CHAPTER 1

INTRODUCTION

1.1 Overview of human hair sample

Some heavy metals are essential to maintain the metabolism in human body. However, at high concentration, they can lead to poisoning. The toxic heavy metals such as nickel, cadmium, lead and chromium have negative effect to human health. Toxic metals imitate the action of essential elements in human body interfering with the metabolic process to cause illness [1-2].

The human biological samples which use in analysis such as blood, urine, nails and hair are generally used for investigation heavy metals. Blood sample is the first choice for toxicological studies. However, blood is very complex matrix, also known to cause interference in the spectrometric determination of trace metals. Generally, urine is considered for the evaluation of such metals because it is simple and easy to collect samples. However, urine is probably the most difficult biological matrix to analyze due to its high concentration of inorganic salts. Salt of alkali elements can cause serious interference in the determination and affect the accuracy of the analysis. For several years, human hair has been used to assess human exposure to heavy metals, drug abuse, health status and many biological parameters because toxic heavy metals can be accumulated within hair for a long time. Moreover, hair can be collected more quickly and easily than specimens of blood, urine or any other tissue and can be stored and transported to the laboratory for analysis easily. Furthermore, the concentrations of elements in this sample is high compared with those in body

tissues or fluids. This characteristic makes hair an attractive biomonitoring substrate. As hair analysis has not been popular in Thailand, it still needs more laboratory for verification to support its use [1-6].

1.1.1 The structure of hair [7]

A hair consists of two parts: a follicle and a shaft (**Figure 1.1**). The follicle is a club-shaped structure in the skin. At the end of the follicle is a network of blood vessels that supply nutrients to feed the hair and help it grow. This is called the *papilla*. Surrounding the papilla is a bulb. A sebaceous gland, which secretes oil that helps keep the hair conditioned, is associated with the bulb. The erector muscle that causes the hair to stand upright attaches to the bulb. Nerve cells wind around the follicle and stimulate the erector muscle in response to changing environmental conditions. The hair shaft is composed of the protein keratin, which is produced in the skin. Keratin makes hair both strong and flexible. Like all proteins, keratin is made up of a chain of amino acids that forms a helical, or spiral, shape. These helices are connected by strong bonds between amino acids. These bonds make hair strong [7].

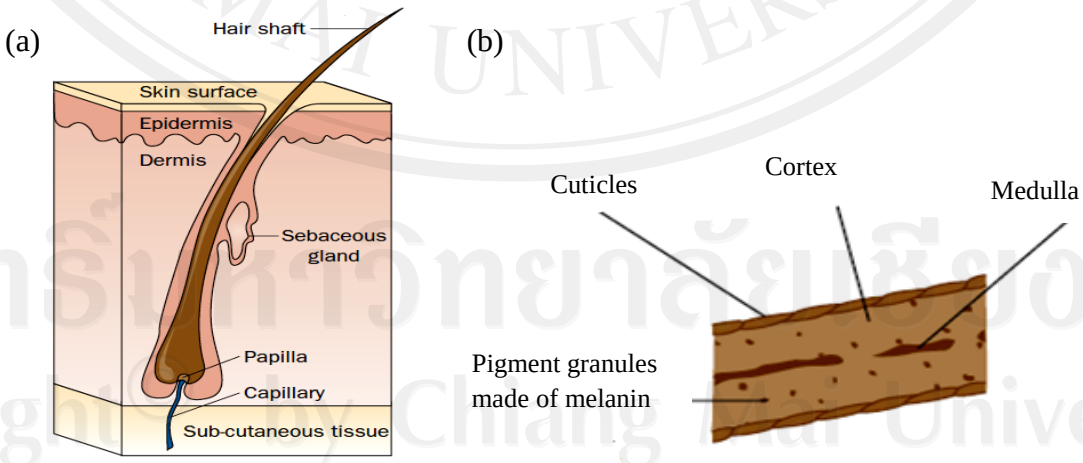


Figure 1.1 (a) cross-section and (b) structure of a hair shaft in a hair follicle in the skin [7]

The hair shaft is made up of three layers: a medulla, a cortex, and a cuticle, as show in **Figure 1.1.** The cuticle is a transparent outer layer of the hair shaft. It is made of scales that overlap one another and protect the inner layers of the hair. The cortex is the largest part of the hair shaft. The cortex is the part of the hair that contains most of the pigment granules (melanin) that give the hair its color. The center of the hair is called the medulla. It can be a hollow tube, or filled with cells. The medulla can contain pigment granules or be unpigmented [7].

1.1.2 Hair growth

Hair grows in cycles, alternating between periods of growth and quiescence. A follicle that is actively producing hair is called the anagen phase. Hair is produced during 4 to 8 years for head hair (<12 months for non–head hair) at a rate of approximately 0.22 to 0.52 mm/day or 0.6 to 1.42 cm/month [8]. After this period, the follicle enters a relatively short transition period of about 2 weeks, known as the catagen phase, during which cell division stops and the follicle begins to degenerate. Following the transition phase, the hair follicle enters a resting or quiescent period, known as the telogen phase, during which the hair shaft stops growing completely and hair growth begins to shut down, as shown in **Figure 1.2**. On the scalp of an adult, approximately 85% of the hair is in the growing phase and the remaining 15% is in a resting stage [7-8, 10].

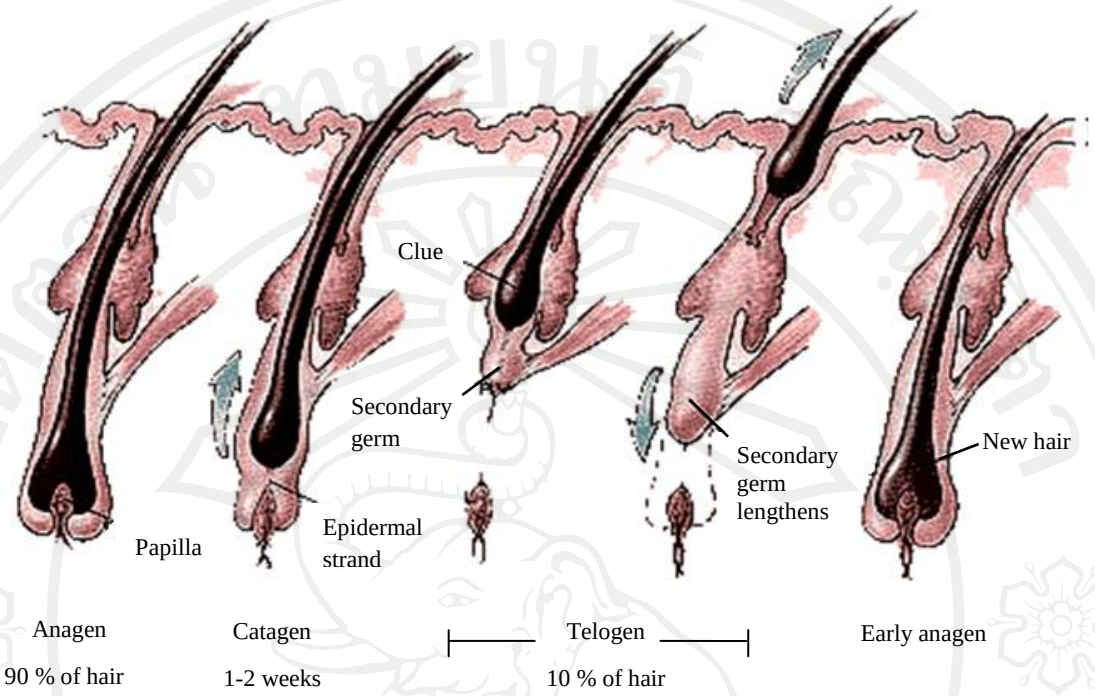


Figure 1.2 Diagram illustrating hair growth sequences

From http://hairtransplantsurgery.ie

1.1.3 Mechanisms of metal incorporation into hair

Metals enter into hair by two processes: contamination from the external environment and incorporation into the growing hair shaft from blood that supplies the hair follicle (**Figure 1.3**). Metals can enter the hair from exposure to chemicals in aerosols, smoke or secretions from sweat and sebaceous glands. The exact mechanism by which chemicals are bound into hair is not known. It has been suggested that passive diffusion may be augmented by metals binding to intracellular components of the hair cells, such as the hair pigment melanin. Another proposed mechanism is the binding of metals with sulfydryl-containing

amino acids that are present in hair. There is an abundance of amino acids, such as cystine, in hair; these form cross-linking SS bonds to stabilize the protein fiber network [10-11].

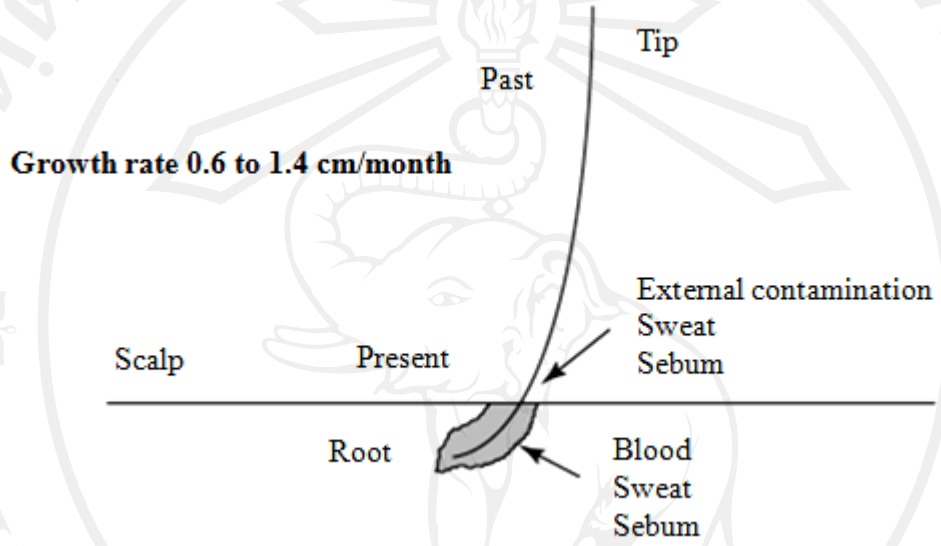


Figure 1.3 Possible model for metal incorporation into hair [10]

1.1.4 The advantages of human hair samples [12]

The use of human hair samples which favored for the determination of heavy metals can be summarised as follows:

(i) sampling is carried out easily and painlessly

(ii) human hair samples can be collected more quickly and easily than samples of blood, urine or any other tissue

(iii) human hair is a stable matrix, samples can be stored at room temperature for a long time and it not necessary to have special storage conditions

(iv) hair is an inert and chemically homogeneous sample

(v) the composition of hair does not change measurably

(vi) most of the toxic elements are accumulated within hair higher than in other human organs

(vii) repeated determinations can be operated easily over time

(viii) serum and urine concentrations provide both an acute index and also over a relatively short time period whereas the concentrations in hair provide a retrospective index of trace element supplies

(ix) hair provides information on the trace element concentrations of the intracellular space

(x) with the knowledge of the growth rate of the hair, it is possible to select the studied previous period (usually 2–3 months)

1.2 Ultrasonic acid digestion [13-19]

1.2.1 History of ultrasonic energy

Ultrasound has found applications in many fields (e.g. remediation, organic synthesis, industrial applications). In the past decade, ultrasonic processors have been recognized as efficient tools in the analytical laboratory to fulfill different goals. From general uses (e.g. degassing and cleaning) to more specific ones (e.g. extraction, derivatization, homogenization, emulsification), ultrasound can be implemented in many ways to enhance analytical processes [13].

Ultrasonic energy (UE) can often be used to quickly and easily perform effective sample dissolutions. For instance, UE has been used successfully to extract organic analytes from environmental matrices and sonication forms the basis of a

United States Environmental Protection Agency (EPA) method for the extraction of organic compounds from soil samples [14]. During the last 20 years we have witnessed an amazing increase in the application of ultrasonic energy in different fields of science. This is especially true for analytical chemistry [15]. The number of manuscripts devoted to almost all kinds of analysis dealing with the uses of ultrasonic energy continues to grow year by year. As the uses of ultrasonication have become increasingly important in analytical chemistry. The effects of interest regarding ultrasonication are related to cavitation. Cavitation causes solute thermolysis along with the formation of highly reactive radicals and reagents, such as hydroxyl radicals and hydrogen peroxide, which induce drastic reactive conditions in the liquid media. In addition, if a solid is present in solution, the sample size of the particles is diminished by solid disruption, thereby increasing the total solid surface in contact with the solvent [15-17].

Generally, ultrasonication aids chemical analysis by

- Enhancing solid–liquid elemental extraction
- Shortening sequential extraction schemes for elemental determination
- Shortening elemental speciation schemes
- Speeding up solid–liquid extraction of organic species
- Speeding up enzymatic reactions
- Accelerating liquid–liquid extraction techniques
- Enhancing the performance in solid-phase extraction and microextraction

1.2.2 Ultrasonic bath

The familiar laboratory ultrasonic bath has been used extensively for sample preparation purposes. Ultrasonic baths are available in a wide range of dimensions and power ratings and many offer temperature controls. Baths are normally constructed so that ultrasonic transducers are fastened to the outer surface of the bottom and sides of the bath's walls, enabling transmission of ultrasonic vibrations into the liquid within the bath. The most common transducers for laboratory ultrasonic baths are comprised of piezoelectric materials. Typical laboratory ultrasonic baths are equipped with transducers providing vibrations in the frequency range of 20 – 40 kHz., as show in **Figure 1.4** [14].

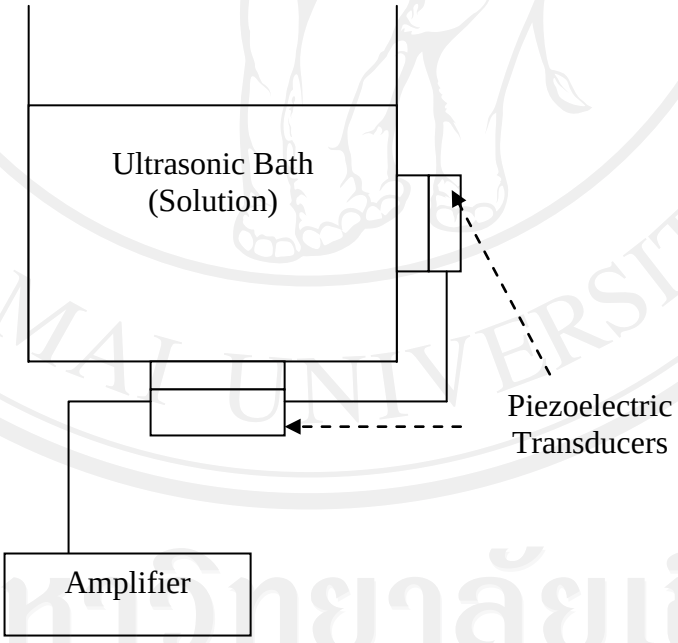


Figure 1.4 Ultrasonic bath for operation at frequencies of 20 – 80 kHz. [14]

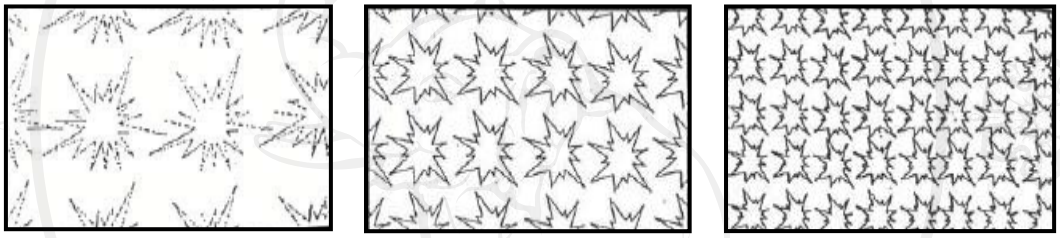
The heart of any ultrasonic system is the transducer. At the present time, the two types of transducers offered are magnetostrictive, made of nickel or its alloys and electrostrictive, made of lead zirconate titanate or other ceramics. Electrostrictive materials change their physical dimensions when placed in an electrical field of varying voltage. This is known as the "piezoelectric effect." Magnetostrictive transducers are made of materials which change dimensions in a varying magnetic field. Regardless of the type of transducer, the common, but primary factor, is the intensity of cavitation produced. Ultrasonic energy, like any sound wave, is a series of pressure points, or rather a series of compressions and rarefactions. If the sound energy is of sufficient intensity, the liquid will actually be pulled apart at the rarefaction stage and small bubbles or cavities will be formed. With the following compression stages, the bubbles collapse or implode throughout the liquid, creating an extremely effective force which is uniquely suited to cleaning. This is the process known as cavitation. Cavitation occurs throughout the liquid if the energy intensity is sufficient and it is for this reason that ultrasonics can effectively clean holes and small crevices. It also accelerates chemical reactions and the rate at which surface films are dissolved [15, 18].

1.2.3 Parameters affecting ultrasonic cavitation

Ultrasonic cavitation is a physical phenomenon whose performance depends upon the parameters described below.

1.2.3.1 Frequency

As the lower operating frequency the implosion bubble becomes larger and releases more energy when they implode but also lower the number or amount of events. As the increase operating frequency, reduce the size of the implosion bubble releasing less energy when they implode but also increase the number or amount of events, as show in **Figure 1.5**.



Cavitation at 25 kHz Cavitation at 40 kHz Cavitation at 68 kHz

Figure 1.5 The cavitation bubble at frequencies of 20 – 80 kHz. [19]

The low frequency of 25 kHz has a cavitation bubble range size of 50 to 200 microns in diameter. These cavitation bubbles have little chance of penetrating into these small irregular areas to remove small micron particles. The higher frequencies of 68 kHz. with its smaller range of cavitation bubbles and higher number of cavitation events have a better chance of penetrating these areas and removing more of the particles [19].

1.2.3.2 Intensity

The intensity of sonication is proportional to the amplitude of vibration of the ultrasonic source and, as such, an increment in the amplitude of vibration will lead to an increase in the intensity of vibration and to an increase in the sonochemical effects. In addition, high amplitudes of sonication can lead to rapid deterioration of the ultrasonic transducer, resulting in liquid agitation instead of cavitation and in poor transmission of the ultrasound through the liquid media. However, the amplitude should be increased when working with samples of high viscosity, such as blood. This is because as the viscosity of the sample increases so does the resistance of the sample to the movement of the ultrasonic device. Therefore, a high intensity (high amplitude) is needed to set the ultrasonic device to obtain the necessary mechanical vibrations so as to promote cavitation in the sample [15].

1.2.3.3 Solvent

The solvent used to perform sample treatment with ultrasonication must be carefully chosen. As a general rule, most applications are performed in water. However, other less polar liquids, such as some organics, can be also used, depending on the intended purpose. Both solvent viscosity and surface tension are expected to inhibit cavitation. The higher the natural cohesive forces acting within a liquid (e.g. high viscosity and high surface tension) the more difficult it is to attain cavitations [15].

1.2.3.4 Temperature

Solvent temperature plays two roles in ultrasonication. On the one hand, the use of high temperatures helps to disrupt strong solute–matrix interactions, which involve Van der Waals forces, hydrogen bonding and dipole attractions between the solute molecules and active sites on the matrix. Moreover, faster diffusion rates occur at higher temperatures. On the other hand, cavitation is better attained at lower temperatures when the ultrasonic power of the generator is constant. This is because as the temperature of the solvent rises so to does its vapor pressure and so more solvent vapor fills the cavitation bubbles, which then tend to collapse less violently, that is, the sonication effects are less intense than expected [15, 19].

1.2.4. Applications of ultrasonic

Ultrasonication can be applied in analytical chemistry in two ways: directly to the sample or indirectly through the walls of the sample container. Direct application is achieved through ultrasonic probes, which are immersed into sample, performing ultrasonication directly over the solution without any barrier to be crossed by the ultrasonication wave other than the solution itself. Indirect application is performed, generally, using an ultrasonication bath. In both cases the ultrasonic wave needs first to cross the liquid inside the ultrasonic device and then to cross the wall of the sample container. Therefore, ultrasonication intensity inside the sample container is lower than expected. As ultrasonic baths are not powerful devices, their applications are greatly limited by the lack of ultrasonic intensity [15]. However, most ultrasonic bath applications are performed for longer than 30 min and as consequence of continuous ultrasonication, the bulk liquid warms up. Endothermic reactions can

take advantage of this warming. In addition, the kinetics of many reactions are accelerated when the temperature is increased. Ultrasonic baths are used without temperature control and since bulk liquid warming is a slow process the final temperature achieved for a given time of sonication depends on the temperature of the laboratory. Thus, it is generally accepted that it is necessary to determine the maximum temperature the bath reaches and maintains, the so-called equilibrium temperature, when operating continuously under ambient conditions. Most reactions can be performed under the equilibrium temperature simply by filling the bath with water heated to that temperature previously [15, 18].

Thus, high local temperatures inside collapsing cavitation bubbles can cause an increase in analyte solubility and solvent diffusivity inside the solid particles. High pressure occurring during microbubble implosion improves solvent penetrability and transport. Surface renewal caused by particle fragmentation makes it possible for more analyte to come in contact with the solvent, as show in **Figure 1.6** [15].

(1) (2) (3)

Figure 1.6 Cavitational collapse at a solid-liquid interface. The sequence (1), (2) and (3) shows a scheme of fragmentation or disruption due to gas trapped on the defects on the solid surface giving rise to particle-size reduction [15]

Ultrasound irradiation aimed at matrix decomposition allows mild conditions (room or nearly room temperature, atmospheric pressure) to be applied. High local temperatures and pressures reached inside the cavitation microbubbles benefit sample digestion. Moreover, acid digestion carried out in open vessels is safer than acid attacks under pressure, although concentrated acids or their mixtures are still needed. Apart from that, sample vessels are subjected to different intensities of ultrasonic energy depending on their positions inside the bath, and that reduces precision. The main analytical techniques include atomic absorption spectrometry (AAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) [13].

1.3 Cloud point extraction (CPE) [20-30]

1.3.1 History of cloud point extraction

The determination of very low concentration of trace elements in complex matrixes has often been a problem for analytical chemists. Separation and preconcentration can solve this problem by offer the ability to isolate the target analytes from the matrix solution, including to control or even eliminate the interferences originally present and increase the opportunity to achieve a high confidence level [20]. The classical liquid–liquid extraction and separation methods are usually time consuming and labor extensive, also require relatively large volumes of high purity solvents. Of additional concern is disposal of the solvent used, which creates a severe environmental problem. Cloud point extraction (CPE) is an attractive technique that very useful for the separation and preconcentration of metal ions in

biological samples. Moreover, it has been successfully demonstrated in the extractions of environmental organic pollutants for further chemical analysis as well [20-21].

1.3.2 Theory of cloud point extraction

In cloud point extraction (CPE), the role of extraction solvent is performed by a micelle (surfactant-rich phase) occurring from a surfactant solution that is added into the sample. Surfactants are amphiphilic molecules, having both a hydrophilic (water soluble) head group and a hydrophobic (oil soluble) tail, usually an alkyl chain. The amphilic character of surfactants also results in unique solution behaviour. At low concentrations, surfactant molocules are soluble as discreet monomers in aqueous solution. At a specific concentration known as the critical micelle concentration (CMC) it becomes energetically more favorable for surfactant monomers to form aggregates known as micelles. In the micellar aggregates the nonpolar (hydrophilic) alkyl tails are oriented towards the center of the micelle and shielded from unfavorable water contacts by the polar head groups as show in **Figure 1.7**. This is ascribed to their formation consisting of a hydrophilic surface and a hydrophobic core. The hydrophobic core can entrap and thus isolate hydrophobic substances. This ability has been extensively used in the past few years under the term cloud point extraction for the preconcentration of hydrophobic compounds. In addition, under certain conditions these areas also can interact electrostatically with amphoteric or even charged substances such as metal ions [20-23].

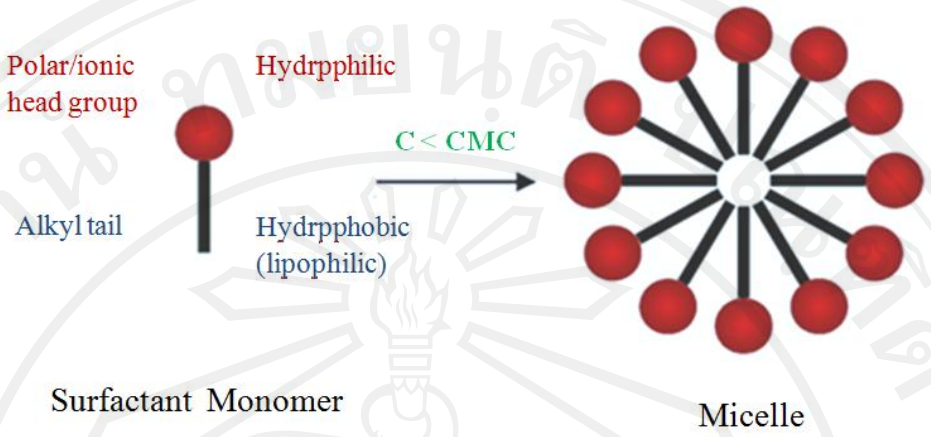


Figure 1.7 Micelle formation with increasing surfactant concentration

From http://science.uwaterloo.ca/~wettig/Research/overview.html

The principle of CPE is based on the property of surfactant in aqueous solutions to from micelles and become turbid when heated to the temperature known as cloud point temperature. Above the cloud point, the micelle solution separated into a supernatant aqueous phase and a surfactant-rich phase at the bottom of the test tubes, in which the surfactant concentration is closed to CMC. Any analyte that solubilized in the non-polar core of the micelles will be separated and become concentrated in the small volume of surfactant-rich phase [22].

1.3.3 The advantages of cloud point extraction [23]

(i) The capacity to preconcentrate an excess of analytes with almost quantitative recoveries

(ii) The preconcentration factors obtained are comparable or superior to other schemes, and they can also be modified on demand by varying the amount of surfactant

(i) Commercial surfactants are cost effective and friendly to environmental

(ii) The amounts of reagents used in CPE are minimal compared to the amounts of organic solvents used in conventional extraction

(v) The mild conditions applied in CPE allow for preconcentration schemes targeted at thermally sensitive analytes such as molecules of biological and environmental interest

(vi) The surfactant-rich phase is compatible with most mobile phases used in hydrodynamic analytical systems, also it increases atomic signal in FAAS and wettability of the graphite surface in ETAAS techniques

1.3.4 Influential parameters [23-25]

In CPE, to achieve such a wide range of applications, several chemical parameters have to be optimized to achieve the quantitative extraction. For organic species, the parameters which susceptible to the optimization stem are the properties of the surfactant medium. However, for inorganic species, where the quantitative formation of a hydrophobic complex is an essential prerequisite for efficient of CPE, properties of the surfactant system have to be optimized more carefully, considering the variables of complex formation. Ordinary parameters for both organic and inorganic species, which have to be optimized to make CPE successful are:

1.3.4.1 pH of the solution

For organic species, pH is the most important factor to partition the target analyte in the surfactant-rich phase. Especially for ionizable species such as phenols and amines, maximum extraction efficiency is achieved at pH values where the uncharged form of the target analyte predominates. In recently development of CPE, schemes based on ionic surfactants were used effectively to extract charged analytes. However, for inorganic species, there was little differentiation in the extraction efficiency of the complexes formed at different pH values, because these complexes are bulky, uncharged and covalent. In any other case, the role of pH is the same as in traditional pH-selective fractional precipitation, where the separation of several metal ions is made practicable by repeatedly adjusting the pH [23].

1.3.4.2 Effect of surfactant concentration

The concentration of surfactant is an important factor which has to discuss on CPE. There is a narrow range of surfactant concentration which accomplished the easy phase separation, maximum extraction efficiency and analytical signal. Increasingly, outside this optimal range, the analytical signal is observed to deteriorate due to the increase in the final volume of the surfactant-rich phase that causes the preconcentration factor (phase–volume ratio) to decrease. However, if surfactant concentration is decreased from that recommended, accuracy and reproducibility would probably suffer because the resultant surfactant-rich phase would not be sufficient to make reproducible measurements of extraction and separation [23].

1.3.4.3 The chelating agent

The chelating agent is the controlling factor for extracting all metal in CPE schemes. Since Watanabe's pioneering application of CPE in metal extraction, several chelating agents have been utilized in order to produce sufficiently hydrophobic complexes to be isolated in the surfactant-rich phase of a micelle solution. Based on their reactivity and formation constants with the target metal species, some of the most widely applicable reagents are carbamates, pyridylazo, quinoline, and naphthol derivatives. These molecules are universal chelators that form hydrophobic compounds with the majority of metal ions and they can be applied when an element-specific detector is available. In any case, a ligand is selected with the requirement that the derived complex is sufficiently hydrophobic, possesses a high partition coefficient, formed quickly and quantitatively with the least possible excess. The thermodynamics parameters such as formation constant (Kf), as well as the kinetics of complex formation and transfer into the micelle phase control the whole procedure, while the contributions of cloud point and micellization parameters are less mentioned. The distribution behavior of metal chelates in the surfactant medium is depended on the nature of the complex and the prevailing conditions. In contrast with organic solvents, the distribution constants are almost independent of the nature of the metallic ions [23].

1.3.4.4 Equilibration temperature

Temperature of the CPE procedure are important factors to complete reactions, and to achieve easy phase separation. Normally, temperature change results in two-phase separation of non-ionic and zwitterionic surfactant

solutions, while other parameters are involved in two-phase separation process of ionic surfactants. The parameter inducing phase separation can limit the types of compound that can be extracted. Thus, CPE at high temperatures cannot be applied to analysis of thermolabile compounds, while acidic solutions are not suitable for weak basic compounds. In addition, high temperatures are not suitable in the proposed analytical method because they could create stability problems for chelates and chelating agents. Therefore, if high temperatures are not required by the experimental conditions, they can be avoided, even if they may give some improvement for extraction efficiency [27-29].

1.3.4.5 Effect of ionic strength

Ionic strength has also been of consideration, although it has proved to has a negligible effect on the performance of CPE. Increasing ionic strength enhances phase separation through sorting out phenomena that also apply to conventional extraction schemes, yielding higher recoveries without by any means deteriorating the analytical performance. In that direction, it is possible to apply this factor directly to difficult matrixes such as water in environmental and biological fluids [26].

1.3.5 Implementation of CPE in metal analysis

Cloud point extraction used for metal determinations is relatively simple. A few milliliters of concentrated surfactant solution are added into the aqueous sample, this volume is in the range of tens of hundreds of milliliters. When necessary, a chelating agent solution is dissolved in an organic solvent or directly in water, depending on its solubility. The solution is then heated above its cloud point

and separation of the phases usually occurs after centrifugation. The discard of bulk aqueous phase after separation of micelle phase is facilitated after an ice bath, because the viscosity of the surfactant-rich phase is increased. CPE efficiency mainly depends on many factors such as the interaction of metal species with surfactant, the formation of complex when a chelating agent takes part, the kinetics of chelating agent to form complex and the phase transfer in the micelle media. It is interesting that the distribution constants of metal chelates in micelle medium depend on the nature of the metal ions with consequent variations in selectivity. Because of the hydrated nature of the surfactant phase, the distribution mechanisms are different from those of conventional solvent extraction, where the distribution constants of chelates are almost independent of the nature of the metal ions [23, 28-30].

Usually, the experimental procedure of CPE can be described as shown in **Figure 1.8**.

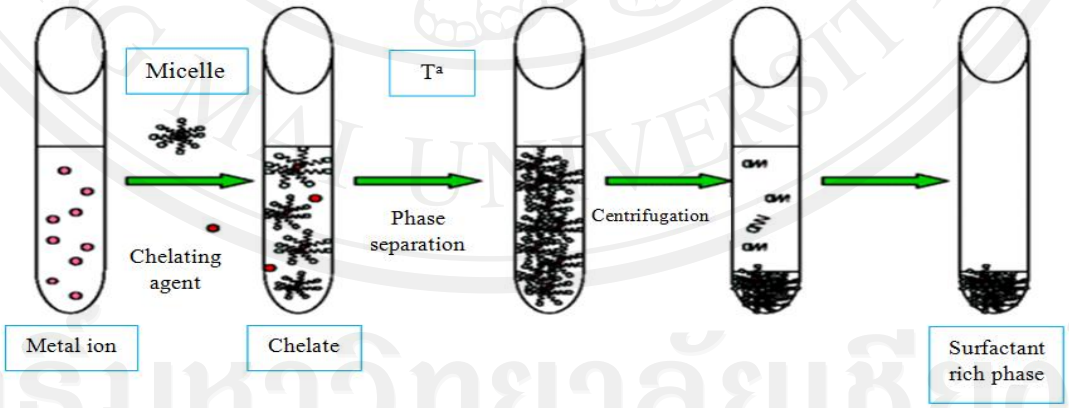


Figure 1.8 The experimental procedure for CPE in metal analysis [28]

(i) The metal reacts with a suitable chelating agent to form a hydrophobic complex

(ii) Clouding is generated by increasing temperature above the cloud point

(iii) The micelles formed entrap the metal complexes inside their hydrophobic core

(iv) The surfactant-rich phase is subsequently separated from the bulk aqueous one by centrifugation

(v) Isolation of the surfactant-rich phase

1.4 Atomic absorption spectrometry (AAS) [31-34]

Atomic absorption spectrometry is an analytical technique based on the absorption of radiant energy by atom. When a dispersion of the sample atoms is produced in a flame, some of these atoms get thermally excited and emit characteristic radiation, as they return to the ground level. Most of them, however, remain in the ground state. When a beam of light is made to pass through the flame, a portion of it will be absorbed by dispersed atoms, in the same manner that a beam of light passing through a solution will be absorbed by the dispersed molecules of a solute. It is possible to find a series of absorption bands corresponding to the energy levels of the atoms sprayed into the flame. The wavelength of the band is characteristic of the atoms of the element concerned and the absorbance of the band is proportional to the concentration of the atoms in the flame [31-33].

1.4.1 Theory of atomic absorption spectroscopy [31]

Lambert's Law

Lambert's Law states that each layer of equal thickness of an absorbing medium absorbs an equal fraction of the radiant energy that traverses it.

Let suppose that I_0 is the incident radiant energy and I is the energy which is transmitted. The ratio of the radiant power transmitted by a sample to the radiant power incident on the sample is known as the transmittance. Lambert's Law is expressed as:

$$\text{Transmittance } T = I/ I_0$$

It is customary to express transmittance as a percentage

$$\% \text{ Transmittance} = I/ I_0 \times 100$$

The logarithm to the base of the reciprocal of the transmittance is known as absorbance.

$$\text{Absorbance} = \log (1/T) = \log_{10} (I_0/I)$$

$$\text{Optical density} = \log_{10} (100/T)$$

Beer's Law

Beer's Law states that the absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium in the light path.

The Beer-Lambert Law

A combination of the two laws, known jointly as the Beer-Lambert Law, defines the relationship between absorbance (A) and transmittance (T). It states that the concentration of a substance in solution is directly proportional to the "absorbance", *A*, of the solution.

$$\text{Absorbance } A = \varepsilon cb$$

where A is the absorbance (no unit of measurement)

ε is the molar absorptivity ($dm^3\ mol^{-1}\ cm^{-1}$)

c is the molar concentration ($mol\ dm^{-3}$)

b is the path length (cm)

Absorptivity is a constant, depending upon the wavelength of the radiation and nature of the absorbing material. Absorbance is the property of a sample, where as absorptivity is the property of a substance and is a constant.

Mathematically, absorbance is related to percentage transmittance T by the expression:

$$A = \log_{10} (I_0/I) = \log_{10} (100/T) = \varepsilon bc$$

1.4.2 Flame atomic absorption spectrometry

The technique of flame atomic absorption spectroscopy (FAAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800 ^oC. During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths [32].

Flame atomic absorption spectroscopy is the most widely used form of atomic spectroscopy. Parts per million (ppm) levels of many metal ions may be readily determined by means of what has now become a relatively simply experimental procedure. Frame atomic absorption component is similar to

spectroscopic instrument. There are a radiation source, atomizer, monochromator and detector. **Figure 1.9** shows the atomic absorption component [33].

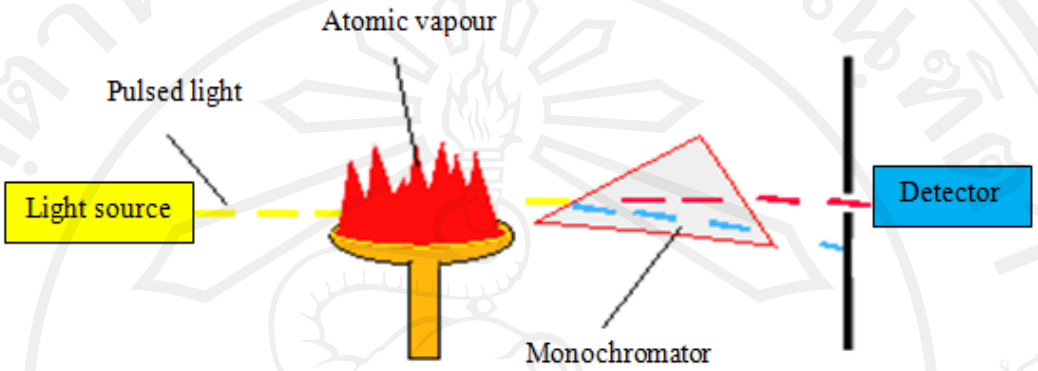


Figure 1.9 Schematic of FAAS

From http://www.dynamicscience.com.au/tester/solutions/chemistry/analytical%20chem/spectroscopy4.htm

The light source

The hollow cathode lamp is the most commonly used source of radiation for atomic absorption spectroscopy. It is a discharge lamp which emits the characteristic light of the element to be analyzed. The cathode is a hollow metal cylinder 10 to 20 mm in diameter constructed of metal that spectrum is desired or it may simply serve as a support for depositing a layer of the element that emit radiation at a specific wavelength [33].

The Flame atomizer

Flame atomizers contains a pneumatic nebulizer, which converts the sample solution into a mists or aeroso that is fed to the flame. The nebulizer is a device operating on the principle of a scent or paint spray. Today, most of the burners

used are those of long-slot designed burning premixed fuel and oxidant gases and fitted with a pneumatic nebulizer [33-34].

The monochromator

The basic requirement of a monochromator is to separate the resonance line from other spectral lines nearby. The function of the monochromator is to select radiation of the correct wavelength and eliminate other radiation from the light path. [33].

The detector

A detector is to produce an electrical current that is depends on the light intensity. Photomultipliers are used exclusively on commercial equipment. [32].

1.5 Literature review

The literature reviews for the determination of heavy metals in human hair samples have been reported with various techniques. The widely used techniques that have been reported for determination of heavy metals in hair are flame atomic absorption spectrometry (FAAS) [39], electrothermal atomic absorption spectrometry (ETAAS) [4], inductively coupled plasma atomic emission spectrometry (ICP-AES) [38] and inductively coupled plasma mass spectrometry (ICP-MS) [35- 36, 44]. Among the spectrometric techniques, ICP-MS provides the greatest sensitivity for determination of metals. Moreover, it is a multi- elemental technique used for determination of many elements in biological samples. However, many methods described in the literature have used AAS as the technique for the detection of metals.

Its popularity is due to the advantages offered by this technique, such as relatively low cost, efficiency and the low quantities of sample required [6, 43-44].

There are some review papers reporting the use of ultrasonic acid digestion (UAD) in chemical analysis. UAD can be used for digesting both liquid and solid samples. This technique has been also applied for trace metal determination in hair samples. Ultrasound energy can be considered as an alternative for solid sample pretreatment because it facilitates and accelerates some steps such as dissolution, fusion and leaching. For this technique, solid samples are deposited in a suitable vial, extracted with acid for 15–30 min in ultrasonic bath and the extract is finally separated from the solid residue [35, 37, 39]. In the case of biological samples, it was reported that oxidation process with high organic matter was usually incomplete when using only HNO_3 but H_2O_2 combined with HNO_3 yielded clear solutions and improved recovery. Moreover, high temperature was necessary for optimum recoveries of heavy metals from all samples as compared to the work reported at room temperature [37]. Furthermore, the sample particle size is a critical parameter for UAD, a small size (e.g. 50 mm) being necessary in order to achieve quantitative extraction [39].

The literature reviews of CPE for human hair samples have reported about the principle as well as advantages of CPE and this method has been interested in recent years. The use of CPE process has been applied for preconcentration of metals, metal chelates, biomolecules and many types of organic species. Many types of chelating reagents such as dithizone, *O*, *O*-Diethyldithiophosphate (DDTP), 1-(2-thiazolylazo)-2-naphthol (TAN) and 1-(2-pyridylazo) naphthol (PAN) and many types of non-ionic surfactant reagent such as TritonX-100, Triton X-114, and Tween 80 are important for

analysis [41-43]. The concentration of surfactant using in CPE is a critical factor because surfactant concentration which is smaller than 0.1% (v/v) Triton X-114 leads to a reduction of the analytical response due to incomplete partitioning of the analytes in the surfactant micelles [43]. Moreover, the effects of equilibration temperature and incubation time were investigated due to the shortest incubation time and the lowest possible equilibration temperature, which compromised efficient separation of the phases [41]. Furthermore, the surfactant rich phase was very viscous, so that ethanol was added to the surfactant-rich phase after CPE to facilitate its into the atomizer of the spectrometer [43].

1.5 Research Objectives

The main proposes of this research are as follows:

(1) To develop the sample preparation method of ultrasonic acid digestion for determination of some heavy metals in human hair

(2) To develop the preconcentration method of cloud point extraction for determination of some heavy metals in human hair

(3) To determine the amount of some heavy metals in human hair sample by FAAS