

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Microextraction Techniques as a Sample Preparation Step for Metal Analysis

Pourya Biparva¹ and Amir Abbas Matin²

¹Department of Nanotechnology, Islamic Azad University, Langaroud Branch, Langaroud,

²Research Department of Analytical Chemistry, Iranian Academic Center for Education,

Culture & Research (ACECR), Urmia

Iran

1. Introduction

Analytical methods consist of several steps including; sampling, sample preparation, analysis, calculations and statistical evaluation of the results. Each step has a direct impact on accuracy, precision and sensitivity of the method. Among these steps, sample preparation is the most time consuming step. Result of studies showed that more than 60% of analysis time is spent for sample preparation. Sample preparation follows two main aims; sample clean-up and concentration. Sample Clean-up is carried out for isolating the target analytes from matrix components which interfere on determination and concentration is done for enrichment of the analytes in sample because despite advances in analytical instrumentation, sensitivities are limited.

Characteristics of an ideal sample preparation technique are listed as below:

- Minimum loss of the sample and maximum recovery of the analyte
- Elimination of accompanying compounds with high yield
- Simple, fast and cheap method
- Capable with analytical instruments
- In agreement with green chemistry

In the case of atomic absorption spectrometry (AAS) which is the subject of this book, there are two principle systems which are familiar with readers of this book, flame and electrothermal AAS. In continue of our discussions about microextraction techniques for metal analysis by AAS, we will emphasis on reduced volumes of extracting phases in the microlitre scale. It is clear that due to consumption of large volumes (in the mililitre scale) of the sample in flame AAS, coupling of microextraction techniques with flame AAS is difficult. But in the case of electrothermal AAS, this is so easy. Because volume of the samples introduced to graphite furnaces are very low and in microlitre scale. So a review on literature show that most of the microextraction methods are capable with electrothermal AAS not with flame AAS.

1.1 Liquid-Liquid Extraction (LLE)

Liquid-Liquid Extraction (LLE) is a versatile classical sample preparation technique. LLE is based on establishment of distribution equilibrium of the analytes between two immiscible

phases, an aqueous and an organic phase. Apparatus for LLE is a separating funnel. If distribution equilibrium constant is enough large, a quantitative extraction of the analytes can be occurred in one step. But most of the LLEs are multistep.

LLE commonly is used for extraction of organic and inorganic compounds. In the case of metal analysis, extraction of them as ammonium pyrrolidin dithiocarbamate (APDC) complexes using methyl isobutyl ketone (MIBK) as extraction solvent is known as Standard method. Other examples for application of LLE in metal analysis are briefly described here; Extraction of As (inorganic and organic) in urine and water samples as their iodide salts extracted in chloroform and re-extracted in dilute dichromate solution for total As determination by electrothermal atomic absorption spectrometry (ET-AAS) was reported (Fitchett et al., 1975). Another method for extraction of As (inorganic and organic) is based on extraction to toluene and back extraction with cobalt nitrate solution (Lauwerys et al. 1979). A simple LLE method for extraction of methylmercury was introduced by using toluene as extraction solvent before analysis by ET-AAS (Saber-Tehrani et al. 2007). Ease of operation and simplicity of the method are advantages of LLE. But important disadvantages such as consumption of large volumes of expensive and toxic solvents, emulsion formation at the interface of the two phases and difficult phase separations and finally low concentration factor lead the analytical chemists to introduce alternative methods for LLE by decreasing volume of the extracting solvent at micro liter scale known as liquid phase microextraction (LPME) which introduced in the late 1990s and early 2000s.

1.2 Solid Phase Extraction (SPE)

One of the alternative methods for LLE is solid phase extraction (SPE) which was introduced in early 1970 and developed during 1980-90. SPE process is based on distribution of analytes between solid sorbent packed in a cartridge and liquid sample which moves through the solid phase. Solid phase usually consists of small porous particles of silica with or without bonded organic phase, organic polymers and ion exchangers. Mechanisms of extractions are based on adsorption, partitioning or ion exchange according to kind of solid phase. SPE is used for extraction of both of the organic and inorganic compounds. A wide group of chemicals which their SPE procedures were reported in the literature is metal ions.

Main SPE methods for metal ions are summarized in Table 1 (Fritz, 1999).

Increasing development of SPE during 1990s continues until recent years by introducing novel solid sorbents such as molecularly imprinted polymers (Lucci et al., 2010) and nanostructured materials (Faraji et al., 2010). SPE has many attractive features in comparison with classical solvent extraction methods. However, it has its limitations. Some of the main limitations of SPE are listed below:

1. Clogging the pores of the solid phase by large biomolecules, oily materials and fine solids in the sample.
2. Despite, decrease in solvent consumption in SPE in comparison with LLE, SPE needs at least 100 μL of the solvent.
3. It is time consuming method due to several steps of operation including; conditioning, sample loading and elution

Despite popularity of the SPE, miniaturization of it caused to introduction of solid phase microextraction (SPME) (Arthur et al. 1990).

Metal ion	Extracted as	Solid Phase
Mo(VI), W(VI), Ta(V), Nb(V), Ti(IV), V(IV), V(V)	Hydrogen Peroxide complexes	Cation- exchanger
+2, +3 and +4 cations	Bromide complexes	Strong-acid cation-exchanger
Al(III), Mo(VI), Nb(V), Sn(IV), Ta(V), Ti(IV), U(VI), W(VI), Zr(IV)	Fluoride complexes	Cation- exchanger
Fe(III)	Chloride complexe	Haloport-F
Mo(VI)	Chloride complexe	Granular polymer impregnated with MIBK
Au(III)	Chloride complexe	Amberchrome resin
Cd(II), Co(II), Cu(II), Fe(II), Ni(II), Pb(II)	pyrrolidin dithiocarbamat complexes	C18
Cu(II), Fe(III), U(VI), Al(III)	Chelate with solid phase	Hydroxamic Acid resins (Chelating resin)
Ag(I), Au(III), Bi(III), Cd(II), Cu(II), Fe(III), Hg(II), Pb(II), Sb(III), Sn(IV), U(VI), Zn(II)	Chelate with solid phase	Thioglycolate resins (Chelating resin)
U(VI)	Chelate with solid phase	Cellulose phosphate ion exchanger (Chelating resin)

Table 1. SPE techniques for metal ion analysis.

2. Microextraction techniques

In order to get rid of limitations of classical sample preparation methods and reach to an ideal method, miniaturization of extraction techniques is necessary. The first step in miniaturization is reducing volume of extraction solvent in LLE (Liquid Phase Microextraction). Different ways of this miniaturization causes various modes of LPME like single drop microextraction, dispersive liquid-liquid microextraction, hollow fiber based supported liquid membrane microextraction and liquid phase microextraction based on solidification of floating organic drop. Miniaturization was also applied to SPE. Product of this process is known as solid phase microextraction. Review of scientific literature shows that design; development and applications of microextraction techniques are growing rapidly. Popularity and applicability of microextraction techniques requires discussing these subjects in books.

2.1 Solid Phase Microextraction (SPME)

Solid phase microextraction as a solvent free alternative method for conventional sample preparation methods was introduced (Arthur et. al., 1990). In SPME, a small volume of extraction phase (usually less than 1 μL) coated on fused silica support is mounted in a modified Hamilton 7000 series syringe. Extraction phase could be a high molecular weight polymeric liquid or a solid porous sorbent with high surface area. Fig. 1 illustrates the structure of a SPME device manufactured by Supelco Company. A stainless steel tube was

replaced with inner wire of syringe needle and fiber was installed on inner tube. With pulling the syringe plunger in, the fiber is protected in the needle and with pulling out; the fiber is exposed to the sample. SPME process is carried out in three modes. Headspace mode which fiber is exposed into the headspace of the sample suitable for volatile analytes, direct mode which fiber is immersed directly in the sample suitable for nonvolatile analytes and direct mode with membrane protection suitable for biological or dirty samples.

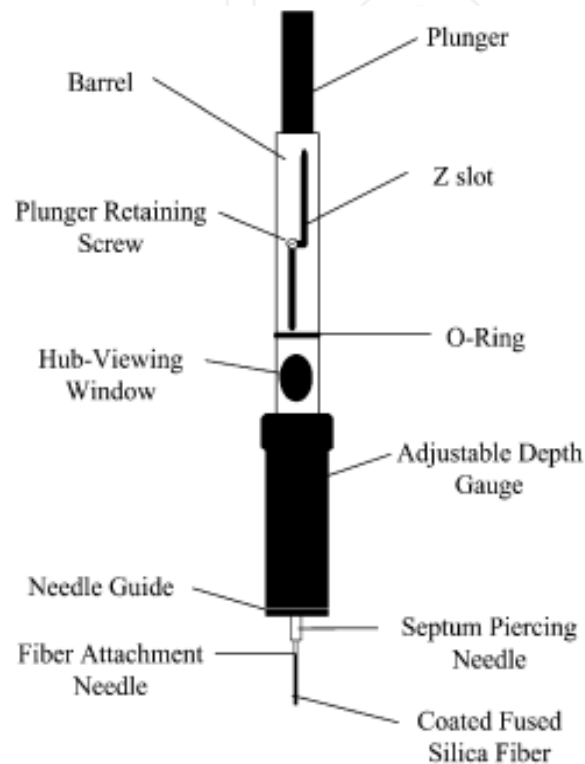


Fig. 1. SPME device manufactured by Supelco Co. (Mester et. al., 2005)

2.1.1 Theoretical aspects of SPME

SPME is based on distribution of the analytes between the sample and the fiber. Based on nature of fiber coating, mechanism of microextraction differs. Mechanism of microextraction for polymeric liquid phases and solid sorbents are partitioning and adsorption, respectively. Amount of extracted analytes depend on distribution constant (D) of them.

$$D = K_d \frac{V_2}{V_1} \quad (1)$$

Fraction of extracted analyte from aqueous sample to the fiber is calculated from Eq. 2

$$F_{ex} = \frac{D}{1+D} = \frac{K_d V_2}{K_d V_2 + V_1} \quad (2)$$

2.1.2 Factors affecting SPME

In order to reach equilibrium conditions in SPME, factors such as nature of fiber coating, time and temperature of extraction, time and temperature of desorption, salt addition,

sample agitation and solution pH must be optimized. One of the most advantages of SPME is ability for hyphenation with various analytical instruments. The most capable instrument with SPME is gas chromatograph. But HPLC, CE and AAS had been coupled with SPME. According to subject of this book, we will focus on coupling of SPME with AAS. Direct coupling of SPME with AAS can be carried out using hydride generation apparatus, including quartz tube equipped with electric heater as flow cell. So due to this system carrier gas flow is another parameter which needs to investigate.

2.1.2.1 Fiber coating selection

The first step in SPME is selection of appropriate fiber. SPME fiber coatings with different natures including polar, non-polar and semi polar are available. According to chemical nature of the analytes, the best fiber must be selected. Polydimethylsiloxane (PDMS) is the most useful coating for SPME fibers which is commercially available. Now a days, in addition to commercial fibers, various coating compositions are made at laboratories. Therefore analysts can select appropriate available fibers or design novel coatings suitable for their aims.

2.1.2.2 Microextraction temperature

Temperature has a major effect on efficiency of SPME. Increasing the temperature causes an increase in distribution coefficient of the analytes between the sample and the fiber. In the case of headspace SPME, it causes increase in distribution coefficients between the sample and the headspace and between headspace and the fiber. But temperature is a limiting parameter, because increasing the temperature more than a certain value causes a significant decrease in distribution coefficient of the analytes between the sample (direct SPME) or headspace and the fiber and results decrease in amount of extracted analytes.

2.1.2.3 Microextraction time

Exposure time of the fiber in the headspace of samples is usually kept long enough to achieve equilibrium between the headspace and the adsorbent in order to maximize the extraction efficiency.

2.1.2.4 Desorption temperature and time

In order to transfer the analytes from the fiber to the analytical instruments like GC and AAS, thermal desorption is the best method. So desorption temperature and time of fiber duration in the desorption chamber (injection port and heated quartz tube for GC and AAs, respectively) must be studied.

2.1.2.5 Sample agitation

Various modes of agitation could be applied to the sample for faster achieving the equilibrium. Magnetic stirring and sonication are usual methods for transporting the analytes from bulk of the sample solution to the surface of the fiber. Power and time of sonication or rate of magnetic stirrer must be adjusted at different levels for selection of optimum condition.

2.1.2.6 Salting out effect

Addition of an inorganic salt has often been used in order to enhance the activity coefficients of volatile components in aqueous solutions, increasing the concentration in the headspace

vapor. The salts are also added to equalize the activity coefficients of analytes in different matrices (Zuba et al., 2001). For this purpose, microextraction processes are carried out in presence of various salt concentrations and also in salt less solution. Results demonstrates role of salt addition in microextraction of target analytes.

2.1.3 Application of SPME for extraction of metallic analytes before atomic absorption spectrometric determination

SPME can be coupled with AAS easily via heated quartz tube. Quartz tube flow cells equipped with electric heater usually used for mercury determination and hydride generation techniques. Quartz tube AAS (QT-AAS) not only is used for direct coupling of SPME with AAS but also it can be used as gas chromatographic detector (SPME-GC-QT-AAS). This technique is mostly reported for determination of organometallic compounds or derivatized metals as organometallics. A method for determination of organomercury species based on solid phase microextraction after hydride generation using KBH_4 and determination using GC-QT-AAS was reported. As mentioned above, in the case of mercury hydrides commercial fibers are not suitable and researchers had to design a novel fiber based on acid treated fused silica. Suitable capillary column for separating mercury hydrides is CPL-SIL 5CB (10 m \times 0.25 mm) under 40 °C isothermal condition (He, et al., 1998). This method was applied for determination of methylmercury in biological samples and sediments. Another similar method was reported for determination of methyl, ethyl and phenyl mercury species in soil samples using above mentioned method (He, et al., 1999). Recently successful efforts were done for direct coupling of SPME with QT-AAS (Fragueiro et al., 2004). In this method direct coupling between headspace SPME and QT-AAS was evaluated for speciation of methylmercury in seafood after volatilization of its hydride or chloride derivatives. The best limit of detection (LOD) for methyl mercury obtained by using PDMS/DVB fiber coating as 0.06 ng mL⁻¹.

2.2 Single Drop Microextraction (SDME)

The LPME is a miniaturized version of the LLE in which the extraction solvents, volume reduced to about 1-10 μL . Various techniques are known as LPME. Single drop microextraction (SDME) is one of these methods in which extraction solvent is a single drop. Jeannot and cantwell was reported the SDME for first time by suspending an 8 μL organic solvent drop at the end of Teflon rod immersed in the stirring sample. After extraction, solvent drop was removed from the end of the Teflon rod using a micro syringe and injected to analytical instrument (Jeannot and cantwell, 1996). Fig. 2 shows the schematic SDME system.

Some modifications were made by He and Lee on primary reported method. In newer version of SDME, teflon rod was replaced by a micro syringe (Fig. 3). A one μL immiscible extracting solvent drop is exposed into the sample (liquid or gaseous) from a micro syringe. After establishment of distribution equilibrium the organic drop is retracted back into the micro syringe and is injected to the analytical instrument for determination of the analytes (He and Lee, 1997). SDME is suitable for coupling with various instrumental methods such as gas and liquid chromatography (GC & HPLC), atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP).

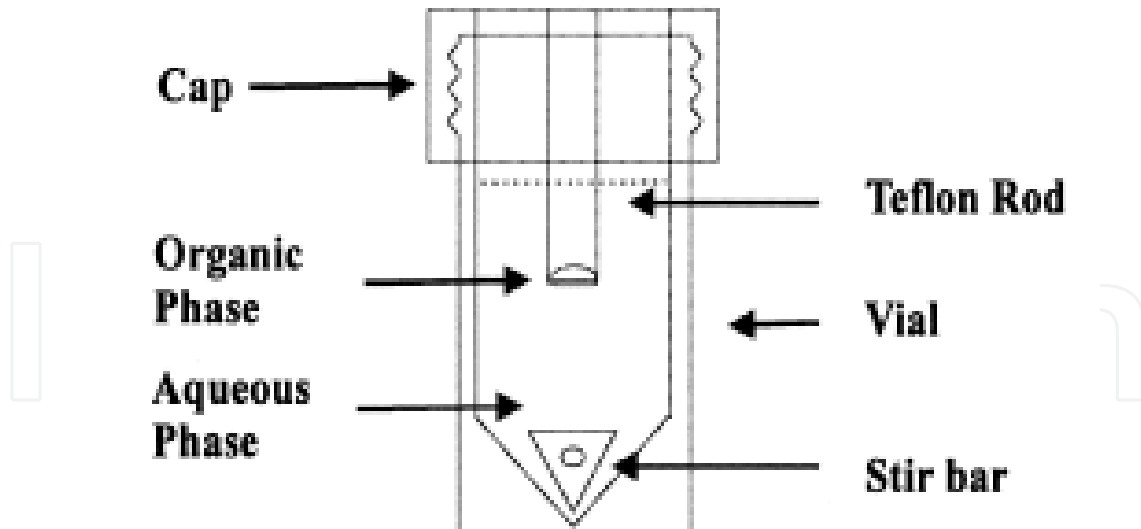


Fig. 2. Illustration of SDME method reported by Jeannot and cantwell (Jeannot et. al., 1996)

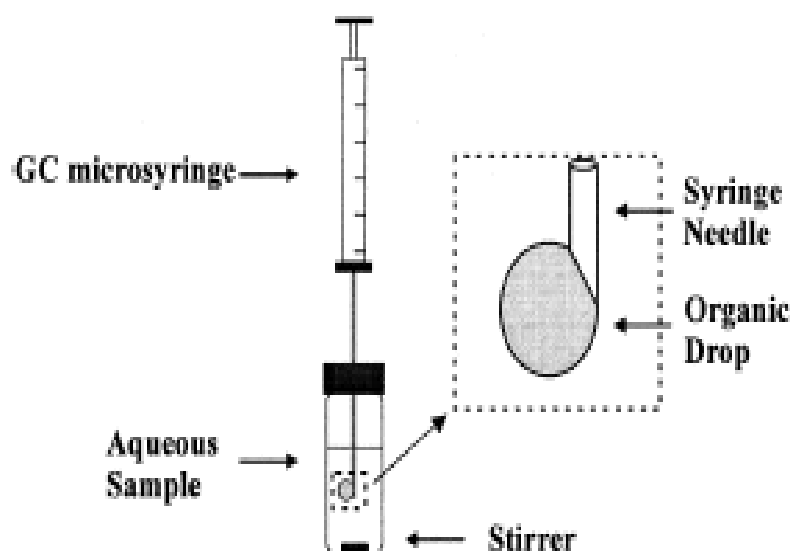


Fig. 3. Schematic diagram of SDME using micro syringe (He et. al., 1997)

Some of the advantages of the SDME are:

- SDME is a cheap technique
- SDME needs simple equipment
- Its operation is easy
- Use of minimum amounts of solvents which introduces the technique as a green approach to sample preparation
- In situ derivatization of the analytes is possible

In addition to above mentioned advantages, instability of the drop, small surface of the drop and slow kinetics of extraction are disadvantages of the method (Dadfarnia and Haji Shabani, 2010). Like SPME, SDME can be operated in various modes known as direct immersed SDME (DI-SDME), headspace SDME (HS-SDME) and three phases SDME.

2.2.1 Theoretical aspects of SDME

Extraction of the analytes via SDME process is an equilibrium phenomenon. Microextraction conditions must be optimized for establishment of thermodynamic equilibrium of analyte partitioning between the sample and extracting phase. In order to better understanding of microextraction process a model was introduced. This model is based on dynamic mass balances for the analytes in each phase or both. Mass balance equation for SDME is presented as equation (3).

$$C_A^{aq}V^{aq} + C_A^oV^o = C_{A,o}^{aq}V^{aq} \quad (3)$$

Where C_A^{aq} and C_A^o are the concentrations of the analyte in the sample and the microdrop respectively; V^{aq} is the sample volume and V^o is the microdrop volume, and $C_{A,o}^{aq}$ is the initial concentration of the analyte in the aqueous sample. The dynamic mass balance of the analyte in the microdrop is given by following equation:

$$\frac{d(C_A^oV^o)}{dt} = k_{tot}^o A_i [K_A C_A^{aq} - C_A^o] \quad (4)$$

Where A_i is the interfacial area (the surface area of the microdrop). K_A is the equilibrium partition coefficient and k_{tot}^o is the total mass transfer coefficient of the analyte with respect to the organic phase. If the two-film theory is considered, k_{tot}^o is given by:

$$\frac{1}{k_{tot}^o} = \frac{1}{k^o} + \frac{K_A}{k^{aq}} \quad (5)$$

Where k^o and k^{aq} are the mass transfer coefficients for the analyte in the film of the organic and the aqueous phases respectively. If we consider the volume of microdrop constant, then A_i is also constant. So we can obtain Eq. 4 as result of combination of Eq. 1 and 2. Eq. 6 presents C_A^o as a function of time.

$$C_A^o(t) = C_{A,eq}^{o,aq} [1 - e^{-\lambda t}] \quad (6)$$

Where $C_{A,eq}^{o,aq}$ is the analyte concentration in the microdrop at equilibrium and λ is the rate constant.

2.2.2 Factors affecting SDME

In order to reach equilibrium status, all the factors affecting microextraction process must be optimized. Kind and amount of organic solvent drop, extraction time and temperature, salting out and agitation rate are important factors.

2.2.2.1 Kind and volume of extraction solvent

In SDME solvent selection is the most important parameter. Selectivity and extraction efficiency directly depends on solvent's nature. Solvents with different polarities must be examined for extraction of studied analytes. Volume of the selected solvent is also important. Solubility of the analytes and partitioning of them in extraction solvent depends on volume of solvent microdrop. Increase in solvent volume, increases the amount of

extracted analytes but after reaching quantitative recoveries, increasing the solvent volume causes a significant decrease in concentration factor due to dilution of the analytes. On the other hand, hanging of large volumes of organic solvents made the microdrop unstable. So in SDME solvent volume is a limited parameter.

2.2.2.2 Extraction time

Exposure time of the microdrop to the samples is an important parameter in achieving distribution equilibrium of analytes between solvent drop and sample; it is a decisive factor for improving the extraction efficiency. So it is necessary to be optimized. But according to difficulty of microdrop expose to the sample in this technique, long extraction times are not preferred.

2.2.2.3 Extraction temperature

As mentioned before, SDME process is a thermodynamic equilibrium, so effect of temperature is not negligible. But extraction temperature in SDME, strongly limited by solvent's boiling point.

2.2.2.4 Salt addition

Addition of salt to the sample increases the ionic strength of the solution. Depending on the solubility of the target analytes, extraction is usually enhanced with increased salt concentration (salting out effect). But in the case of SDME, presence of salt changes the physical properties of the extraction film and reduces the diffusion rates of the analytes into the solvent drop. So, salt addition has a negative effect on method efficiency.

2.2.2.5 pH Adjustment

Effect of pH on extraction efficiency, depends on analyte nature. Extraction of analytes with weak acidity and basicity is strongly pH sensitive. Solution pH easily adjusted by appropriate buffers according to the pKa values of the analytes.

2.2.2.6 Sample agitation

Similar to the other extraction methods, sample agitation has a significant effect on enhancement of extraction yield. Increase in agitation rate, decreases the extraction time due to faster establishment of the distribution equilibrium. But despite positive effect of higher agitation rates, it is so critical parameter in SDME because instability of microdrop on syringe tip.

2.2.3 Application of the SDME for extraction of metal ions before atomic absorption spectrometric determination

Due to low volumes of extraction solvents in SDME, electrothermal atomic absorption spectrometry is suitable for determination of metals after extraction with SDME technique. The first coupling of SDME with ET-AAS was reported for extraction of As in aqueous samples. Total arsenic species were converted to As (III) using NaBH_4 and extracted by a 4 μL organic drop consisting pyridine and benzyl alcohol containing silver diethyldithiocarbamate (AgDDC) as complexing agent with arsine (Chamsaz et al., 2003). Palladium is a good sorbent for arsenic and act as matrix modifier in ET-AAS. So a green headspace SDME method for extraction of As (III) and total As was presented using a 3 μL aqueous drop of Pd (30 mg L^{-1}) as extraction solvent prior to determination by ET-AAS

(Fragueiro et al, 2004). This method is faster than previously reported method by Chamsaz et al, but both of the concentration factor and detection limit of previous method is better.

Another green method using aqueous micro drop of Pd (II) and Pt (IV) was reported for determination methylmercury in fish samples. Volatilization of methylmercury was performed using hydride generation reaction and after extraction analysis was done by ET-AAS (Gil et al., 2005). Microextraction of Se using SDME prior to ET-AAS analysis was reported by photogeneration of volatile hydride and alkyl Selenium derivatives. Aqueous microdrop containing Pd (II) was used as extracting phase (Figueroa et al., 2005). A series of SDME methods were reported for lead determination in various samples by ET-AAS. A microdrop of benzene containing 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP) was used for extraction of Pb (II) from biological samples and its determination by ET-AAS (Liang et al., 2008). Dithizone is another extractant in SDME which was used for microextraction of lead from water samples prior to assay by ET-AAS (Liu and Fan, 2007). Application of ionic liquids as novel extractants in SDME was reported for determination of manganese and lead. Complex of manganese with 1-(2-thiazolylazo)-2-naphthol (TAN) was extracted into a microdrop of $[C_4MIM][PF_6]$ (Manzoori et al., 2009). The same group extracted lead ions after complexation with ammonium pyrrolidine dithiocarbamate (APDC) into the same ionic liquid (Manzoori et al., 2009). Simultaneous direct SDME of Cd and Pb after complexation with dithizone in aqueous samples into toluene microdrop followed by determination with ET-AAS was reported (Jiang and Hu et al., 2008). Ag, Tl, Cr and Sb are another metals which the SDME-ET-AAS method was reported for them.

2.3 Dispersive liquid-liquid microextraction (DLLME)

Dispersive liquid-liquid microextraction (DLLME) is a newer mode of LPME (Rezaee, et al., 2006). DLLME is a ternary solvent system consisting of aqueous sample solution, extraction solvent and disperser solvent. Extraction solvent must be immiscible with aqueous sample solution and disperser solvent must be soluble in both of the extraction solvent and aqueous sample solution. In DLLME, 5-10 mL of sample solution is placed in a test tube with conical bottom, then optimized volumes of extraction solvent (μL) and disperser solvent (mL) are mixed and mixture of these solvents is injected rapidly in the sample solution. After injection of solvents mixture a stable cloudy suspension consisting of fine droplets of extraction solvent is appeared which can be easily separated by centrifugation (Fig. 4). Aqueous phase is discarded and sedimented organic phase is transferred to an analytical instrument using a micro syringe. Early works on DLLME were based on denser solvents than water (mostly chlorinated solvents) but later DLLME methods based on lighter extraction solvents than water were reported (Farajzadeh, et al., 2009).

Some of the advantages of DLLME are listed below:

- Simple, fast and cheap
- High preconcentration factor
- High recovery of the analytes
- Small volumes of the sample are needed
- Minimum volumes of the organic solvents (μL) are used
- Easy coupling with most of the instrumental methods

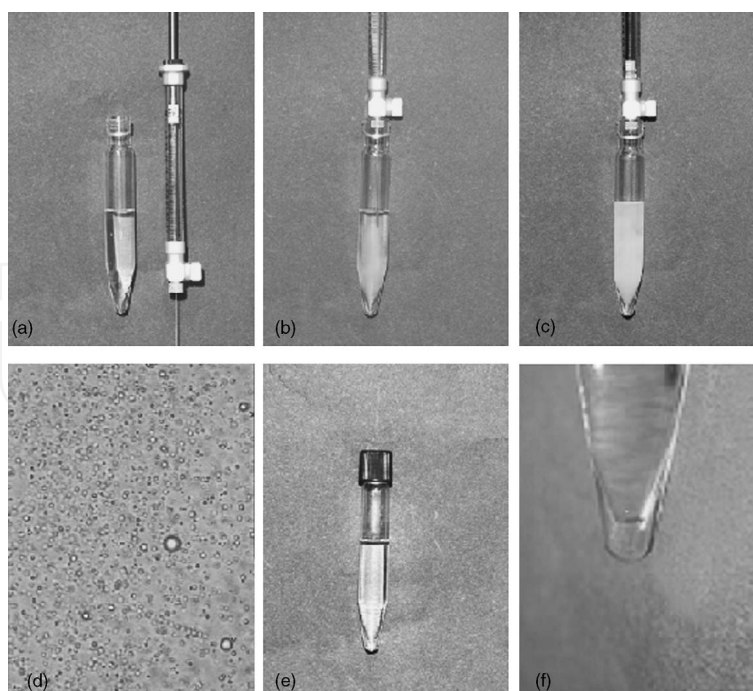


Fig. 4. Schematic illustration of a DLLME process: a) prior to injection of extraction and disperser solvents b) starting the injection c) end of injection, cloudy suspension was made d) fine droplets of solvents in solution bulk e) after centrifugation f) conical bottom of the test tube with sedimented phase (Rezaee et. al., 2006)

2.3.1 Theoretical aspects of DLLME

In order to design an accurate DLLME process, some terms must be identified:

Preconcentration or enrichment factor is the ratio of the analyte concentration in sedimented phase (C_{sed}) to analyte concentration in the sample (C_0)

$$EF = \frac{C_{sed}}{C_0} \quad (7)$$

Extraction recovery of the analyte (%) is the percentage of extracted analyte in sedimented phase, where n_0 is the amount of analyte in the sample prior to extraction and n_{sed} amount of analyte in sedimented phase.

$$ER = \frac{n_{sed}}{n_0} \times 100 = \frac{C_{sed} \times V_{sed}}{C_0 \times V_0} \times 100 \quad (8)$$

$$ER = \left(\frac{V_{sed}}{V_{aq}} \right) EF \times 100 \quad (9)$$

2.3.2 Factors affecting DLLME

Like every other methods several experimental factors affect DLLME process. For development of a sensitive and accurate DLLME method these parameters including Kind and volume of extraction solvent, kind and volume of disperser solvent, extraction temperature, solution pH and salting out must be optimized.

2.3.2.1 Kind and volume of extraction solvent

It is clear that extraction solvent is the most important parameter in a LPME method. As we discussed above, extraction solvent must be immiscible with water and must be a good solvent for the analytes. Solubility of the extraction solvent in water has an inverse relation with stability of cloudy suspension. Volume of extraction solvent is important too, because it is obvious that with increasing extraction solvent volume, amount of extracted analytes increase. But when we reach to quantitative recoveries, increase of extraction solvent volume causes a significant decrease in an analytical signal due to dilution and reducing enrichment factor. Extraction solvent can be a denser or lighter solvent than water. Usually denser solvents are tetrachloroethylene, trichloroethylene, carbon tetrachloride and etc.

2.3.2.2 Kind and volume of disperser solvent

Disperser solvents usually are polar solvents such as acetone, acetonitrile and methanol. Disperser solvent acts as a bridge between two immiscible phases. The role of an ideal disperser solvent is making more fine droplets of extraction solvent and dispersion of extraction phase in sample bulk. Amount of disperser solvent is also important. Increasing in disperser solvent volume, increases the volume of sedimented phase and naturally lowers the analytical signal due to dilution. So, optimization of disperser solvent volume is as important as extraction solvent volume.

2.3.2.3 Extraction temperature and time

Temperature is an important parameter in all of the equilibrium systems. But in DLLME temperature is a critical parameter. Because boiling point of the extraction and disperser solvents are limited the process. Extraction time is contact time between the sample and extraction solvent. It is clear that extraction time directly depends on extraction efficiency. But in the case of DLLME, time is not so important parameter due to high surface contact between the sample and fine droplets of extraction solvent. So in most of the reports, time has no effect on extraction efficiency.

2.3.2.4 Salting out

Despite other extraction methods, in DLLME salting out has dual effect. Similar to other extraction techniques addition of an inorganic salt, causes an increase in amount of extracted analytes. But in DLLME salt addition causes a significant increase in volume of sedimented phase. So effect of salt addition on extraction efficiency are controlled by these two factors.

2.3.2.5 Complexation of metal ions prior to extraction

Similar to SDME, extraction of metal ions with organic solvent needs to convert them to suitable form. Appropriate chelating agents must be selected and complexation conditions such as solution pH, concentration of ligand and etc, must be adjusted. It is clear that extraction of metal ions after conversion of them to organometallic compounds is possible.

2.3.3 Application of DLLME for extraction of metal ions before determination by AAS

A simple and powerful microextraction technique was used for determination of selenium in water samples using dispersive liquid-liquid microextraction (DLLME) followed by ET-AAS (Bidari et al., 2007). In this study, complex of Se and APDC extracted by a mixture of

ethanol (disperser solvent) and carbon tetrachloride (extraction solvent) from water samples. The concentration of enriched analyte in the sedimented phase was determined by iridium-modified pyrolytic tube graphite furnace atomic absorption spectrometry. Determination of trace levels of lead is possible with DLLME followed by ET-AAS (Liang and sang, 2008). In the proposed approach, 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP) was used as a chelating agent, and carbon tetrachloride and ethanol were selected as extraction and dispersive solvents. Another simple DLLME-Flame AAS method was reported for determination Pd(II) as complex with thioridazine HCl (Ahmadzadeh Kokya and Farhadi, 2009). Ethanol as disperser solvent and chloroform as extraction solvent were used in this study.

Extraction of Co(II) as its complex with Br-TAO via a DLLME method was reported (Baliza et al., 2009). The procedure is based on a ternary system of solvents, where appropriate amounts of the extraction solvent, disperser solvent and the chelating agent Br-TAO are directly injected into an aqueous solution containing Co(II). A cloudy mixture is formed and the ions are extracted in the fine droplets of the extraction solvent. After extraction, the phase separation is performed with a rapid centrifugation, and cobalt is determined in the enriched phase by FAAS.

Use of ionic liquids is a novel development in DLLME. A new ionic liquid based DLLME method was developed for preconcentration and determination of Pb (II) and Cd (II) in aqueous samples containing very high salt concentrations (Yousefi and Shemirani, 2010). This is believed to arise from dissolving of the ionic liquids in aqueous samples with high salt content. In this method, the robustness of microextraction system against high salt concentration (up to 40%, w/v) is increased by introducing a common ion of the ionic liquid into the sample solution. The proposed method was applied satisfactorily to the preconcentration of lead and cadmium in saline samples. After preconcentration, the settled IL-phase was dissolved in 100 μ L ethanol and introduced to Flame-AAS.

2.4 Liquid phase microextraction based on solidification of floating organic drop (LPME-SFO)

Liquid phase microextraction based on solidification of floating organic drop (LPME-SFO) is one the newest versions of LPME (Khalili-Zanjani et al., 2007) in which the extraction solvent with lower density than water, low toxicity and proper melting point near room temperature (in the range of 10–30 °C) was used. In this method, small volume of an extraction solvent is floated on the surface of aqueous solution. The aqueous sample solution is agitated for an optimized time. After the extraction, tube containing the sample is transferred in the ice bath and the floated extractant droplet solidified at low temperature. The solidified organic solvent can be separated from the sample and melted quickly at room temperature, which is then determined by appropriate analytical methods (Fig. 5).

In comparison with other methods, LPME-SFO has some advantages which is listed below:

- Simplicity of operation
- Small amount of low toxic solvent used
- Good repeatability
- Low cost
- High preconcentration factors
- More suitability for the analysis of complex matrix samples.

LPME-SFO is only applicable for the analytes with high or moderate lipophilic property and can not be used to those neutral analytes with high hydrophilic property and this is the main disadvantages of the method.

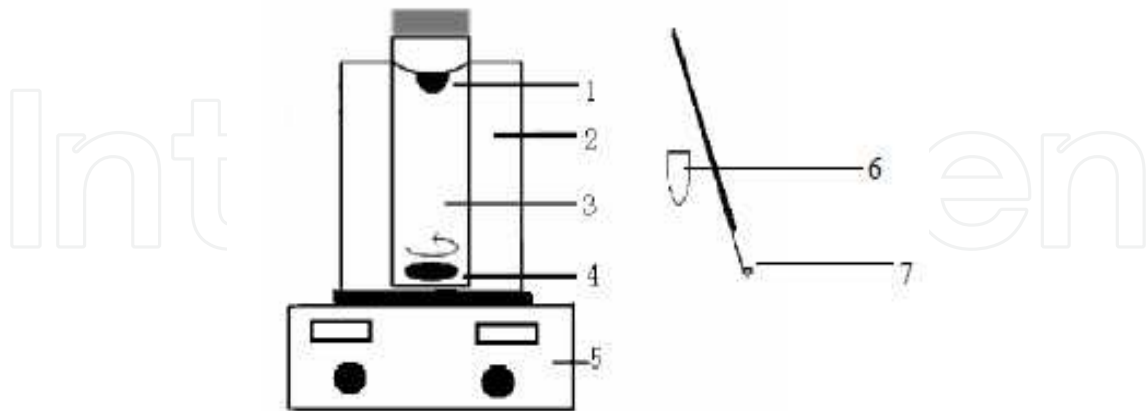


Fig. 5.1 Floated organic solvent, 2) Water bath, 3) Sample, 4) Stirring bar, 5) Heater-Stirrer, 6) Conical vial, 7) Spatula (Ying-Ying et. al., 2010)

2.4.1 Theoretical aspects of LPME-SFO

LPME-SFO is based on the distribution of the analytes between floated extraction solvent and the aqueous sample matrix. As mentioned previously, the organic extraction solvent of LPME-SFO must have the melting point near room temperature (in the range of 10–30 °C) and lower density than water. It should be readily solidified at low temperatures, and thus its droplet can be collected easily. Maximum sensitivity and precision were obtained by stirring the sample solution until the equilibrium was obtained.

Distribution coefficient (K) is defined as the ratio between the analyte concentration in extraction solvent and sample solution. The enrichment factor (ER) and extraction recovery (ER) are calculated as follows:

$$EF = \frac{C_{o,F}}{C_{aq}} \quad (10)$$

Where $C_{o,F}$ and C_{aq} are the analyte concentration in the organic solvent, and the initial concentration of the analyte in the aqueous sample, respectively.

$$ER(\%) = 100 \times \frac{C_{o,F} V_{o,F}}{C_{aq} V_{aq}} \quad (11)$$

Where $V_{o,F}$ and V_{aq} are the volume of the organic phase and the volume of the aqueous sample, respectively.

2.4.2 Factors affecting LPME-SFO

The partitioning of the analytes between two phases (extraction solvent and the aqueous solution) was affected by various parameters, such as the type and volume of the organic solvent, aqueous sample volumes, the extraction time, the stirring rate, and salt addition. In order to design an accurate method, every parameter must be optimized.

2.4.2.1 Selection of extraction solvent

The selection of an appropriate extraction solvent is of major importance for the optimization of the LPME-SFO process. The selected extraction solvent must satisfy several requirements. First, it should be immiscible with water, have low volatility, low density, low melting point near room temperature and be able to extract the desired analytes. According to these considerations, limited number of the organic extraction solvents commonly used in LPME-SFO which are listed below. 1-Undecanol (13-15 °C), 1-Dodecanol (22-24 °C), 2-Dodecanol (17-18 °C), 1-Bromo hexadecane (17-18 °C), n-Hexadecane (18 °C) and 1,10-Dichlorodecane (14-16 °C).

2.4.2.2 Effect of extraction temperature

Generally, increasing sample solution temperature has a positive effect on extraction efficiency. Based on the extraction kinetics, higher temperatures would facilitate the diffusion and mass transfer of the analytes from sample solution to the organic solvent, and the time required to reach the equilibrium would be decreased. However, at high temperatures, the over-pressurization of the sample vial could make the extraction system unstable. On the other hand in LPME boiling point of the solvents is a limiting factor.

2.4.2.3 Effect of organic solvent volume

Similar to other LPME methods, increasing organic solvent volume increases extraction recovery. But after reaching quantitative recoveries, increasing solvent volume causes a significant decrease in enrichment factor. Usually in LPME-SOF, 5–100 µL of extraction solvent is selected.

2.4.2.4 Effect of stirring rate

For the SFO-LLME, sample agitation is an important parameter that influences the extraction efficiency. According to the film theory of convective-diffusive mass transfer for LPME system, high stirring speed can decrease the thickness of the diffusion film in the aqueous phase, so the aqueous phase mass-transfer coefficient will be increased with increased stirring speed (rpm). But increasing stirring rate must be controlled, because it may be cause to sputtering of the solvent drops and influence the extraction efficiency.

2.4.2.5 Effect of extraction time

LPME is not an exhaustive extraction, so extraction time has great effect on extraction efficiency, precision, sensitivity, and the repeatability of the LPME-SFO. It is necessary to choose an appropriate extraction time to guarantee the equilibrium between aqueous and organic phases and the maximum extraction of analytes.

2.4.2.6 Salting out

As described before, salt addition can improve the extraction efficiency of the analytes due to salting out effect. But higher salt concentration can be affect physical properties of the extraction film. This causes reducing in the diffusion rates of the analytes into the organic phase. Therefore, the amount of salt should be optimized in LPME-SFO.

2.4.3 Application of LPME-SFO for extraction of metal ions before determination by AAS

LPME-SFO can be used for extraction of metal ions from aqueous solution in combination with ET-AAS. A LPME-SFO technique was used for the extraction of lead in water samples (Dadfarnia et al., 2008) 1-undecanol containing dithizone as the chelating agent was used as

floated organic drop for extraction of lead ions. After stirring the sample for a certain time, the sample vial was cooled in an ice bath for 5 min. The solidified extract was transferred into a conical vial where it melted immediately, and then 10 μL of it was analyzed by ET-AAS. Another LPME-SFO method was designed for extraction of Co(II) and Ni (II) as complexes with 1-(2-Pyridylazo)-2-naphthol (PAN) as chelating agent (Bidabadi et al., 2009). A highly efficient LPME-SFO method for the determination of arsenic by electrothermal atomic absorption spectrometry (ETAAS) was reported (Ghambarian et al., 2010). In this method extraction of As(III) as its complex with APDC was carried out in ppb level. Another literature reported the same method for the determination of trace lead and cadmium in water samples (Rivas et al., 2010). When LPME-SFO is coupled with flame atomic absorption spectrometry (FAAS), direct injection analysis cannot be performed, since the volume of the extraction solvent is too little for Flame-AAS. For this purpose a LPME-SFO method was proposed for extraction of cadmium ions in different water samples for determination by Flame-AAS (Dadfarnia et al., 2009). In the method, the extraction solution was first diluted with ethanol to 250 μL and then 100 μL of it was analyzed by flow injection flame atomic absorption spectrometry (FI-FAAS).

2.5 Hollow fibre based liquid phase microextraction

An alternative concept for LPME was developed using hollow fiber membranes (Pedersen-Bjergaard, et al., 1999). This technique is based on the use of hollow fibers, typically made of polypropylene. This form of LPME consists of a donor phase (the sample), an acceptor phase (in the lumen of the hollow fiber) and the hollow fiber between them. Pores of the hollow fiber membrane are impregnated with organic solvent and the system is called as supporting liquid membrane. This configuration can be used in two modes; two phase and three phase. In two phase system donor phase is aqueous; acceptor phase is organic with organic solvent in hollow fiber pores. But in three phase system, donor phase and acceptor phase are both aqueous with hollow fiber impregnated with organic solvent (Fig. 6)

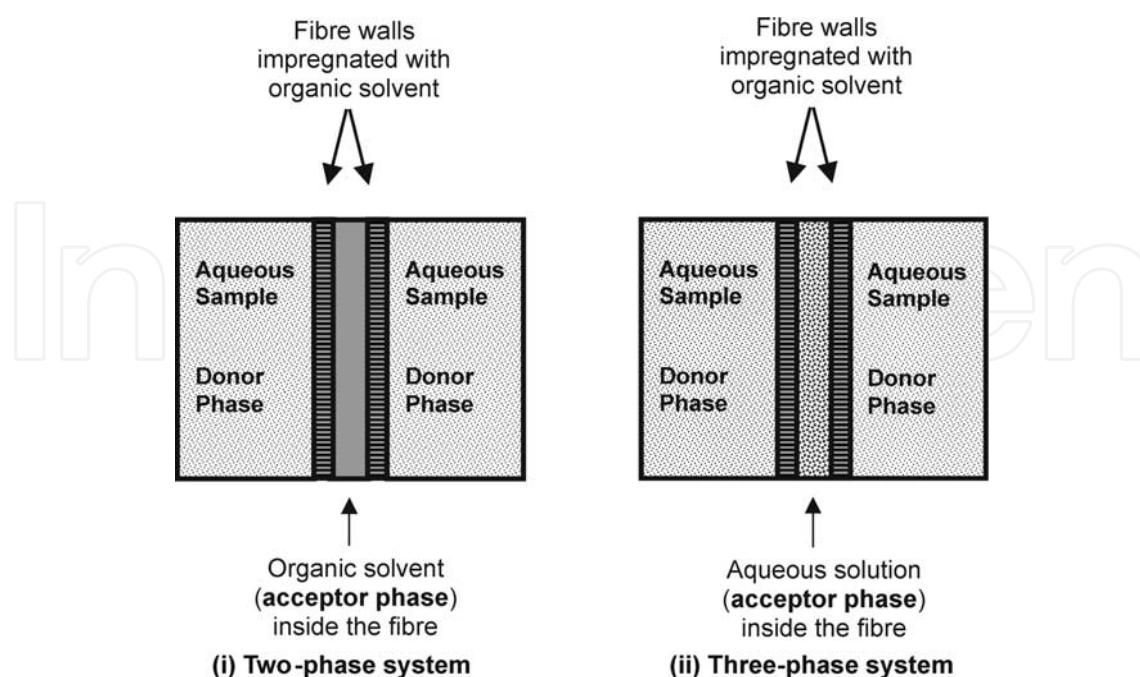


Fig. 6. Illustration of Hollow fiber based LPME (i) two phase (ii) three phase system (Psillakis et. al., 2003)

2.5.1 Theoretical aspects of hollow fiber based LPME

Two phase extraction mode: Extraction through hollow fiber membranes by passive diffusion of analytes from donor phase (the sample) into acceptor phase (extractant) is based on a partitioning equilibrium. Partition coefficient is presented by:

$$K_{a/d} = C_{eq,a} / C_{eq,d} \quad (12)$$

Where $C_{eq,a}$ and $C_{eq,d}$ are analyte concentration at equilibrium in acceptor and donor phases, respectively. Extraction recovery and enrichment factor are given by:

$$R = (100K_{a/d}V_a) / (K_{a/d}V_a + V_d) \quad (13)$$

$$E = (V_dR) / (100V_a) \quad (14)$$

Where V_a and V_d are volume of acceptor and donor phase solutions.

Three phase extraction mode: Similar to two phase mode, this system is based on passive diffusion of analytes through the membrane, too. But two partitioning equilibrium system must be established. The first equilibrium between donor phase and organic phase (impregnated in membrane pores) and second one between organic phase and donor phase.

$$K_{org/d} = C_{eq,org} / C_{eq,d} \quad (15)$$

$$K_{a/org} = C_{eq,a} / C_{eq,org} \quad (16)$$

$$K_{a/d} = C_{eq,a} / C_{eq,d} = K_{org/d} \cdot K_{a/org} \quad (17)$$

Where $C_{eq,org}$ is the analyte concentration in organic phase at equilibrium also $K_{a/d}$ as partition coefficient of the analytes between acceptor and donor phases is the main force for performing the microextraction. Extraction recovery (R) in three phase system can be given as:

$$R = (100K_{a/d} \cdot V_a) / (K_{a/d} \cdot V_a + K_{org/d} \cdot V_{org} + V_d) \quad (18)$$

2.5.2 Factors affecting hollow fiber based LPME

Several parameters affects hollow fiber based LPME. Kind of hollow fiber, nature of organic phase, volume of donor, acceptor and organic phases, pH of donor and acceptor phases, salt addition and sample agitation must be optimized.

2.5.2.1 Kind of hollow fiber

The hollow fibers used in LPME should be compatible with the organic solvent. Polypropylene hollow fibers are the most popular fibers for this purpose. Common dimensions of employed hollow fibers are as listed below. Inner diameter 600 μm , wall thickness 200 μm , nominal and maximum pore sizes are 0.2 and 0.64 μm , respectively. Length of the hollow fiber is based on optimum volume of acceptor phase according to volume of fibers lumen.

2.5.2.2 Organic solvent

An important step in method development for different modes of hollow fiber based LPME is organic solvent selection. Water solubility and polarity are the main characteristics of the

organic solvent which must be considered. To prevent dissolution of it in aqueous phase, it should have minimum solubility. Low volatility is also another important characteristic of the selected organic solvent which will restrict solvent evaporation during extraction. Solubility of the analytes in organic solvents and high partition coefficients of the analytes between two phases are other principle considerations in solvent selection.

2.5.2.3 Agitation of the sample

In order to improve extraction kinetics, use of factors increasing convection of the analytes from solution bulk into the extracting phase is usual. In this method sample and extracting phase are not in direct contact with each other and sample agitation facilitates transfer of the analytes through the supported liquid membrane. Stirring, vibration and sonication are popular agitation techniques. Suitability of the selected agitation method must be considered. Rate and power of agitation must be optimized to prevent air bubble formation around the membrane or accelerate solvent evaporation which causes repeatability and imprecision problems in analysis.

2.5.2.4 Salt addition

In both of the two phase and the three phase LPME methods, salting out effect must be investigated by adding optimized amounts of selected salt to donor phase. Positive effect of salt addition on extraction efficiency depends on nature of the analyte and ionic strength of the sample solution.

2.5.2.5 Volumes of donor and acceptor solutions

It's clear that, the main aim of all preconcentration techniques is reaching to higher enrichment factors. This is available with increasing the ratio of donor phase volume to acceptor phase volume. In this case kind of analytical instrument which LPME is coupled is important. Because various volumes are required for analyte introduction. In the case of the atomic absorption spectrometry (AAS) it depends on the mode of the instrument. For Flame-AAS appropriate minimized volume of the analyte solution is about one mL which is large enough in comparison to the volume of membrane lumen. But electrothermal-AAS is more compatible with this technique due to lower volumes (μL scale) of the samples need to be introduced to graphite furnace.

2.5.2.6 Adjustment of pH

pH of donor and acceptor phases must be adjusted according to the nature of the analytes. In both of the two phase and three phase modes of this technique extraction efficiency has great dependence to solution pH. If the analytes are dissociable in different pHs (weak acids and bases) effect of pH on extraction efficiency must be investigated.

2.5.2.7 Extraction time

In all of the extraction methods, extraction time is an important parameter. Because sample preparation is rate determining step of an analytical method. Mass-transfer of the analytes between two phases is a time consuming process. As discussed before sample agitation is one of the methods which is applied for faster extraction and lower extraction times.

2.5.3 Application of the hollow fiber based LPME for extraction of the metal ions before atomic absorption spectrometric determination

One decade after introduction of hollow fiber based LPME for enrichment of the organic compounds, the first application of this technique for extraction of inorganic compounds

was reported (Xia, et al., 2006). In this study extraction of Se (IV) and Se (VI) from water samples before determination by ICP-MS was investigated. The first report on use of hollow fiber based LPME before AAS is about determination of Cd (II) in sea water using electrothermal AAS (Peng, et al., 2007). In this three phase extraction system dithizone/oleic acid in 1-octanol - HNO₃ was used as extraction phase 0.8 ng L⁻¹ and 387 are LOD and preconcentration factor of the method, respectively. Other two phase and three phase methods for extraction of organomercury and As before electrothermal AAS were reported and summarized in a review article (Dadfarnia, et al., 2010).

2.6 Cloud point extractin (CPE)

Cloud point extraction (CPE), known also as phase separation extraction and surfactant (or micelle)-mediated phase separation is based on the phase behavior of non-ionic surfactants in aqueous solutions, which exhibit phase separation after an increase in temperature or the addition of a salting-out agent. Separation and preconcentration based on cloud point extraction (CPE) are becoming an important and practical application of surfactants in analytical chemistry. The technique is based on the property of most nonionic surfactants in aqueous solutions to form micelles and to separate into a surfactant-rich phase of a small volume and a diluted aqueous phase when heated to a temperature known as the cloud point temperature.

The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap, highly efficient, fast and environmental friendly in comparison with classical methods. CPE might be an interesting and efficient alternative, once it eliminates or reduces consumption of organic solvents significantly. Trace elements can be extracted to the surfactant-rich phase usually after formation of a hydrophobic complex with an appropriate chelating agent (ghaedi et al., 2009).

2.6.1 Surfactants and micelles

Surfactants are compounds that lower the surface tension of a liquid, the interfacial tension between two liquids or between a liquid and a solid. The term surfactant is a blend of surface active agent (Rosen, 2010). Surfactants are amphiphilic molecules, one of whose parts (the head) is polar or hydrophilic in nature and the other (the tail) hydrophobic (Fig. A). This latter part is generally a hydrocarbon chain with different numbers of carbon atoms and may be linear or branched. It may also contain aromatic rings. Therefore, a surfactant molecule contains both a water insoluble (oil soluble component) and a water soluble component. Surfactant molecules migrate to the water surface, where the insoluble hydrophobic groups may extend out of the bulk water phase, either into the air (Fig. B) or if water is mixed with an oil, into the oil phase, while the water soluble head group remains in the water phase. Surfactants in dilute aqueous solutions arrange on the surface. With increasing the concentration of the surfactant the solution surface becomes completely loaded with surfactant and any further additions must arrange as micelles (Fig. C). Therefore, when the surfactant concentration is increased above a certain threshold, called the critical micellar concentration (CMC), the surfactant molecules become dynamically associated to form molecular aggregates of colloidal size. These aggregates containing 60 to 100 monomers are at equilibrium with surfactant molecules in solution with concentration near to CMC.

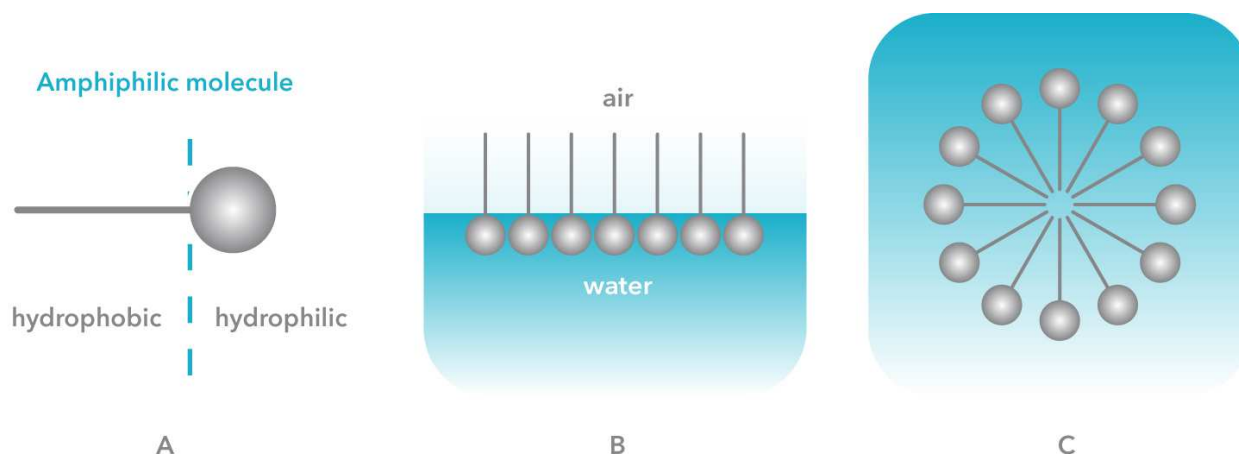


Fig. 7. Schematic representation of the surfactant and formation of a micelle from its monomers beyond its critical micellar concentration (CMC).

2.6.2 Phase separation in CPE

When a micellar solution of a non-ionic surfactant is heated, it becomes turbid over a narrow temperature range, which is referred to as its cloud-point temperature (Hinze & Pramauro, 1993). Above the cloud-point temperature, the system initially in an isotropic phase is separated into two isotropic phases, one of them surfactant-rich phase which is separated from the bulk aqueous solution; and the other aqueous phase, in which the surfactant concentration will be approximately equal to the critical micelle concentration. The phenomenon is reversible and upon cooling, a single phase is again obtained. The mechanism by which separation occurs is poorly understood but some authors have explained the cloud point phenomenon on the basis of the dehydration process that occurs in the external layer of the micelles of non-ionic surfactants when temperature is increased (Hinze, 1987).

2.6.3 Experimental procedure of CPE in metal analysis

The extraction process of CPE for metal ions is very simple and is shown in Fig. 7. First, a few ml of the surfactant or a concentrated surfactant solution is added to some tens to hundreds of millilitres of an aqueous sample containing the metal ions. The final surfactant concentration must exceed its CMC in order to ensure formation of micelle aggregates. The chelating agent is added, where necessary, along with the surfactant, dissolved in an organic solvent or directly to the water, depending on its solubility. Next, the solution is heated above the cloud point and separation of the phases usually takes place after centrifugation. Any analyte solubilized in the hydrophobic core of the micelles, will separate and become concentrated in the small volume of the surfactant-rich phase. After cooling in an ice bath, the surfactant-rich phase became viscous and retain at the bottom of the tube. The supernatant aqueous phases can readily be discarded by inverting the tube.

Finally, a volume (microliter) of nitric acid in aqueous or organic solvent can be added to the surfactant-rich phase to reduce its viscosity and to facilitate sample handling prior to AAS assay.

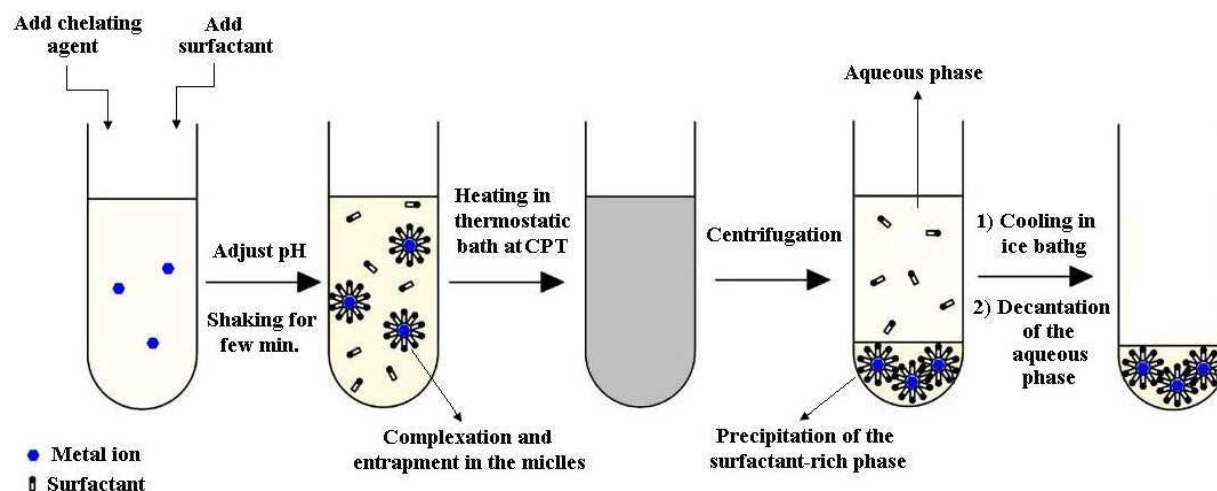


Fig. 8. Experimental schemes for cloud point extraction of metal ions prior to atomic absorption spectrometry.

2.6.4 Calculations

The CPE of metal ion from micellar solutions was evaluated in terms of extraction recovery (ER), distribution coefficient (D), selectivity $S_{X/A}$ and the concentration factor CF that are defined as follows:

The distribution coefficient is a parameter used to describe the degree of analyte partitioning from the aqueous to the surfactant-rich phase, is given by

$$D = \frac{[A]_s}{[A]_w} \quad (19)$$

where $[A]_s$ and $[A]_w$ are the final analyte concentrations in the surfactant-rich phase and in the aqueous phase, respectively. $[A]_w$ can be calculated from the mass balance equation:

$$[A]_0 V_0 = [A]_w V_w + [A]_s V_s \quad (20)$$

where $[A]_0$ refers to the analyte concentration in the original aqueous solution prior to the extraction step, and V_0 is the volume of original aqueous solution. V_w and V_s are the volumes of aqueous solution and surfactant rich phase obtained after the extraction step, respectively.

The extraction recovery (percent of analyte extracted) can be expressed as:

$$ER = \frac{[A]_0 - [A]_w}{[A]_0} \times 100 \quad (21)$$

Selectivity of analyte in the CPE process is described as:

$$S_{X/A} = \frac{D_X}{D_A} \quad (22)$$

Where D_X and D_A are distribution coefficient of foreign ion and analyte, respectively. Finally the concentration factor is given by:

$$CF = \frac{m_S}{m_W} = \frac{[A]_S V_S}{[A]_W V_W} = \frac{[A]_O V_O - [A]_W V_W}{[A]_W V_W} \quad (23)$$

Where m_S and m_W are total mass of analyte in the surfactant rich phase and aqueous phase, respectively.

2.6.5 Factors affecting the CPE efficiency

In CPE, extraction needs to be carried out under optimal conditions in order to maximize the preconcentration factor and extraction recovery. It is well known that the extraction/preconcentration process can be altered by pH, complexing agent, the types and concentration of surfactant, ionic strength (additive), equilibration temperature and incubation time (Quina & Hinze, 1999).

2.6.5.1 Effect of solution pH

The separation of metal ions by CPE involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of surfactant-rich phase. The formation of metal-ligand complexes and its chemical stability are two important factors for CPE. The pH, which plays a unique role on formation of the complex and subsequent extraction, is proved to be a main parameter for CPE. Extraction yield depends on the pH at which complex formation is carried out (Biparva & Hadjmohammadi, 2007).

2.6.5.2 Effect of the complexing agent

Generally, CPE of a metal ion is taken place via a complex formation of the analyte with a lipophilic ligand. These complexes interact with the micellar aggregate and can be extracted from the aqueous solution into the surfactant-rich phase. The selectivity and efficiency of the method depend directly on the hydrophobicity of the ligand and the complex formed, the apparent equilibrium constants in the micellar medium, the kinetics of the complex formation, and the transference of the complex between the phases (Constantine, 2002; Carabias-Martinez, et al., 2000).

2.6.5.3 Effect of surfactant type and concentration

Depending on the nature of the hydrophilic group, surfactants are classified as non-ionic, zwitterionic, cationic, and anionic. Up to now, non-ionic, zwitterionic and anionic surfactants are most widely used for CPE of metal ions. However, it is very important to select an appropriate surfactant for a successful CPE analysis since it can directly affect the extraction and preconcentration, and accuracy of the final analytical results. Reports showed that Triton X-114 and PONPE-7.5 (cloud point temperature, near room temperature) (Paleologos et al., 2000, 2001) are proper surfactants to perform CPE for trace elements because of its commercial availability in a high purified homogeneous form, low toxicity and cost. Also, low cloud point temperature (23–26 °C) and high density of the surfactant-rich phase facilitates phase separation by centrifugation. A successful cloud point extraction should maximize the extraction efficiency by minimizing the phase volume ratio ($\frac{V_S}{V_W}$). This

shows that the smaller surfactant concentration provides higher preconcentration factor; but when the volume of surfactant-rich phase is small, the extraction process becomes more difficult and the accuracy and reproducibility probably suffer (Eiguren Fernandez et al., 1999; Moreno Cordero et al., 1993). However, since the volume of the surfactant-rich phase

must be manageable, a compromise must be reached so that the surfactant concentration will allow a high phase ratio and a manageable surfactant-rich phase.

2.6.5.4 Effect of Ionic strength

The cloud point of micellar solutions can be controlled by addition of salts, alcohols, non-ionic surfactants and some organic compounds (salting-out effects). To date, most of the studies conducted have shown that ionic strength has no appreciable effect on the extraction efficiency. An increase in the ionic strength in the CPE does not seriously alter the efficiency of extraction of the chemical forms. Moreover, the addition of a salt can markedly facilitate the phase separation process. As demonstrated with some non-ionic surfactant systems, it alters the density of the bulk aqueous phase.

2.6.5.5 Effects of equilibration temperature and incubation time

Optimal incubation time and equilibration temperature are necessary to complete the reaction, and to achieve easy phase separation and preconcentration as efficient as possible. The greatest analyte preconcentration factors are thus expected under conditions where the CPE is conducted using equilibration temperature that are well above the cloud point temperature of the surfactant. It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature, which compromise completion of the reaction and efficient separation of phases.

2.6.6 Applications of CPE in atomic absorption spectrometry

The use of CPE coupled with atomic absorption spectrometry (AAS) offers a conventional alternative to more traditional extraction systems and permits the design of extraction schemes that are simple, cheap, of high efficiency, reducing in extraction time and environmentally clean methodology due to low consumption of a solvent, apart from the results that comparable to those obtained with other separation procedures. CPE technique has been successfully employed for the preconcentration of micro amounts of several metals in different matrices, as prior step before their determinations by Atomic Absorption Spectrometry.

Watanabe et al. used this unconventional liquid-liquid separation to extract metal ions for the first time (Watanabe & Tanaka, 1978). They extracted Ni using Triton X-100 but this surfactant has a relatively high cloud point, around 70 °C. Later, to extract Zn, they used PONPE 7.5, which requires a lower temperature (around 5 °C) for the phase separation. This surfactant and Triton X-114 which has a very convenient cloud point were used in other studies of metal ion extraction. Cu (Kulichenko et al., 2003), Mn (Doroschuk et al., 2004), Co (Nascentes & Arruda, 2003), Cd and Ni (Manzoori & Karim-Nezhad, 2004), Ag and Au (Mesquita da Silva et al., 1998) were determined by FAAS. CPE was also applied for extraction of Fe(III) (Ohashi et al., 2005) and As(III) and As(V) (Shemirani et al., 2005) before determination by ET-AAS. Cd, Cu, Pb, and Zn (Chen & Teo, 2001) were simultaneously extracted as complex with 1-(2-thiazolylazo)-2-naphthol (TAN) using TritonX-114 prior to determination by FAAS. Several ligands such as 1-(2-pyridylazo)-2-naphthol (PAN), 2-(2-thiazolylazo)-4-methylphenol (TAC), dialkyldithiophosphates (DDTP), 4-(2-pyridylazo) resorcinol (PAR), 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP) have been used in CPE of metal ions.

3. Conclusion

Analysis of metallic analytes is so important in environmental, biological and food samples. Due to complex matrix of real samples and trace concentration of them use of sample preparation methods is necessary. Development of efficient sample preparation methods in agreement with green chemistry is one of the most exciting research fields for analytical chemists. Growing number of scientific publishing in this area shows emerging needs for newer methods with higher concentration factors, higher recoveries, more cheap and simple and environmental friendly methods. All of the techniques discussed in this chapter have some advantages and some limitations. An analyst must be able to choose the best method according to his problem. Kind of analyte and sample, complexity of the sample, range of concentration which analyte exist in the sample are some of the parameters which must be considered.

4. Acknowledgment

Financial supports of Iranian Academic Center for Education, Culture and Research (ACECR) from our research projects gratefully acknowledged. Also the authors express their deep sense of gratitude to INTECH publishing company for their efforts in order to development of science and technology.

5. References

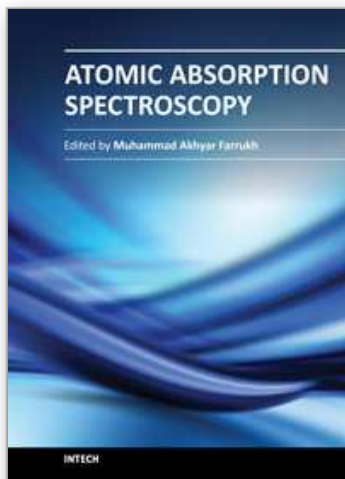
- Ahmadzadeh Kokya, T. & Farhadi, K. (2009). Optimization of Dispersive liquid-liquid Microextraction for the Selective Determination of Trace Amounts of Palladium by Flame Atomic Absorption Spectroscopy. *Journal of Hazardous Materials*, Vol 169, pp. 726-733
- Arthur, C. L. & Pawliszyn, J. (1990). Solid Phase Microextraction with Thermal Desorption. *Analytical Chemistry*, Vol 62, pp. 2145-2148.
- Baliza, P. X., Teixeira, L. S. G. & Lemos, V. A. (2009). A Procedure for Determination of Cobalt in Water Samples after Dispersive liquid-liquid Microextraction. *Microchemical Journal*, Vol 93, pp. 220-224.
- Bidabadi, M. S., Dadfarnia, S. & Shabani, A. M. H. (2009). Solidified Floating Organic Drop Microextraction (SFODME) for Simultaneous Separation/Preconcentration and Determination of Cobalt and Nickel by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). *Journal of Hazardous Materials*, Vol 166, pp. 291-296.
- Bidari, A., Zeini Jahromi, E., Assadi, Y. & Milani Hosseini, M. R. (2007). Monitoring of Selenium in Water Samples using Dispersive liquid-liquid Microextraction Followed by Iridium-modified Tube Graphite Furnace Atomic Absorption Spectrometry. *Microchemical Journal*, Vol 87, pp. 6-12.
- Biparva, P. & Hadjmohammadi, M. R. (2007). Cloud Point Extraction Using NDTT Reagent for Preconcentration and Determination of Copper in Some Environmental Water Samples by Flame Atomic Absorption Spectroscopy. *Acta chimica Slovenica*, Vol 54, pp. 805-810.
- Chamsaz, M., Arbab-Zavar, M. H. & Nazari, S. (2003). Determination of Arsenic by Electrothermal Atomic Absorption Spectrometry using Headspace Liquid Phase Microextraction after in situ Hydride Generation. *Journal of Analytical Atomic Spectrometry*, Vol 18, pp. 1279-1282.

- Carabias-Martinez, R., Rodriguez-Gonzalo, E., Moreno-Cordero, B., Perez-Pavon, J. L., Garcia Pinto, C. & Fernandez Laespada, C. E. (2000). Surfactant Cloud Point Extraction and Preconcentration of Organic Compounds prior to Chromatography and Capillary Electrophoresis. *Journal of Chromatography A*, Vol 902, pp. 251-265.
- Chen, J. & Teo, K. C. (2001). Determination of Cadmium, Copper, Lead and Zinc in Water Samples by Flame Atomic Absorption Spectrometry after Cloud Point Extraction. *Analytica Chimica Acta*, Vol 450, pp. 215-222.
- Constantine, D.S. (2002). Micelle-mediated Extraction as a Tool for Separation and Preconcentration in Metal analysis. *Trends in Analytical Chemistry*, Vol 21, pp. 343-355.
- Dadfarnia, S. & Shabani, A. M. H. (2010). Recent Developments in Liquid Phase Microextraction for Determination of Metals-A Review. *Analytica Chimica Acta*, Vol 658, pp. 107-119.
- Dadfarnia, S., Salmanzadeh, A. M. & Shabani, A. M. H. (2008). A Novel Separation/Preconcentration System based on Solidification of Floating Organic Drop Microextraction for Determination of Lead by Graphite Furnace Atomic Absorption Spectrometry. *Analytica Chimica Acta*, Vol 623, pp. 163-167.
- Dadfarnia, S., Shabani, A. M. H. & Kamranzadeh, E. (2009). Separation/Preconcentration and Determination of Cadmium Ions by Solidification of Floating Organic Drop Microextraction and FI-AAS. *Talanta*, Vol 79, pp. 1061-1065.
- Doroschuk, V. O., Lelyushok, S. O., Ishchenko, V. B. & Kulichenko, S.A. (2004). Flame Atomic Absorption Determination of Manganese(II) in Natural Water after Cloud Point Extraction. *Talanta*, Vol 64, pp. 853-856.
- Eiguren Fernandez, A., Sosa Ferrera, Z. & Santana Rodriguez, J. J. (1999). Application of Cloud-point Methodology to the Determination of Polychlorinated Dibenzofurans in Sea Water by High-Performance Liquid Chromatography. *Analyst*. Vol 124, pp. 487-491.
- Faraji, M., Yamin, Y., Saleh, A., Rezaee, M., Ghambarian, M. & Hassani, R. (2010). A Nanoparticle-based Solid-Phase Extraction Procedure Followed by Flow Injection Inductively Coupled Plasma-Optical Emission Spectrometry to Determine some Heavy Metal Ions in Water Samples. *Analytica Chimica Acta*, Vol 659, pp. 172-177.
- Farajzadeh, M. A., Seyedi, S. E., Shalamzari, M. S. & Bamorovat, M. (2009). Dispersive Liquid-Liquid Microextraction using Extraction Solvent Lighter than Water. *Separation Science*, Vol 32, pp. 3191-3200.
- Figueroa, R., Garcia, M., Lavilla, I. & Bendicho, C. (2005). Photoassisted Vapor Generation in the Presence of Organic Acids for Ultrasensitive Determination of Se by Electrothermal-Atomic Absorption Spectrometry Following Headspace Single-Drop Microextraction. *Spectrochimica Acta Part B: Atomic Spectroscopy*, Vol 60, pp. 1556-1563.
- Fitchett, A. W., Hunter Daughtrey, E., & Mushak, P. (1975). Quantitative Measurements of Inorganic and Organic Arsenic by Flameless Atomic Absorption Spectrometry. *Analytica Chimica Acta*, Vol 79, pp. 93-99.
- Fragueiro, S., Lavilla, I. & Bendicho, C. (2004). Headspace Sequestration of Arsine onto a Pd(II)-containing Aqueous Drop as a Preconcentration Method for Electrothermal Atomic Absorption Spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, Vol 59, pp. 851-855.

- Fragueiro, S., Lavilla, I. & Bendicho, C. (2004). Direct Coupling of Solid Phase Microextraction and Quartz Tube-Atomic Absorption Spectrometry for Selective and Sensitive Determination of Methylmercury in Seafood: an Assessment of Chloride and Hydride Generation. *Journal of Analytical Atomic Spectrometry*. Vol 19, pp. 250-254.
- Fritz, J. S. *Analytical Solid Phase Extraction*, WILEY-VCH, ISBN 0-471-24667-0, New York, USA
- Ghaedi, M., Niknam, K., Niknam, E. & Soylak, M. (2009). Application of Cloud Point Extraction for Copper, Nickel, Zinc and Iron Ions in Environmental Samples. *Journal of the Chinese Chemical Society*, Vol 56, pp. 981-986.
- Ghambarian, M., Khalili-Zanjani, M. R., Yamini, Y., Esrafil, A. & Yazdanfar, N. (2010). Preconcentration and Speciation of Arsenic in Water Specimens by the Combination of Solidification of Floating Drop Microextraction and Electrothermal Atomic Absorption. *Talanta*, Vol 81, 197-201.
- Gil, S., Fragueiro, S., Lavilla, I. & Bendicho, C. (2005). Determination of Methylmercury by Electrothermal Atomic Absorption Spectrometry using Headspace Single-Drop Microextraction with in situ Hydride Generation. *Spectrochimica Acta Part B: Atomic Spectroscopy*, Vol 60, pp. 145-150.
- He, B., Jiang, G. B. & Ni, Z. (1998). Determination of Methylmercury in Biological Samples and Sediments by Capillary Gas Chromatography Coupled with Atomic Absorption Spectrometry after Hydride Derivatization and Solid Phase Microextraction. *Journal of Analytical Atomic Spectrometry*. Vol 13, pp. 1141-1144.
- He, B. & Jiang, G. B. (1999). Analysis of Organomercuric Species in Soils from Orchards and Wheat Fields by Capillary Gas Chromatography on-line Coupled with Atomic Absorption Spectrometry after in situ Hydride Generation and Headspace Solid Phase Microextraction. *Fresenius Journal of Analytical Chemistry*. Vol 365, pp. 615-618.
- He, Y. & Lee, H. K. (1997). Liquid-Phase Microextraction in a Single Drop of Organic Solvent by Using a Conventional Microsyringe. *Analytical Chemistry*. Vol 69, pp. 4634-4640.
- Hinze, W. L. *Ordered Media in Chemical Separations*, ACS Symposium Series, Vol 342, 1987.
- Hinze, W. L. & Pramauro, E. (1993). A Critical Review of Surfactant-Mediated Phase Separations (Cloud-Point Extractions): Theory and Applications. *Critical Reviews in Analytical Chemistry*. Vol 24, pp. 133-177.
- Jeannot, M. A. & Cantwell, F. F. (1996). Solvent Microextraction into a Single Drop. *Analytical Chemistry*. Vol 68, pp. 2236-2240.
- Jiang, H. & Hu, B. (2008). Determination of Trace Cd and Pb in Natural Waters by Direct Single Drop Microextraction Combined with Electrothermal Atomic Absorption Spectrometry. *Microchimica Acta*, Vol 161, pp. 101-107.
- Khalili-Zanjani, M. R., Yamini, Y., Shariati, S. & Jonsson, J. A. (2007). A New Liquid-Phase Microextraction Method based on Solidification of Floating Organic Drop. *Analytica Chimica Acta*, Vol 585, pp. 286-293.
- Kulichenko, S. A., Doroschuk, V. O. & Lelyushok, S. O. (2003). The Cloud Point Extraction of Copper(II) with Monocarboxylic Acids into Non-ionic Surfactant Phase. *Talanta*, Vol 59, pp. 767-773.
- Lauwerys, R. R., Buchet, J. P. & Roels, H. (1979). The Determination of Trace Levels of Arsenic in Human Biological Materials. *Archives of Toxicology*, Vol 41, pp. 239-247.

- Lucci, P., Derrien, D., Alix, F., Perollier, C. & Bayouhd, S. (2010). Molecularly Imprinted Polymer Solid-Phase Extraction for Detection of Zearalenone in Cereal Sample Extracts. *Analytica Chimica Acta*, Vol 672, pp. 15-19.
- Liang, P., Liu, R. & Cao, J. (2008). Single Drop Microextraction Combined with Graphite Furnace Atomic Absorption Spectrometry for Determination of Lead in Biological Samples. *Microchimica Acta*, Vol 160, pp. 135-139.
- Liang, P. & Sang, H. (2008). Determination of Trace Lead in Biological and Water Samples with Dispersive Liquid-Liquid Microextraction Preconcentration. *Analytical Biochemistry*, Vol 380, pp. 21-25.
- Liu, X. & Fan, Z. (2007). Determination of Trace Pb in Water Samples by Electrothermal Atomic Absorption Spectrometry After Single-Drop Microextraction. *Atomic Spectroscopy Journal*, Vol 6, pp. 215-219.
- Manzoori, J. L., Amjadi, M. & Abulhassani, J. (2009). Ionic Liquid-Based Single Drop Microextraction Combined with Electrothermal Atomic Absorption Spectrometry for the Determination of Manganese in Water Samples. *Talanta*, Vol 77, pp. 1539-1544.
- Manzoori, J. L., Amjadi, M. & Abulhassani, J. (2009). Ultra-trace Determination of Lead in Water and Food Samples by using Ionic Liquid-Based Single Drop Microextraction-Electrothermal Atomic Absorption Spectrometry. *Analytica Chimica Acta*, Vol 644, pp. 48-52.
- Manzoori, J. L. & Karim-Nezhd, G. (2004). Development of a Cloud Point Extraction and Preconcentration Method for Cd and Ni prior to Flame Atomic Absorption Spectrometric Determination. *Analytica Chimica Acta*, Vol 521, pp. 173-177.
- Mesquita da Silva, M. A., Azzolin Frescura, V. L., Nome Aguilera, F. J. & Curtius, A. J. J. (1998). Determination of Ag and Au in Geological Samples by Flame Atomic Absorption Spectrometry after Cloud Point Extraction. *Anal. At. Spectrom.* Vol 13, pp. 1369-1373.
- Mester, Z. & Sturgeon, R. (2005). Trace element speciation using solid phase microextraction. *Spectrochim. Acta. Part B*, Vol 60, 1243-1269.
- Moreno Cordero, B., Perez Pavon, J. L., Garcia Pinto, C. & Fernandez Laespada, E. (1993). Cloud Point Methodology: A New Approach for Preconcentration and Separation in Hydrodynamic Systems of Analysis. *Talanta*, Vol 40, pp. 1703-1710.
- Nascentes, C. C. & Arruda, M. A. Z. (2003). Cloud Point Formation Based on Mixed Micelles in the Presence of Electrolytes for Cobalt Extraction and Preconcentration. *Talanta*, Vol 61, pp. 759-768.
- Ohashi, A., Ito, H., Kanai, C., Imura, H. & Ohashi, K. (2005). Cloud Point Extraction of Iron(III) and Vanadium(V) using 8-quinolinol Derivatives and Triton X-100 and Determination of 10^{-7} mol dm⁻³ level Iron(III) in River Water Reference by a Graphite Furnace Atomic Absorption Spectroscopy. *Talanta*, Vol 65, pp. 525-530.
- Paleologos, E. K., Stalikas, C. D., Tzouwara-Karayanni, S. M., Pilidis, G. A. & Karayannis, M. I. (2000). Micelle-Mediated Methodology for Speciation of Chromium by Flame Atomic Absorption Spectrometry. *Journal of Analytical Atomic Spectrometry*, Vol 15, 287-291.
- Paleologos, E. K., Stalikas, C. D. & Karayannis, M. I. (2001). An Optimised Single-Reagent Method for the Speciation of Chromium by Flame Atomic Absorption Spectrometry Based on Surfactant Micelle-Mediated Methodology. *Analyst*. Vol 126, pp. 389-393.

- Pedersen-Bjergaard, S. & Rasmussen K. E. (1999). Liquid–Liquid–Liquid Microextraction for Sample Preparation of Biological Fluids Prior to Capillary Electrophoresis. *Anal. Chem*, Vol 71, 2650-2656.
- Peng, F., Liu, J. F., He, B., Hu, X. L. & Jiang, G. B. (2007). Ultrasensitive Determination of Cadmium in Seawater by Hollow Fiber Supported Liquid Membrane Extraction Coupled with Graphite Furnace Atomic Absorption Spectrometry. *Spectrochim. Acta. Part B*, Vol 62, 499-503.
- Psillakis, E. & Kalogerakis, N. (2003). Developments in liquid-phase microextraction. *Trends Anal. Chem*, Vol 22, 565-574.
- Quina, F.H. & Hinze, W.L. (1999). Surfactant-Mediated Cloud Point Extractions: An Environmentally Benign Alternative Separation Approach. *Industrial & Engineering Chemistry Research*, Vol 38, 4150-4168.
- Rezaee, M., Assadi, Y., Milani Hosseini, M. R., Aghaee, E., Ahmadi, F. & Berijani, S. (2006). Determination of Organic Compounds in Water using Dispersive Liquid–Liquid Microextraction. *Journal of Chromatography A*, Vol 1116, pp. 1-9.
- Rivas, R. E., Garcia, I. L. and Cordoba, M. H. (2010). Microextraction Based on Solidification of a Floating Organic Drop Followed by Electrothermal Atomic Absorption Spectrometry for the Determination of Ultratraces of Lead and Cadmium in Waters. *Analytical Methods*, Vol 2, pp. 225-230.
- Rosen, M. J. *Surfactants and Interfacial Phenomena* (3rd ed.) John Wiley & Sons, Hoboken, New Jersey, (2010).
- Saber-Tehrani, M., Givianrad, M. H. & Hashemi-Moghaddam, H. (2007). Determination of Total and Methyl Mercury in Human Permanent Healthy Teeth by Electrothermal Atomic Absorption Spectrometry after Extraction in Organic Phase. *Talanta*, Vol 71, pp. 1319-1325.
- Shemirani, F., Baghdadi, M. & Ramezani, M. (2005). Preconcentration and Determination of Ultra Trace Amounts of Arsenic(III) and Arsenic(V) in Tap Water and Total Arsenic in Biological Samples by Cloud Point Extraction and Electrothermal Atomic Absorption Spectrometry. *Talanta*, Vol 65, 882-887.
- Watanabe, H. & Tanaka, H. (1978). A Non-Ionic Surfactant as a New Solvent for Liquid–Liquid Extraction of Zinc(II) with 1-(2-pyridylazo)-2-naphthol. *Talanta*, Vol 25, 585-589.
- Xia, L., Hu, B., Jiang, Z., Wu, Y., Chen, R. & Li, L. (2006). Hollow Fiber Liquid Phase Microextraction Combined with Electrothermal Vaporization ICP-MS for the Speciation of Inorganic Selenium in Natural Waters. *J. Anal. At. Spectrom*, Vol 21, 362-365.
- Ying-Ying, W., Guang-Ying, Z., Qing-Yun, C., Xiao-Huan, Z., Chun, W. & Zhi, W. (2010). Developments in liquid-phase microextraction method based on solidification of floating organic drop. *Chinese J. Anal. Chem*, Vol 38, 1517-1522.
- Yousefi, S. R. & Shemirani, F. (2010). Development of a Robust Ionic Liquid-Based Dispersive Liquid–Liquid Microextraction Against High Concentration of Salt for Preconcentration of Trace Metals in Saline Aqueous Samples: Application to the Determination of Pb and Cd. *Analytica Chimica Acta*, Vol 669, pp. 25-31.
- Zuba, D., Parczewski, A. & Rozanska, M. (2001). 39th Annual TIAFT meeting, Prague, Czech Republic.



Atomic Absorption Spectroscopy

Edited by Dr. Muhammad Akhyar Farrukh

ISBN 978-953-307-817-5

Hard cover, 258 pages

Publisher InTech

Published online 20, January, 2012

Published in print edition January, 2012

Atomic Absorption Spectroscopy is an analytical technique used for the qualitative and quantitative determination of the elements present in different samples like food, nanomaterials, biomaterials, forensics, and industrial wastes. The main aim of this book is to cover all major topics which are required to equip scholars with the recent advancement in this field. The book is divided into 12 chapters with an emphasis on specific topics. The first two chapters introduce the reader to the subject, its history, basic principles, instrumentation and sample preparation. Chapter 3 deals with the elemental profiling, functions, biochemistry and potential toxicity of metals, along with comparative techniques. Chapter 4 discusses the importance of sample preparation techniques with the focus on microextraction techniques. Keeping in view the importance of nanomaterials and refractory materials, chapters 5 and 6 highlight the ways to characterize these materials by using AAS. The interference effects between elements are explained in chapter 7. The characterizations of metals in food and biological samples have been given in chapters 8-11. Chapter 12 examines carbon capture and mineral storage with the analysis of metal contents.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Pourya Biparva and Amir Abbas Matin (2012). Microextraction Techniques as a Sample Preparation Step for Metal Analysis, Atomic Absorption Spectroscopy, Dr. Muhammad Akhyar Farrukh (Ed.), ISBN: 978-953-307-817-5, InTech, Available from: <http://www.intechopen.com/books/atomic-absorption-spectroscopy/microextraction-techniques-as-a-sample-preparation-step-for-metal-analysis>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen