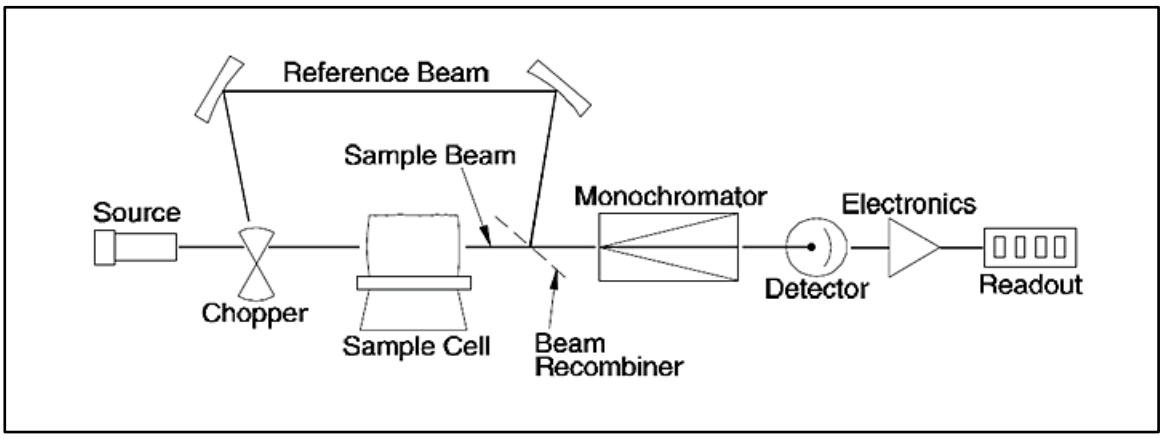

Figure 2.24. Constructs of double -Beam type of atomic absorption instrument.

3. EXPERIMENTAL

3.1. Reagents and Chemical Material

All the reagents utilized had been of attested evaluation and required no more purification. 1000 mg L^{-1} standard solution of Pb(II) was prepared from $Pb(NO_3)_2.3H_2O$, Cd(II) was prepared from $CdCl_2.2H_2O$, Cu(II) was prepared from $Cu(NO_3)_2.3H_2O$, and Ni(II) was prepared from $Ni(NO_3)_2.6H_2O$ all the reagents were purchased from (Merck, Germany). $1x10^{-3}$ M Dithizone ($C_{13}H_{12}N_4S$) utilized as a chelating agent was obtained from Riedel-de Haen, Germany, and dissolved in an aprotic solvent tetrahydrofuran (THF) that was purchased from (Merk, Germany).

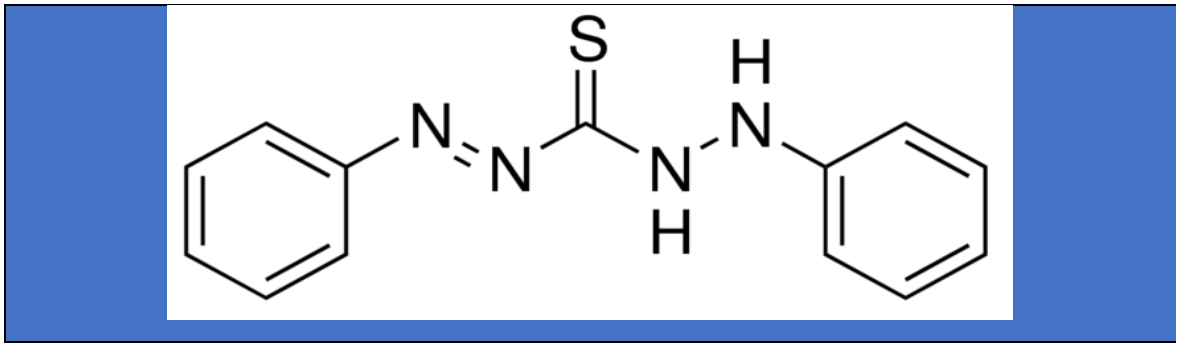

Figure 3.1. Chemical structure of Dithizone ($C_{13}H_{12}N_4S$).

Buffer solutions of CH_3COOH/CH_3COONa, $KH_2PO_4/NaOH$, Na_2HPO_4/HCl, and $Na_2CO_3/NaHCO_3$ were used for adjusting the pH of the analyte solutions (all obtained from Sigma Aldrich, USA). In the preparation of extraction solvent DES, choline chloride (ChCl) and phenol (Ph) were used as hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), respectively. In addition, urea (U) and oxalic acid dihydrate (OA) were also tested as HBD. All chemicals used as HBA and HBD were obtained from (Sigma-Aldrich, Germany). HCl (Merck, Germany) and Ethanol (Merck, Germany) were used for preparing 1% (v/v) acidic ethanol to dissolve the separated DES phase before FAAS determination.

Preparation of all solutions was performed by using deionized water. In purpose to prevent possible contamination, all glassware was submerged into the diluted hydrochloric acid for at least 24 h, and then rinsed three times with deionized water.

For the microwave digestion system, concentrated HNO_3 and 35% (v/v) H_2O_2 purchased from (Merck, Germany) were utilized for dissolving the real samples. Concentrated NH_3 purchased from (Riedel-de Haen, Germany) was used for pH adjustment of the real samples obtained after microwave digestion. For verification of the method accuracy, the NCS ZC73013 (spinach) certified reference material (CRM) was used and purchased from National Analysis Centre, China.

For application of the proposed method to the real samples, five different hair dyes and fourteen different henna samples (ten powder henna for hair dying and four paste henna for tattoo) having different shades purchased from Turkey markets from various origins (Turkey, India, Pakistan).

3.2. Apparatus

All pH measurements were performed using a pH electrode attached digital pH meter (IsoLab GmbH, Germany). An ultrasonic water bath from (Kudos, SK3310LHC, Shanghai) was used for producing micro-sized or nanosized emulsion in water phase to facilitate microextraction process. A microwave digestion system MARS 6 One-Touch model (Cem, USA) was used for dissolving the CRM and real samples [164]. The quantitative analyses of enriched metal ions were performed by the flame atomic absorption spectrophotometer with a deuterium background correction method (Perkin-Elmer A-Analyst 800 Model, USA). Air/acetylene flame having 17.0 / 2.0 L min^{-1} flow rate was used in the measurements. During the analyses, an element-specific hollow cathode lamp (HCL) has been used as the beam source. For Cu(II), the lamp current was 15 mA, the wavelength was 324.8 nm and the slit width was 0.7 nm. For Cd(II) the lamp current was 4 mA, the wavelength was 228.8 nm and the slit width was 0.7 nm. For Pb(II), the lamp current was 10 mA, the wavelength was 283.3 nm and the slit width was 0.7 nm. For Ni(II) the lamp current was 25 mA, the wavelength was 232 nm and the slit width was 0.2 nm. In order to the preparation and dilution for all solutions, ultrapure deionized water obtained by Millipore Simplicity deionized water system (Germany) was utilized. In order to facilitate the separation of organic and aqueous phase a centrifuge device (Hettich-Eba 21, Germany) was utilized.

3.3. Preparation of DES

The most important advantage of deep eutectic solvents is that they are prepared in simple laboratory conditions without the need for special equipment for preparation. DESs with 3 different compositions (ChCl:Ph, ChCl:U and ChCl:OA) and different molar ratios (1:1, 1:2, 1:3, 1:4 and 1:5), were prepared in a suitable beaker on a magnetic stirrer till clear solution is formed.

3.4. Procedure of DES-UA-LPME

DES-UA-LPME technique was applied by taking a 25 mL of analyte solution that containing one of the metal ions studied. 2 mL of acetic acid/sodium acetate as a pH 6 buffer solution, 500 µL M DTZ as a complexing agent, and 1000 µL of DES as extraction solvent was added to the tube that contains sample solution. Then the tube was placed into an ultrasonic bath 3 min. Thus, aggregated DES droplets were gradually broken into nanosized particles due to the irradiation caused transient cavitation near the interface of DES droplets. Afterwards, the mixture was centrifuged at 6000 rpm for about 4 min to accelerate the separation of biphasic system of the DES rich phase and aqueous phase. Subsequently, upper aqueous phase was ejected by a dropper. The volume of DES phase remained in the tube was completed to 0.5 mL 1% (v/v) acidic ethanol and analyzed directly by home-made micro-sampling system attached flame atomic absorption spectrometer. In order to record the absorbance value, peak height was used. The entire procedure applied to the samples was also implemented to the blank solutions. The scheme of the DES-UA-LPME method is shown in Figure 3.2.

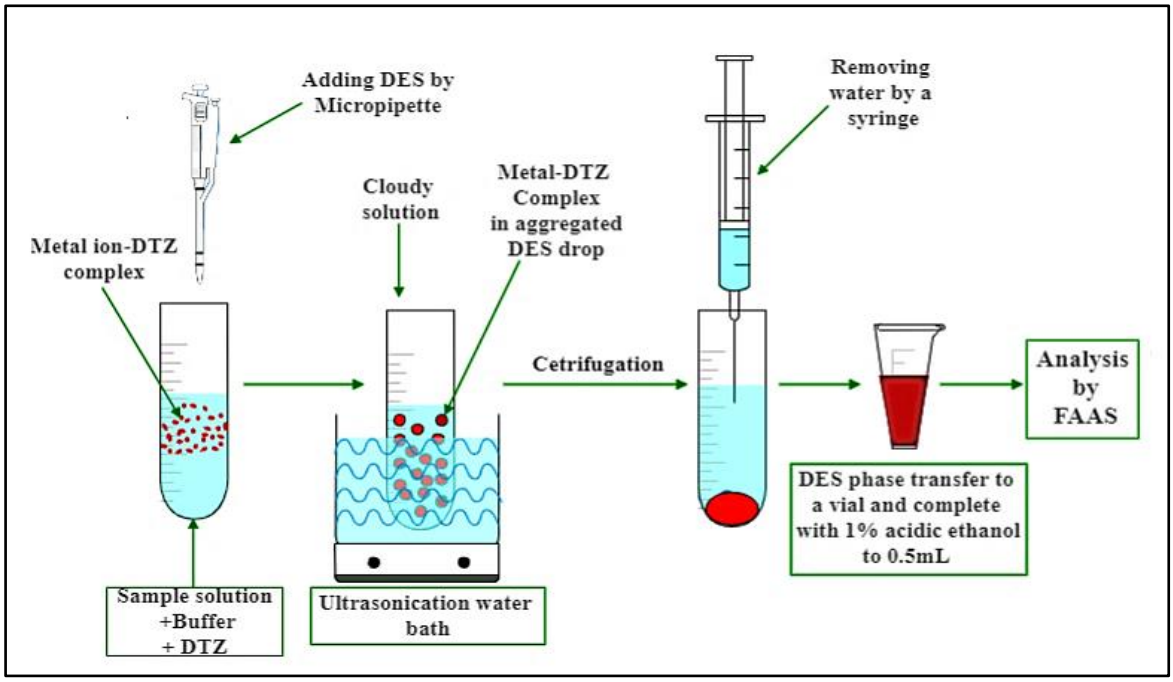

Figure 3.2. Schematic representation of the DES-UA-LPME.

3.5. Digestion and Preparation of CRM and Real Samples

For the dissolution of CRM (NCS ZC 73013, Spinach), hair dye or henna, 0.5 g of sample was digested by adding 1 mL of concentrated H_2O_2 and 7 mL of concentrated HNO_3 at the microwave-heating program that was applied as starting 0 W to 290 W with ramp for 6 min, then at 440 W for 6 min, at 550 W for 6 min, at 290 W for 6 min and at 0 W for 10 min for cooling. A blank digest was carried out in the same way. Digestion vessels were cleaned with concentrated HNO_3 (6 mL) by holding in the microwave system at 1000 W during. Afterwards holding at 0 W for 20 min to chill.

After the digestion process, the dissolved sample solution transferred to volumetric flasks with 25 mL volume and the volume was filled up to the line of flask with deionized water. Then a 2.5 mL portion was taken for each metal ion studied. The pH of the sample solutions was set to be around 6 by using NH_3 solution, and finally, the volumes were completed to 25 mL with deionized water. After that proposed method was applied to this solution.

4. RESULTS AND DISCUSSION

In this study, various parameters (such as pH, composition of DES, molar ratios of DES components, volume of extraction solvent, dithizone amount, ultrasonication time, etc.,) affecting the DES-UA-LPME method were optimized separately for all studied elements in order to obtain maximum extraction efficiency for the quantitation of Pb(II), Ni(II), Cd(II), and Cu(II). All optimization studies were carried out by changing only one of the parameters and keeping the others constant. All data presented in the study were obtained as the average of at least three repetitive analyzes.

4.1. Complexation of Heavy Metals Ions

In order to ensure the extraction of inorganic ions into an organic extraction solvent, it is necessary to convert it into a water-immiscible complex, and various complexing agents can be used for this purpose. In this thesis, dithizone (DTZ) ($C_{13}H_{12}N_4S$) was chosen as the complexing agent, it has a molecular weight of 256.33 g $mol^{-1,}$ and a melting point of 168^{o} C. DTZ does not form complex with alkaline and alkali earth metals. DTZ is more selective and sensitive to transition metals in general and can form water insoluble and stable-colored complexes with these metal ions. The interaction accrues with a cation such as Fe, Mn, Co, Cu, Ni, Zn, Pd, Cd, Ag, Sn, Au, Pt, Pb and Hg via bond donor S and N atoms on the DTZ molecule [165]. The complex consisted from 2:1 of DTZ to metal ion mole ratio as seen in Figure 4.1 where X refers to metal ion [166].

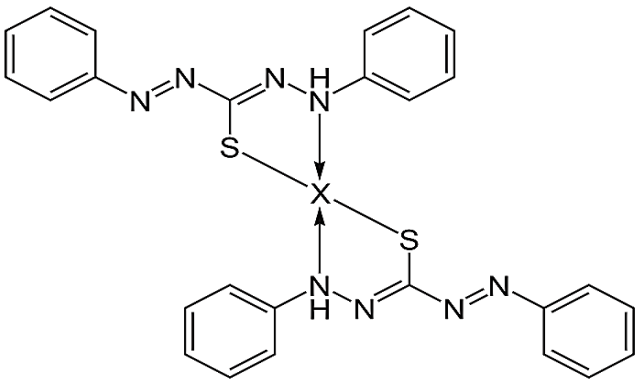

Figure 4.1. The structure of the DTZ-metal ion complex [166].

4.2. Selection of the DES Composition and Mole Ratio

As explained in the theoretical part of the thesis, DESs are formed by mixing a hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) in certain proportions, and there are many compounds that can be used as HBA and HBD. For example, ammonium salts such as ethylammonium chloride, tetraethylammonium bromide, choline chloride can be used as HBA, and phenols, carboxylic acids, amides and amines can be used as HBD. Although there are many alternatives that can be used as HBA, ChCl was chosen because of its easy accessibility, cheapness and environmental friendliness. Three different HBDs (phenol, urea and oxalic acid) were selected to form DES with ChCl. The optimum DES composition was determined by mixing the selected HBA and HBDs at different mole ratios. For this purpose, different DESs were formed by mixing [ChCl:OA] , [ChCl:U] and [ChCl:Ph] in mole ratios of 1:1, 1:2, 1:3, 1:4 and 1:5. All obtained DESs were used only in Pb(II) microextraction, representing all other ions studied. As can be seen from the graph presented in Figure 4.2, the highest extraction efficiency was obtained when using DES obtained with ChCl and Ph. It is also seen from the graph that the optimum ChCl:Ph mole ratio is 1:3. The increase in the Ph ratio in DES caused an increase in the extraction efficiency as it significantly reduced the viscosity of DES and thus facilitated the transfer of metal complexes to the DES phase. However, the increased amount of Ph led to a further increase in viscosity, thus decreasing the self-aggregation of DES and decreasing the phase separation and thus the extraction efficiency.

In addition, ChCl:Ph was easily obtained at room temperature without the need for any external energy, unlike the preparation of ChCl:OA and ChCl:U, which were prepared by mixing at high temperature for a long time. Therefore, DES obtained using ChCl:Ph was selected as the extraction solvent for subsequent studies. After that, experimental studies were continued for each metal ion to determine the optimum ChCl:Ph mole ratio. According to the results presented in Figure 4.3, the maximum yield for each metal ion was obtained when the ChCl:Ph ratio was 1:3.

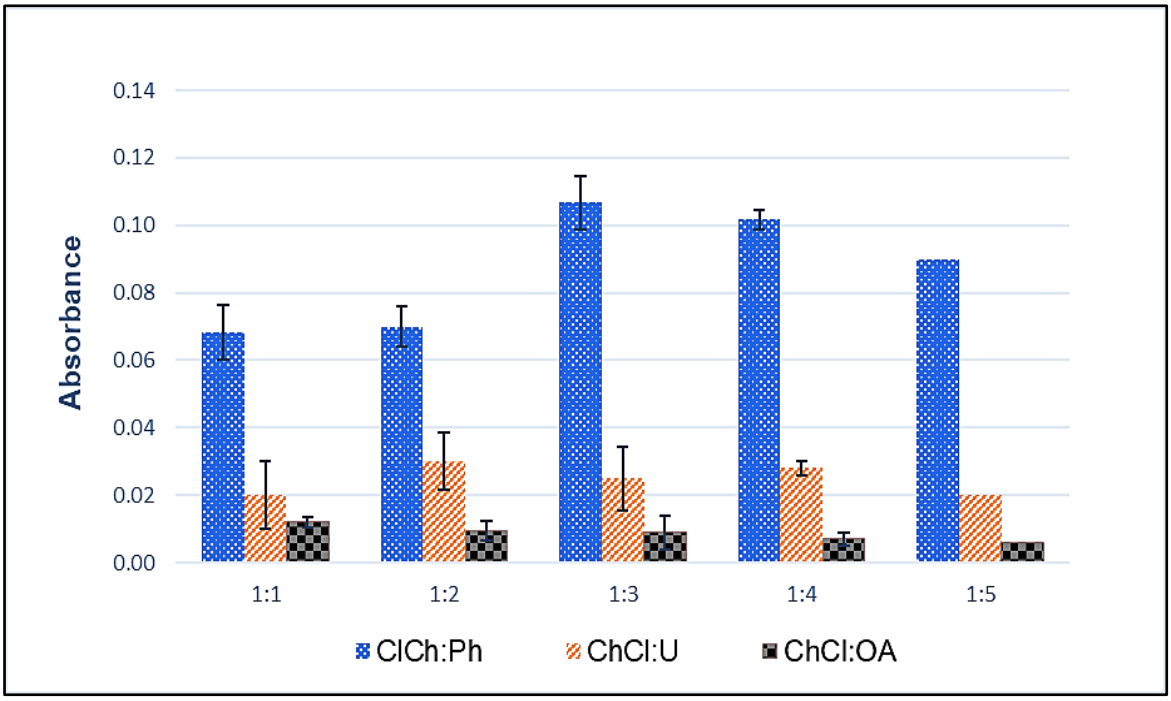

Figure 4.2. Optimization of DES composition.

Microextraction conditions: (For ChCl:Ph composition; 25 mL sample solution of Pb (II) 100 µg L^{-1}, pH: 6, DTZ 500 µL, ChCl:Ph 1000 µL from different mole ratios (1:1, 1:2, 1:3, 1:4 and 1:5), Ultrasonication time of 3 min., and centrifugation time of 4 min.;

For ChCl:U composition; 25 mL sample solution of Pb (II) 100 µg L^{-1}, pH: 6, DTZ 500 µL, ChCl:U 1000 µL from different mole ratios (1:1, 1:2, 1:3, 1:4 and 1:5), Ultrasonication time of 3 min., and centrifugation time of 4 min.;

For ChCl:OA composition; 25 mL sample solution of Pb (II) 100 µg L^{-1}, pH: 6, DTZ 500 µL, ChCl:OA 1000 µL from different mole ratios (1:1, 1:2, 1:3, 1:4 and 1:5), Ultrasonication time of 3 min., and centrifugation time of 4 min.

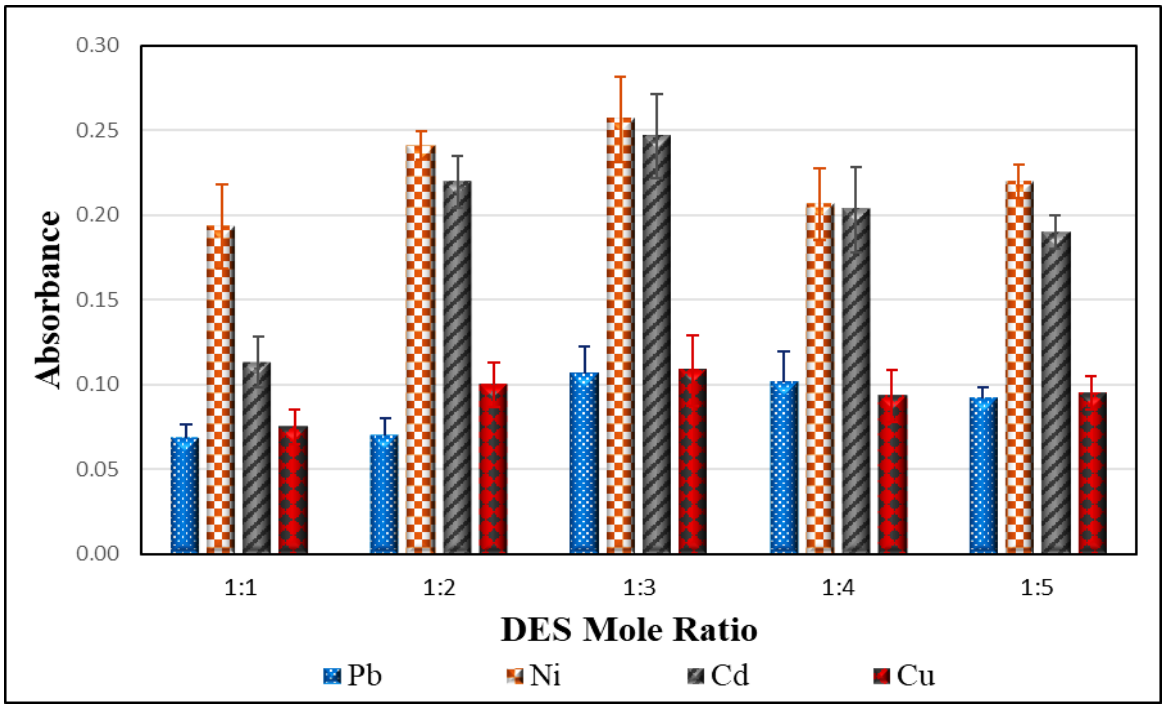

Figure 4.3. Effect of ChCl:Ph mole ratio on the microextraction efficiency of Pb(II), Ni(II), Cd(II), and Cu(II) analyte ions.

Microextraction Conditions: For Cd(II): 25 µg L^{-1} Cd(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of ChCl:Ph (1:1, 1:2, 1:3, 1:4 and 1:5) mole ratios pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Cu(II): 25 µg L^{-1} Cu(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of ChCl:Ph (1:1, 1:2, 1:3, 1:4 and 1:5) mole ratios, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Pb (II): 100 µg L^{-1} Pb(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of ChCl:Ph (1:1, 1:2, 1:3, 1:4 and 1:5) mole ratios, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Ni (II): 100 µg L^{-1} Ni(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of ChCl:Ph (1:1, 1:2, 1:3, 1:4 and 1:5) mole ratios, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.

4.3. Optimization of DES-UA-LPME Operating Conditions for Analyte Ions

After it was decided that the extraction solvent was ChCl:Ph, the effects of parameters such as pH, DES volume, DTZ volume, ultrasonication and centrifugation time on the extraction efficiency were examined for each metal ion studied and their optimum values were determined.

4.3.1. Effect of pH

During the extraction of metal ions, pH directly affects the formation of the metal-ligand complex [167]. Therefore, it is vital to optimize the pH value which affects the extraction efficiency. In order to achieve maximum recovery, model solutions of each studied metal ions were prepared and examined between pH 3 and pH 10. Desired pH values were obtained by using appropriate buffer solutions. From the absorbance values shown in Figure 4.4, it can be interpreted that at low pH, due to the competition of the hydrogen ions in the solution with the analyte ions for the active cites on the ligand, all of the metal ions cannot be complexed and transferred to the extraction solvent, thus reducing the extraction efficiency. Then, with the increase in pH value, the absorbance value for all metal ions increased and reached its maximum value in the pH range of 5-7. Finally, a decrease in the absorption and thus the extraction efficiency was observed because of the analyte's hydrolysis ions in alkaline medium. For these reasons, the optimum pH was decided as 6 for all metal ions studied.

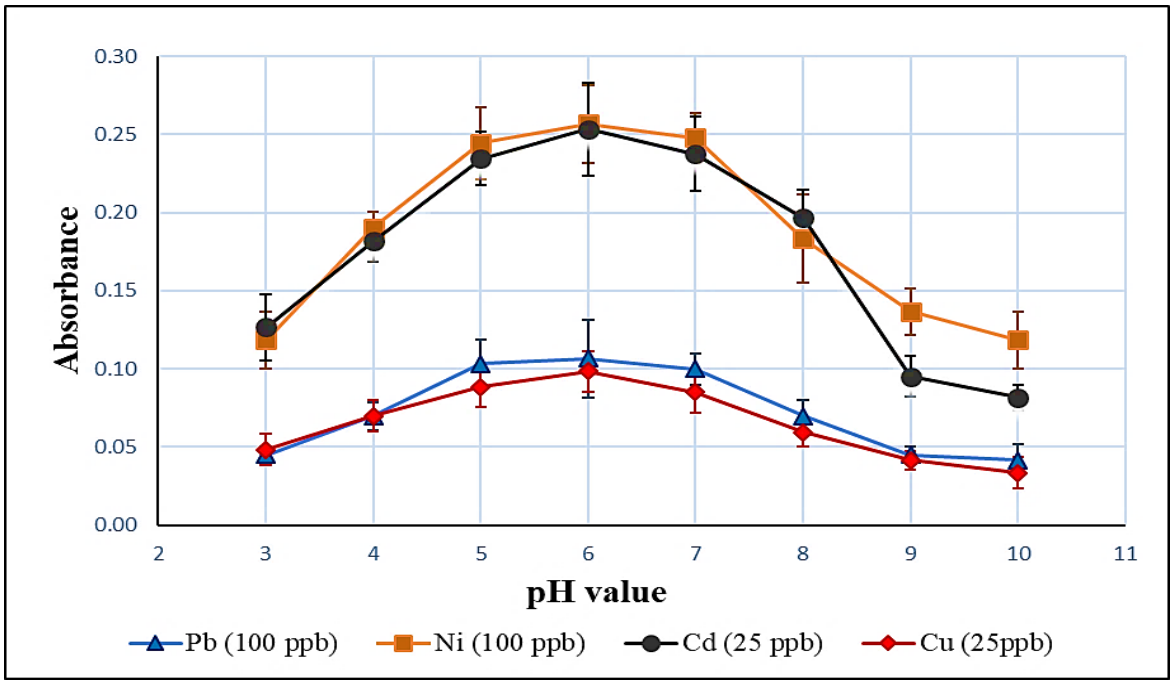

Figure 4.4. Effect of pH on microextraction efficiency.

Microextraction Conditions: For Cd(II): 25 µg L^{-1} Cd(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Cu(II): 25 µg L^{-1} Cu(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Pb(II): 100 µg L^{-1} Pb(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Ni(II): 100 µg L^{-1} Ni(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.

4.3.2. Effect of DES Volume

In order to obtain the required volume of the extraction solvent, the suggested method was implemented to analyte solutions containing DES in different volumes between 100-1500 µL, keeping other experimental conditions constant. From Figure 4.5 it can be seen that the volume of DES from 100 µL to 750 µL is insufficient to

extract all the analyte ions in the sample solution. The maximum extraction efficiency for all metal ions studied was obtained with a volume of 1000 µL of DES. When the volume of DES is greater than 1000 µL, the extraction efficiency decreased. That was attributed to increasing the acidic ethanol's volume that added for dilution the DES phase having high viscosity, and dilution resulted a drop in the extraction efficiency. For these reasons, the optimum volume of DES was chosen as 1000 µL in subsequent studies.

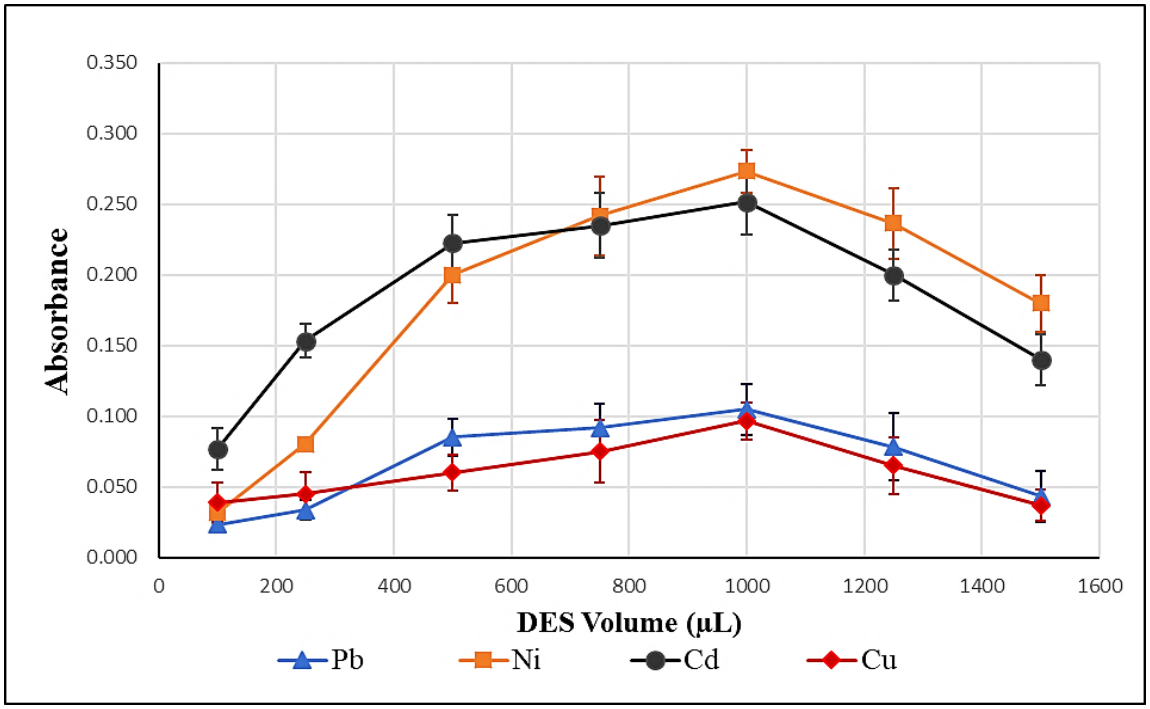

Figure 4.5. Effect of DES volume on the extraction efficiency.

Microextraction Conditions: For Cd(II): 25 µg L^{-1} Cd(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Cu (II): 25 µg L^{-1} Cu (II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Pb (II): 100 µg L^{-1} Pb (II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Ni (II): 100 µg L^{-1} Ni (II) sample solution, 500 µL of 1x10^{-3} M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.

4.3.3. Effect of the Complexing Agent Amount

In order for the polar metal ions to be extracted into the hydrophobic DES, it is necessary to form a hydrophobic complex of metal ions. Therefore, the amount of complexing agent (DTZ) is a parameter that affects the extraction efficiency and its optimum value should be determined. For optimization, DTZ was added in varying volumes between 100 and 1000 µL into the prepared model analyte solutions and the developed DES-UA-LPME method was applied. The results showed that 500 µL of DTZ was sufficient for quantitative extraction (Figure 4.6). Therefore, 500 µL was decided as the appropriate DTZ volume for further studies.

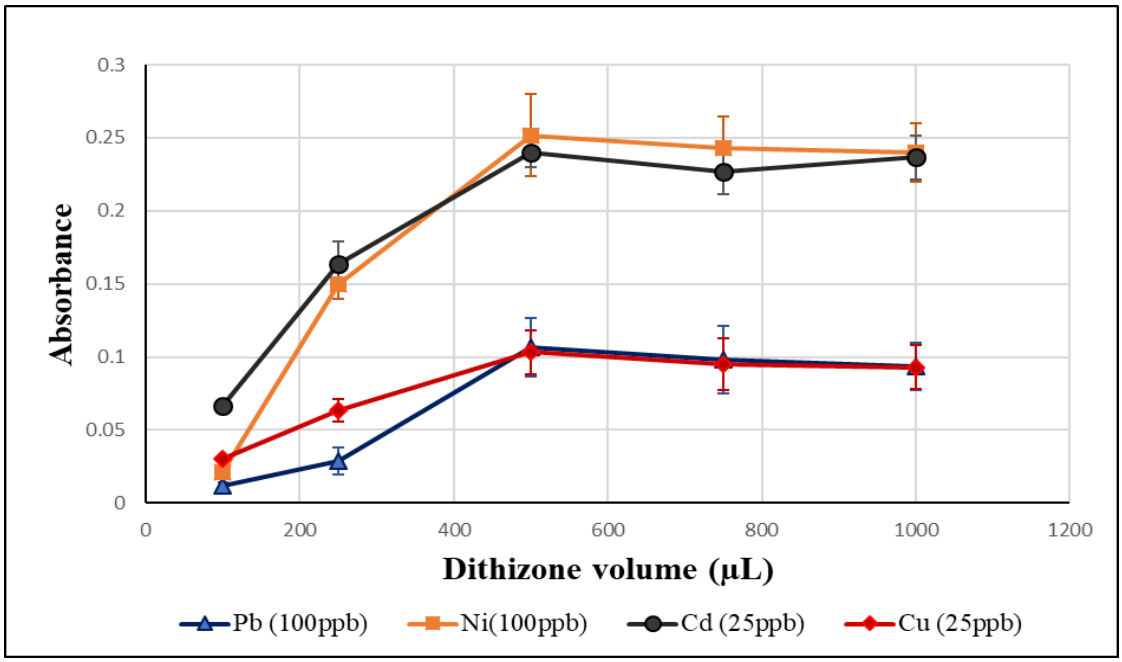

Figure 4.6. Effect of DTZ amount on the microextraction efficiency of analyte ions.

Microextraction Conditions: For Cd (II): 25 µg L^{-1} Cd (II) sample solution, 500 µL of 1x10^{-3} M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Cu (II): 25 µg L^{-1} Cu (II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Pb (II): 100 µg L^{-1} Pb (II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Ni (II): 100 µg L^{-1} Ni (II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl: Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.

4.3.4. Effect of the THF Amount

Aprotic solvent tetrahydrofuran (THF) was used as emulsifier to obtain quantitative extraction efficiency in DES-UA-DLPME. When THF is added to the sample solution, the water molecules interact with the THF and separate the DES molecules from the aqueous phase. In other words, increased water and THF interaction decreases water and DES interaction, which leads to self-aggregation of DESs. The effect of THF volume on microextraction efficiency was evaluated between 0 and 1000 µL, as shown in Figure 4.7. Quantitative results showed that the absorption signal was close to zero when THF was not used, increased with increasing amount of THF up to 500 µL and not changed anymore thereafter. Therefore, 500 µL was chosen as an appropriate amount of THF for subsequent experiments.

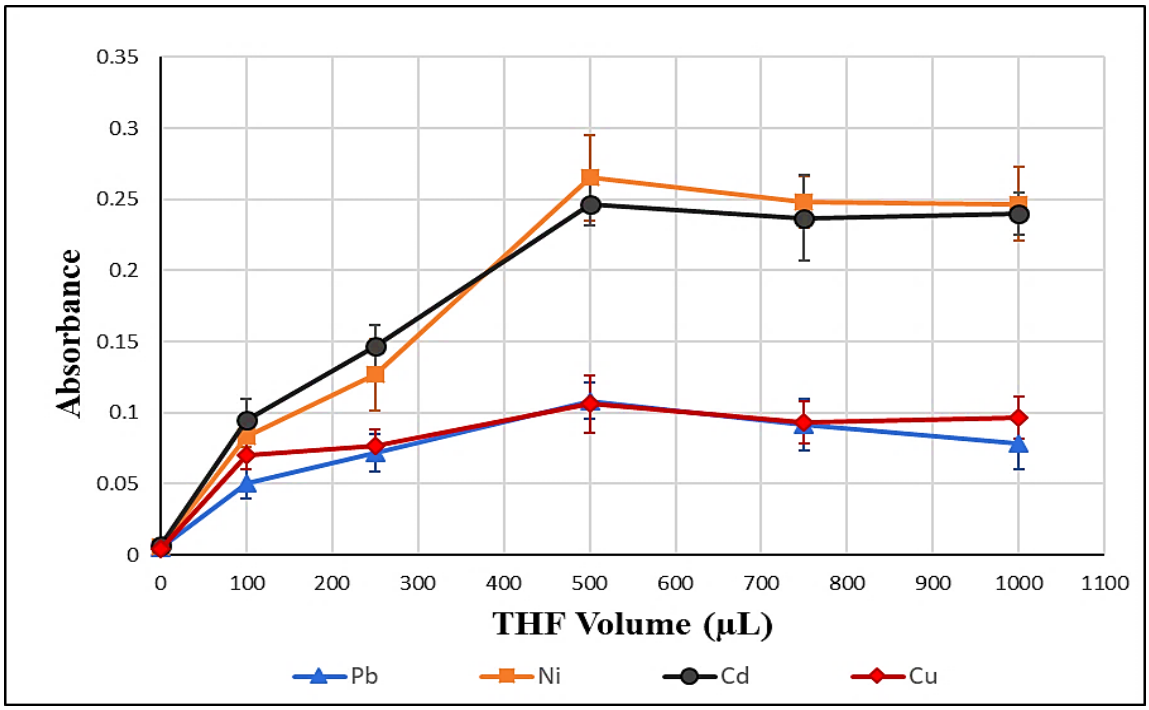

Figure 4.7. Effect of THF volume on microextraction efficiency.

Microextraction Conditions: For Cd(II): 25 µg L^{-1} Cd(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Cu(II): 25 µg L^{-1} Cu(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6,, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Pb(II): 100 µg L^{-1} Pb(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.;

For Ni(II): 100 µg L^{-1} Ni(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min., Centrifugation time of 4 min.

4.3.5. Effect of Ultrasonication Time

The application of ultrasonic radiation during extraction causes an increase in mass transfer by increasing the interaction between the analyte in the sample solution and the extraction solvent. In the thesis study, ultrasonic water bath was used to increase the extraction efficiency by obtaining micro and nano sized aggregates.

The effect of ultrasonication time was controlled in the range of 1 to 10 minutes, keeping other experimental conditions constant. The extraction efficiency remained constant between 2 and 5 minutes, and maximum recovery was achieved during this time (Figure 4.8,). Therefore, in further studies, samples were exposed to a 3-minute ultrasonic radiation, taking into account sufficient time, to achieve maximum extraction efficiency.

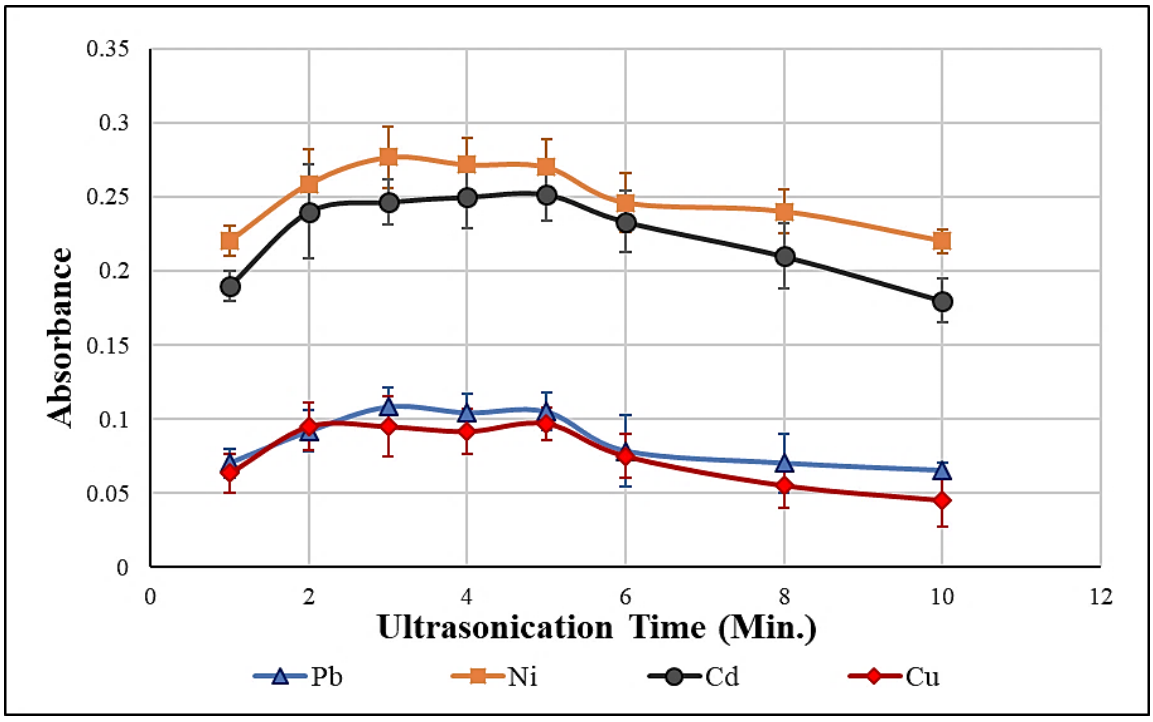

Figure 4.8. Effect of the ultrasonication time on the microextraction efficiency.

Microextraction Conditions: For Cd(II): 25 µg L^{-1} Cd(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Centrifugation time of 4 min.;

For Cu(II): 25 µg L^{-1} Cu(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Centrifugation time of 4 min.;

For Pb(II): 100 µg L^{-1} Pb(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Centrifugation time of 4 min.;

For Ni(II): 100 µg L^{-1} Ni(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Centrifugation time of 4min.

4.3.6. Effect of Centrifuging Time

Centrifugation was used to separate the extraction phase and the aqueous phase, which have different densities. The effect of centrifugation time on DES-UA-LPME was tested in the range of 1-6 minutes at 6000 rpm, keeping all other parameters constant (Figure 4.9). The results showed that the centrifugation time of 4 minutes was sufficient for complete phase separation. Therefore, the optimum centrifugation time was selected as 4 minutes for further studies.

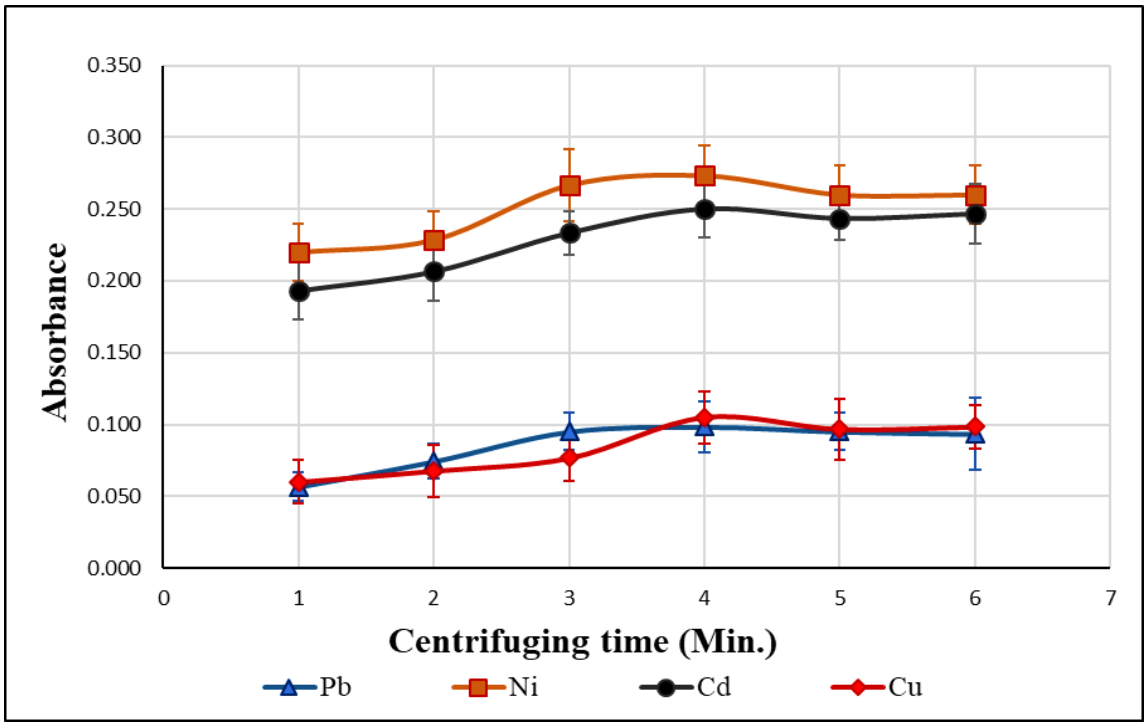

Figure 4.9. Effect of the centrifuging time on microextraction efficiency.

Microextraction Conditions: For Cd(II): 25 µg L^{-1} Cd(II) sample solution, 500 µL of 1x10^{-3} M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min.;

For Cu(II): 25 µg L^{-1} Cu(II) sample solution, 500 µL of 1x10^{-3} M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min.;

For Pb(II): 100 µg L^{-1} Pb(II) sample solution, 500 µL of 1x10^{-3} M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min.;

For Ni(II): 100 µg L^{-1} Ni(II) sample solution, 500 µL of $1x10^{-3}$ M DTZ, 1000 µL of (1:3) ChCl:Ph, pH: 6, Ultrasonication time of 3 min.

4.3.7. Effect of Interfering Ions on Extraction Efficiency

Since aqueous model solutions are utilized while performing the optimization of the DES-UA-LPME method, the effect of matrix ions that can be found in real samples on the extraction efficiency should be known. Interfering ions in the medium may cause a sensitivity decrease and thus an extraction efficiency decrease. For this purpose, the interference effects of matrix ions that can be found in hair coloring cosmetics and ions (mainly Co, Cr, Mn, Fe and Zn) that can be complexed with DTZ have been investigated. Therefore, the tolerability of the suggested DES-UA-LPME was studied by addition of likely interferents in increasing concentrations to 25 mL of individual standard analyte solutions (25 µg L^{-1} Cd(II), 25 µg L^{-1} Cu(II), 100 µg L^{-1} Pb(II) and 100 µg L^{-1} Ni(II)). A foreign ion is considered an interfering species if it results in a alteration in extraction recovery of the analyte higher than ±5%. The results are summarized in Tables 4.1 and 4.2. As can be seen from the tables, under optimized experimental conditions, possible interfering ions have no significant interference.

Table 4.1. Effect of interfering ions on Cd(II) and Cu(II) microextraction efficiency.

Foreign ion	**Added as**	**Cd(II)**		**Cu(II)**	
		Tolerable concentration (mg L^{-1})	Recovery (%)	Tolerable concentration (mg L^{-1})	Recovery (%)
Na^{+}	NaCl	200	96.2 ± 1.6	400	97.7 ± 1.8
K^{+}	KCl	200	98.1 ± 1.1	400	98.8 ± 1.7
Cl^{--}	NaCl	200	97.6 ± 1.3	200	95.8 ± 2.1
NO_3^{--}	KNO_3	200	99.2 ± 2.0	200	97.6 ± 1.3
SO_4^{--2}	Na_2SO_4	200	97.7 ± 1.8	200	97.4 ± 1.6
Cd^{2+}	$CdCl_2$	-	-	50	98.8 ± 1.3
Mn^{2+}	$MnCl_2$	200	96.9 ± 1.9	200	98.7 ± 1.7
Cu^{2+}	Cu $(NO_3)_2$	250	95.8 ± 2.1	-	-
PO_4^{3--}	$(NH_4)_3PO_4$	200	97.8 ± 1.8	400	97.5 ± 1.4
Zn^{2+}	Zn $(NO_3)_2$	100	96.3 ± 1.3	300	96.4 ± 1.2
Co^{2+}	Co $(NO_3)_2$	150	97.4 ± 1.6	200	98.1 ± 1.1
Ni^{2+}	Ni $(NO_3)_2$	100	98.1 ± 1.7	100	97.8 ± 1.7

Mg^{2+}	$Mg(NO_3)_2$	50	97.8 ± 1.7	200	96.9 ± 1.9
Al^{3+}	$Al(NO_3)_3$	50	98.8 ± 1.7	200	96.2 ± 1.6
Cr^{3+}	$CrCl_3$	50	98.7 ± 1.7	200	98.1 ± 1.7
Fe^{3+}	$Fe(NO_3)_3$	50	96.4 ± 1.2	200	99.2 ± 2.0
Pb^{2+}	$Pb(NO_3)_2$	25	99.2 ± 1.8	100	99.2 ± 1.8
CrO_4^{2-}	K_2CrO_4	100	97.5 ± 1.4	200	97.8 ± 1.8
CO_3^{2-}	$CaCO_3$	100	98.8 ± 1.3	200	98.2 ± 1.7
SCN^-	KSCN	100	98.2 ± 1.7	200	96.3 ± 1.3

Table 4.2. Effect of interfering ions on Pb(II) and Ni(II) microextraction efficiency.

Foreign ion	**Added as**	**Pb(II)**		**Ni(II)**	
		Tolerable concentration (mg L^{-1})	Recovery (%)	Tolerable concentration (mg L^{-1})	Recovery (%)
Na^+	NaCl	500	98.3 ± 1.3	100	96.8 ± 1.8
K^+	KCl	500	96.4 ± 2.2	100	97.8 ± 1.7
Cl^{--}	NaCl	500	99.1 ± 1.3	200	96.3 ± 1.3
NO_3^{--}	KNO_3	400	97.6 ± 1.8	100	96.2 ± 1.6
SO_4^{--2}	Na_2SO_4	300	95.8 ± 1.7	100	99.2 ± 1.8
Cd^{2+}	$CdCl_2$	200	99.0 ± 2.1	50	98.6 ± 2.1
Mn^{2+}	$MnCl_2$	300	97.2 ± 1.7	200	96.4 ± 2.2
Cu^{2+}	$Cu(NO_3)_2$	200	96.5 ± 1.5	200	96.9 ± 1.9
PO_4^{3--}	$(NH_4)_3PO_4$	200	97.5 ± 1.6	200	97.6 ± 1.3
Zn^{2+}	$Zn(NO_3)_2$	200	97.1 ± 1.8	50	98.1 ± 1.1
Co^{2+}	$Co(NO_3)_2$	200	98.7 ± 1.5	50	97.7 ± 1.8
Ni^{2+}	$Ni(NO_3)_2$	200	96.5 ± 1.3	-	-
Mg^{2+}	$Mg(NO_3)_2$	300	95.9 ± 1.4	100	99.2 ± 2.0
Al^{3+}	$Al(NO_3)_3$	50	96.2 ± 1.6	100	96.5 ± 1.5
Cr^{3+}	$CrCl_3$	100	98.5 ± 1.5	100	97.2 ± 1.7
Fe^{3+}	$Fe(NO_3)_3$	200	97.7 ± 2.0	100	97.5 ± 1.6
Pb^{2+}	$Pb(NO_3)_2$	-	-	50	97.6 ± 1.8
CrO_4^{2-}	K_2CrO_4	200	96.8 ± 2.2	200	98.3 ± 1.3
CO_3^{2-}	$CaCO_3$	400	98.6 ± 1.9	200	99.0 ± 2.1
SCN^-	KSCN	400	99.1 ± 1.9	200	99.1 ± 1.3

4.4. Analytical Performance Characteristics of the DES-UA-LPME Method

The enrichment factor (EF) is one of the most important analytical performance features of DES-UA-LPME, which was developed for the determination of Cd (II), Cu (II), Pb (II) and Ni (II) ions. EF is calculated from the ratio of the slope of the linear calibration curve obtained after the application of the proposed method to the

analyte solution of known concentration, to the slope of the linear calibration equation obtained with aqueous standard solutions. The linear concentration range, linear equation and correlation coefficient, R^2, of the calibration curve obtained with standard aqueous solutions are presented in Table 4.3.

Table 4.3. Calibration data for standard aqueous solutions of Cd(II), Cu(II), Pb(II) and Ni(II).

Metal İon	Linear Concentration Range (µg L^{-1})	Calibration curve linear equation*	Correlation coefficient (R^2)
Cd (II)	250- 3000	A= 0.00014 C + 0.016	0.9990
Cu (II)	500-8000	A= 0.00063 C + 0.00098	0.9996
Pb (II)	2500-50,000	A= 0.000082 C + 0.0023	0.9997
Ni (II)	500-8000	A= 0.000039 C + 0.0046	0.9991

*A: Absorbance, C: metal ion concentration.

In Table 4.4, the linear concentration range, linear equation and correlation coefficient of the calibration curve obtained after applying DES-UA-LPME to the model solutions are presented.

Table 4.4. Calibration data for Cd(II), Cu(II), Pb(II) and Ni(II) model solutions after applying DES-UA-LPME.

Metal İon	Linear Concentration Range (µg L^{-1})	Calibration curve linear equation*	Correlation coefficient (R^2)
Cd (II)	2.5 - 50	A= 0.0082 C + 0.039	0.9997
Cu (II)	5 - 150	A= 0.0032 C + 0.031	0.9993
Pb (II)	10 - 250	A= 0.00075 C + 0.012	0.9999
Ni (II)	2.5 - 150	A= 0.0023 C + 0.037	0.9996

*A: Absorbance; C: metal ion concentration

The EFs calculated using the data in the tables are 57 for Cd(II), 50 for Cu(II), 92 for Pb(II), and 58 for Ni(II).

Other analytical performance characteristics of the DES-UA-LPME method were calculated in accordance with the definitions given in Table 4.5 and their values are presented in Table 4.6.

Table 4.5. Definition and calculation formula of some performance feature of the proposed DES-UA-LPME method [168].

Feature	Definition	Calculation
Enrichment factor (EF)	The enrichment factor EF is defined as the ratio of the liquid mass-transfer coefficients for absorption with and without a chemical reaction. The enhancement factor can be determined by calculating the slope ratio of calibration curve after (S2) and before (S1) extraction.	EF= S2/S1
Limit of Detection (LOD)	The LOD is generally defined as the lowest amount of an analyte in a sample that can be detected by a particular analytical method. According to IUPAC it is the smallest concentration or absolute amount of analyte that has a signal significantly larger than the signal arising from a reagent blank and practically measured by calculating the precision estimated (S) that should be based on at least 6 independent complete determinations of analyte concentration in a typical matrix blank, with no censoring of zero or negative results, and the approximate limit of detection calculated as 3S.	LOD = 3*S*
Limit of Quantification (LOQ)	The (LOQ) is the lowest analyte concentration that can be quantitatively detected with a stated accuracy and precision. LOQ is similar to the LOD based on technique and sample matrix. LOQ can be determined by basing the level of 10 times the standard deviation of method blanks to compensate for the matrix effects.	LOQ= 10*S*
Correlation Coefficient (R^2)	Correlation coefficients are used to measure how strong a relationship is between two variables. A correlation coefficient of 1 means that for every positive increase in one variable, there is a positive increase of a fixed proportion in the other.	Calculated from the calibration curve
Linear Range	It is the range of concentrations where the signals are directly proportional to the concentration of the analyte in the sample.	Calculated from the calibration curve

Precision or Repeatability (RSD%)	Repeatability is the closeness of the agreement between the results of successive measurements of the same measuring carried out under the same conditions. It is usually definite in terms of relative standard deviation and expressed in terms of the deviation of a set of results from the arithmetic mean of the set.	RSD%= 100x *S*/ $\overline{X}$

Table 4.6. Analytical performance characteristics of the DES-UA-LPME method developed for Cd (II), Cu (II), Pb (II) and Ni (II) ions.

Analytical Feature	**Metal ions**			
	Cd (II)	Cu (II)	Pb (II)	Ni (II)
Enhancement factor (EF)	57	50	92	58
Sample volume (µL)	500	500	500	500
Linear Range (µg L^{-1})	2.5-50	5-150	10-250	2.5-150
Limit of detection (LOD) (µg L^{-1}) (n= 10)	0.7	1.4	2.3	0.8
Limit of quantitation (LOQ) (µg L^{-1}) (n = 10)	2.3	4.7	7.6	2.5
RSD (%) (n=10, 25 µg L^{-1} Cd (II), 25 µg L^{-1} Cu (II), 100 µg L^{-1} Pb (II), 100 µg L^{-1} Ni (II))	2.3	1.8	1.7	2.7

4.5. Accuracy of DES-UA-LPME

The accuracy of the DES-UA-LPME method was proved in two ways. First, the method was applied to CRM (NCS ZC 73013-spinach leaves). Since there is no CRM available for hair dye and henna, a certified spinach sample was used, assuming that it has a plant matrix similar to henna. Secondly, different known amounts of the studied ions were spiked to the hair dye and henna samples, and the recoveries were calculated after the implementation of the DES-UA-LPME method.

4.5.1. Verification of the Method Accuracy by Using CRM

The accuracy of the suggested DES-UA-LPME method was detected via analyzing a CRM having 0.15 ± 0.02 µg g^{-1} Cd, 8.9 ± 0.40 µg g^{-1} Cu, 11.10 ± 0.90 µg g^{-1} Pb and 0.92 ± 0.12 µg g^{-1} Ni. The results are given in Table 4.7. As seen from this table, percent recovery values for analytes are in the range of 94-99%. Recovery values for spiking-recovery experiments performed with CRM prove that the presented DES-UA-LPME method has been applied successfully.

Table 4.7. Certified reference material NCS ZC 73013 (Spinach) analysis results for Cd(II), Cu(II), Pb (II) and Ni (II) metal ions (n = 4).

Analyte	Certificate Value (µg g^{-1})	Added (µg g^{-1})	Found (µg g^{-1})	Recovery (%)
Cd (II)	0.15 ± 0.02	25	24.2 ± 0.2	96 ± 3
Cu (II)	8.90 ± 0.40	-	8.5 ± 0.3	95 ± 2
Pb (II)	11.10 ± 0.90	-	11.0 ± 0.5	99 ± 1
Ni (II)	0.92 ± 0.12	25	24.6 ± 0.5	94 ± 2

At this stage of the thesis, a t-test (Equation 4.1) was applied in order to understand whether there is a significant difference between the certificate values of certified reference materials and the values found, and the results obtained are presented in Table 4.8.

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

X: Average of analysis results

µ: Value of certified reference material

S: Standard deviation of the analysis results

n: Number of analyses performed.

To make a comparison with the result, the t_{table} value was calculated according to the n^{-1} degree of freedom at the α = 0.05 significance level (95% confidence level). The fact that the calculated $t_{cal.}$ value was smaller than the t_{table} value has proved there was no remarkable difference between the results.

Table 4.8. T-test results for the CRM NCS ZC 73013 (Spinach) for Cd(II), Cu(II), Pb(II) and Ni(II) ions (n = 4)

CRM	Analyte	$T_{cal.}$	T_{table}	Evaluation
NCS ZC 73013 (Spinach)	Cd (II)	1.22	3.18	$t_{cal.} \leq t_{table}$
	Cu (II)	1.54	3.18	$t_{cal.} \leq t_{table}$
	Pb (II)	2.16	3.18	$t_{cal.} \leq t_{table}$
	Ni (II)	0.63	3.18	$t_{cal.} \leq t_{table}$

4.5.2. Analysis of Hair Dyes and Henna Samples

At this stage of the thesis, spiking-recovery experiments of the studied metal ions were carried out on various hair dyes and henna samples having varied shades and

origins, and the amounts of heavy metal ions in the original samples were calculated. The obtained results are presented in Table 4.9 for Cd(II), Table 4.10 for Cu(II), Table 4.11 for Pb(II) and Table 4.12 for Ni(II). The high recoveries obtained prove the accuracy of the DES-UA-LPME method and its suitability for the determination of these metal ions in hair coloring cosmetics.

Table 4.9. Analysis results for Cd (II) in real samples of hair dyes and henna samples and spike solution recoveries.

Sample ID*	**Color**	**Origin Country**	**Added (μgL^{-1})**	**Found (μgL^{-1})**	**Recovery%**	**Calculated Cd Amount ($\mu g\ g^{-1}$)**
D1	Light Blond	Germany	0	0.48	–	0.24 ± 0.06
			10	10.18	97 ± 3	
			25	24.12	94 ± 1	
			50	47.77	94 ± 3	
D2	Deep Blond	Germany	0	1.09	–	0.55 ± 0.05
			10	11.39	102 ± 1	
			25	24.73	94 ± 3	
			50	48.98	95 ± 2	
D3	Blond	Germany	0	0.72	–	0.36 ± 0.04
			10	10.18	94 ± 2	
			25	24.73	96 ± 2	
			50	48.37	95 ± 1	
D4	Deep Brown	Germany	0	0.48	–	0.23 ± 0.08
			10	10.18	97 ± 2	
			25	26.55	104 ± 1	
			50	47.77	94 ± 2	
D5	Red	Germany	0	2.90	–	1.45 ± 0.08
			10	12.61	97 ± 3	
			25	28.36	101 ± 4	
			50	50.19	94 ± 2	
H1	Natural	Turkey	0	0.24	–	0.12 ± 0.06
			10	10.54	105 ± 2	
			25	25.94	102 ± 3	
			50	48.98	97 ± 3	
H2	Black	India	0	1.69	–	0.84 ± 0.02
			10	11.34	97 ± 5	
			25	25.94	97 ± 3	

			50	50.19	97 ± 2	
H3	Brown	India	0	4.12	–	2.06 ± 0.04
			10	13.81	97 ± 4	
			25	28.36	97 ± 2	
			50	51.40	95 ± 3	
H4	Red	India	0	5.33	–	2.66 ± 0.02
			10	15.03	98 ± 2	
			25	29.58	97 ± 2	
			50	53.83	97 ± 3	
H5	Natural	Turkey	0	0.48	–	0.24 ± 0.02
			10	10.18	101 ± 1	
			25	25.94	101 ± 2	
			50	47.77	94 ± 2	
H6	Red	India	0	1.09	–	0.54 ± 0.06
			10	11.39	102 ± 2	
			25	28.28	108 ± 4	
			50	48.98	95 ± 3	
H7	Violet	India	0	1.69	–	0.84 ± 0.02
			10	11.39	97 ± 3	
			25	24.73	92 ± 5	
			50	49.58	95 ± 3	
H8	Black	India	0	0.48	–	0.24 ± 0.02
			10	10.30	102 ± 2	
			25	24.72	97 ± 5	
			50	48.98	97 ± 4	
H9	Brown	India	0	0.24	–	0.12 ± 0.04
			10	10.42	101 ±1	
			25	24.73	98 ± 2	
			50	47.77	95 ± 2	
H10	Natural	India	0	0.47	–	0.24 ± 0.06
			10	10.18	97 ± 3	
			25	24.73	97 ± 5	
			50	50.19	99 ± 2	
HT11	Bright Blue	India	0	3.51	–	1.74 ± 0.15
			10	13.57	100 ± 2	
			25	27.15	95 ± 3	
			50	51.40	96 ± 4	
HT12	Brown	India	0	3.51	–	1.63 ± 0.20
			10	13.57	100 ± 3	
			25	28.36	99 ± 2	
			50	51.40	96 ± 4	
HT13	Brown	Pakistan	0	1.69	–	0.83 ± 0.12

			10	11.39	97 ±5	
			25	25.69	96 ± 3	
			50	51.40	99 ± 3	
HT14	Black	Pakistan	0	0.48	–	0.23 ± 0.06
			10	10.18	97 ± 2	
			25	25.94	101 ±3	
			50	48.98	97 ± 3	

*D: hair dyes, H: henna, HT; Henna Tattoo Paste, n=3.

Table 4.10. Analysis results for Cu(II) in real samples of hair dyes and henna samples and spike solution recoveries.

Sample ID*	Color	Origin Country	Added (μgL^{-1})	Found (μgL^{-1})	Recovery%	Calculated Cu Amount ($\mu g\ g^{-1}$)
D1	Light Blond	Germany	0	3.10	–	1.49 ± 0.15
			10	12.49	95 ± 2	
			25	28.04	100 ± 3	
			50	54.76	103 ± 1	
D2	Deep Blond	Germany	0	1.53	–	0.73 ± 0.06
			10	10.93	94 ± 2	
			25	27.52	103 ± 1	
			50	50.06	97 ± 3	
D3	Blond	Germany	0	1.53	–	0.75 ± 0.03
			10	10.93	94 ± 5	
			25	25.01	94 ± 2	
			50	53.19	103 ± 1	
D4	Deep Brown	Germany	0	4.66	-	2.27 ± 0.22
			10	14.37	97 ± 3	
			25	27.83	93 ± 5	
			50	56.33	103 ± 2	
D5	Red	Germany	0	6.23	–	2.94 ±0.06
			10	15.62	96 ± 3	
			25	29.71	95 ± 5	
			50	59.46	105 ± 1	
H1	Natural	Turkey	–	–	–	n.d.*
H2	Black	India	0	9.36	–	4.68 ± 0.04
			10	18.75	96 ± 4	
			25	34.41	100 ± 2	
			50	56.33	94 ± 3	
H3	Brown	India	0	12.49	–	6.24 ± 0.14
			10	21.88	97 ± 3	
			25	36.91	98 ± 2	

			50	59.46	95 ± 4	
H4	Red	India	0	14.06	–	7.07 ± 0.52
			10	24.39	101 ± 1	
			25	37.54	96 ± 3	
			50	65.72	102 ± 1	
H5	Natural	Turkey	0	9.36	–	4.68 ± 0.30
			10	18.75	96 ± 5	
			25	33.78	98 ± 3	
			50	59.46	100 ± 2	
H6	Red	India	0	10.93		5.41 ± 0.05
			10	21.26	101 ± 2	
			25	34.41	95 ± 4	
			50	59.46	97 ± 3	
H7	Violet	India	0	9.36	–	4.58 ± 0.04
			10	18.75	96 ± 4	
			25	33.78	98 ± 3	
			50	59.48	99 ± 3	
H8	Black	India	0	10.93	–	5.46 ± 0.25
			10	21.88	104 ± 2	
			25	34.40	95 ± 4	
			50	62.59	102 ± 1	
H9	Brown	India	0	12.49		6.24 ± 0.14
			10	21.88	97 ± 4	
			25	36.91	98 ± 5	
			50	59.46	95 ± 2	
H10	Natural	India	0	10.93		5.46 ± 0.05
			10	20.32	97 ± 6	
			25	35.97	100 ± 4	
			50	62.59	102 ± 1	
HT11	Bright Blue	India	–	–	–	n.d.*
HT12	Brown	India	0	9.67	–	4.48 ± 0.34
			10	18.75	95 ± 5	
			25	34.41	99 ± 3	
			50	62.59	104 ± 1	
T13	Brown	Pakistan	0	9.36	-	4.51 ± 0.41
			10	18.75	96 ± 2	
			25	32.84	95 ± 5	
			50	56.33	94 ± 3	
HT14	Black	Pakistan	0	9.67	-	3.48 ± 0.20
			10	18.12	101 ± 2	
			25	31.72	95 ± 3	
			50	59.46	102 ± 1	

*D: hair dyes, H: henna, HT; Henna Tattoo Paste, n.d.: not detected, n=3.

Table 4.11. Analysis results for Pb(II) in real samples of hair dyes and henna samples and spike solution recoveries.

Sample ID*	Color	Origin Country	Added (μgL^{-1})	Found (μgL^{-1})	Recovery %	Calculated Pb Amount ($\mu g\ g^{-1}$)
D1	Light Blond	Germany	0	16.88	–	8.39 ± 0.50
			50	64.76	96 ± 5	
			100	113.96	97 ± 3	
			250	256.26	96 ± 2	
D2	Deep Blond	Germany	0	14.22	–	7.22 ± 0.34
			50	61.43	95 ± 3	
			100	109.97	96 ± 1	
			250	256.26	96 ± 2	
D3	Blond	Germany	0	12.89	–	6.49 ± 0.24
			50	63.43	100 ± 4	
			100	109.97	97 ± 3	
			250	249.61	94 ± 2	
D4	Deep Brown	Germany	0	30.18	–	14.24 ± 0.30
			50	79.39	99 ± 4	
			100	123.27	94 ± 2	
			250	269.56	96 ± 1	
D5	Red	Germany	0	23.53	–	11.77 ± 0.41
			50	72.74	98 ± 1	
			100	116.62	94 ± 2	
			250	260.25	95 ± 3	
H1	Natural	Turkey	0	16.88	–	8.28 ± 0.32
			50	64.09	95 ± 3	
			100	109.97	94 ± 1	
			250	262.91	98 ± 3	
H2	Black	India	0	16.88	–	8.39 ± 0.13
			50	66.09	98 ±2	
			100	116.62	99 ± 2	
			250	270.89	101 ± 1	
H3	Brown	India	0	12.89	–	6.42 ± 0.31
			50	64.76	102 ± 1	
			100	116.62	103 ± 1	
			250	258.92	98 ± 3	
H4	Red	India	0	16.88	-	8.43 ± 0.22
			50	64.76	96 ± 3	
			100	115.29	98 ± 2	
			250	269.56	101 ± 4	
H5	Natural	Turkey	0	7.58	–	3.79 ± 0.14
			50	56.78	98 ± 3	
			100	109.97	102 ± 1	
			250	249.61	96 ± 2	
H6	Red	India	0	30.18	–	15.09 ± 0.25
			50	76.73	95 ± 3	

			100	123.27	94 ± 2	
			250	269.56	96 ± 2	
H7	Violet	India	0	19.54	–	9.75 ± 0.34
			50	70.08	100 ± 3	
			100	119.28	99 ± 2	
			250	265.57	98 ± 2	
H8	Black	India	0	8.91	–	4.45 ± 0.51
			50	58.11	98 ± 4	
			100	108.64	99 ± 3	
			250	261.58	101 ± 2	
H9	Brown	India	0	9.57	–	4.79 ± 0.20
			50	56.78	95 ± 3	
			100	103.32	94 ± 2	
			250	256.26	98 ± 2	
H10	Natural	India	0	12.89	–	6.45 ± 0.62
			50	63.43	100 ± 3	
			100	113.96	100 ± 1	
			250	258.92	98 ± 2	
HT11	Bright Blue	India	0	31.51	–	15.60 ± 0.41
			50	79.39	97 ± 2	
			100	125.93	95 ± 1	
			250	265.57	94 ± 2	
HT12	Brown	India	0	26.19	-	12.13 ± 0.62
			50	73.80	96 ± 3	
			100	125.93	99 ± 1	
			250	262.91	95 ± 4	
HT13	Brown	Pakistan	0	16.88	-	8.28 ± 0.43
			50	66.09	98 ± 3	
			100	113.96	97 ± 2	
			250	256.26	96 ± 2	
HT14	Black	Pakistan	0	7.58	-	3.58 ± 0.21
			50	56.78	98 ± 2	
			100	104.66	97 ± 1	
			250	246.95	95 ± 3	

*D: hair dyes, H: henna, HT; Henna Tattoo Paste, n=3.

Table 4.12. Analysis results for Ni(II) in real samples of hair dyes and henna samples and spike solution recoveries.

Sample ID*	Color	Origin Country	Added (μgL^{-1})	Found (μgL^{-1})	Recovery%	Calculated Ni Amount ($\mu g\ g^{-1}$)
D1	Light Blond	Germany	0	13.14	-	6.31 ± 0.42
			10	23.97	103 ± 1	
			25	39.12	102 ± 1	
			50	62.94	99 ±3	
D2	Deep Blond	Germany	0	4.48	-	2.13 ± 0.28
			10	14.44	99 ± 2	
			25	30.46	103 ± 1	
			50	52.54	96 ± 3	
D3	Blond	Germany	0	5.78	-	2.83 ± 0.45
			10	15.31	97 ± 4	
			25	30.89	100 ± 2	
			50	54.28	97 ± 3	
D4	Deep Brown	Germany	0	6.65	-	3.24 ± 0.05
			10	16.17	97 ± 3	
			25	32.63	103 ± 1	
			50	54.28	95 ± 3	
D5	Red	Germany	0	2.32	-	1.16 ± 0.02
			10	11.84	96 ± 3	
			25	27.43	100 ± 2	
			50	49.95	95 ± 2	
H1	Natural	Turkey	0	5.78	-	2.82 ± 0.41
			10	15.31	97 ± 3	
			25	30.46	99 ± 2	
			50	54.28	97 ± 3	
H2	Black	India	0	6.65	-	3.25 ± 0.22
			10	17.04	102 ± 1	
			25	32.63	103 ± 1	
			50	57.74	101 ± 2	
H3	Brown	India	0	6.65	-	3.25 ± 0.25
			10	17.47	105 ± 1	
			25	32.63	103 ± 1	
			50	54.28	95 ± 3	
H4	Red	India	0	8.81	-	4.30 ± 0.54
			10	19.20	102 ± 1	
			25	32.63	96 ± 3	
			50	56.44	96 ± 3	
H5	Natural	Turkey	0	2.32	-	1.16 ± 0.72
			10	12.28	99 ± 2	
			25	26.13	95 ± 3	
			50	51.25	98 ± 2	
H6	Red	India	0	6.65	-	3.32 ± 0.65
			10	16.17	97 ± 3	

			25	32.63	103 ± 2	
			50	54.28	95 ± 3	
H7	Violet	India	0	4.48	-	2.20 ± 0.30
			10	14.44	99 ± 2	
			25	28.30	96 ± 3	
			50	54.28	99 ± 2	
H8	Black	India	0	4.48	-	2.24 ± 0.41
			10	14.44	99 ± 3	
			25	28.30	96 ± 2	
			50	55.14	101 ± 2	
H9	Brown	India	0	14.44	-	7.22 ± 0.32
			10	23.97	98 ± 2	
			25	39.12	99 ± 1	
			50	62.94	97 ± 3	
H10	Natural	India	0	13.14	-	6.57 ± 0.24
			10	23.97	103 ± 1	
			25	36.96	96 ± 2	
			50	62.07	98 ± 1	
HT11	Bright Blue	India	0	6.65	-	3.25 ± 0.35
			10	16.17	97 ± 2	
			25	32.63	103 ± 1	
			50	54.28	95 ± 2	
HT12	Brown	India	0	6.65	-	3.08 ± 0.15
			10	17.47	104 ± 1	
			25	30.46	96 ± 3	
			50	56.01	98 ± 3	
HT13	Brown	Pakistan	0	15.31	-	7.36 ± 0.34
			10	23.97	94 ± 3	
			25	41.29	102 ± 2	
			50	62.07	95 ± 3	
HT14	Black	Pakistan	0	13.14	-	5.86 ± 0.61
			10	21.80	94 ± 2	
			25	36.96	96 ± 3	
			50	62.07	98 ± 1	

*D: hair dyes, H: henna, HT; Henna Tattoo Paste, n=3.

When the results were examined, it was seen that the metal with the highest concentration was lead and the metal with the lowest concentration was cadmium in the analyzed hair dyes and henna samples. The amount of studied metal ions is in decreasing order as Pb> Ni> Cu> Cd.

The highest concentrations detected for cadmium metal were 2.66 ± 0.02 µg g^{-1} in the red henna (H4) sample, 1.74 ± 0.15 µg g^{-1} in the bright blue color henna paste (HT11) sample, and 1.45 ± 0.08 µg g^{-1} in the red hair dye (D5) sample.

It is seen that the highest concentration detected for copper metal is approximately 7.07 ± 0.52 µg g^{-1} in the red colored henna (H4) sample and there is a higher amount of copper ions in all henna samples than in hair dye samples.

The highest concentrations determined for lead metal were 15.60 ± 0.41 µg g^{-1} in the bright blue colored henna paste (HT11) sample, and a similar value was found in the red henna (H6) sample (as 15.09 ± 0.25 µg g^{-1}). The dark brown colored hair dye (D4) sample was also among the samples containing high copper metal with 14.24 ± 0.30 µg g^{-1}.

The highest concentrations detected for nickel metal were 7.36 ± 0.34 µg g^{-1} and 7.22 ± 0.32 µg g^{-1}, respectively, in brown colored henna paste (HT13) and brown henna (H9) samples.

5. CONCLUSIONS

The determination of metals is very important due to their essentiality and toxic effects in living organisms. In this thesis; A highly accurate and sensitive microextraction technique has been developed for the determination of Cd, Pb, Cu and Ni elements in hair coloring cosmetics at trace levels with the FAAS system. With this developed method, it is aimed to use the FAAS system, which is easy to use and has low operating cost, instead of instruments with expensive and difficult applications. For this purpose, studies have been carried out to improve the analytical performance properties of FAAS.

The DES-UA-LPME method has been developed in which a deep eutectic solvent, one of the new generation solvents that has become popular in recent years, is used as the extraction solvent. While developing the method, the parameters affecting the extraction efficiency were optimized first, and then this improved method was applied to real samples.

Compared to conventional dispersive liquid phase extraction techniques, the volume of extraction solvent used in this technique is at the level of microliters, making the method practical, economical, safe for human health and also environmentally friendly.

In the developed DES-UA-LPME method, all parameters affecting the extraction efficiency were optimized.

The first parameter to be optimized was pH because the pH of the sample solution has a significant influence on the metal ion complexing with DTZ and subsequent extraction into an organic phase. Optimum working pH was determined by changing the pH of the aqueous solutions containing analyte ions between 3-10. As a result, appropriate pH was selected as 6 for all ions.

It is very important to have a sufficient amount of complexing agent in the complex formation of metal ions. For this purpose, DTZ at a concentration of $1x10^{-3}$ M was selected and added to the analyte solution in varying volumes to find the volume in

which the highest yield was obtained. As a result, the optimal amount of DTZ was determined as 500 µL.

Another work done during the optimization is the mole ratio and amount of DES used as the extraction solvent. The highest absorbance values for the four metal ions were obtained when DES had a mole ratio of ChCl:Ph of 1:3 and a volume of 1000 µL.

In the proposed method, an aprotic solvent, was utilized to separate DESs from the aqueous phase as well as to dissolve DTZ. The effect of THF volume on extraction efficiency was evaluated in the range of 0 -1000 µL. Quantitative results indicated that 500 µL of THF was suitable for this study.

Another important parameter that affects the extraction efficiency and reduces the time required to collect the metal ion complex molecules into the DES phase is ultrasonication. This technique has been used to increase fine droplets and facilitate mass transfer between the two liquid phases. For this purpose, the time required to obtain the maximum extraction efficiency was examined and it was seen that 3 minutes was sufficient to complete the dispersion.

The final optimized parameter is the time of centrifugation applied to separate the aqueous and extraction phase. At the end of the studies, the centrifugation time was selected as 4 minutes. Optimum conditions of DES-UA-LPME method were summarized in Table 5.1.

Under optimum conditions, for Cd(II), Cu(II), Pb(II), and Ni(II), the enhancement factor (EF) of 57, 50, 92, and 58, the limit of detection (LOD) of 0.7, 1.4, 2.3 and 0.8 µg L^{-1}, the limit of quantitation (LOQ) of 2.3, 4.7, 7.6 and 2.5 µg L^{-1}, the relative standard deviation (n= 10) of 2.3, 1.8, 1.7 and 2.7, were calculated, respectively.

The NCS ZC 73013 spinach certified reference material was used to control the accuracy of the results obtained with the developed DES-UA-LPME method. As a result of the 4 replicates analysis, the recovery values were calculated as 96% ± 3 for Cd (II), 95% ± 2 for Cu (II), 99% ± 1 for Pb (II) and 94% ± 2 for Ni (II). The method

has also been successfully applied to various hair dye and henna samples, and quantitative results have been obtained in spiking-recovery studies.

Table 5.1 Optimum conditions of DES-UA-LPME method developed for the determination of Cd (II), Cu (II), Pb (II) and Ni (II) ions.

Parameters	**Optimum conditions**			
	Cd (II)	Cu (II)	Pb (II)	Ni (II)
pH value	6	6	6	6
DTZ volume (µL)	500	500	500	500
DES mole ratio	1:3	1:3	1:3	1:3
DES volume (µL)	1000	1000	1000	1000
THF volume (µL)	500	500	500	500
Ultrasonication time (min)	3	3	3	3
Centrifugation time (min)	4	4	4	4
Sample volume (mL)	25	25	25	25
Extracted sample volume (mL)	0.5	0.5	0.5	0.5

The performance of the DES-UA-LPME method was compared with the performance of other microextraction techniques reported for determination of the studied metal ions in different samples. During this comparison, studies having FAAS and more responsive detection techniques (ICP and ET-AAS) than FAAS were chosen. As comparison data, EF, LOD, LR and %RSD were selected. According to the data in the Table 5.2 to Table 5.5, at least 1 of the 5 parameters used to compare the proposed method with those in the literature has the same or better value than those presented in the tables for the proposed method.

Examining the FAAS-containing methods in the tables, the DES-UA-LPME is either better or equal in terms of enrichment factor, linear operating range, accuracy, and precision. In addition, the presented method is also comparable in terms of repeatability with most of the methods using ICP-OES and ET-AAS in the tables.

Considering all the advantages described so far, the DES-UA-LPME method developed during this thesis study is a method with high accuracy, high extraction efficiency, high sensitivity, good reproducibility and no matrix effects for the determination of Cd, Pb, Cu and Ni in hair dye and henna sample.

Table 5.2. Comparison of the DES-UA-LPME method for Cd (II) metal ion detection with the other reported methods.

Analytical method	Detection type	Analytical sample	EF/PF	LOD (µg L^{-1})	Linear Range (µg L^{-1})	RSD (%)	Ref.
I**L- SPME**	ICP-OES	Human hair	10	0.33	1–100	5.1	[169]
DLPME	FAAS	Food, vegetation and water samples	55	0.4	5 - 100	1.9	[170]
SPs-LPME	FAAS	Water, vegetable, fruit and cigarette samples	28	0.16	0.53–157	5.4	[171]
UASEME	FAAS	Water and sediment samples	14	0.23	1.0– 30	3.4	[172]
TSIL - DLPME	ETAAS	Biological samples	10	0.005	0.02–1.42	2.3	[173]
SHS- LPME	GFAAS	Environmental and biological samples	52	0.0039	0.01- 0.25	6.2	[174]
IL-VADLPME	FAAS	Groundwater and hair	72.2	0.13	5-15	4.2	[175]
DLPME	FAAS	Water samples	26	1.2	5-150	2.1	[176]
DI-SDME	ETAAS	Water samples	65	0.0007	0.01 - 1.0	7.4	[177]
VALPME	FAAS	Tap water, apple and rice samples	35	2.9	10 -250	4.1	[178]
DES-UA-LPME	FAAS	Henna and hair dyes	57	0.7	2.5-50	2.3	This study

Table 5.3. Comparison of the DES-UA-LPME method for Cu (II) metal ion detection with the other reported methods.

Analytical method	Detection type	Analytical sample	EF/PF	LOD (μg L^{-1})	Linear Range (μg L^{-1})	RSD (%)	Ref.
IP-DLPME	FAAS	Soil, multivitamin tablet, tea and water samples	10	3.7	6.0-100	1.9	[179]
RP-SHS-LPME	FAAS	Oil sample	22.7	6.9	23 –1000	9.4	[180]
SS-LPME	FAAS	Water, food and hair samples	25	1.80	-	3.8	[181]
UA-DES-DB-ELPME	FAAS	Liver samples	10	4	-	3.2	[182]
GA-DLPME	UV-vis-Spectro.	Water samples	122	0.07	0.30–2.00	3.9	[183]
DLPME	UV-vis Spectro.	Environmental aqueous samples	63.6	0.33	1-200	4	[184]
SA-DLPME	UV-vis Spectro.	Marine brown algae	104	0.033	0.1-100	-	[185]
USAE-SFODME	FAAS	Water samples	12.5	0.76	20- 600	3.8	[186]
DSDME	FAAS	Environmental waters, fruits, vegetables	25	1.84	25-1200	2.1	[187]
HLLLE	FAAS	Water samples	25	1.74	10–2000	7.6	[188]
DES-UA-LPME	FAAS	Henna and hair dyes	50	1.4	5-150	1.8	This study

Table 5.4. Comparison of the DES-UA-LPME method for Pb (II) metal ion detection with the other reported methods.

Analytical method	Detection type	Analytical sample	EF/PF	LOD (µg L^{-1})	Linear Range (µg L^{-1})	RSD (%)	Ref.
UA-DES-LPME	FAAS	Cosmetics	71.6	0.66	1- 10	1.2	[189]
MADLPME	GFAAS	Lipsticks and hair dyes	96	0.1	0.3- 50	8.3	[190]
LL-DLPME	FAAS	Water samples	15.5	0.5	0.007 - 6	1.6	[191]
HF-IL- LPME	ETAAS	Environmental and biological samples	75	0.02	0.04- 1.0	5	[192]
AA-DES-LPME	GFAAS	Food and water samples	60	0.0006	0.12–2.5	2.9	[193]
TIL-DLME	FAAS	Blood samples	93	0.13	5–20	4.3	[194]
DLPME	GFAAS	Human urine and water samples	78	0.039	0.0001–0.02	3.2	[116]
VA-DES-LPME	SQT-FAAS	Milk samples	48	8.7	50 -1000	3.1	[195]
SHS- LPME	GFAAS	Water, tea and human hair samples	49	0.016	0.04–2	4.2	[174]
SDILNDµE	ETAAS	Eye makeup products	50	0.086	1.0-20	< 4.2	[196]
DES-UA-LPME	FAAS	Henna and hair dyes	92	2.3	5-150	1.7	This study

Table 5.5. Comparison of the DES-UA-LPME method for Ni (II) metal ion detection with the other reported methods.

Analytical method	Detection type	Analytical sample	EF/PF	LOD (µg L^{-1})	Linear Range (µg L^{-1})	RSD (%)	Ref.
DES- DLPME	FAAS	Water and fruit juice samples	24	0.30	0.80–50	4.1	[197]
HF–LPME	ETAAS	Environmental and biological samples	60	0.03	0.08- 2	4.2	[192]
PV-IS-DLPME	FAAS	Chocolate samples	17	2	6.7–100	4.8	[198]
LL-SHS-LPME	FAAS	Food and cigarette samples	10	5.2	17–500	6	[199]
IL-DLPME	FAAS	Wastewater and alloy samples	61.2	0.93	4-180	2.7	[200]
DLPME	ICP-OES	Seafood	20	0.12	0.12–100	≤3.6	[201]
IL-USE-AALLME	FAAS	Food and Biological samples	21	7	7.0 –667	9.1	[202]
pH-MS-HLPME	FAAS	Water Samples	45	3.2	10.0- 450	2	[203]
UA-CPE	FAAS	Milk-based samples	48.6	0.56	2–160	3.6	[204]
IL-DLPME	ETAAS	Serum and tap water samples	100	0.005	0.02-0.7	0.02	[205]
DES-UA-LPME	FAAS	Henna and hair dyes	58	0.8	2.5-150	2.7	This study

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