CHAPTER 1

INTRODUCTION

1.1 Overview [1-5]

Rice is the staple food for over half of the world's population. In addition, it is the major source of some micronutrients for Thai people. Iron is an essential nutritional element for all life forms, e.g. it is a cofactor in many enzymes and essential for oxygen transport and electron transfer. The fractional absorption of iron was correlated with its oxidation state. Also, Fe^{2+} is required for proper transport and storage of oxygen in higher animal by means of hemoglobin and myoglobin, while oxidized forms methemoglobin and metmyoglobin, which contain Fe^{3+}, will not bind oxygen. Iron absorption refers to the amount of dietary iron that the body obtains from the intake food. Insufficient iron level is causally related to iron-deficiency anemia (IDA). Anemia is a condition in which the blood cannot carry enough oxygen because there are less red blood cells than normal.

The speciation analysis in analytical chemistry is used to indicate the analytical activity of identifying chemical species and measuring the quantities of one or more individual chemical species in a sample. Metal speciation is important in a variety of environmental, biological, geological and medical applications. The chemical and physical properties of metal species highly depend on its oxidation state, hence an accurate determination of each species is important to evaluate the potential risk of some metals. It is evident that speciation determines the behavior of trace elements in a system, and in the human organism speciation has a profound effect on

bioavailability, distribution, and toxicity. Consequently, iron determination and speciation methods in food samples have been developed with high sensitivity.

1.2 Speciation [4, 6-7]

Trace elements play an important role in the functioning of life on our planet. Some elements can be highly toxic to various life forms; others are considered essential, but can become toxic at higher doses. Many of these effects depend strongly on the particular form in which the element is present in the system.

Often these different chemical forms of a particular element or its compounds are referred to as "species". The notion that the distribution among its various species will have a major effect on the behavior of a particular element has been accepted in such diverse fields as toxicology, clinical chemistry, geochemistry, and environmental chemistry. New developments in analytical instrumentation and methodology now often allow us to identify and measure the species present in a particular system. Because of these possibilities, numerous publications have appeared in which the term "speciation" is employed. However, this term has been used in a number of different ways, including the transformation of species, the distribution of species, or the analytical activity to determine the concentrations of species.

The International Union of Pure and Applied Chemistry (IUPAC) has defined elemental in chemistry as follows:

i. *Chemical species*. Chemical elements: specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex or molecular structure

ii. *Speciation analysis*. Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample

iii. *Speciation of an element; speciation.* Distribution of an element amongst defined chemical species in a system.

The fractionation is in use when elemental speciation is not feasible.

iv. *Fractionation.* Process of classification of an analyte or a group of analytes from a certain sample according to physical (*e.g.*, size, solubility) or chemical (*e.g.*, bonding, reactivity) properties.

1.3 Iron [8-10]

Iron is a shiny and malleable metal with a density of 7.9 g/ml, melting point of 1535 °C and boiling point of 2750 °C. It is the second most abundant metal on earth. Iron is a transition metal with atomic number 26 and mass number 55.8. Naturally occurring iron is composed of three isotopes ^{56}Fe (92%), ^{54}Fe (6%) and ^{57}Fe (2%). In compounds founded in the environment it usually has a valence of +2 and +3 but +6 oxidation state is rarely formed. Iron is a necessary mineral for body function and good health. Every red blood cell in the body contains iron in its hemoglobin, the pigment that carries oxygen to the tissues from the lungs. Iron is a constituent of a number of enzymes. It is the only nutrient for which women have a higher daily requirement than men. The U.S. Recommended Daily Allowance (RDA) of iron for men is 8-11 mg per day and 15-18 mg per day for women. For pregnant women, the RDA increases to 27 mg per day. It is potentially toxic in excess concentrations because of its pro-oxidant activity. Hence, its concentrations in body samples should

be frequently controlled. But, the attention to the iron is sourced from its essential properties for the living than its toxicity.

1.3.1 The importance of iron speciation [7, 10-13]

One of the most important properties of iron is its ability to change valence. It has the possibility to access various oxidation states (from –2 to +4), the principal ones being ferrous (Fe^{2+}) and ferric (Fe^{3+}). Whereas Fe^{2+} is water soluble, Fe^{3+} is relatively insoluble in water although significant concentrations of water soluble Fe^{3+} species can be attained by complex formation. The interaction between Fe^{2+} and Fe^{3+} and ligand donor atoms will depend on the strength of the chemical bond formed between them. An idea of the strength of such bonds can be got from the concept of hard and soft acids and bases. Soft bases have donor atoms of high polarisability with empty, low energy orbitals; they usually have low electronegativity and are easily oxidised in contrast to hard bases. Metal ions are soft acids if they have a low charge density, a large ionic radius and have easily excited outer electrons in contrast to hard acid metal ions. In general, hard acids prefer hard bases and soft acids form more stable complexes with soft bases.

Iron plays a central role in the biosphere, serving as the active center of proteins responsible for O_2 and electron transfer and of metalloenzymes such as oxidases, reductases, and dehydrases. In higher animal, hemoglobin and myoglobin are importants proper for storage oxygen and acting as electron transport between Fe^{2+} (reduced form) and Fe^{3+} (oxidized form; methemoglobin and metmyoglobin). In human body iron is an essential mineral. The average adult has 4-5 g of iron, of which 60-70% is present as haem in the circulating haemoglobin, and the remainder present

in various enzymes (*e.g.* catalase, cytochrome oxidase), in muscle myoglobin or stored. About 15% of the iron is stored in the liver as ferritin, in the other tissue as haemosiderin, and as the blood transport complex called tranferin (average blood level 50-180 μg per 100 ml plasma). A human body loses iron 0.5-1.5 mg per day (in faeces 0.3-0.5 mg per day, in sweat as skin cells 0.5 mg, traces in hair and urine) so the sufficient amount should be intaken (12 mg per adults, 15 mg during pregnancy and lactation and for adolescents, 7.5-10.5 mg for children, rising to 13.5 mg in 11-14 years old group). The human body cannot absorb all of intaken iron. Only 0.5-1.5 mg from a diet containing 10-15 mg can be absorbed.

The main source of iron is red meat. Other sources of iron include meats, some fish, egg yolks, beans, dried fruit and spinach. Iron can also be found in breakfast cereals that are fortified with iron. Iron absorption refers to the amount of dietary iron that the body obtains from the intake food. Meat is a better source of iron because haem is more readily absorbed. Absorption of iron in the body is aided by vitamin and reduced by phosphate and phytic acid. The components such as phytate, phosphate, polyphenols containing alkyl groups, oxalic acid, casein, phosphoprotein, albumin and minerals such as Ca, Cu, Zn and Mn decrease the fractional iron absorption.

Insufficient iron level is causally related to iron deficiency anemia (IDA). Iron deficiency anemia is the most common of all nutritional deficiencies. Anemia is a condition in which the blood cannot carry enough oxygen, either because there is a low number of red blood cells or because each red blood cell is able to carry less oxygen than normal. Some of the symptoms are pale skin color, fatigue,

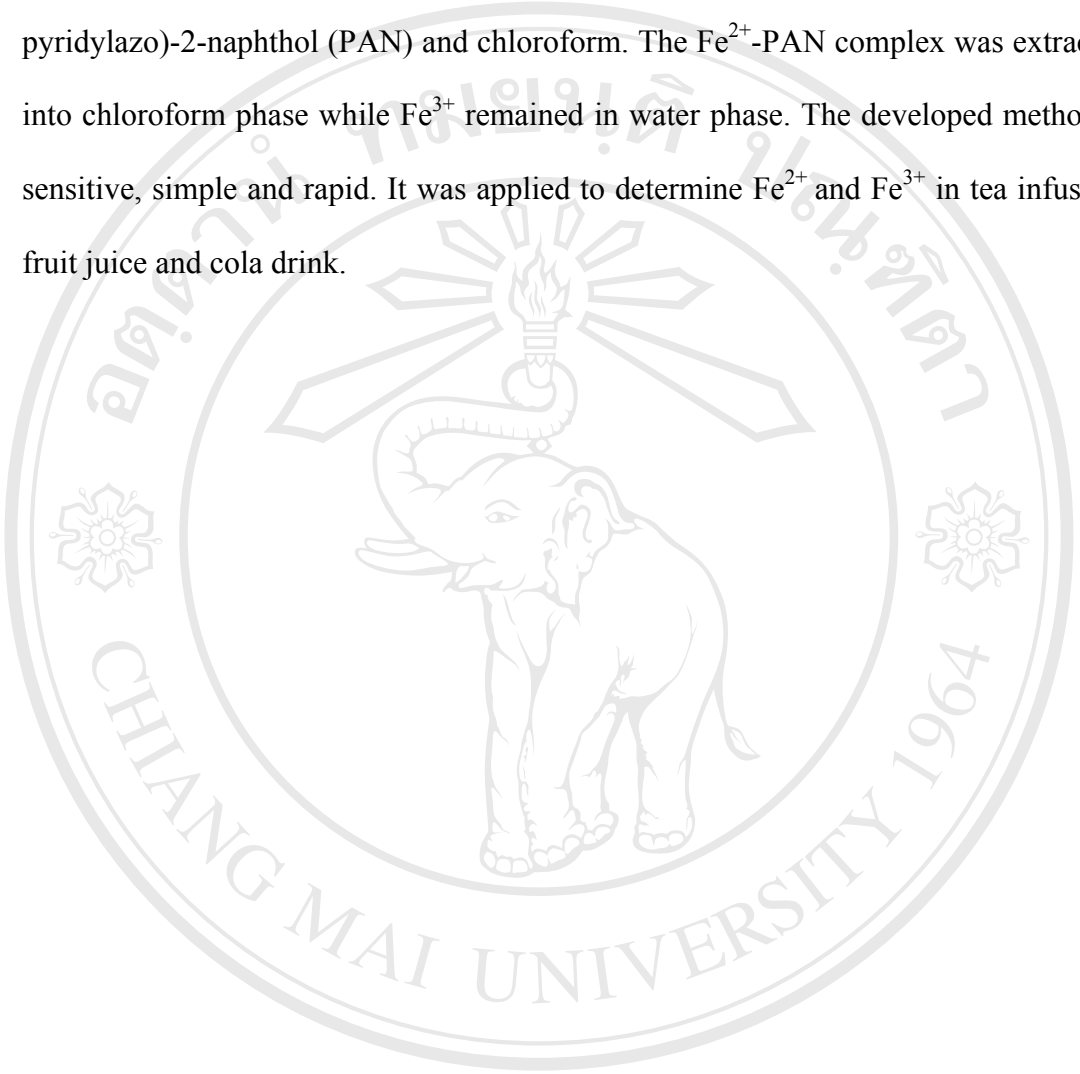
irritability, dizziness, weakness, shortness of breath, sore tongue, brittle nails, decreased appetite and often cold hands and feet.

1.3.2 Methods for iron speciation

There are several techniques for the speciation of iron in various samples such as inductively coupled plasma-mass spectrometry (ICP-MS) [14], ion chromatography (IC) [15], complexation with specific chelating agent followed by spectrophotometric measurement [13, 16-20] and solvent extraction combined with atomic absorption spectrometry (AAS) [13]. The common chelating agents are used for Fe^{2+} such as 1,10-phenantroline [17], bathophenantroline (4,7-diphenyl-1,10-phenantroline) [16, 18], ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine). After the determination of Fe^{2+} and Fe^{3+} is reduced to Fe^{2+} using proper reducing agent such as ascorbic acid or hydroxylamine hydrochloride and the total iron is determined. Fe^{3+} is then calculated by subtraction the concentration of Fe^{2+} from total iron. Most of methods are lack of sufficient sensitivity for iron speciation at μgL^{-1} or sub-μgL^{-1} levels. The high selectivity and sensitivity of atomic absorption methods overcome the problems [19].

Many reports have been studied for the speciation of iron in foods. Camara *et. al.* [18] studied the speciation of Fe^{2+} and Fe^{3+} from school menus, and used bathophenantroline as complexing agent and determined by spectrophotometric method. Quinteros *et. al.* [16] studied the speciation of soluble iron, Fe^{2+} and Fe^{3+} from vegetal foods using water extraction with spectrophotometric detection. Adewusi *et. al.* [20] studied the effect of cooking on soluble iron. The report reviewed that cooking increased soluble iron contents. Yaman *et. al.* [13] developed speciation

method for separation of Fe^{2+} and Fe^{3+} using solvent extraction and flame atomic absorption spectrometry (FAAS). The complexing agent for Fe^{2+} and Fe^{3+} were 1-(2-pyridylazo)-2-naphthol (PAN) and chloroform. The Fe^{2+}-PAN complex was extracted into chloroform phase while Fe^{3+} remained in water phase. The developed method is sensitive, simple and rapid. It was applied to determine Fe^{2+} and Fe^{3+} in tea infusion, fruit juice and cola drink.



1.4 Graphite furnace atomic absorption spectrometry (GFAAS) [21-23]

Graphite furnace atomic absorption spectrometry (GFAAS) is also known by various other acronyms, including electrothermal atomic absorption spectrometry (ETAAS). The technique is based on the fact that free atoms will absorb light at frequencies or wavelengths characteristic of the element of interest. In this technique, a tube of graphite is located in the sample compartment of atomic absorption spectrometer, with the light path passing through it. A small volume of sample solution is quantitatively placed into the tube, normally through a sample injection hole located in the center of the tube wall. The tube is heated through a programmed temperature sequence until finally the analyte present in the sample is dissociated into atoms and atomic absorption occurs. As atoms are created and diffuse out of the tube, the absorbance rises and falls in a peak-shaped signal. The peak height or integrated peak area is used as the analytical signal for quantization.

The sensitivity of graphite furnace atomic absorption makes it the obvious choice for trace metal analysis applications. Routine determinations at the μg/l level for most elements make it ideal for environmental applications. Advances in instrumentation and techniques have made it possible to analyze very complex sample matrices, such as those frequently found in biological and geological samples. The microliter sample volume used offer additional benefits where the amount of sample available for analysis is limited, as in many clinical analyses.

1.4.1 Components of the graphite furnace system

The major components of GFAAS are light source, atomizer, monochromator and detector

A block diagram of a graphite furnace atomic absorption spectrometer is shown in **Figure 1.1**.

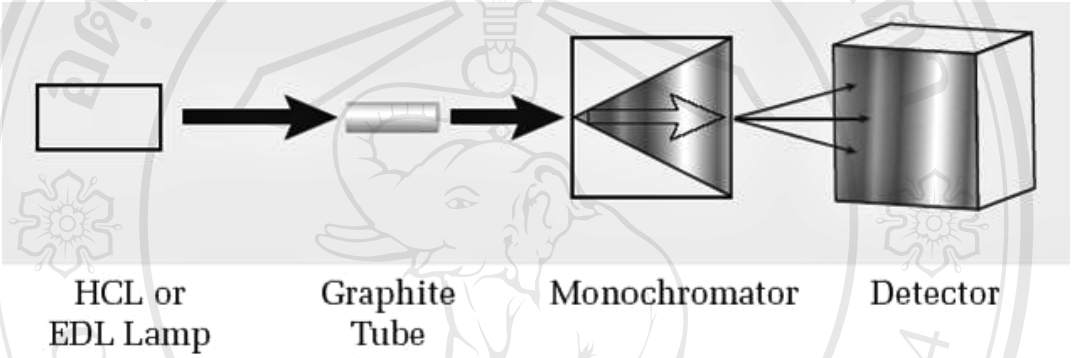


Figure 1.1 Block diagram showing basic components of a graphite furnace atomic absorption spectrometer [24].

1.4.1.1 The light source

An atom absorbs light at discrete wavelengths. In order to measure this narrow light absorption with maximum sensitivity, it is necessary to use a line source, which emits the specific wavelengths which can be absorbed by the atom. Narrow line sources not only provide high sensitivity, but also make atomic absorption a very specific analytical technique with few spectral interferences.

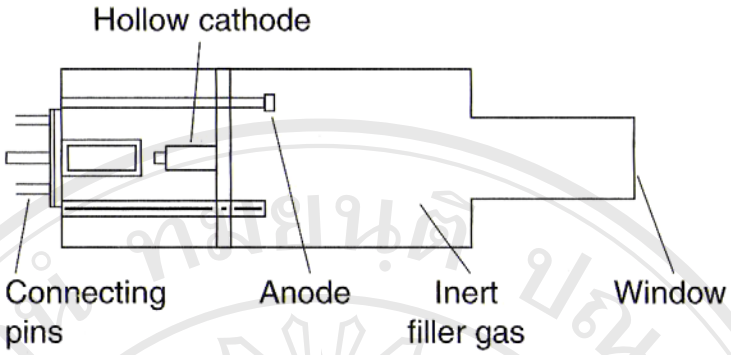


Figure 1.2 Hollow cathode source for atomic absorption spectrometry [25].

The common source of light is a hollow cathode lamp as shown in **Figure 1.2**. Hollow cathode lamps basically consist of a glass cylinder that contains a cathode and an anode. The glass cylinder itself is filled with neon or argon with a pressure of a few millibars. The cathode has the shape of a hollow cylinder and either consists of, or is filled with the element of interest. Applying a voltage of several hundred volts, a glow discharge develops between the electrodes. Sputtering atoms from the cathode surface are excited and emit the spectrum of the cathode material by a flow of positive gas ions (Ne^+ or Ar^+) impacts on its surface. Because of the lower pressure and lower temperature in a HCL, compared to that in the atomizer, the width of the lines emitted by the radiation source is significantly smaller than that of the absorption lines. Depending on the wavelength of the main analytical line the exit window of the lamp is made of silica or glass. The filled gas is selected in a way that no spectral interferences are encountered between the spectrum of the filled gas and the analytical line, and to achieve the highest possible emission intensity of the analyte spectrum.

Hollow cathode lamps have a limited life time. Firstly, sputtered atoms are deposited in part on colder parts of the lamp, *e.g.* the glass cylinder, forming a metal film; secondly, the fill gas is absorbed slowly by the metal film and the glass. Hollow

cathode lamps can be manufactured for a wide variety of elements. For certain combinations of elements it is also possible to make so-called multi-element lamps, which contain an alloy or a mixture of several metals. These lamps have the advantage of being more economic than single element lamps. In addition they shorten the change-over time if more than one element has to be determined.

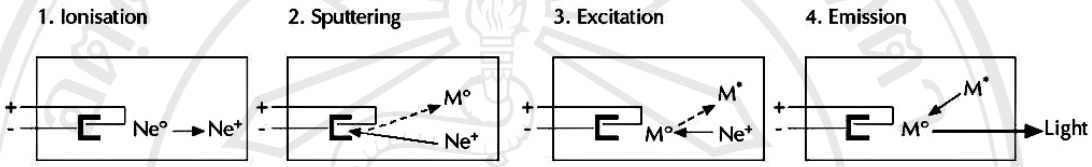


Figure 1.3 Hollow cathode lamp process [25].

A typical atomic absorption instrument holds several lamps each for a different element. The lamps are housed in a rotating turret so that the correct lamp can be quickly selected.

1.4.1.2 The atomizer

A basic graphite furnace atomizer is comprised of the following components:

(1) Graphite tube

(2) Electrical contacts

(3) Enclosed water cooled housing

(4) Inert purge gas controls

A graphite tube is normally the heating element of the graphite furnace. The cylindrical tube is aligned horizontally in the optical path of the spectrometer and serves as the spectrometer sampling cell. A few microliters (usually 5-50 μl) of

sample are measured and dispensed through a hole in the center of the tube wall onto the inner tube wall or a graphite platform. The tube is held in place between two graphite contact cylinders, which provide electrical connection. An electrical potential applied to the contacts cause current to flow through the tube, the effect of which is heating of the tube and the sample.

The entire assembly is mounted within an enclosed, water-cooled housing. Quartz windows at each end of the housing allow light to pass through the tube. The heated graphite is protected from air oxidation by the end windows and two streams of argon. An external gas flow surrounds the outside of the tube, and a separately controllable internal gas flow purges the inside of the tube. The system should regulate the internal gas flow so that the internal flow is reduced or, preferably, completely interrupted during atomization. This helps to maximize sample residence time in the tube and increase the measurement signal.

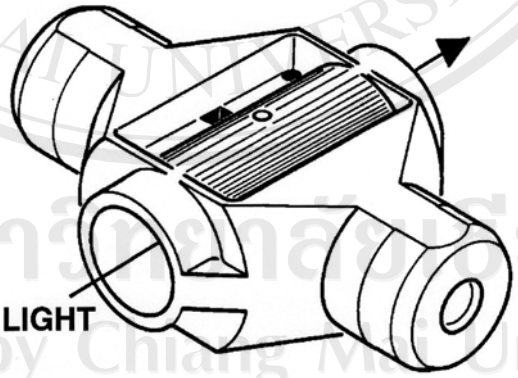


Figure 1.4 A graphite tube for a transversely-heated furnace [26].

The graphite tube of the transversely-heated furnace, shown in **Figure 1.4**, includes integral tabs which protrude from each side. These tabs are inserted into the

electrical contacts. When power is applied, the tube is heated across its circumference (transversely). By applying power in this manner, the tube is heated evenly over its entire length, eliminating or significantly reducing the sample condensation problem seen with longitudinally-heated furnace systems.

A graphite furnace consists of measuring and dispensing a known volume of sample into the furnace. The sample is than subjected to a multi-step temperature program. When the temperature is increased to the point where sample atomization occurs, the atomic absorption measurement is made. Variables under operator control include the volume of sample placed into the furnace and heating parameters for each step. These parameters include:

(1) temperature	final temperature during step
(2) ramp time	time for temperature increases
(3) hold time	time for maintaining final temperature
(4) internal gas	gas type and flow rate

In additional to the above, spectrometer control functions can be programmed to occur at specified times within the graphite furnace program. While the number of steps within each program is variable, 4 steps make up the typical graphite furnace program. These steps include:

(1) The drying step

The purpose of the drying step is to remove the solvent from the sample. After the sample is placed in the furnace, it must be dried at a sufficiently low temperature to avoid sample spattering, which would result in poor analytical precision. Temperature around 100-120 °C are common for aqueous solutions. During

the drying process, the internal gas flow normally is left at its default maximum value (250-300 ml per minute) to purge the vaporized solvent from the tube.

(2) The pyrolysis step

The purpose of the pyrilysis step (sometimes referred to as the ashing, char or pretreatment step) is to volatilize inorganic and organic matrix components selectively from the sample, leaving the analyte element in a less complex matrix for analysis. During this step, the temperature is increased as high as possible to volatilize matrix components but below the temperature at which analyte loss would occur.

The temperature selected for the pyrolysis step will depend on the analyte and the matrix. The internal gas flow is again left at 250-300 ml per minute in the pyrilysis step, to drive off volatilized matrix materials. For some sample types, it may be advantageous to change the internal gas, *e.g.*, to air or oxygen, during the pyrolysis step to aid in the sample decomposition.

(3) The atomization step

The purpose of the atomization step is to produce an atomic vapor of the analyte elements, thereby allowing atomic absorption to be measured. The atomization temperature is a property of the analyte element. The temperature in this step is increased to the point where dissociation of volatilized molecular species occurs.

(4) The clean step

After atomization, the graphite furnace may be heated to still higher temperatures to burn off any sample residue which may remain in the furnace. An optional cool down step then allows the furnace to return to near ambient temperature prior to the introduction of the next sample.

1.4.1.3 The monochromator

A monochromator is used to select the specific wavelength of light *i.e.* spectral line, which is absorbed by the sample, and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others. The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube. This produces an electrical signal proportional to the light intensity. A typical monochromator is diagrammed in **Figure 1.5**.

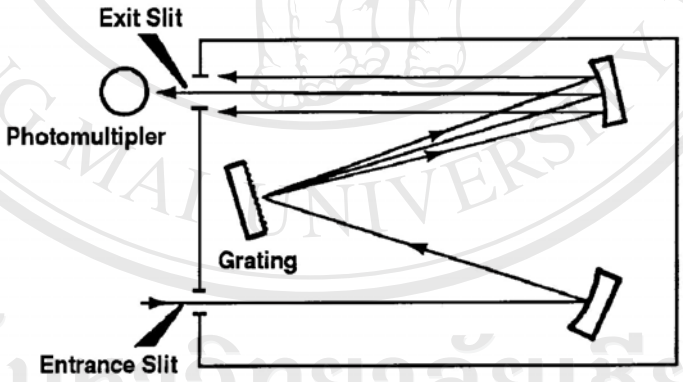


Figure 1.5 A monochromator [26].

1.4.1.4 The detector

The detection of radiation in conventional atomic absorption spectrometers is typically accomplished by a photomultiplier tube (PMT). A PMT is an electronic tube that is capable of converting a photon current into an electrical signal and of

amplifying this signal. A PMT consists of a photo cathode and a secondary electron multiplier. The photons impact on the photo cathode and sputter electrons from its surface. These electrons are accelerated in an electrical field and impact on other electrodes, so-called dynodes, from the surface of which each impacting electron sputters several secondary electrons. This cascade effect results in a significant increase in the number of electrons. In order to function this way, the dynodes have to be on an increasingly positive potential. At the end the electrons impact on an anode and flow off to the mass. The resulting current is measured. The amplification factor increases exponentially with the number of dynodes. Typical PMT have some 10 dynodes, which corresponds to an amplification factor of about 10^7.

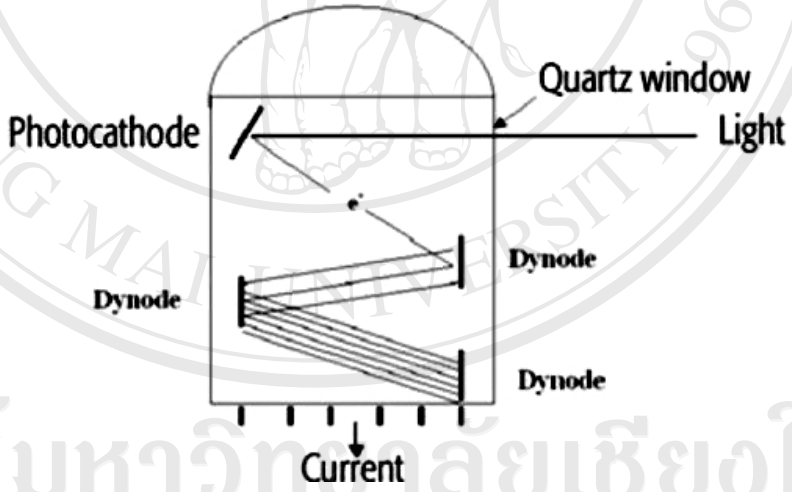


Figure 1.6 Operation principle of a photomultiplier [26].

1.4.3 Matrix modification

The main function of matrix modifiers is to react with the analyte to stabilize the element so that higher pyrolysis temperatures can be used. When the element compound is more stable, it will remain on the platform until later in the atomization step, allowing a closer approach to isothermal conditions. Alternatively, matrix modifiers may react with the matrix to make it more volatile so it can be removed more effectively.

1.4.4 Quantitative analysis by atomic absorption

The atomic absorption process is illustrated in **Figure 1.7**. Light at the resonance wavelength of initial intensity, (I_o), is focused on the flame cell containing ground state atoms. The initial light intensity is decreased by an amount determined by the atom concentration in the flame cell. The light is then directed onto the detector where the reduced intensity, (I), is measured. The amount of light absorbed is determined by comparing I to I_o.

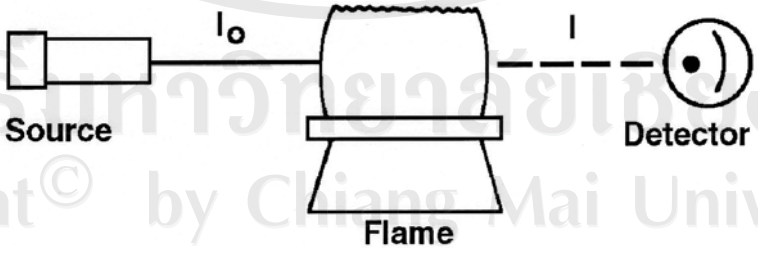


Figure 1.7 The atomic absorption process [26].

Several related terms are used to define the amount of light absorption. The "transmittance" is defined as the ratio of the final intensity to the initial intensity, by the indication of the fraction of I_o passed through the cell to fall on the detector.

$$T = I/I_o$$

The "percent transmission" is simply the transmittance expressed in percentage terms.

$$\%T = 100 \times I/I_o$$

The "percent absorption" is the complement of percent transmission defining the percentage of the initial light intensity which is absorbed in the flame.

$$\%A = 100 - \%T$$

The "absorbance", is purely a mathematical quantity of visualization on a physical basis.

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

Beer's Law defines this relationship; as this quantity follows a linear relationship with concentration.

$$A = abc$$

Where "A" is the absorbance; "a" is the absorption coefficient, a constant which is characteristic of the absorbing species at a specific wavelength; "b" is the length of the light path intercepted by the absorption species in the absorption cell; and "c" is the concentration of the absorbing species.

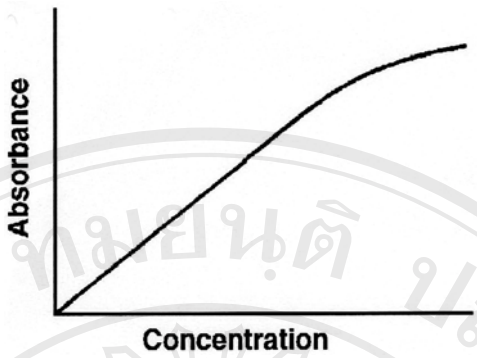


Figure 1.8 Concentration versus absorbance [26].

This directly proportional behavior between absorbance and concentration is observed in atomic absorption. The absorbance data are plotted against the standard concentration, a calibration relationship similar to that in **Figure 1.8** is established. Over the region where the Beer's Law relationship is observed, the calibration yields a straight line. As the concentration and absorbance increase, nonideal behavior in the absorption process can cause a deviation from linearity, as shown.

After such a calibration is established, the absorbance of unknown solutions may be measured and the concentration determined from the calibration curve.

1.4.5 Zeeman effect background correction

1.4.5.1 Principle

Background correction systems measure the background absorption signal and subtract it from total absorbance signal to give a corrected signal. Background correction is required for all furnace work.

When subjected to a strong magnetic field, atom lines, either emission or absorption, are split into three or more polarized components. These extra lines arise through the splitting of electron energy levels within the atoms. In the simplest case this splitting produces three spectral lines, the π component at the original analyte wavelength and the σ^+ and σ^- components symmetrically shifted either side of the original line. The magnitude of the splitting depends on the strength of the magnetic field. This phenomenon is call the Zeeman effect. Because it only effects atomic species it can be used to separate atomic from non-atomic (background) absorption.

The π component is linearly polarized, with the electric vector (polarization direction) parallel to the magnetic field. The two shifted lined (σ components) are circularly polarized, in opposite directions, with the axes of polarization parallel to the magnetic field. The non-shifted line has twice the intensity of the shifted line due to quantum selection rules governing the probability of transitions between the energy levels available to the electrons. With more complicated (anomalous) Zeeman patterns the π and σ lines are split into several components.

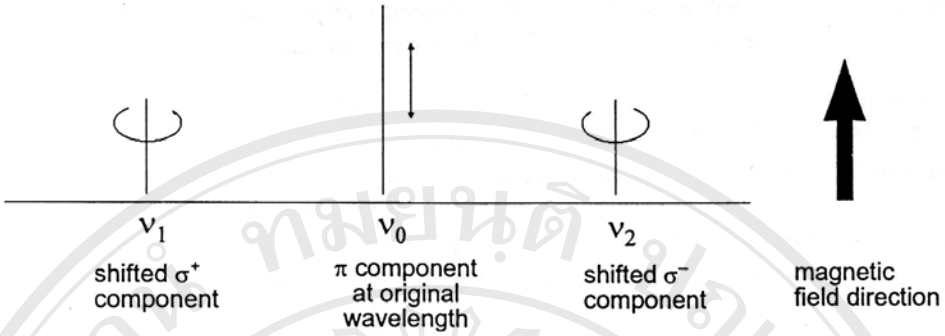


Figure 1.9 Simple Zeeman effect splitting of a spectral line [26].

1.4.5.2 The longitudinal configuration

In this arrangement only the two shifted lines are seen. These appear to be, and are, circularly polarized, because of the direction of viewing, which is parallel to the axis of circular polarization. The nonshifted line cannot be seen because its electric vector is aligned with the radiation beam (an electromagnetic wave does not propagate in the direction of its electric vector). With the longitudinal configuration no polarizer is needed.

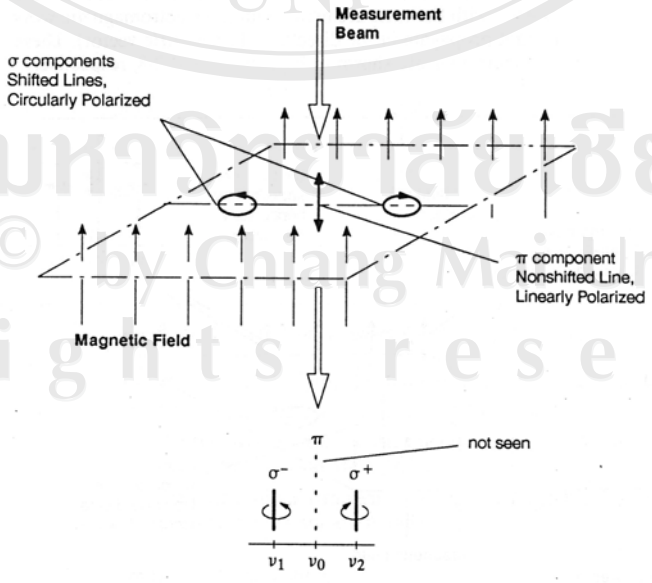


Figure 1.10 The Zeeman effect observed in the longitudinal direction [26].

1.4.5.3 Analyte signal and background correction

Each measurement cycle contains two light phases. The magnetic field is on during one of them and off during the other. With the magnetic field on, the analyte signal is split into the two shifted σ components and the 'unseen', nonshifted π component, The two σ components cannot absorb energy at the analytical wavelength, and the π component, not having an effect in longitudinal direction, also cannot absorb energy at the analytical wavelength. Any attenuation of radiation at the analytical wavelength is thus due only to the background (structured and/or continuous). With the magnetic field off, any attenuation of radiation at the analytical wavelength is due to absorption by the single analyte signal in addition to any attenuation due to the background; structured, continuous, or both.

This procedure of rapidly alternating measurement of [analyte + background] and [background only] at exactly the same wavelength during the measurement cycle gives the system its ideal double-beam characteristics.

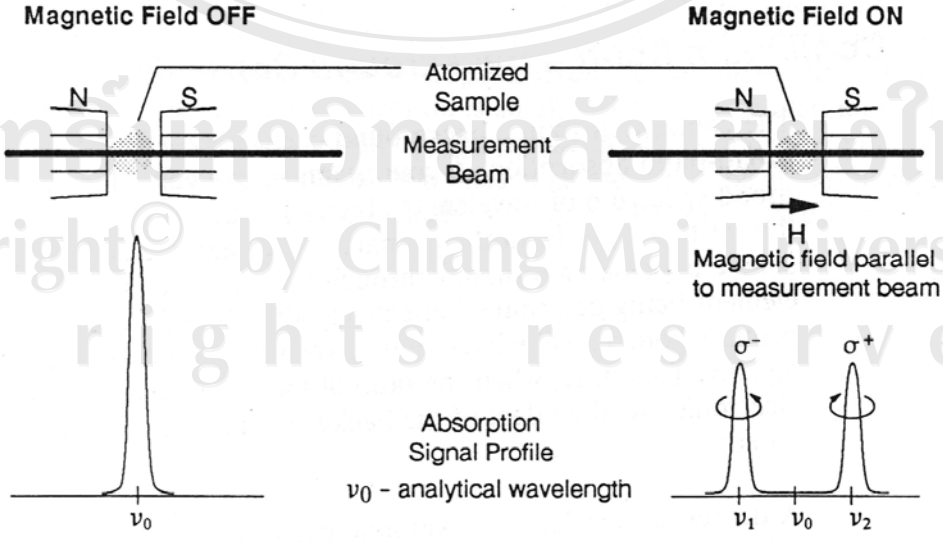


Figure 1.11 The Zeeman effect observed in the longitudinal direction [26].

1.4.6 Advantages of the graphite furnace technique

Detection limits for the graphite furnace fall in the ng/l range for most elements. The sample is atomized in a very short period of time, concentrating the available atoms in the heated cell and resulting in the observed increased sensitivity. Even though this technique used only microliter sample volumes, the small sample size is compensated by long atom residence times in the light path. This provides detection limits similar to the techniques discussed above which use much larger samples.

The graphite furnace is much more automated than the other technique. Even though heating programs can be very sophisticated, the entire process is automated once the sample has been introduced and the furnace program initiated. Automatic samplers make completely unattended operation for graphite furnace atomic absorption possible.

Early experiences with the graphite furnace were plagued with interference problems, requiring detailed optimization procedures for every sample to obtain accurate results. However, extensive studies into the history of the furnace technique combined with the development of improved instrumentation have changed furnace atomic absorption into a highly reliable, routine technique for trace metal analysis.

The final and most obvious advantage of the graphite furnace is its wide applicability. The graphite furnace can determine most elements measurable by atomic absorption in a wide variety of matrices.

1.5 Solvent extraction [6, 27-28]

Solvent extraction, or liquid-liquid extraction, is one of the oldest and most widely used techniques in the preparation of samples for qualitative and quantitative analysis. As for any sample preparation technique, sample clean up and analyte preconcentration are the most common goals of solvent extraction.

The basic idea behind solvent extraction is the separation of components (solutes) from mixture by partitioning them between two immiscible solvents (phases). The solutes partition between the two phase according to their relative solubilities and an equilibrium is established. In most solvent extraction, one of the phases is aqueous and one of the phases is an immiscible organic solvent. The concept "like dissolves like" works well in solvent extraction. A hydrophobic molecule prefers an organic medium whereas an ionic compound prefers to remain in aqueous solution.

The organic solvent is chosen to have a low solubility in water, possess physical properties that cause analytes to have a greater affinity for it than for the water, have volatility for easy removal and concentration after extraction, and be compatible with the analytical measurement technique.

Extraction of various metal ions using acetylacetone, benzoylacetone, dibenzoylmethane, diethyldithiocarbamate, diphenyl-thiocarbazone, α-nitroso-β-naphthol, 8-hydroxyquinoline, N-nitroso-N-phenylhydroxylamine, 1-(2-pyridylazo)-2-naphthol**,** β-isopropyltropolone, 2-thenoyl-1,1,1-trifluoroacetone etc. as chelate forming agent.

Thus, extraction of metal ions with a chelate forming agent is represented as:

$$M^{2+} + 2\,\overline{HL} \quad \rightleftharpoons \quad \overline{ML_2} + 2\,H^+$$

Where M^{2+} is the divalent metal ion in the aqueous solution and HL the chelate forming agent in its protonated form dissolved in the organic solvent; the bars refer to the organic phase.

The advantage of solvent extraction derives from the vast body of solvent extraction literature, accumulated over many decades, which provides information on choice of organic solvent, of pH and of type and concentration of reagents.

1.6 Microwave-assisted digestion [29-31]

Microwave ovens began to find widespread use in chemical laboratories in the late 1980's. The use of laboratory microwave units has become increasingly popular because of the significant improvement in chemical reaction rates that are possible using microwave radiation.

The most innovative source of energy for wet digestion procedures is microwaves. Because the heating take place inside the digestion mixture, microwave digestion is more efficient than with conventional means of heating. Using microwaves, both the speed and the efficiency of digestion for some types of samples considered difficult to solubilize are often improved. Additionally, automation becomes possible with some instrumentation.

Although this technique makes use of microwave radiation, the direct effects of this radiation are of minor importance, at most. Microwaves cannot rupture molecule bonds directly because the corresponding energy is too low to excite electronic or vibrational state. Rotational excitation of dipoles and molecular motion associated with the migration of ions are the only processes that are observed in this microwave field. The microwave energy is instantly transferred to the sample by absorptive polarization and not by molecular collision. The internal heating mechanically agitates and ruptures the surface layers of the sample, thereby providing a better contact between the acids and the sample.

The advantages of microwave digestion include faster reaction rates that result from the high temperatures and pressures attained inside the sealed containers. They are generally made of polymers that will not contaminated or adsorb the sample and do not absorb microwave energy. The caps, which are screwed on to the canister with

a torquing device, are design to safely vent container gas in case of an excess internal pressure buildup.

Microwave-assisted digestion has been applied for a variety of sample types, such as geological, biological, clinical, botanical, food, environmental, sludge, coal and ash, metallic, and synthetic materials and mixed samples and present specific experimental conditions as a function of the matrix to digest.

Conventional wet-sample preparation methods for the decomposition of solid samples are usually carried out in vessels containing the sample and a large volume of decomposition reagent, typically 15 to 100 ml. This mixture is heated for long periods of time using a hot plate, heating mantle, or oven. Heating is terminated when the analyst decides that the decomposition of the sample is sufficiently complete. This type of open-vessel digestion has many drawbacks, which include the use of large volumes (and multiple additions) of reagents, a large potential for contamination of the sample by materials and laboratory environment, and the exposure of the analyst and the laboratory to corrosive fumes.

Closed-vessel microwave decomposition uses significantly different technology and fundamentally unique principles to accomplish sample decomposition. Decomposition of most solid samples can be achieved using near stoichiometric quantities of reagents, typically 10 ml, and can usually be completed in 10 to 15 minutes. This decrease in sample preparation time can be attributed to the closed vessels and the rapid heating of the sample mixture.

The use of closed vessel systems for acid digestion, especially with microwaves, has now become routine. In addition, modern microwave ovens are safer

and simpler and provide more controlled and reproducible conditions than hot plate or block digesters.

Advantages	Disadvantages
Achieve higher temperatures	Higher cost
Time greatly reduced	Limited sample weight
Volatile elements during digestion is virtually eliminated	
Possibility of airborne contamination is eliminated or substantially reduced	

1.7 Rice [32-34]

Rice is the staple food for over half of the world's population. Ninety percent of the world crop is grown and consumed in Asia. Rice is classified in the division Magnoliophyta , class Liliopsida, order Cyperales and family Gramineae. In the beginning rice grew wild, but today most cultivated rice belongs to two species, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). The three main strains of *Oryza sativa* are indica, Japonica and Javanica. The majority of the cultivated varieties belong to this species, which is characterized by its plasticity and taste qualities. It is medium to long grain and has a nutty flavor.

In Thailand, rice is the essence of life. The grains, which are the edible part of the plant, are surrounded by a hard indigestible husk which has to be removed by milling or pounding. Rice is an extremely healthy food for a number of reasons. Rice is rich in carbohydrates, the main sources of energy. Complex carbohydrates are digested slowly, allowing the body to utilize the energy released over a longer period which is nutritionally efficient. Rice has low sodium content and contains useful quantities of potassium, the B vitamins, thiamin and niacin. It contains only a trace of fat and no cholesterol. Rice is also gluten free, so suitable for celiac, and it is easily digested.

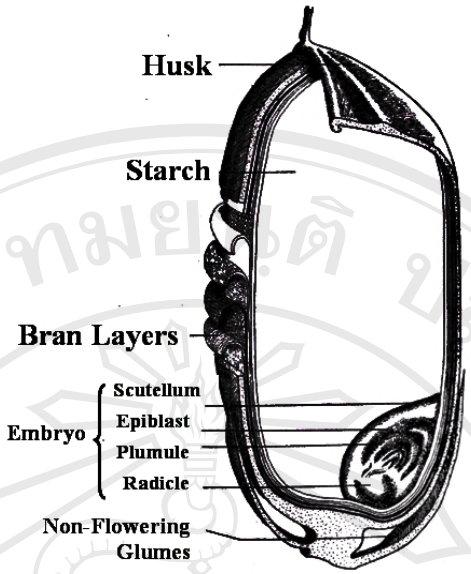


Figure 1.12 Rice grain structure [35].

In general, each rice kernel is composed of the following layer:

- Rice shell, Hull or Husk: encloses the brown rice consists mainly of embryo and endosperm.
- Bran coat (layer): a very thin layer of differentiated tissues. This layer contains fiber, vitamin B, protein and fat. The most nutritious part of rice resides in this layer.
- Embryo: The innermost part of rice consisting mainly of starch called amylose and amylo pectin. The mixture of these two starches determines the cooking texture of rice.

After the husk is removed the remaining product is called brown rice. Brown rice is more nutritious than white rice, but very little rice is consumed in the brown form. Brown rice contains a bran layer that is about 12% of the brown kernel by weight. The protein in white rice is much more nutritious than most cereal grains but has a low level of the essential amino acid, lysine. The protein level in bran layer of

brown rice is higher and has more lysine. The protein in brown rice has one of the most complete essential amino acid profiles of any vegetable crop.

The rice bran layer of brown rice also contains digestible fiber as well as minerals and vitamins not found in milled rice. It was learned that incidences of beriberi were dramatically reduced when diets where switched from white milled rice to brown rice or even parboiled rice which contained more thiamin. The oil content of the rice bran contains several vitamin E components and valuable nutrients. Because minerals and vitamins are lost when the bran is removed, years ago many states passed laws requiring white rice to be fortified with vitamins and minerals. Despite its nutritional value, consumption of brown rice is low because it takes almost one hour to cook and many people do not care for the taste and texture. Unfortunately, once the husk is removed from rice, the bran layer starts going rancid and this contributes to the bitter taste of brown rice. Milled rice is the most common form of rice found in market. It is also called white rice or polished rice. It has all husk, bran and germ layers removed, leaving only the core of rice which is starch.

1.8 Research objectives

The main propose of this research are as follows:

1. To extract iron from rice samples using suitable extracting solvents
2. To separate Fe^{2+} and Fe^{3+} in rice samples by solvent extraction technique and determine by graphite furnace atomic absorption spectrometry (GFAAS)