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Determination and Pre- Concentration of Iron and Copper in Urine of Occupational Workers Samples by Using Cloud Point Extraction

A Thesis

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By

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بسم الله الرحمن الرحيم

قَالُواْ سُبۡحَٰنَكَ لَا عِلۡمَ لَنَآ إِلَّا مَا عَلَّمۡتَنَآۖ إِنَّكَ أَنتَ
ٱلۡعَلِيمُ ٱلۡحَكِيمُ ٣٢

صدق الله العلي العظيم

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Mouyed Khudhair Hassan Al-Assady
2017

Supervisor Certification

I certify that this thesis **" Determination and Pre- Concentration of Iron and Copper in Urine of Occupational Workers Samples by Using Cloud Point Extraction**" was conducted under my supervision at the department of chemistry, College of science, University of Kerbala, as a partial fulfillment of the requirements for the degree of Master in chemistry.

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Abstract

This thesis contains four main chapters. They relate to extract and determine of some trace elements such as; iron (III) and copper (II) in urine of occupational workers samples in kerbala-Iraq by using cloud point extraction method coupling with UV-Vis spectrophotometric and atomic absorption spectrometric methods. **Chapter one** includes an introduction of the major lines for project title content. **Chapter two** comprises a full description of chemical compounds, preparation methods, in addition to the most important tools that were used to pre-concentrate and determine trace elements. As well as, that will be given all the descriptions for the sampling process and the full measurement conditions. **Chapter three** indicates the most important results which obtained from the quantitative analysis of trace elements. Iron (III) responded with 4, 4-biphenyl diamine (Benzidine) to produce a green complex that was extracted in a nonionic surfactant Triton X-114 at pH 4.20 . The light green copper(II) complex extraction method was based on the reaction of copper(II) with the salicylaldoxime (SAO) reagent and extracted by Triton X-114 as nonionic surfactant .The main factors affecting cloud point extraction efficiencies, such as pH of sample solution, the concentration of Benzidine reagent, type of surfactant effect, the concentration of Triton X-114, the effect of salt out, the influence of interferences and impact of equilibration temperature and time of were studied. The calibration curve of iron (III) ion was linear in the range of 0.25-3.0 µg. mL^{-1} with r = 0.9825 for UV-Vis spectrophotometer at λmax 425 nm. The limit of detection was 0.05 µg. mL^{-1}. The relative standard deviation for six replicates was 2.955%. While the curve of copper (II) ion was linear in the range of 0.50-16.00 µg. mL^{-1} with r =0.9979 for UV-Vis spectrophotometer at λmax 380 nm. The limit of detection was 0.10 μg. mL^{-1}. The relative standard deviation for six replicates was 4.887%. These methods were applied successfully to determination iron (III) and copper (II) in 44 urine of occupational worker samples by UV-Vis spectrophotometry and FAAS techniques. The data of the application results were statistically treated. The significant tests t- Test, F-Test and One-Way ANOVA Test were applied. The statistical results showed that a significant difference between the concentration of iron and copper in urine samples in comparison with the concentration in literature reviews. While there is no significant difference between the measuring results by using the UV-Vis spectrophotometry method and FAAS method to measure iron (III) and copper (II) in this work. Also the measuring by UV-Vis spectrophotometry coupling with the cloud point extraction will increase the efficiency, accuracy and sensitivity of this technique, which can be used as alternative methods for determination of copper and iron in urine sample.

Arabic Summary

List of abbreviations

Abbreviation	**Full Name**
3-NBT	3-nitro benzaldehyde thiosemicarbazone
5-Br-PADAP	2-(5-bromo-2-pyridylazo)-5- (diethylamino) phenol
Aaq	Absorbance of an aqueous layer
ANOVA	Analysis of variance
ANPAP	Amino-4-(3-nitrophenylazo) pyridine-3-ol
A_O	Absorbance of an original solution
APDC	Ammonium pyrolidinedithiocarbamate
As	Absorbance of a rich phase surfactant
ATAP	2-amino-4-(mtolylazo) pyridine-3-ol
BDAP	2-(2′-benzothiazolylazo)-5-(N,N-diethyl)aminophenol
Benzidine	4,4-biphenyldiamine
BIYPYBI	2-(6-(1H-benzo[d]imidazol-2-yl)pyridin-2-yl)-1H- benzo[d] Imidazole
BPG	Bromopyrogallol red
C.F	Concentration factor
C.I	Confidence interval
CMC	Critical micelle concentration
CPE	Cloud Point Extraction
CPT	Cloud Point Temperature
CTAB	Cetyl tri methyl ammonium bromide
D	Distribution coefficient
E%	Extraction percentage
ECD	Electrochemical Deposition
EDBOCA	N,N -(2,2 - (ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(2-chloroacetamide)
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
Er%	Relative Error percent
FAAS	Flame atomic absorption spectrometry
HEPTS	4-Ethyl-1-(pyridinyl) thiosemicarbazide
HMO8Q	2-methyl-5-octyloxymethyl-8- quinolinol
IARC	International Agency for Research on Cancer
ISO	International organization for standardization
IUPAC	International Union of Pure and Applied Chemistry
LLE	Liquid-Liquid Extraction

MPKO	methyl-2-pyridylketone oxime
NDDT	6-(2-naphthyl)-2,3-dihydro-as-triazine-3-thione
OP-10	polyoxyethylated phenol
PAN	1-(2-Pyridylazo)-2-naphthol
PDBDM	4-(phenyl diazenyl) benzene-1,3-diamine
PHBI	2-phenyl-1H-benzo[d] Imidazole
PHBI	2-phenyl-1H-benzo[d] Imidazole
PONPE 7.5	polyethyleneglycol-mono-*p*-nonylphenylether
PUF	Polyurethane foam
r	Correlation coefficient
r^2	Coefficient of determination
r^2%	Relative coefficient of determination
RSD	Relative standard deviation
SAO	Salicylaldehyde oxime
SD	Standard deviation
SDS	Sodium Dodecyl Sulfate
SPAR	Sulfapyridylazo resorcinol
SPE	Solid Phase Extraction
SPP	Surfactant-poor phase
SRP	Surfactant-rich phase
TAN	1-(2-thiazolylazo)-2-naphthol
Triton X-114	Polyethelyene glycol Tert-octyl phenyl ether
Triton X-100	Polyoxyethelyene octyl phenyl ether
Tween 80	Polyoxyethylene sorbitan mono oleate
UV-Vis	Ultraviolet–visible spectrophotometry
V_M	Volume of metal
V_T	Total Volume
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Introduction of Pre-Concentration Methods

The increase in the concentration of trace components relative to the total component concentration can be achieved by pre-concentration technique [1]. The need for pre-concentration of trace elements or ions in aqueous solution results from the fact that instrumental analytical methods often do not have the necessary selectivity, sensitivity, or free of matrix interferences. There are multiple methods of pre-concentration for metals, such as: solid phase extraction (SPE), liquid-liquid extraction (LLE), co-precipitation, precipitation, electrochemical deposition (ECD) and cloud point extraction (CPE) [2- 5].

1.1.1 Solid Phase Extraction

Solid Phase Extraction (SPE) is one of the techniques of pre-concentration for trace elements that have been used in early 1970 and developed during 1980s. Where, SPE operation is depending on the distribution of analytes between solid sorbent filled in a column and liquid sample which moving through the solid phase [1, 6]. SPE usually consists of small, porous particles of silica with a bonded organic phase or of an organic polymer, for, example cross-linked polystyrene. The extraction can take place in a batch mode in which the solid extractant is intimately mixed with the liquid sample solution. Chemically, analysis is the most common to fill the solid extractant into a small column and passes the liquid sample during the column [7]. Its selective transfer of material in sub-microgram to milligram quantities between a solid sorbent and a liquid phase. The separations depend on different relative affinities for the two phases based on adsorption, size or charge, selectivity achieved by pH control, solvent composition and surface chemistry of the sorbent [8]. There are many types of solid materials such as modified polyurethane foam (PUF) [9], silica gel [10], cellulose [11], activated carbon [12] and microcrystalline naphthalene [13]. The surface can be improved chemically by using chemical reaction or physically by adsorption, in order to improve the selectivity toward certain particle or a certain group of components [14]. SPE has many benefits compared to traditional methods of solvent extraction. One of the most important disadvantages of this method is time consumption due to many practical steps [15]. The disadvantage of this technique also is the repeated use of columns or cartridges will lead to the consumption of these columns and thus affect the selectivity and may contaminate the sample and the disadvantage is expensive [16].

1.1.2 Liquid-Liquid Extraction

One of the most important methods of pre-concentration which is widely used for the extraction and identification of trace elements is Liquid-Liquid Extraction (LLE) method [5], which includes distribution of metal between two non-miscible liquid phases (often both aqueous and organic phases) [17]. It presents many applications related to the determination of elements at very low concentration in complex matrices like environmental or biological samples [3], and depends on the variance in the solubility of trace elements and their compounds in two non-miscible liquid phases. Generally, the first phase is an aqueous solution and the second phase is an organic solvent non-miscible with water [4], to separate the analyte by using liquid–liquid extraction technique it is desirable that the analyte be amount excluding from aqueous matrix sample by adding non-miscible solvents. The effectiveness of this technique depends primarily on the tendency of materials with extraction solvents, a ratio between the phases and number of extractions [18]. This technique has some advantages. One of the advantages is the ease of working with this technique and its simplicity. The disadvantages are the use of large quantities of expensive and sometimes toxic solvents, so they are not environmentally friendly, emulsion formation at the interface of the two phases and not easy phase separations as well as low concentration factor [2, 6].

1.1.3 Co-Precipitation and Precipitation Methods

This technique is consider as the oldest method used for pre-concentration of ions, where the use is limited to those analytical techniques requiring liquid samples [5].The basis of the work of this technique is precipitation of metal ions in solution. The technique involves the conversion of metal ions into non-soluble varieties where this step is achieved by the chemical reaction between soluble ions and the precipitating reagent. One of the precipitating reagents that can be added for this purpose is alum, salts and other organic polymers [19]. This technique was used when the concentration of metal ions is very low in solutions, but direct precipitation is impossible. The co-precipitation can be related with metal adsorption on the precipitate surface or due to metal incorporation onto the precipitate structures after precipitation; the precipitate can be removed or excluded by centrifuge and then dissolve it by using acid to be measured [4]. Sedimentation agents used in this method are usually either organic or inorganic. Co-precipitation technology often uses the organic precipitation agents for several reasons, including its ability to bond with metal ions. The carrier element is precipitated to co-precipitate trace elements in sample solutions. Organic co-precipitants, usual dithiocarbamates of bismuth and copper have been generally used as efficient collectors of trace elements [4, 20, 21]. The Co-precipitation technology has some disadvantages, including the production of large quantities of sediments containing toxic compounds [19].

1.1.4 Electrochemical Deposition

Electrochemical deposition (ECD) is one of the techniques used for pre-concentration of metal ions; the technique gives the desired characteristics and specifications for the pre-concentration method. Electrochemical deposition method has been broadly used for many years and has been used extensively used in electrogravimetry [5]. Electro deposition technique is based mainly and following the laws of electrolysis, which include the deposition or collection of ions on the electrodes by using an electric cell consisting of three electrodes (reference, work, and auxiliary) [22]. Electrolysis can be performed or achieved in some cases by preparing a galvanic cell using the sample solution with an inert electrode as a first cell and using the most positive electrode as a second cell, if high currents are forced through the solution to speed up the deposition. One of the most important advantages of this technique does not need to a large number of reagents in addition to the simplicity of the devices and very little operator attention is required during the deposition. A common defects in this technique is being slow to a large extent; in addition, an inert supporting electrolyte must be added when fresh water is used, so that an increase the current-carrying capability of the solution [5].

1.1.5 Cloud Point Extraction

Cloud point extraction (CPE) is one of the pre-concentration methods based on the use of non-ionic surfactant in aqueous solutions. Cloud point refers to the temperature over which solutions of non-ionic surfactants become turbid or cloudy [23]. The process of the cloud point extraction involves three basic steps: the formation of the micelles, the micellar aggregation to form a cloudy solution and the final step is phase separation for analysis, where the micelles are connected with the organic complexions in the solutions and extracted to the surfactant-rich phase [24]. This technique can be considered as a way alternative to traditional extraction systems for several reasons, the most important is low cost and simplicity of high efficiency and is one of the ways in which the safety conditions are available [25]. This technique also gives high recovery efficiency and a high concentration coefficient. This is due to the small amounts of the surfactant-rich phase that resulting from the correlation of the micellar associated with the complex and obtained from the original solution [26]. Cloud point extraction techniques can be considered a method that is compatible with green chemistry for several reasons: (a) The medium used to extract dilute solutions from the surfactants is inexpensive, due to less consuming reagent and less production of chemical residues; (b) The surfactant used is safe, non-volatile, and non-toxic [27,28]. Green chemistry can be defined as steps or actions that reduce or eliminate the generation of toxic substances for human health and for the environment [29]. The general steps for the cloud point extraction method are shown in Figure 1.1.

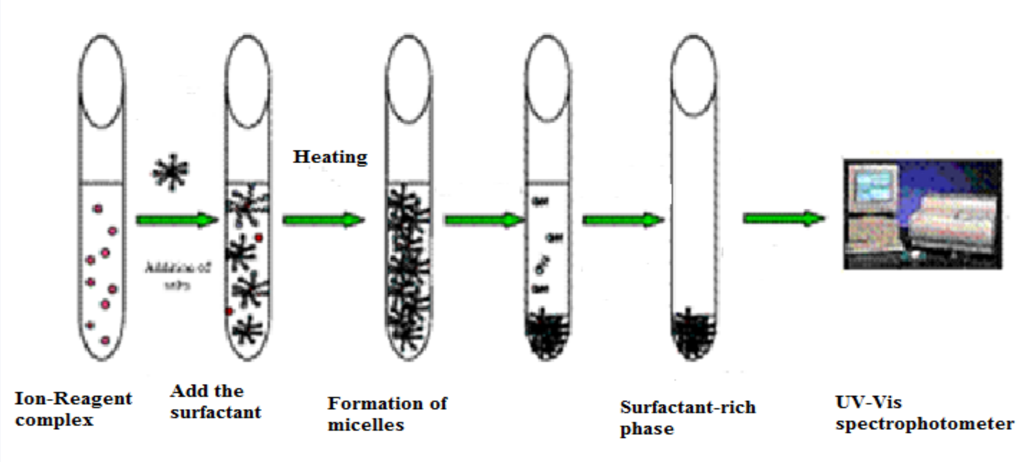


Figure 1.1 The General procedure of cloud point extraction

Cloud point extraction is an efficient method for its ability to extract and pre-concentrate metal ions and organic molecules from different complex matrices. In general, the mechanism of the extraction method at the cloud point is somewhat similar to the traditional liquid-liquid extraction method, which separates the preferred analytes by their affinity in two non-miscible (homogeneous) liquid phases. But, in the CPE procedure, the only differentiation being the “organic” phase (micelles) is generated in the aqueous phase, changing a formerly homogeneous solution into a heterogeneous one by essentially gathering its previously scattered hydrophobic suspension [23, 30]. The formation of metals-reagents complexes with micelles is more stable than the one without the presence of micelles. This technique has achieved many successes in identifying many of the elements in various samples, making it a major focus in the past years [31].

This technique can be coupled to many different instruments for the determination of metals. Table 1.1 optimize the comparison between the old extraction method and some new extraction procedures [32].

Table 1.1 Comparison of different extraction methodologies for liquid samples

Parameter	Pre-concentration methods			
	LLE	**SPE**	**ECD**	**CPE**
Brief description	Analyte is partitioned between two immiscible solvents shaking/ continuous process	Analyte is retained on a solid adsorbent filtration process.	Collection of ions on the electrodes by using an electric cell	Analyte is partitioned between two phases "surfactant rich phase'' and ''aqueous phase''
Extraction time	Takes 1-2 hours/sample	20–30 min	Very slow	20 min
Solvent usage	200-500 mL organic solvent	2-20 mL organic solvent	Few reagents need be added	Surfactant solution
Cost	cost	Relatively low cost	Relatively low cost	Low cost
Ease of operation	Easy	Relatively easy	Relatively easy	Easy
Disadvantages	-Time consuming - Expensive labor and materials -Poor results -Little selectivity	When the repeated use of columns will lead to the consumption of these columns and thus affect the selectivity and may contaminate the sample .	This technique is time consuming.	It is required to optimize of operating conditions.

1.2 Surfactants

Surfactants can be defined as surface active substances that have the ability to bond and correlating to the formation of micelles. Surfactant is one of the most common materials and is widely used in various applications for its obvious effect on the properties of surfaces [33]. Surfactants are "surface-active-agents” which are typically organic compounds that are hydrophilic. It has two sides or groups hydrophobic groups (tails) which is usually a long-chain hydrocarbon or aromatic ring. It works to reduce solubility in water and, hydrophilic groups (heads) that contain functional group which is often ionizable and has the opposite effect [34]. If the surfactants are diluted in aqueous solutions, it behaves like normal electrolytes, while its behavior is different in the case of high concentration [33]. Figure 1.2 illustrated the structure of surfactant molecule [35].

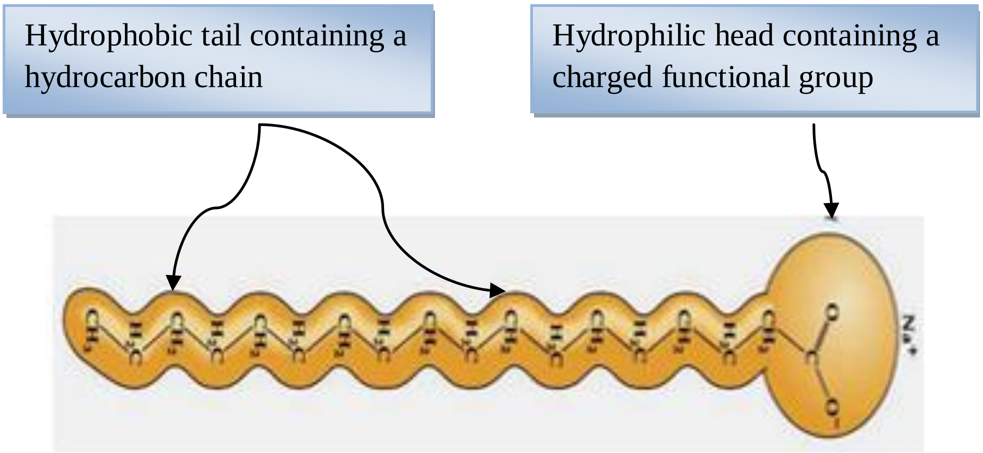


Figure 1.2 Structure of Surfactant Molecule

The surfactants are play an important role in their ability to form colloidal-sized clusters in solution and are therefore widely used in many applications of basic science and contemporary pharmaceutical biotechnology each of which contain some molecules. For the amphiphilic nature and surfactants are able to solubilize hydrophobic compounds in water at certain conditions. There are several factors that affect the function of the surfactants, including the shape and size of these molecules of surfactant in solutions which in turn depend on the surfactants concentration and temperature, pH, and ionic strength. These molecules can be arranged in different shapes and sizes. Figure 1.3 illustrates the most common shapes of surfactant micellar aggregates [36-38]. These properties allow surfactants to be soluble in water in aqueous solutions. It is possible to clarify the structure of the surfactant-micelles in aqueous solution in Figure 1.4 [39].

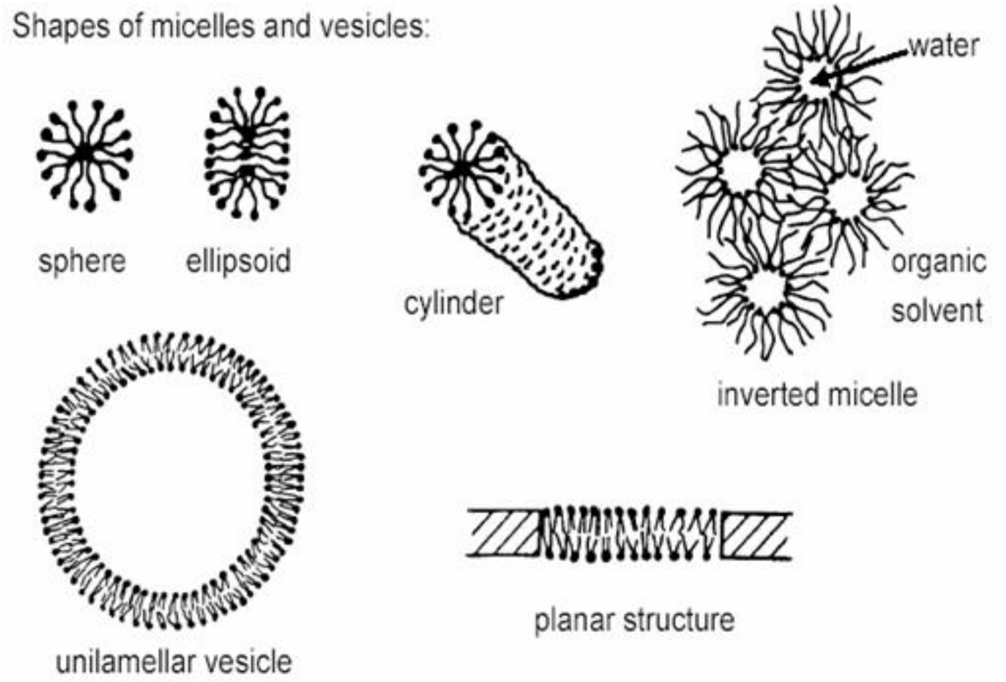


Figure 1.3 Shapes of surfactant micelles aggregates.

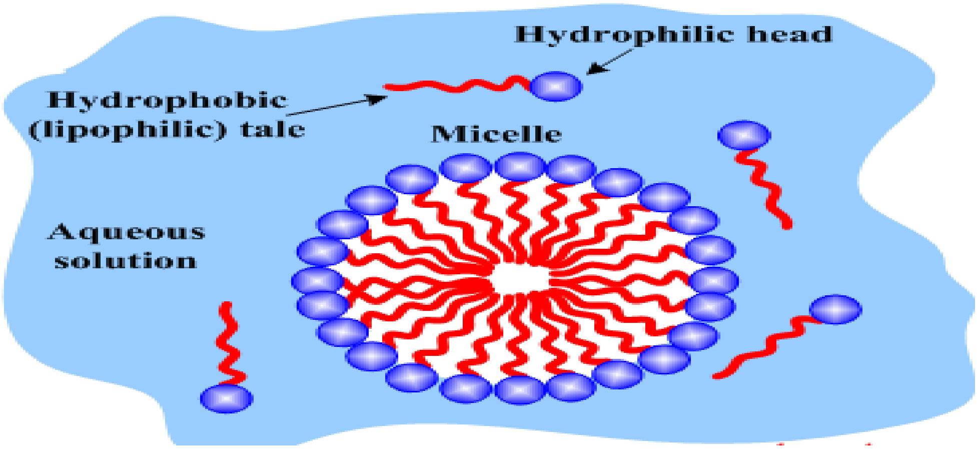


Figure 1.4 Surfactant-micelles structure in aqueous solution

The typical properties of aqueous surfactant solutions may be certified to the presence of a hydrophilic head group and a hydrophobic tail in the molecule. The polar or ionic head group usually interaction powerfully with aqueous surroundings, in either case it is solved by dipole-dipole or ion-dipole interactions [33]. As it works to reduce tension between the surfaces that are resolved according to the system of polar group [40]. The surfactants are divided into the following main types**.**

1.2.1 Anionic Surfactants

Normally anionic surfactants include alkaline minerals such as K^+, Na^+ and quaternary ammonium, So, this type is of surfactants dissociated in water in an amphiphilic anion, and a cation Such as: alkaline carboxylic salts (soap), alkaline sulfonic salts (detergents), alkyl – aromatic sulfonic salts and sodium dodecyl sulfate (SDS) [41, 42].

1.2.2 Cationic Surfactants

It is dissociated in water into an amphiphilic a cation and an anion, most often of the halogen type. For example, cetyl-trimethyl ammonium bromide (CTAB), this type contains cationic functional groups such as quaternary ammonium cation and amine salts [41, 42]. Surfactants are safe and harmless when it comes to skin, but can be used to clean wounds. Its water solutions can also be used for cleaning contaminated utensils [43].

1.2.3 Amphoteric or Zwitterionic

Amphoteric or zwitterionic surfactants are molecules that have ionic and cationic centers, for example sulfonate, carboxylate, long chain amino acid salts and sulfobetanie [41, 42].

1.2.4 Nonionic Surfactants

This type does not ionize in an aqueous solution because their hydrophilic groups are non-dissociable, such as: Triton X-114 and Triton X-100 [41, 42]. This type of surfactants can be classified into several major classes are alcohols, polyether, esters [40]. Table 1.2 tabulated some surfactants compounds. The four main types of the surfactants can be illustrated in

Figures 1.5 and 1.6 depending on the main components of their head and structures of several surfactants [35].

Table 1. 2 Types of surfactants compounds

Surfactant	Chemical formula
Anionic surfactant	
Sodium stearate (soap)	$CH_3(CH_2)_{16}COO^- \ Na^+$
Sodium dodecyl sulfate (SDS)	$CH_3(CH_2)_{11}SO_4^- \ Na^+$
Sodium dodecyl benzene sulfonate	$CH_3(CH_2)_{11}C_6H_4SO_3^- \ Na^+$
Cationic surfactant	
Laurylamine hydrochloride	$CH_3(CH_2)_{11}NH_3^+ \ Cl^-$
Trimethyl dodecylammonium chloride	$C_{12}H_{25}N^+(CH_3)_3Cl^-$
Cetyl trimethylammonium bromide	$CH_3(CH_2)_{15}N^+(CH_3)_3Br^-$
Nonionic surfactant	
Polyoxyethylene alcohol	$C_nH_{2n+1}(OCH_2CH_2)_mOH$
Alkylphenol ethoxylate	$C_9H_{19}{-}C_6H_4{-}(OCH_2CH_2)_nOH$
Octylphenoxypolyethethanol (Triton X-114), (Triton X-100)	$C_{14}H_{22}O(C_2H_4O)_n$ n= (7-8), (9-10)
Zwitterionic surfactant	
Dodecyl betaine	$C_{12}H_{25}N^+(CH_3)_2CH_2COO^-$
Lauramidopropyl betaine	$C_{11}H_{23}CONH(CH_2)_3N^+(CH_3)_2CH_2COO^-$
Cocoamido-2-hydroxy-propyl sulfobetaine	$C_nH_{2n+1}CONH(CH_2)_3N^+(CH_3)_2CH_2CH(OH)CH_2SO_3^-$

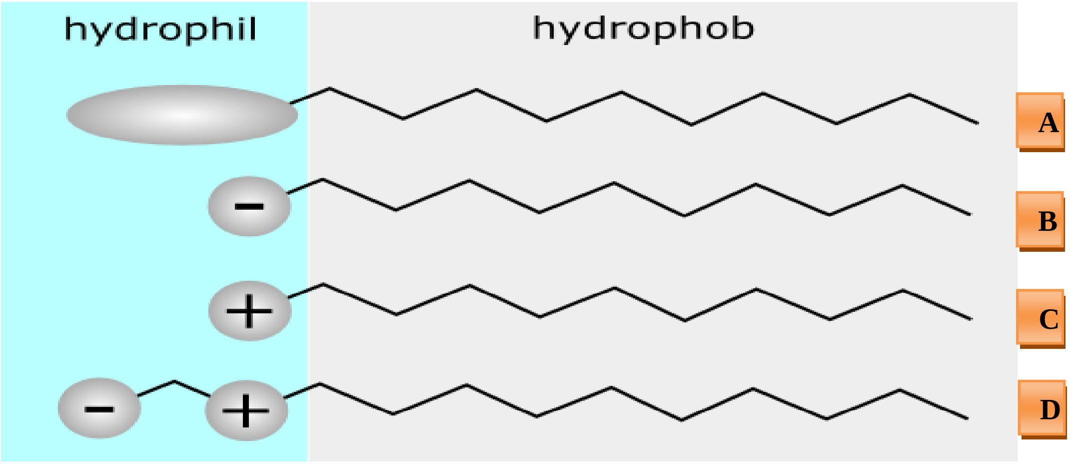


Figure 1.5 Surfactant classification according to the composition of their head (A) nonionic, (B) anionic, (C) cationic, (D) amphoteric or zwitterionic

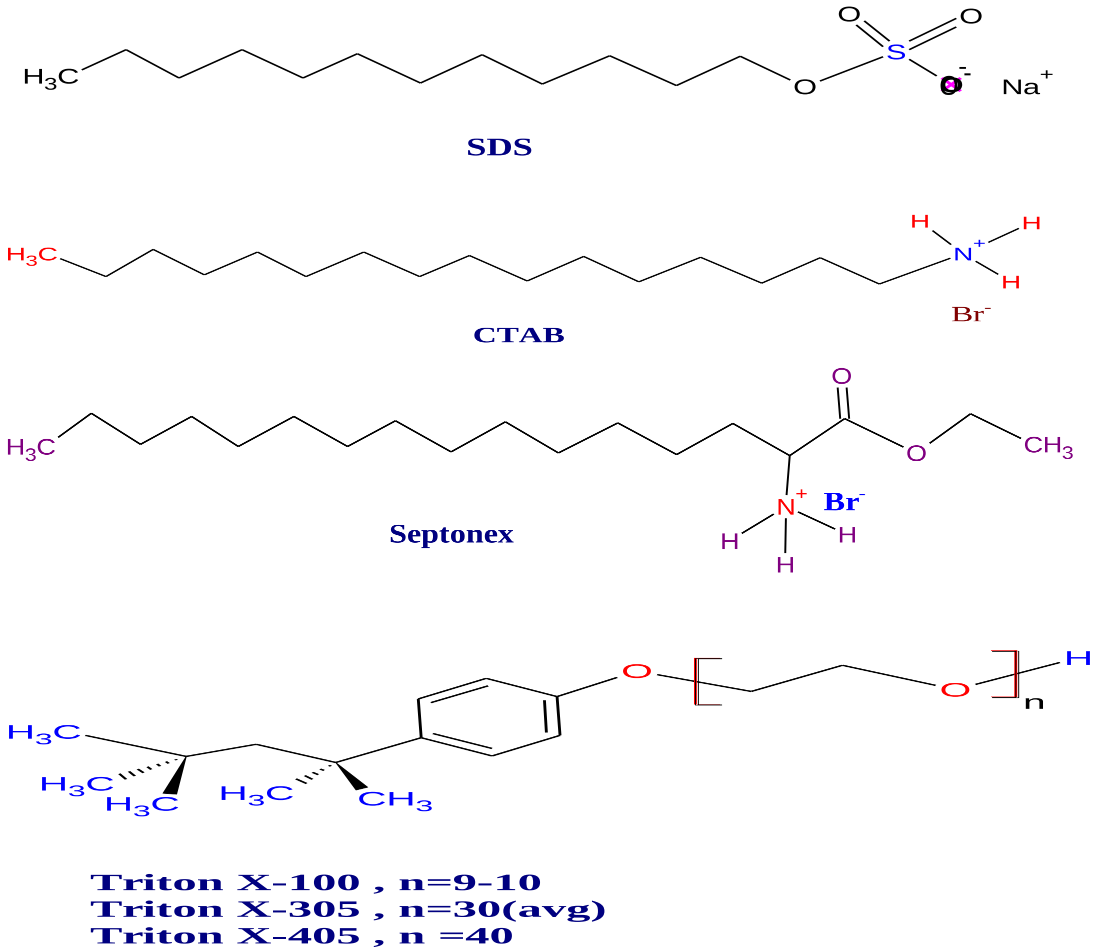


Figure 1.6 Typical Structures of several surfactants

The appropriate temperature helps to convert solutions containing non-ionic surfactants into cloudy solutions. As temperature increases, aqueous solution of a non-ionic surfactant becomes turbid where micellar growth resulting from the dehydration of the polyoxyethylene chain of the hydrophilic group and increased intermicellar attraction causes the formation of large particles and the solution becomes visibly turbid [44]. When heating aqueous solutions containing nonionic surfactants and when reaching the cloud point temperature, these solutions become turbidity or cloudy. The homogeneous surfactant solution separates easily into two immiscible phases: one contains most of the surfactant which consists of a large hydrated micelles of a small volume, called surfactant-rich phase (SRP) or coacervate phase while the other excess water phase, called surfactant-poor phase (SPP). This layer is completely devoid of surfactant. This temperature is known as cloud point temperature (CPT) of the surfactant [45, 46]. There are two type of structure of micelles are called normal micelles and reverse micelles. Figure 1.7 shows typical of micelles structure [35].

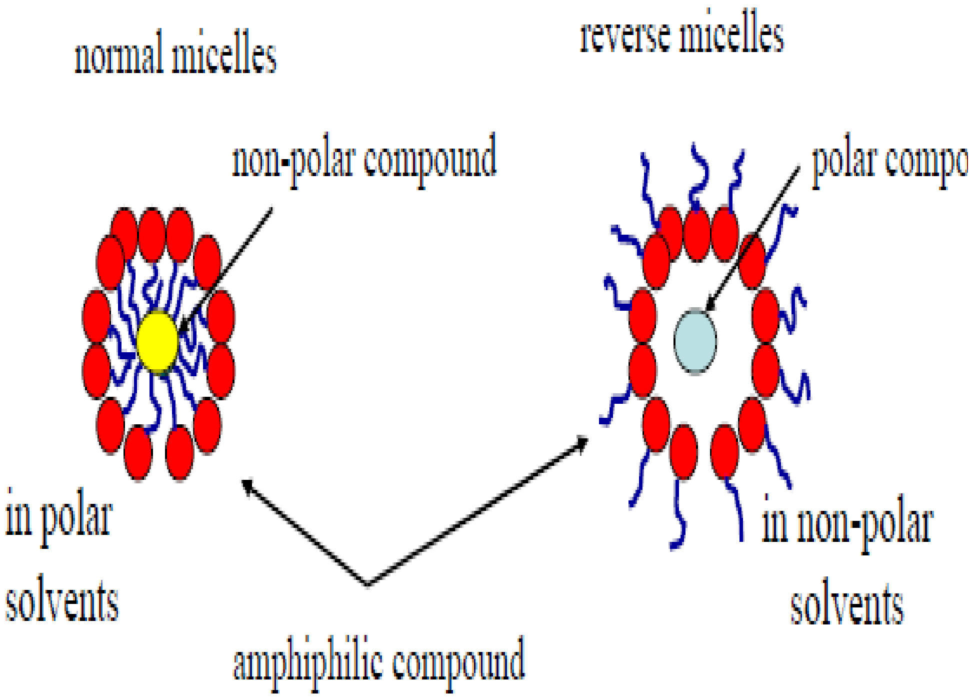


Figure 1.7 Micelles structure

1.2.4.1 Triton X-114

One of the most commonly used species of non-ionic surfactants in the cloud point extraction is Triton X-114. The fact that this type has many physical and chemical properties such as ; low cloud point temperature (CPT), high density of the surfactant-rich phase, which facilitates phase separation by centrifugation, business accessibility, generally low cost, and low toxicity. Triton X-114 is an Polyethelyene glycol Tert-octyl phenyl ether its average molecular weight is 536 g.mol^{-1} [34, 47]. Figure 1.8 shows the structure of Triton X-114.

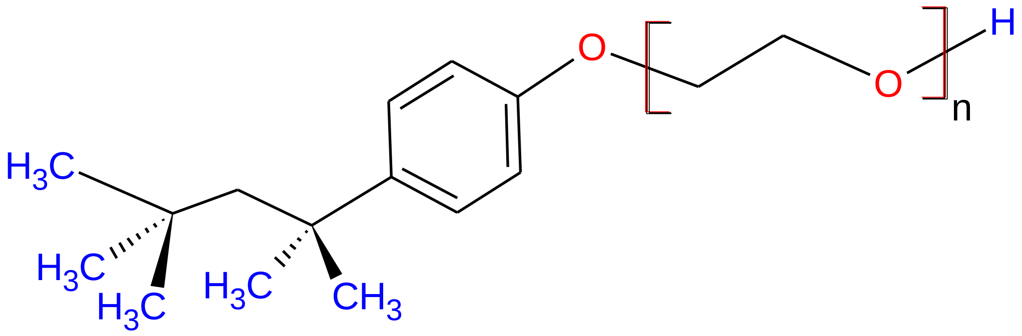


Figure 1.8 Chemical structure of Triton X-114

1.3 Trace Elements and Chemical of Metals

The presence of heavy elements is considered a source of pollution to the environment, although dangerous, but the low concentration of some of them is considered beneficial for health, but when increasing concentrations may harm health [48, 49, 50]. These elements cause us anxiety because of residential or occupational exposure, such as : antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc [51]. As mentioned above, the increase in the concentration of heavy metals makes them toxic as they reduce energy levels and affect the brain, lungs, kidneys, liver and other organs of the body as well as in the long term. These elements may cause some diseases, including sclerosis, Alzheimer's and muscular dystrophy and may even go beyond injury cancer [51, 52]. Some of these elements can accumulate in the body as a result of continuous and direct exposure to these elements. The degree of mineral toxicity depends on the absorbed dose, the method of exposure and the duration of exposure [53]. There are some trace elements in the human body at low levels and varying rates from one element to another and have some benefits and disadvantages on human health such as, chromium, iodine, iron, manganese, cobalt, copper, molybdenum, selenium, and zinc [54]. Due to the many effects of heavy elements, so the concentrations of these elements in the environmental and biologic samples should be determined,therefore can apply many analytical chemistry techniques to estimate these elements [48]. The most common heavy elements in wastewater include arsenic, cadmium, chromium, and copper, lead, nickel, and zinc, all of which cause risks for human health and the environment [52]. Heavy metals are found in the environment in several forms and through several sources include natural weathering of the earth's crust, mining, soil erosion, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others [55]. Where environmental and occupational toxicology are applied through biologic monitoring as well as environmental studies to identify these factors and other factors affecting the varying toxicity of these elements. Usually, urine is a marker of past exposure. High concentration of an element in the urine indicates past and chronic long-term exposure to, for example: arsenic, cadmium, nickel, chromium, cobalt as well as copper or a delay in medical treatment after high occupational [56]. Trace elements such as iron and copper are concentrations in living organisms less than 0.001%.These elements are essential elements in human life but it is toxic if it increases its concentration in the body. Table 1.3 and Figure 1.9 show classifications of trace elements according to the beneficial and effect of trace elements concentration on health respectively [57].

Table 1.3 Classifications of trace elements according to the beneficial

Nutritionally essential metals	Metals with possible beneficial effects	Metals with no known beneficial effects
Co, Cr, Cu, Fe, Mn, Zn, Mo, Se	B, Ni, Si, V, I	Al, Ba, Cd, Sb, As, Be, Pb, Hg, Ag, Sr, Tl

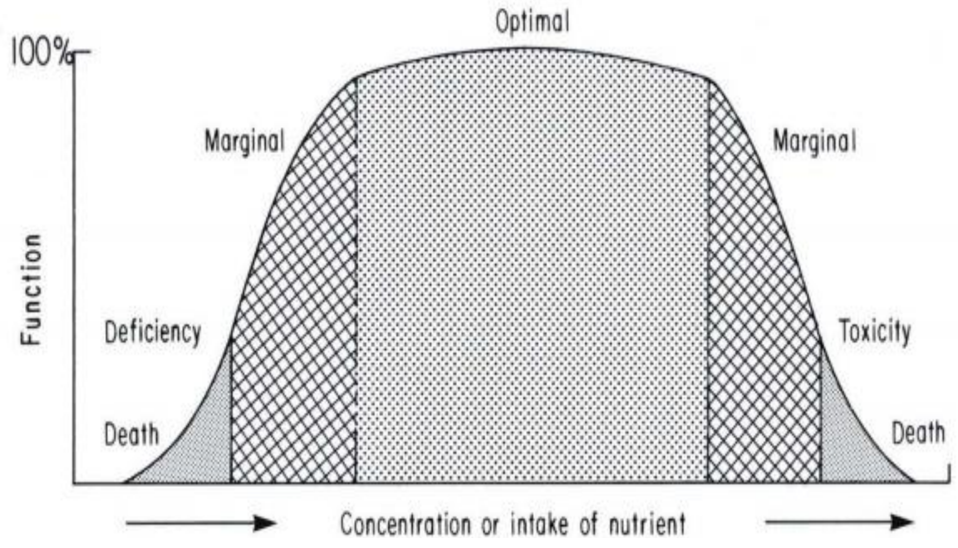


Figure 1.9 Effect of trace elements concentration on human health

1.3.1 Trace Iron Element

Iron is one of the elements of the first transitional series. It is the fourth most common element in the earth's crust and the second most abundant metal on earth. Iron is abundant in the earth [58]. It occupies the 26th position in the periodic table and the iron element is of great importance for the growth of almost all living organisms [59]. It is an essential element for microbes, plants, and higher animals. It is a component of heme and iron-sulfur centers in many key redox enzymes and is an essential component of oxygen storage and transport proteins such as hemoglobin and myoglobin. In humans, iron is strictly conserved, in large part, by recycling the iron (about 20 mg/day) from the hemoglobin of senescent red blood cells to provide iron for new red blood cells. Smaller amounts of iron from myoglobin and various redox enzymes are also recycled. In most circumstances, human diets contain more iron than is necessary to replace the small daily losses (1–2 mg/day) [60, 61]. Iron is one of the most important essential elements of most living organisms as a result of its entry into most metabolic processes, including oxygen and electron transport, in the ferrous state (Fe^{2+}), iron acts as an electron donor, while in the ferric state (Fe^{3+}) it acts as an acceptor and DNA synthesis [58].

1.3.1.1 Iron Deficiency

Deficiency of concentration of iron affects mainly the storage of iron in the body, since iron is important for hemochlobin, the primary clinical manifestation of iron deficiency is iron deficiency anemia [62]. Iron deficiency is due to the following reasons: Nutritional deficiency, decrease some vitamins such as vitamin A and B12 and decrease the riboflavin, folic acid [63].

1.3.1.2 The increasing of Iron

The increasing of iron concentration in the body may lead to injury of several disorders and diseases, for example endocrine problems, arthritis, diabetes, lung cancer, and liver disease [58, 64-66]. Short-term health effects causes erosion in the intestinal mucosa, while long-term health effects causes stomach and bowel erosion and ulcers [57].

1.3.1.3 Concentration of Iron

The concentration of iron in urine for non-occupationally exposed people was 40-150 µg/24h creat [56]. In addition, the mediam and range concentration of iron in urine was 4.9 ng. mL^{-1} and 2.1-16.4 ng. mL^{-1} [56, 67].

1.3.1.4 Previous Literature of Iron

Table 1.4 shows the literature review for Fe (III) by using cloud point extraction coupled with varies technique.

Table 1.4 Review studies of cloud point extraction of iron (III) in various samples coupling with varies analytical methods

Type of sample	Analytical Reagent	Type of Surfactant	LOD Linearity RSD%	Ref.
Flame Atomic Absorption Spectrophotometry (FAAS)				
Environmental samples	N,N -(2,2 - (ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(2-chloroacetamide) (EDBOCA)	Triton X-114	1.22 µg L^{-1} 0.2-5.0 µg L^{-1} 4.2%	68
Waters red wine	Ammonium pyrolidinedithiocarbamate	Triton X-114	19 µg L^{-1} ---	69

and sewage	(APDC)		2.41%	
Human or cow's milk,	8-hydroxy-7-iodoquinoline-5-sulfonic acid (Ferron)	Triton X-114	1.7µg L^{-1} 5-150 µg L^{-1} 2.1%	70
Food samples	Eriochrome cyanine R (ECR)	Triton X-114	0.33 µg L^{-1} 1.5-25 µg L^{-1} --	71
Spice samples.	3-amino-7-dimethylamino-2-methylphenazine (Neutral Red,NR)	Triton X-114	0.7 µg L^{-1} 2.50-200 µg L^{-1} 2.1%	64
Biological and Environmental samples	2-phenyl-1H-benzo[d] Imidazole (PHBI)	Triton X-114	2.8 µg L^{-1} --- 2.0%	72
Environmental samples	2-(6-(1H-benzo[d]imidazol-2-yl)pyridin-2-yl)-1H-benzo[d]Imidazole (BIYPYBI)	Triton X-114	2.2 µg L^{-1} -- 1.4%	73
environmental and biological samples	7-iodo-8-hydroxyquinolin-5-sulphonic acid (Ferron)	Triton X-114	0.4 µg L^{-1} 0.01-0.40 µg mL^{-1} 2.40%	74
UV-Vis Spectrophotometry				
Biological and river water samples	(Drug) doxycycline hydrochloride (DOX.HCl)	Triton X-114	7 µg L^{-1} 20-200 µg L^{-1} 0.84-2.60 %	75
Vegetables	Bromopyrogallol red (BPG)	CTAB	0.020 µg L^{-1} 0.05-43.0 µg L^{-1} ----	76
Beer samples	2-(5-bromo-2-pyridylazo)-5-(diethylamino) phenol (5-Br-PADAP)	Triton X-114	1.0 µg L^{-1} 5.0-112 µg L^{-1} 2.6%	77
Graphite Furnace Atomic Absorption Spectrometry (GFAAS)				
River water	2-methyl-5-octyloxymethyl-8- quinolinol (HMO8Q)	Triton X-100	1.9 µgL^{-1} --- 2%	78
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)				
Water	8-Hydroxyquinoline	Triton X-114	0.055µg L^{-1} 1-800 µgL^{-1} 2.68 %	79
Capillary Electrophoresis (CE)				
Urine	2-(5-bromo-2-pyridylazo)-5-(diethylamino) phenol (5-Br-PADAP)	(PONPE 7.5)	0.48 µg L^{-1} -- 1.0%	80

1.3.2 Trace Copper Element

Copper is one of the basic elements which has several important roles in the human body [81]. Copper is usually found in the human body in two oxidation states: Cu (I), Cu (II). However, the most common form is Cu (II) [82]. Copper has important and effective roles, including its important role in maintaining the function of the immune system [83]. Copper is a functional component of several essential enzymes, known as copper enzymes – cytochrome c oxidase, lysyl oxidase. It is one of the basic elements that contribute to the preservation of human health by considering it as a catalyst or structural component of large molecules [82, 84, 85]. Copper content in foods is highly variable, depending on environmental conditions (soil, geographic location, season, water source, use of fertilizers, and methods of handling, processing, and cooking food) [84]. Copper is found in the air, in the water and in the food but the latter one is the principal source of human exposure. World Health Organization (WHO) recommends a copper intake for adults to range from 1 to 5 mg/day where just the range of 0.1-1.0 mg/day is contributed by drinking water (WHO 2004) [86].

1.3.2.1 Copper Deficiency

One of the main reasons for copper deficiency is copper malabsorption, excessive zinc ingestion and gastric surgery. The decrease of copper concentration in human body causes anemia because the copper is necessary for human nutrition for normal iron metabolism and the formation of red blood cells, problems in the nervous system [84, 87]. The decrease and increase in the concentration of copper in the body has negative effects on human health and these effects are the effect on the skeleton, anemia, leucopenia, liver and blood and brain formation system where the effects of the lack of concentration of copper was identified for the first time in infants recovering from malnutrition in conjunction with protein energy deficit and chronic diarrheal disease [83, 84].

1.3.2.2 The Increasing of Copper

The increasing of copper concentration may cause cirrhosis [88]. A high concentration of copper in the water leads to health risks, especially on the digestive system [84]. Also high rates of copper may cause liver, kidney damage and genetic abnormalities [31, 89]. The presence of high concentrations of copper in the water may cause diarrhea [90]. Short-term health effects: the inhalation of copper fumes leads to irritation of the mucous membranes of the nose, eye irritation, nausea and hemolytic anemia, while the exposure to long-term copper fumes may cause liver and kidney damage. It also causes Wilson's disease, which is caused by the accumulation of copper in the liver and the difficulty of getting rid of it [57, 91].

1.3.2.3 Concentration of Copper

The copper concentration in serum or plasma of healthy individuals covers a range from 0.8 to 1.4 $mg.L^{-1}$ [92]. Also the median and range concentration of copper in urine was 8.3 ng. mL^{-1} and 1.9-15.9 ng. mL^{-1} respectively [67]. So in other studies the concentration of copper in urine for non-occupationally exposed people was < 50 μg/g creat [56].

1.3.2.4 Previous Literature of Copper

Table 1.5 lists the literature review for Cu (II) by using cloud point extraction coupled with different technique.

Table 1.5 Literature survey of cloud point extraction of Cu (II) by coupling with varies analytical technique

Type of Sample	Analytical reagent	Type of Surfactant	LOD Linearity RSD%	Ref.
Flame Atomic Absorption Spectrophotometry (FAAS)				
Environment samples	6-(2-naphthyl)-2,3-dihydro-as-triazine-3-thione (NDDT)	Triton X-114	0.22 μg L^{-1} 0.22-100 μg L^{-1} 2.1%	93
Water and fish muscle samples	1,5-diphenylthiocarbazone (dithizone)	Triton X-114	0.140 μg L^{-1} 2-50 μg L^{-1} 3.5%	94
River and sea	1,5-diphenyl-benzoin	Triton X-114	0.04 μg L^{-1}	95

water	(Cupron)		0.05-50.0 μg L^{-1} 1.9%	
Apple ,Spinach and tomato leaves	2-(2′-benzothiazolylazo)-5-(N,N-diethyl)aminophenol (BDAP)	Triton X-114	0.4μg L^{-1} 1.0-100.0 μg L^{-1} 6.4%	96
Water samples	Sulfathiazolylazo resorsin (SPAR)	Triton X-100	0.64 μg L^{-1} 4-400 μg L^{-1} 3.5%	97
Tap water	1-(2-thiazolylazo)-2-naphthol (TAN)	Triton X-114	0.27 μg L^{-1} 0.27-100 μg L^{-1} 1.6%	98
Spice samples.	3-amino-7-dimethylamino-2-methylphenazine (Neutral Red,NR)	Triton X-114	0.3 μg L^{-1} 1.00-200 μg L^{-1} 1.8%	64
Environmental sample	Sulfapyridylazo resorcinol (SPAR)	Triton X-100	1.64 μg L^{-1} 4-450 μg L^{-1} 3.4%	31
Biological and Environmental samples	2-phenyl-1H-benzo[d] Imidazole (PHBI)	Triton X-114	1.8 μg L^{-1} 3.6%	72
Various environmental and Biological samples.	4-(phenyl diazenyl) benzene-1,3-diamine (PDBDM) (chrysoidine)	Triton X-114	0.6 μg L^{-1} 0.01-0.26 μg L^{-1} 1.0%	99
Water samples	1,2-dihydroxyanthraquinone-3-sulfonic acid sodium salt	Triton X-114	1.07 μg L^{-1} 5-200 μg L^{-1}	100
Biological, natural and waste water, soil and blood samples.	methyl-2-pyridylketone oxime (MPKO)	Triton X-114	1.6 μg L^{-1} 0.01-0.25 μg L^{-1} 1.2%	101
Several natural water samples	3-[(8-f[(E)-2-hydroxyimino-1-methylpropylidene] aminog-1-naphthyl) imino]-2-butanone oxime (H2mdo)	Triton X-114	0.14 μg L^{-1} 0.4-150 μg L^{-1} 2.2%	102
Environmenta samples	2-(6-(1H-benzo[d]imidazol-2-yl)pyridin-2-yl)-1H-benzo[d]Imidazole (BIYPYBI)	Triton X-114	1.4 μg L^{-1} -- 1.3%	73
Water samples	Bis (2-hydroxynaphthaldehyde)-1,2-propanediimine	Triton X-114	0.3 μg L^{-1} 1-400 μg L^{-1} 1.0%	103
River water samples	1,5-diphenylthiocarbazone (dithizone)	Triton X-114	----	104

Water samples	monocarboxylic acids with amines	OP-10	0.01 μg mL^{-1} 2%	105
Water, food and pharmaceutical samples	Without ligand	Tween 80	0.4 μg L^{-1} 0.2-0.6μg mL^{-1}	106
UV-Vis Spectrophotometry				
Water	4-Ethyl-1-(pyridinyl) thiosemicarbazide (HEPTS)	Triton X-114	0.1 μg L^{-1} 0.1-25 μg L^{-1} 0.985%	107
Environmental, Biological and Water Samples	amino-4-(3-nitrophenylazo) pyridine-3-ol (ANPAP)	Triton X-114	1.20 μg L^{-1} 4.0-115 μg L^{-1} 1.25%	108
Food, water and biological samples	2-amino-4-(mtolylazo) pyridine-3-ol (ATAP)	Triton X-114	0.03 μg L^{-1} 2.2%	109
Water samples	4-(2-pyridylazo) resorcinol	Triton X-114	9.8 μg L^{-1} 0.02-0.10 μg L^{-1} 3.71%	110
Inductively Coupled Plasma (ICP-MS and ICP-OES)				
Oil effluents	2-(5-bromo-2-pyridylazo)-5- (diethylamino) phenol (5-Br-PADAP)	Triton X-114	0.38 μg L^{-1} -- 3.5% μg L^{-1}	111
River, mineral	3-nitro benzaldehyde thiosemicarbazone (3-NBT)	Triton X-114	0.48 μg L^{-1} 5-120 μg L^{-1} 1.8%	112
Water sample	8-Hydroxyquinoline	Triton X-114	0.012μg L^{-1} 1-800 μgL^{-1} 1.29 %	79
Electrothermal Atomic Absorption Spectrometry (ETAAS)				
Water	1-(2-Pyridylazo)-2-naphthol (PAN)	Triton X-114	0.005 μg L^{-1} 0-2.0 μg L^{-1} 3.1%	113
Capillary Electrophoresis (CE)				
Tap water, snow water and flavor wines.	1-(2-Pyridylazo)-2-naphthol (PAN)	Triton X-114	0.26 μg L^{-1} 3-100 μg L^{-1} 1.8%	114
Spectrofluorimetry				
water samples and parenteral solutions	Thiamine	Triton X-114	0.29 μg L^{-1} 1.0–250 μg L^{-1} 2.12%	115

1.4 Analytical Chemistry of Reagent

1.4.1 Benzidine Reagent

Benzidine is an organic compound that possesses the chemical formula $(C_6H_4NH_2)_2$ and has several labels, which are known as 1,1'-biphenyl-4,4'-diamine, 4,4-biphenyldiamine (Benzidine) and is considered the aromatic amines, while the derivatives of benzidine have wide uses in the production of dyes. The benzidine is a carcinogen, especially cancer of the bladder and pancreas [116]. It has been dealt with and used with great caution. The previous studies on the uses of benzidine and its derivatives in the estimation of some elements, use 3, 3′, 5, 5′-tetramethylbenzidine to determination trace amount of copper [117]. Also use 3,3’-diaminobenzidine to estimate selenium [118, 119]. Figure 1.10 shows the structure of benzidine reagent.

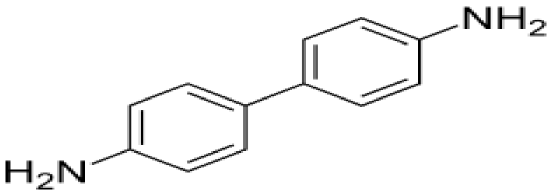


Figure 1.10 Structure of Benzidine reagent

1.4.2 Salicylaldehyde Oxime

Salicylaldoxime (SAO) is an organic compound described by the formula $C_7H_7NO_2$. It is the oxime of salicylaldehyde. This crystalline, colorless solid is a chelator and sometimes used in the analysis of samples containing transition metal ions, with which it often forms brightly colored coordination complexes [120]. The literature Review for SAO reagent for used to determine some elements such as, Co-extraction of cations and anions in base metal recovery [121]. Also used to determine Copper in sea water [122]. Figure 1.11 shows the structure of SAO reagent. Table 1.6 shows some properties of SAO and benzidine reagents.

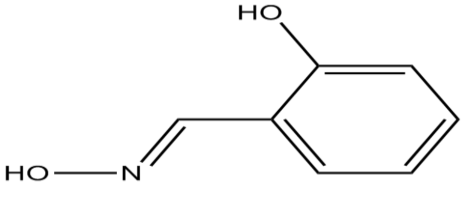


Figure 1.11 Structure of salicylaldehyde oxime SAO reagent

Table 1.6 Physical and chemical properties of SAO and benzidine reagents

Parameter	Type of reagent	
Name of reagent	4,4-biphenyldiamine (Benzidine)	Salicylaldoxime (SAO)
Chemical formula	$C_{12}H_{12}N_2$	$C_7H_7NO_2$
Solubility in water	0.94 g/100 mL at 100 ℃	25 g / L
Molar mass	184.24 g.moL^{-1}	137.14 g.moL^{-1}
Appearance	Grayish – yellow, reddish- gray	White to off-white crystals
Density	1.25 g. cm^3	1.42 g. cm^3
Melting point	122-125 ℃	59-61 ℃
Boiling point	400 ℃	---
Main hazard	Carcinogenic	Harmful if swallowed, Irritating to the eyes, Irritating to the, respiratory system, irritating to the skin

1.5 Urine Samples

Urine is produced as a result of the metabolic process that occurs in the human body, where it is removed by the kidneys and passes through the ureter up to the bladder and then takes it out of the body [123]. The pH value of urine usually is (5.50 – 7.00) with an average of 6.20. Human urine has a specific gravity of (1.003–1.035) [123, 124]. So the urine contains many nitrogen wastes such as urea, creatinine and uric acid, which can be eliminated by urination and can detect some chemicals in urine by careful analysis of urine [123]. The urine is generally composed of 91-96 % water, also contains low concentrations of heavy elements, some chemical compounds, organic and inorganic salts, as well as contains proteins and hormones. Therefore, concentration of these components varies from person to person depending on several factors including food, drink, nature of work and others. The normal average of urine in adults is 1 liter per day and these amounts vary depending on several factors, including the amount of water consumed by the person and the environmental factors also affect this average, the other factors such as weight, level of activity and health status of the individual [124]. Table 1.7 shows the average chemical composition of fresh urine (literature values) and stored urine and concentration of some trace elements in urine [67, 124, 125].

Table 1.7 Concentration of chemical composition of fresh urine (Literature values) and stored urine and concentration of some trace elements in urine.

Parameter	Fresh urine	Stored urine
pH	6.2	9.1
Total nitrogen, TN (mg/L)	8830	9200
Ammonium/ammonia NH4 + and NH3 (mgN/L)	460	8100
Nitrate/nitrite NO_3 + NO_2 (mgN/L)	0.06	0
Chemical oxygen demand, COD (mg/L)	6,000	10.000
total phosphorus, TP (mg/L)	800 – 2000	540
Potassium, K (mg/L)	2740	2200
Sulphate, SO4 (mgSO4/L)	1500	1500
Sodium, Na (mg/L)	3450	2600
Magnesium, Mg (mg/L)	120	0
Chloride, Cl (mg/L)	4970	3800
Calcium, Ca (mg/L)	230	0
Cadmium ng/ml	0.044-0.358 (0.145)	
Cobalt ng/ml	0.060-0.510 (0.170)	
Chromium ng/ml	0.060-0.260 (0.180)	
Copper ng/ml	1.900-15.900 (8.30)	
Iron ng/ml	2.10-16.40 (4.9)	
Nickel ng/ml	0.270-3.680 (1.7)	
Lead ng/ml	0.300-2.000 (1.1)	
Zinc ng/ml	170.00-780.00 (430)	

1.6 Occupational Worker

The industrial workers are treating with many type of chemical compounds that possible to concentrated of trace elements in their bodies, where many types of diseases causing [126]. Industrial occupations produce very small particles that may be harmful for health because of the different chemicals and occupational exposure to these vapors particles may cause respiratory cancer over time, most of the epidemiological evidence for this has been gathered from high occupational exposures in the past [57, 127]. As a result of air pollution, about 2.4 million people die every year, according to the WHO report [128].

1.6.1 Welding

Several studies have been conducted on welding techniques and the effect of welding fumes and the duration of exposure to human health. These studies have shown that prolonged exposure to welding causes several types of disease, its effect on the respiratory tract, bronchitis, airway irritation and its impact on the lung. Usually, the welding aerosol is formed by coating the electrode and the base metals, the metal oxides are generated by the reaction of evaporating metals with the air, which in turn condenses to formation the smoke containing the metal particles [129]. So that the major components of the fume are oxides of metals, some metal constituents of the fumes may pose more potential hazards than others, the most common welding fume components are discussed as follows: Chromium [130], Nickel [131], iron, copper, Cadmium [129]. Table 1.8 demonstrates some welding components [130].

Table 1.8 hazardous by products of welding

Fume	Gases	Radiant energy	Other hazard
Aluminum, Iron, Copper, Cadmium, Magnesium, Lead, Nickel, Manganese, Silica, Chromium, Fluoride, Zinc Titanium	Carbon dioxide Carbon monoxide Nitrogen oxide Nitrogen dioxide Ozone	Ultraviolet Visible Infrared	Heat Noise Vibration

1.6.2 Dyeing

Dyes can be defined as solutions consisting of a substance or a group of chemicals, sometimes dyes are exposed to chemical changes as a result of changes in the surrounding conditions, which leads to the transformation of the basic materials of dyes to other substances that may be more toxic and dangerous than the original component [132]. Dyes include several different types, including acidic, basic, disperse, azo, anthraquinone based and metal complex dyes among others. The textile industry is the largest consumer of dyestuffs [133].

1.6.3 Petroleum Workers

A large number of epidemiologic studies of workers in the petroleum industry have been conducted to address the issue of carcinogenicity of petroleum chemicals. Exposure to hydrocarbons in the petroleum industry includes inhalation of vapors and dermal contact of crude oil, feedstock, intermediate products such as benzene during refining, and end products such as gasoline in marketing or distribution (International Agency for Research on Cancer (IARC) [134].

1.6.4 Casting Alloys

Alloys are substance usually consisting of a mixture or solutions of one or several metals. There are several types of metal alloys such as: aluminum, copper, nickel, Tin, zinc and stainless steel alloys and others [135]. Usually alloys can be classified into ferrous and non-ferrous [136].

1.7 Aim of the Study

The occupational workers handle and breath with varies chemical compounds that possible cause increased a concentration of trace elements in their bodies, where causing many types of diseases.

- The low level of trace elements and complicated matrices of urine samples. It is impossible to directly evaluate them by sensitive instrumental methods; therefore, it should use one of pre-concentration methods.
- In this study, a new and efficient technique was used to enrich and extract trace elements from biologic samples.
- Through previous studies using cloud point extraction technique, it has many advantages that distinguish it from other techniques such as high concentration coefficient, small amounts of the surfactant-rich phase; this technique can be considered a method that is compatible with green chemistry, a high concentration coefficient, low cost, low amount of reagent.
- The main objective of this study is to open a new path and increase the analytical properties for extracting and enriching the elements in different models after installing the optimal conditions for the specific determination and extraction of each element.

CHAPTER TWO

EXPERIMENTAL PART

2. Chemical and Apparatuses

2.1. Chemicals and Reagents

Analytical grade chemicals and reagents were used unless otherwise stated. Table 2.1 listed all chemicals and reagents that were used in this study.

Table 2.1 Chemicals and reagents were used throughout research study

Name of Compound	Formula Molar mass (g.mol^{-1}) Purity%	Supplier	(w/v) or (v/v) of prepared solution	Remarks
Copper(II) 1000 µg.mL^{-1}	$Cu(NO_3)_2.3H_2O$ 241.602 Analar	BDH	0.3802g /100 mL	---
Nickel(II) 1000 µg.mL^{-1}	$Ni(NO_3)_2$ 182.703 Analar	BDH	0.3112 g /100 mL	---
Iron(III) 1000 µg.mL^{-1}	$FeCl_3$ 162.204 Analar	BDH	0.2904 g /100 mL	---
Aluminum (III) 1000 µg.mL^{-1}	$Al(NO_3)_3.9H_2O$ 375.133 99.5%	MERCK	1.3903 g /100 mL	---
Chromium (III) 1000 µg.mL^{-1}	$Cr(NO_3)_3$ 238.011 98.0%	BDH	0.4577 g /100 mL	---
Cobalt (II) 1000 µg.mL^{-1}	$Co(NO_3)_2$ 182.943 97.0%	BDH	0.3104 g /100 mL	---
Lead (II) 1000 µg.mL^{-1}	$Pb(NO_3)_2$ 331.209 99.0%	BDH	0.1598 g /100 mL	---
Zinc (II) 1000 µg.mL^{-1}	$Zn Cl_2$ 136.315 97.0%	MERCK	0.2084 g /100 mL	---
Polyethelyene glycol Tert-octylphenyl ether Triton X-114 20% (v/v)	$C_{14}H_{22}O(C_2H_4O)_n$ (n=7-8) 536.000 98.0%	AMRESCO	20.00 mL /100 mL	Prepared in hot deionized water

Polyoxyethylene octyl phenyl ether Triton X-100 20% (v/v)	$C_{14}H_{22}O(C_2H_4O)_n$ (n=8-9) 602.796 98.0%	HIMEDIA	20.00 mL /100 mL	Prepared in hot deionized water
Sodium Hydroxide 0.1 mol. L^{-1}	NaOH 40.000 96.0%	BDH	0.4000 g /100 mL	---
Hydrochloric acid 3 mol. L^{-1}	36% HCl (11.736 mol L^{-1}) (sp.gr. 1.19) 36.460 98.5%	BDH	25.56 mL /100 mL	---
Nitric acid 2%(V/V)	70% HNO_3 (sp.gr. 1.42) 63.010 99.0%	BDH	2.00 mL /100 mL	---
Sodium chloride 15% (w/v)	NaCl 58.442 98.5%	BDH	3.7500 g /25 mL	---
Potassium chloride 15% (w/v)	KCl 74.551 99.5%	GCC-ENGLAND	3.7500 g /25 mL	---
Sodium Sulfate 15% (w/v)	Na_2SO_4 142.042 99.5%	MERCK	3.7500 g /25 mL	---
Sodium Carbonate 15% (w/v)	Na_2CO_3 105.988 99.7%	FLUKA	3.7500 g /25mL	---
Sodium Fluoride 10% (w/v)	NaF 41.979 99.5%	BDH	5.0000 g /50 mL	---
Ascorbic acid (Vitamin C) 1% (w/v)	$C_6H_8O_6$ 176.120 99.5%	FLUKA	1.0000 g /100 mL	---
Ethylenediamine-tetraacetic acid (EDTA) 1% (w/v)	$C_{10}H_{16}N_2O_8$ 292.240 98.0%	BDH	1.0000 g /100mL	---
Aminoacetic acid (Glycine) 1% (w/v)	$C_2H_5NO_2$ 75.070 98.0%	BDH	1.0000 g /100mL	---
Salicylaldehyde oxime (SAO) 1% (w/v)	$C_7H_7NO_2$ 137.135 98.0%	BDH	1.0000 g /100mL	Dissolved in 5mL of ethanol then added hot deionized water.

4,4-biphenyldiamine (Benzidine) 1% (w/v)	$C_{12}H_{12}N_2$ 184.237 98.5%	FLUKA	1.0000 g /100mL	Dissolved in 1-2mL of DMSO then added ethanol.
Sodium dodecyl sulfate (SDS) 1% (w/v)	$NaC_{12}H_{25}SO_4$ 288.372 99.0%	BDH	1.0000 g /100 mL	--
Hydrogen peroxide 50%	H_2O_2 34.010	PANREAK	--	--
Standard solution of iron 1000 $\mu g.mL^{-1}$	---	Buck Scientiific	---	Prepared in 2% HNO_3 and is equipped with a device FAAS where a series of solutions for the calibration work of the iron element are brought.
Standard solution of copper 1000 $\mu g.mL^{-1}$	---	Buck Scientiific	---	Prepared in 2% HNO_3 and is equipped with a device FAAS the series of solutions for the calibration work of the copper element are brought.
Absolute Ethanol	C_2H_6O 46.070 99.0%	GCC-ENGLAND	---	---

2.2 Instruments

2.2.1 UV-Vis Spectrophotometer

All absorption spectra were recorded on Shimadzu double beam UV- Vis. Spectrophotometer, (UV-1800, Japan) equipped with Deuterium and tungsten Lamps and using 1cm quartz cells (0.5 mL) were used for all determination.

2.2.2 Flame Atomic Absorption Spectrophotometer

The metals ions have been determined by using Flame atomic absorption spectrophotometer (Buck scientific model 210 VGP, USA) with deuterium background correction equipped with 10.0 cm of air / acetylene flame burner head and hollow – cathode lamp that can be changed according to the metal determined. The instrumental parameters used depended on the manufacturer's manual book as listed in Table 2.2.

Table 2.2 Operating parameters of the metals by FAAS

Parameter	Fe(III)	Cu(II)
Wavelength (nm)	248.3	324.7
Slit band pass (nm)	0.2	0.7
Lamp current (mA)	1.5	1.5
Sample energy	2.499	2.401
Back ground energy	3.50	3.13
Oxidant and fuel	Air-acetylene	Air-acetylene
Pressure of air (Psi)*	50-60	50-60
Pressure of acetylene (Psi)*	10-13	10-13

***Psi: Pascal = (Newton / square meter) N·m^{-2}**

2.2.3 pH Meter

The pH adjustment used pH meter WTW (model 720) with a combined glass electrode.

2.2.4 Centrifuge

The phase separation of a rich phase from liquid phase was used N m. A Hettich centrifuge (model EBA – 20, Germany) with 10 mL calibrated centrifuge tubes.

2.2.5 Balance

The chemical compounds were weighed by using a Kern analytical Balance ABS. has four digits.

2.2.6 Water Bath

A Water bath (Lab companion BS-11 Shaking Water bath, Korea) with a good temperature control was used to accelerate CPE experiments.

2.2.7 Micro Pipettes

A Micro pipettes 10-100 μL and 100 – 1000 μL (Dragon Lab) were used to deliver the accurate volumes of chemical solutions.

2.2.8 Refrigerator

A Refrigerator at 5 ℃ was used to keep all samples fresh and cool till the analysis.

2.2.9. Super Mixer (Vortex)

A Lab Line Super mixer (model 129, U.S.A) Hitech Trader, U.S.A. Was used to mix solutions.

2.2.10. Oven

A Memmert Oven (model UN-110 plus) was used to dry glassware.

2.2.11. Millipore Filters

The samples were filterated before analyses through a cellulose membrane, type EMD Millipore of 0.45 μm pore size.

2.2.12 Hot Plate

Electronically controlled stirring from 60 to 1500 rpm type Labtech Hot plate stirrer LMS-1003 Specification, Korea, temperature range: ambient (5 to 380) ℃ max.

2.3 Cloud Point Extraction Method

Cloud point extraction is a separation and pre-concentration technique that has been extensively applied for trace element determination in different samples [137]. CPE is based on the property of most non-ionic surfactants in aqueous solutions to form micelles and become turbid when heated to a temperature known as the cloud point temperature. When reaching above this temperature, the solution will be separated into two layers, one aqueous and the other small volume of organic layer or surfactant-rich layer [138, 139]. Cloud point extraction (CPE) is attractive techniques that reduce the consumption of the solvent, extraction time and the disposal costs. CPE methodology has been used for the extraction and pre-concentration of metal ions after the formation of sparingly water soluble complexes [140]. CPE uses low toxic, environmental friendly surfactant and avoid the use of large amount of toxic and flammable organic solvent [141].

2.3.1 Pre-concentration of Iron (III) Procedure

The cloud point extraction method for iron (III) was used as pre-concentration procedure which was based on aliquots of 10 mL of 2 μg mL^{-1} Fe (III) solution mixed with 0.5 mL of 1% (w/v) of 4, 4-biphenyldiamine Benzidine solution reagent. Green color solution product was formed which has λmax 425 nm, depending on the absorption spectra of UV-Vis spectrometry as shown in Figure 3.2, the pH value of solution was adjusted to 4.20 by using 0.1 M of HCl and 0.1 M of NaOH solutions. Then, a 0.1 mL of 20% (v/v) Triton X-114 surfactant was added to the mixture. The produced mixture was placed on the super mixer to mix and heat in a water bath at 70 ℃ for 5 min. After that, it was centrifuged at 3600 rpm for 5 min to separate the solution into two phases and cooled down in an ice bath (0-5) ℃ for 5 min in order to increase the viscosity of surfactant-rich phase to separate easily by converting tube. The remaining micellar phase which was containing Fe (III)-Benzidine complex, it was dissolved in 0.75 mL of absolute ethanol and transferred into 1.0 cm quartz cell (1mL). Finally, the absorbance of the solution was measured at λmax 425 nm against a blank solution that prepared in the same way. The proposed reaction mechanism for Fe (III)-Benzidine complex formation and the CPE of iron (III) separation procedure are illustrated in Figures 2.1 and 2.2 respectively.

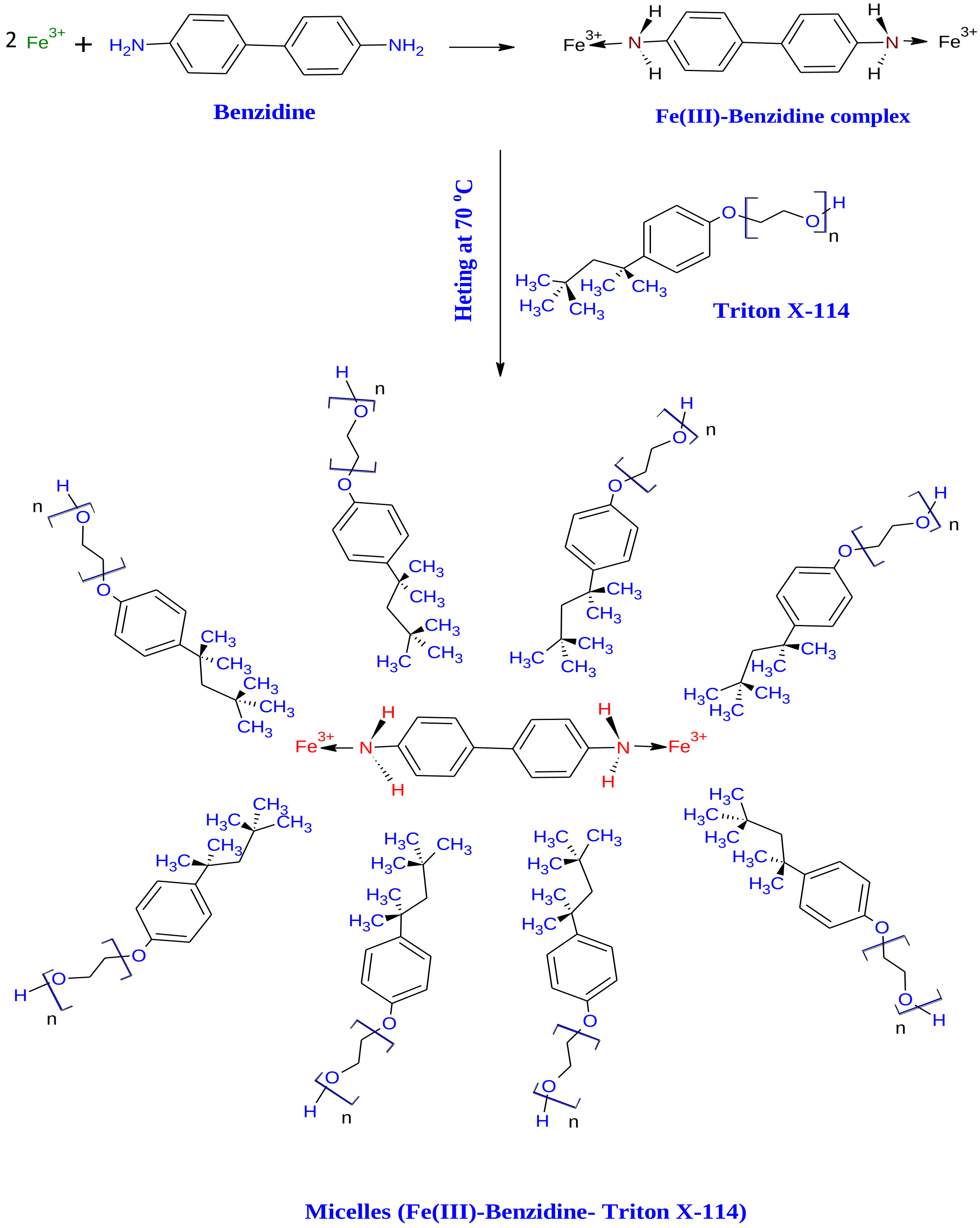


Figure 2.1 Proposal mechanism of Fe (III)-Benzidine complex separation by CPE method

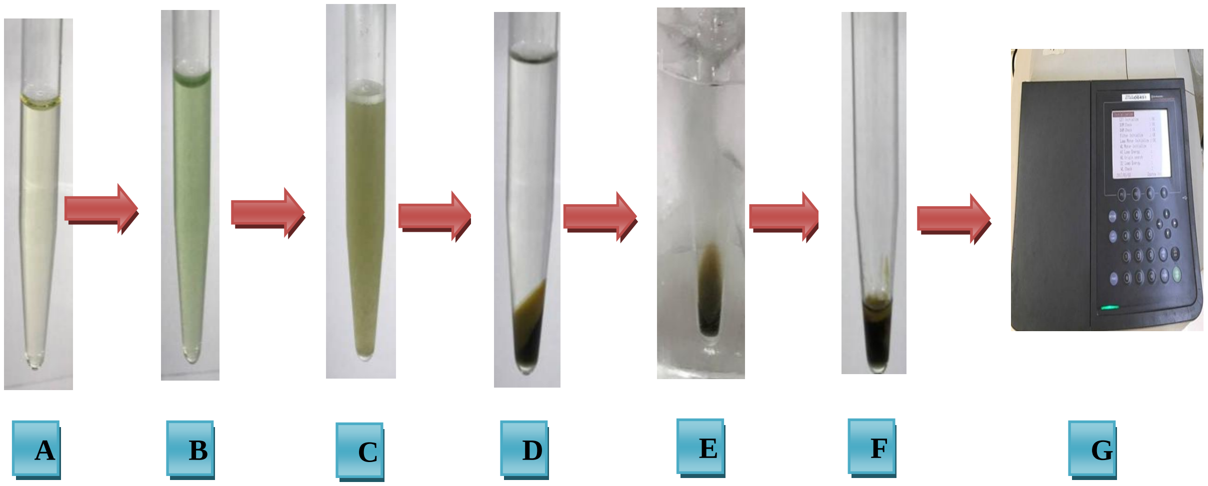


Figure 2.2 Scheme of CPE Procedure for iron (III), (A) Iron(III) solution, (B) Iron (III) mixed with 4, 4-biphenyldiamine benzidine reagent, (C) Addition surfactant Triton X-114 to formation cloud solution ,(D) Phase separation after centrifugation, (E) Cooling by ice-bath ,(F)A Surfactant rich phase ,(G) Measurement by UV-Vis spectrophotometer

2.3.2 Pre-concentration of Copper (II) Procedure

According to the general procedure of cloud point extraction method, aliquots of a 10 mL of 10 µg mL^{-1} of Cu (II) was added to a 0.1 mL of 1% (w/v) of salicylaldehyde oxime (SAO) reagent, the light green color solution has λmax 380. A 0.5 mL of 20% (v/v) Triton X-114 was added and diluted to about 10 mL by de-ionized distilled water. The mixture was placed on the super mixer to mix the solution. The mixture was kept in a water bath at 75 ℃ for 10 min. for equilibrium the separation into two phases was achieved by centrifugation for 5 min. at 3500 rpm and cooled in an ice-bath at (0-5) ℃ for 5 min to increase the viscosity of the surfactant-rich phase separated easily by converting tube. The surfactant –rich phase was dissolved and diluted to 0.75 mL by using absolute ethanol and transferred into 1.0 cm quartz cell (1 mL).The absorbance of the solution was measured against a blank solution prepared in the same method. Figures 2.3 and 2.4 were demonstrated the proposal mechanism of Cu (II)-SAO complex formation and the scheme of CPE of copper (II) separation method respectively.

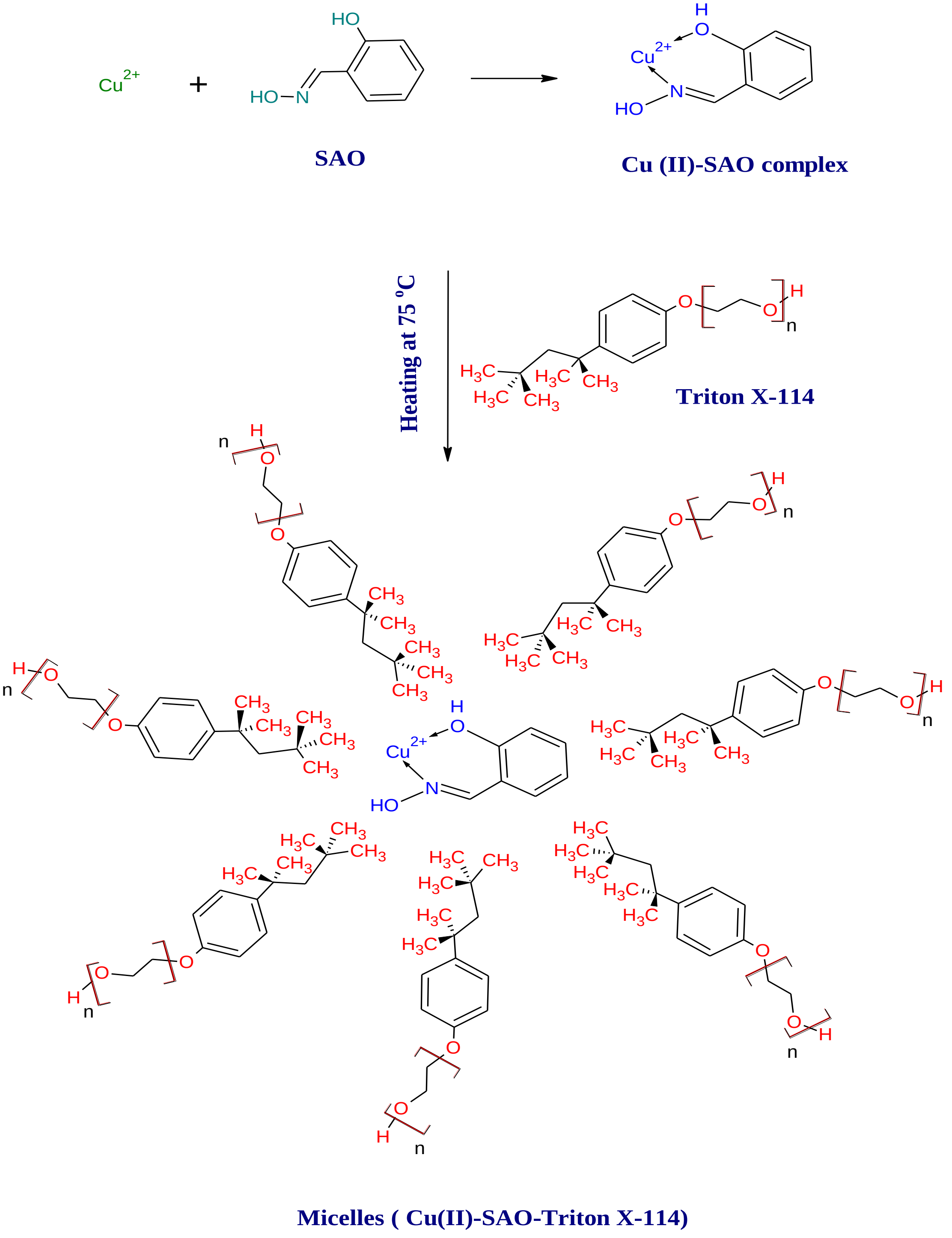


Figure 2.3 Proposal mechanism of Cu (II)-SAO complex separation by CPE method

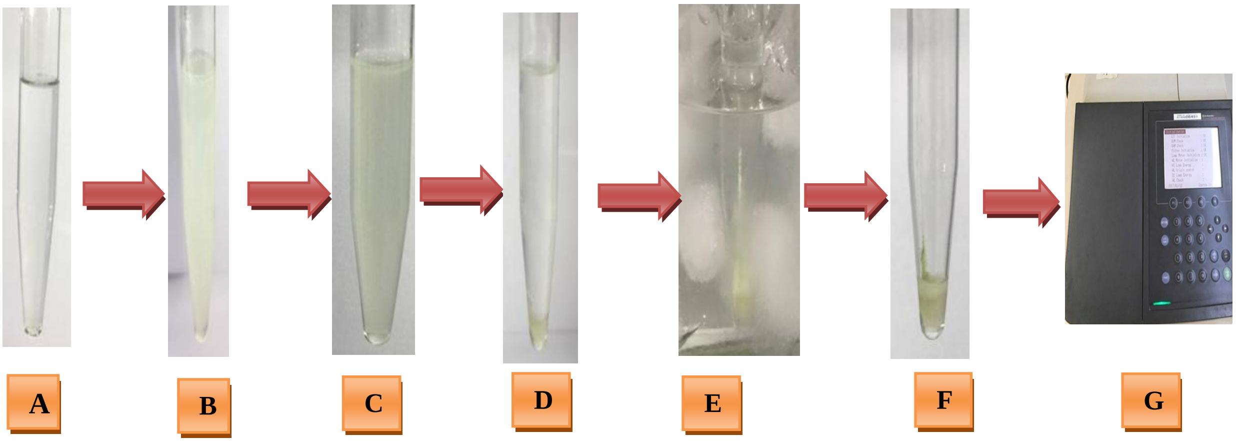


Figure 2.4 CPE Procedure for Cu(II) : (A) Cu(II)solution, (B) addition SAO reagent and mixing, (C) addition Triton X-114 surfactant to cloud solution formation, (D) phase separation after centrifugation, (E) cooling by ice-bath ,(F) separation a rich phase ,(G) measuring by UV-Vis spectrophotometer.

2.4 Sampling

Urine samples were a collected from people working in different types of occupations as tabulated in Table 2.3, such as dyeing, welding, Casting alloys, workers in oil refineries and terminals to provide fuel. The urine samples were collected in a polyethylene bottles (120 mL) and stored in a fridge at (0-5) ℃. To keep the models from damage after that were initialized for subsequent operations, where all samples of urine were taken from workers who lived in Kerbala city, where the concentration of iron and copper in urine samples would be determined and listed the results in chapter three. So that the results obtains were compared with the concentrations of iron and copper in literature values.

Table 2.3 Occupational workers urine samples

Occupational worker type	**No. of sample**
Oil refineries	16
Terminals to provide fuel	6
Dyeing	5
Welding	12
Casting alloys	5
Total	44

2.4.1 Digestion of Urine Sample

The urine samples were digested obey literature procedure [142, 143]. A 60 mL of urine sample was accurately measured and treated with a mixture of 12 mL H_2O_2 and 6 mL of concentrated HNO_3. Then it was placed on a hot plate. The sample was moderately heated up to the disappearance of the amber color and evaporated to dryness. Thereafter, a fresh portion of 2.5 mL of conc. HNO_3 was added to residue the dark with heating until the dryness of the residual sample. This procedure was repeated until a white ash obtain. Finally, the residual urine sample was dissolved in 2.5 mL of 3.0 mol L^{-1} of HCl and diluted to 30 mL with deionized water. After this step, these samples were stored in the refrigerator at a temperature range (0-5) ^oC in order to prepare them and determine the iron (III) and copper (II) elements after an action procedure of cloud point extraction under the optimum conditions to be studied in section 3.2, and then the determination of iron (III) and copper (II) elements by using the UV-Vis spectrophotometry and FAAS methods. Table 2.4 and Figure 2.5 show the concentration of iron and copper element in urine samples in according with the values adopted by the published scientific research for a collected reviews and the procedure of urine samples digestion respectively.

Table 2.4 Summary of Literature concentrations of Iron (III) and Copper (II) in urine samples by the published scientific research for a collected reviews

Metal	Range of conc. $\mu g.L^{-1}$	Mean $\mu g.L^{-1}$	Reference
Fe (III)	16.3-115.0	65.65	144
	100.0-150.0	125.00	145
Cu (II)	5.8-47.4	26.60	144
	6.7-16.3	23.00	146

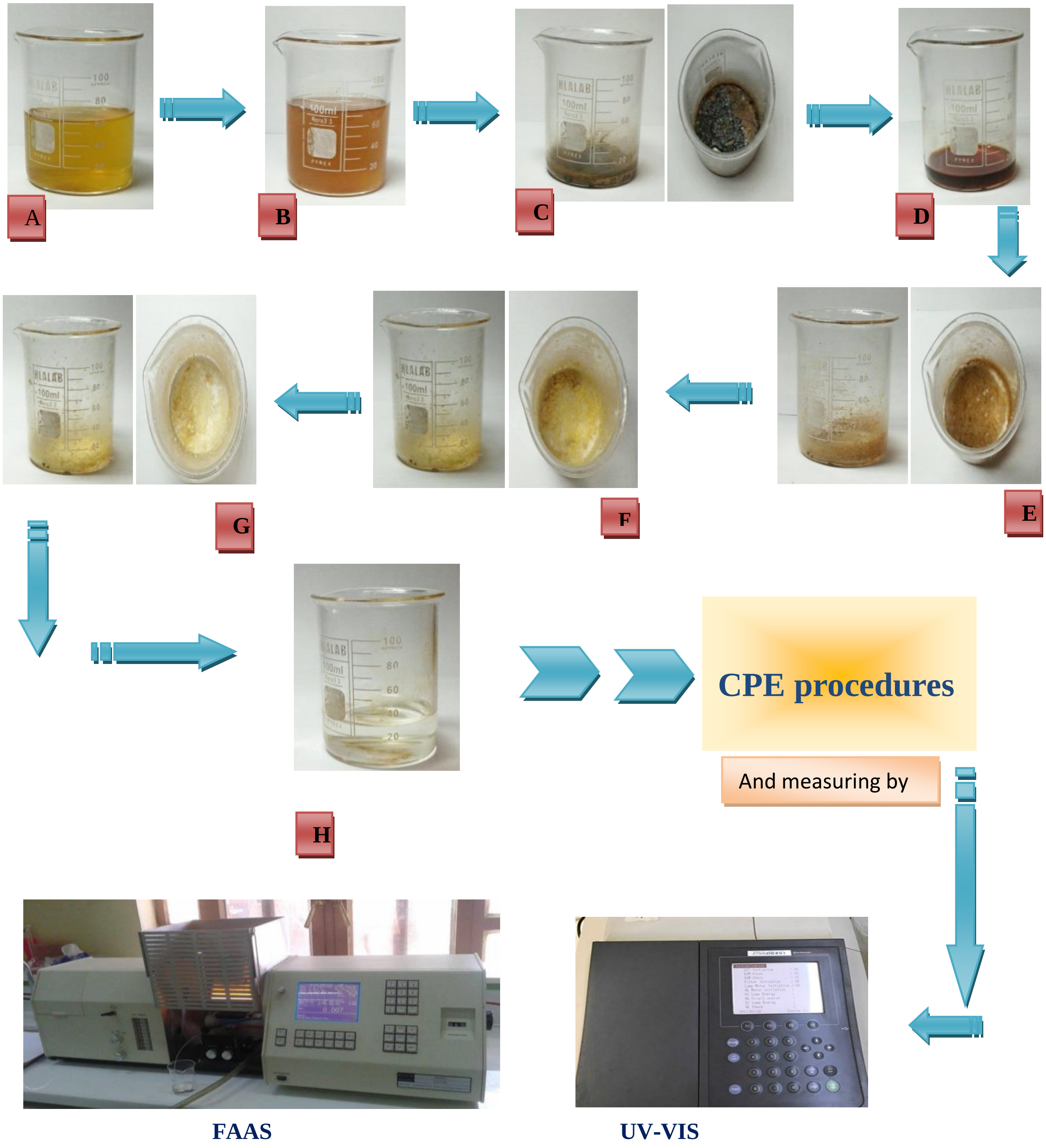


Figure 2.5 Digestion procedure of the urine samples, (A) a 60 mL urine sample, (B) adding a mixture H_2O_2 with conc. HNO_3, (C) Heating to form a dark residue, (D) adding of conc. HNO_3, (E),(F) and (G). These steps are repetition of the step D until obtain a white ash, (H) white ash dissolving in 3M HCl and diluting to 30 mL with deionized water and then conducting CPE steps.

CHAPTER THREE

RISULTS & DISCUSSION

3.1. Determination of Iron (III) and Copper (II) Elements in Urine samples using UV-Vis Technique

3.1.1 Absorption Spectra

The absorbance spectra for the reagent 4, 4- Diamino Diphenyl (Benzidin), Iron (III) solution and the Fe (III)-Benzidine complex in surfactant rich phase were domenstrated in Figure 3.1 (A, B, C and D). The absorbance spectra of Benzidine reagent shown a λmax of 288 nm, while Iron (III) solution has λmax of 320 nm, whereas Fe (III) –Benzidine complex has λmax of 425 nm, it is shifting of about larger than 100 nm comparison with Benzidine reagent and Iron (III) solution spectra.

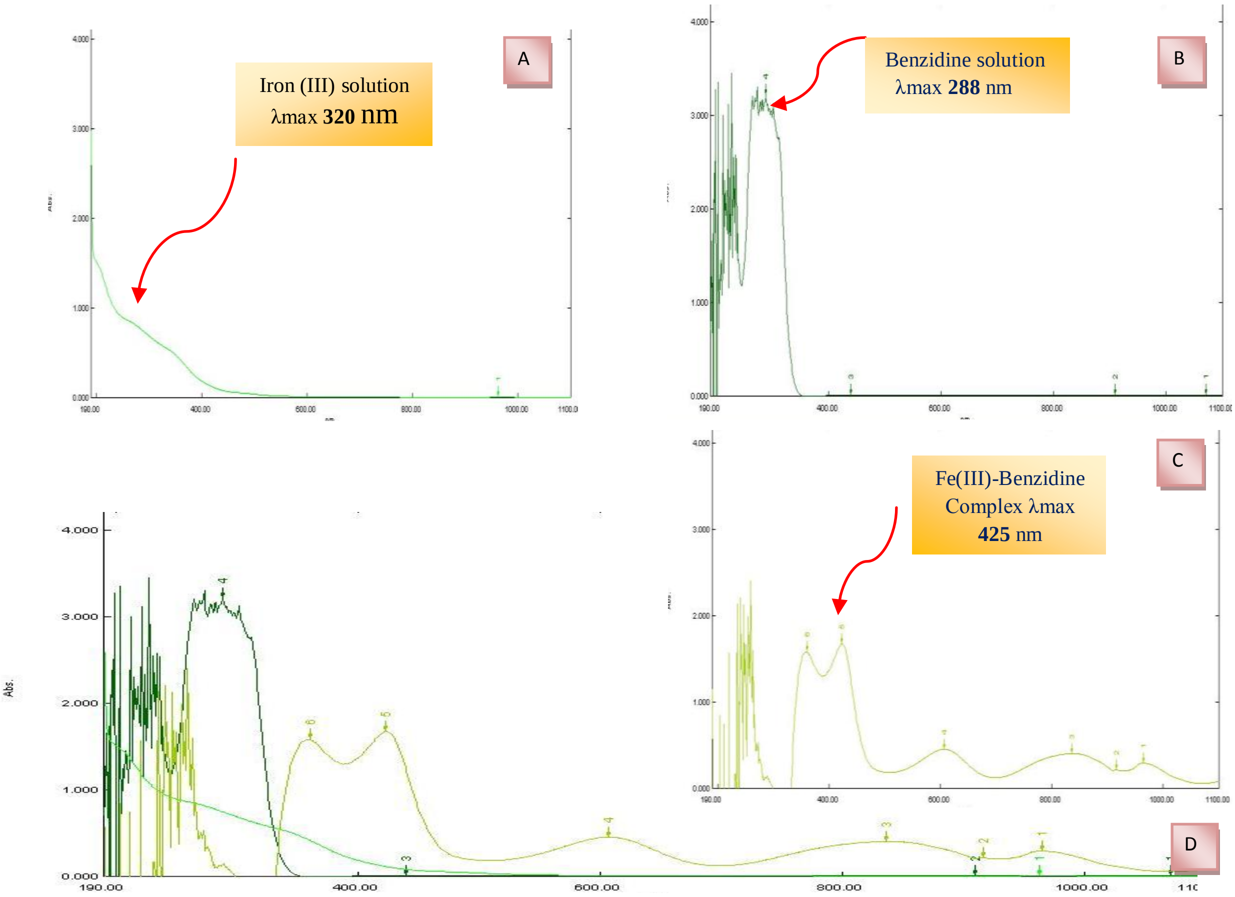
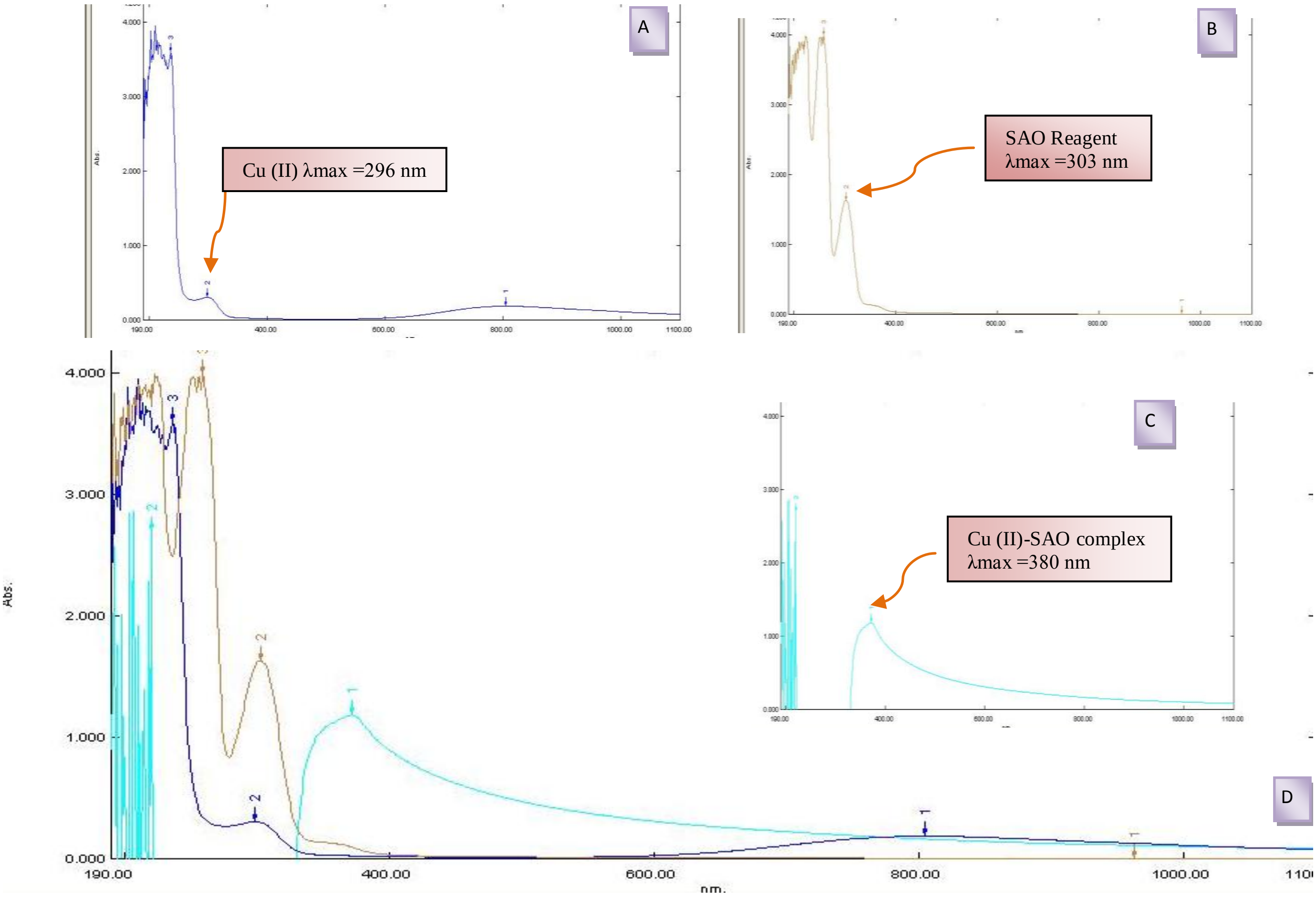


Figure 3.1 Absorption spectra for (A) Fe (III) metal solution, (B) 4, 4-Diamino Diphenyl (Benzidine) reagent, (C) Fe (III) – Benzidine complex and (D) overlapping for Fe (III) – Benzidine complex.

In order to find out the wavelength of maximum signal of Cu (II)-SAO complex surfactant rich phase as shown in Figure 3.2 (A, B, C and D). The maximum absorption wavelength for salicylaldehyde oxime (SAO) reagent was at λmax of 303 nm, while copper (II) solution has a λmax of 296 nm, whereas the absorbance spectra of Cu (II)-SAO complex was at 380 nm. It is shifting of about larger than 80 nm comparisons with SAO reagent and copper (II) solution spectra.


Cu (II) λmax =296 nm

SAO Reagent
λmax =303 nm

Cu (II)-SAO complex
λmax =380 nm

Figure 3.2 Absorption spectra for (A) Cu (II) metal solution, (B) salicalylaldehyde oxime (SAO) reagent, (C) Cu (II) – SAO complex and (D) overlapping for Cu (II) – SAO complex.

3.2 Optimization of the Experimental Conditions

To achieve the best performance for the cloud point extraction procedure, the effects of the experimental conditions studied include: order addition, pH , concentration of reagent, types of surfactant, concentration of surfactant ,equilibrium temperature, time of incubation, salt out and interfering ions were evaluated and optimized.

3.2.1 Order of Addition

The most important factors that have been studied was the order of additions where it changed the order of Fe (III) solution (2 µg. mL^{-1},10 mL) and Benzidine reagent (1% w/v, 0.5mL) with 0.5mL of 20% v/v Triton X-114 was added to the complex formation as indicated through the results in Table 3.1. The best absorbance value at the second test was chosen. While the order additions affect copper complex formation managements by using 10 mL of 10 µg. mL^{-1} of Cu (II) solution and 0.1mL of 1% w/v of the SAO reagent with 0.5mL of 20% v/v Triton X-114 was adedd as listed in Table 3.1, that showing the greatest absorbance value has been at the first test .

Table 3.1 Effect of the order of addition on the absorbance of Fe (III)- Benzidine and Cu(II)- Salicalylaldehyde oxime complex.
(M: Fe (III), Cu(II) ; R:Benzidine, Salicalylaldehyde oxime reagent ; T:Triton X-114)

Type of complex	Order addition	Aaq	As
Fe(III) complex	M + R + T	0.000	0.295
	R + M +T	0.000	0.301
Cu(II) complex	M + R + T	0.036	2.071
	R + M +T	0.099	1.823

Aaq: Absorbance of an aqueous layer, **As: Absorbance of a rich phase surfactant**

3.2.2 Effect of pH

The pH value is one of the most important factors affecting on the efficiency of extraction that based on the cloud point extraction method because of the pH factor plays a vital role in formation of complexes and chemical stability of solutions. The pH of solution was adjusted in the range from 2.70 to 10.40 by using 0.1M NaOH and 0.1 M HCl solution and measured using pH meter. The results in Figure 3.3 show increasing of the absorbance when the pH value was equal to 4.2, but the complexation reaction at pH values lower than 4.2 leads to incomplete probably, which due to the protonation of benzidine. Moreover, at pH value higher than 4.2 precipitate of iron hydroxide was formed. These changes in urinary metal levels can be due to differences in the binding strength of the complex of these metals with organic molecules as a consequence of the variations of pH [75, 144].

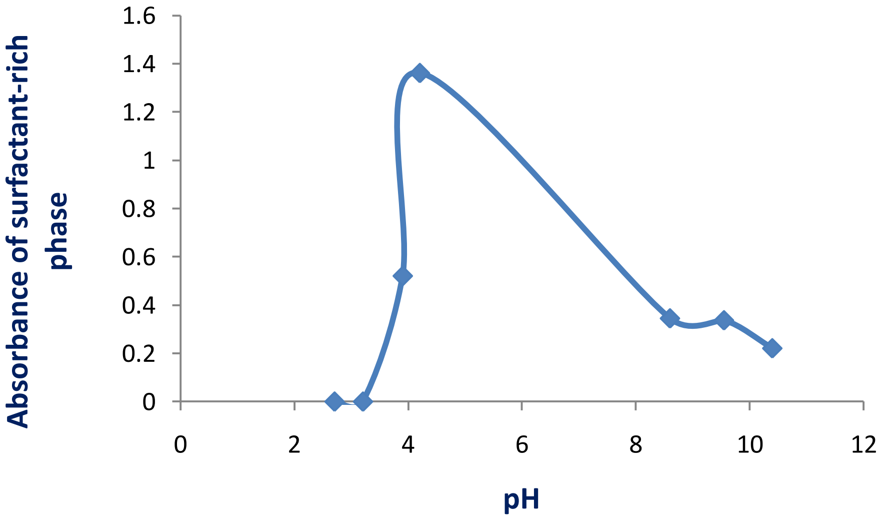


Figure 3.3 Influence of the pH on the absorbance of Fe (III)-Benzidine complex

In this item, the effects of different types of acids were studied as depeded in the Figure 3.4 below by using 0.1 M of HCl, H_2SO_4 and CH_3COOH solutions. The effect of hydrochloric acid on the Fe (III)-benzidine complex formation is stronger than other types of acids, since it gives the highest intensity absorbance signal. Also the best concentration of HCl was estimated with various volume of 0.1M HCl from (0.05 to 1.00) mL(0.5-10) mmoL.L^{-1} as shown in Figure 3.5, and the optimum volume was 0.75 mL (7.5) mmoL.L^{-1} of HCl.

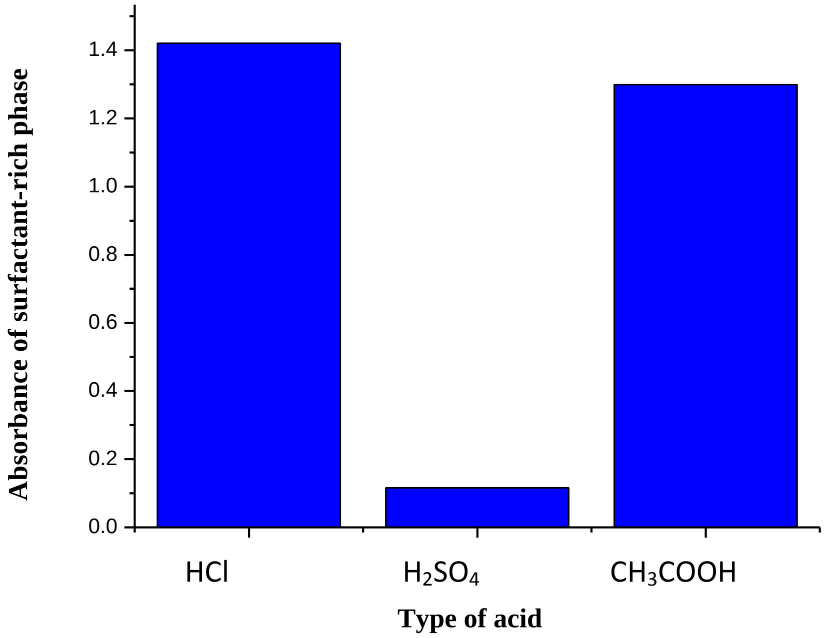


Figure 3.4 Effect of the type of acids on Fe (III)-Benzidine complex.

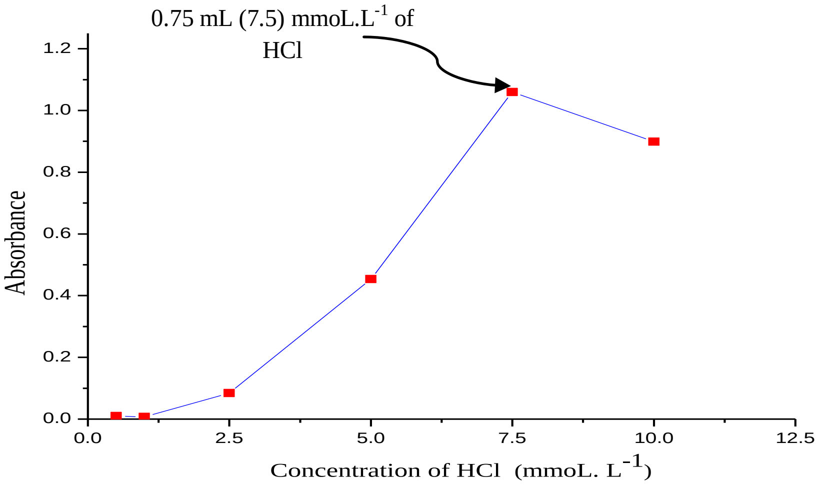


Figure 3.5 Effect of the concentration of HCl on the Fe (III)-Benzidine complex.

Straight away, cloud point extraction of copper (II) was studied in the range of pH from 3.50 to 11.50. Figure 3.6 explains the effect of pH on the absorbance of Cu (II) - SAO complex, therefore, a 0.1M NaOH and 0.1 M HCl solutions were used to fix the pH of the solution by using pH meter. The best result of absorbance of Cu (II)-SAO complex was found at pH 4.20, without using acid or base added to the complex solution. Complexation reaction between Cu(II) and SAO at pH values lower than 4.20 was incomplete and decomposition of the complex formation was probably due to the protonation of SAO, but at the pH value higher than 4.2 precipitate of Copper hydroxide was formed.

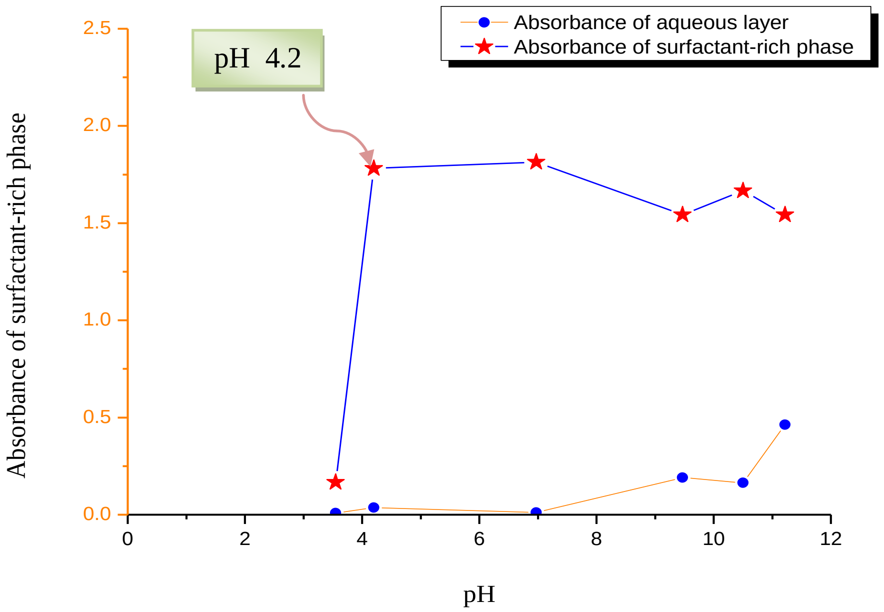


Figure 3.6 Effect of pH on the absorbance of Cu (II)-SAO complex

3.2.3 Effect of Reagent Concentration

The concentration effect of chelating agent 4, 4- diamino diphenyl (benzidine) on absorbance value was studied using various volumes of (0.05 to 1.00) mL of 1% w/v of benzidine and kept the other chemical and physical parameter constant. Figure 3.7 discribs, that the absorbance value was increased with increasing the amount of benzidine reagent added. The best result was relied upon when the amount of the reagent was 0.50 mL (2.55 mmol. L^{-1}) of benzidine. Addition of large quantity of benzidine reagent causes a precipitation of the complex.

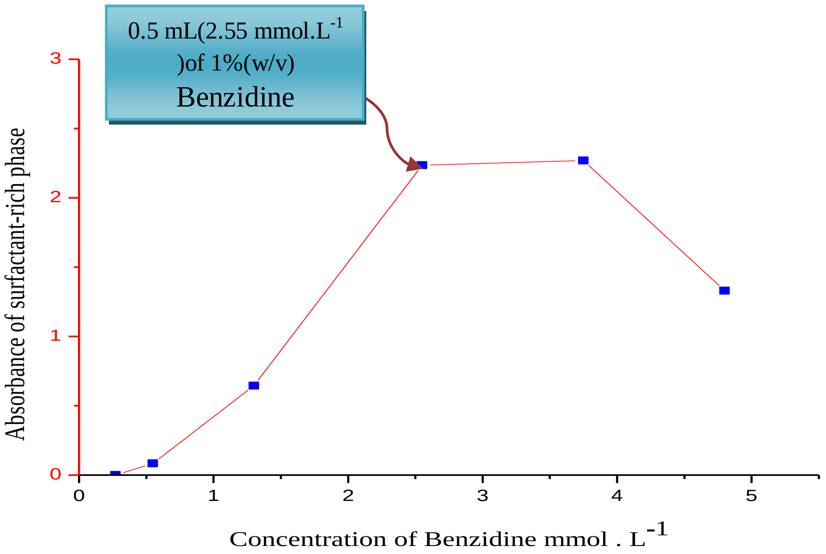


Figure 3.7 Effect of volume of benzidine on the absorbance of Fe (III)-Benzidine complex.

A series of salicylaldehyde oxime (SAO) reagent (0.01-1.00) mL of 1% (w/v) of SAO reagent were prepared. A 10mL of 10 µg.mL^{-1} of Cu (II) ion was used and at pH 4.20. The results of the influence of SAO concentration on cloud point extraction of copper (II) were shown through results in Table 3.2. So the best result was marked at 0.10 mL (0.688 mmol L^{-1}) of SAO reagent.

Table 3.2 Concentration of SAO effect on the absorbance of extracting Cu (II)-SAO complex

Volume of SAO added mL	SAO conc. mmol.L^{-1}	Ao	Aaq	As	*D	*E%
1.00	6.339	1.454	0.165	2.71	16.425	94.26
0.50	3.313	1.599	0.169	2.39	14.153	93.40
0.30	2.025	1.770	0.122	2.503	20.550	95.35
0.10	0.688	1.791	0.088	2.452	28.066	96.55
0.05	0.345	1.651	0.110	2.064	18.860	94.96
0.03	0.208	1.089	0.153	1.67	10.920	91.61
0.01	0.069	0.405	0.063	0.857	13.668	93.18

A_O: **Abs. of an original solution, Aaq: Abs. of an after extraction aqueous layer, As: Abs. of a rich phase surfactant, *D: Distribution coefficient= (conc. of metal in organic layer / conc. of metal in aqueous layer), *E%: Extraction percent = (D/1+D)*100**

3.2.4 Type and Concentration of Surfactant Influence

The efficiency of the cloud point extraction depends on the type of surfactant used, in this study certain concentration of various types of surfactant such as Triton X-100, Triton X-114 and sodium dodecyl sulfate (SDS) were used. The results in Table 3.3 and Figure 3.8 show the effect of surfactant on the absorbance of Fe (III)-Benzidine complex.

Table 3.3 Type of surfactant effect on the absorbance of complex formation

Type of Complex	Type of Surfactant	Ao	Aaq	As
Fe (III)-Benzidine	SDS	0.000	0.000	0.063
	Triton X-100	0.000	0.000	0.091
	Triton X-114	0.000	0.000	1.060
Cu (II)-SOA	SDS	2.017	0.865	2.063
	Triton X-100	2.017	0.294	2.530
	Triton X-114	2.017	0.198	2.741

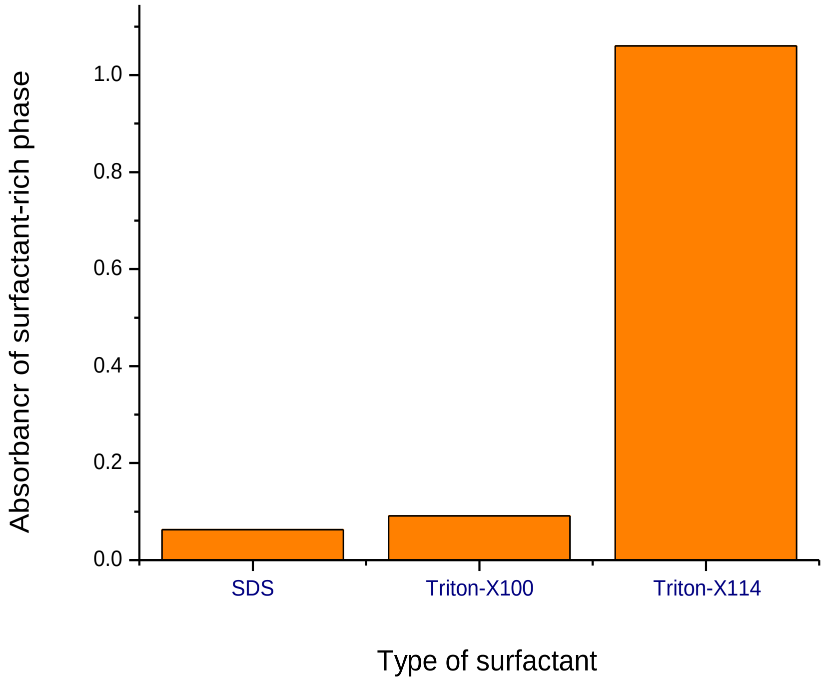


Figure 3.8 Effect of type of surfactant on Fe (III)-Benzidine complex.

The Triton X-114 is one of the non-ionic surfactant was extensively used in CPE. This is due to its advantages such as commercial availability with high purity, relatively low cloud point temperature, low toxicity and cost and high density of the surfactant- rich phase which facilitates phase separation by centrifugation [147, 148]. Figure 3.9 shows the effect of the amount of non-ionic surfactant of 20% (v/v) of Triton X-114 from (0.02 to 1.00) mL on extracting iron (III). It was found that the absorbance of the complex was increased with the increase in the volume of Triton X-114 until 0.1mL was added from surfactant, while the absorbance was relatively decreased with the large quantity of Triton X-114 surfactant.

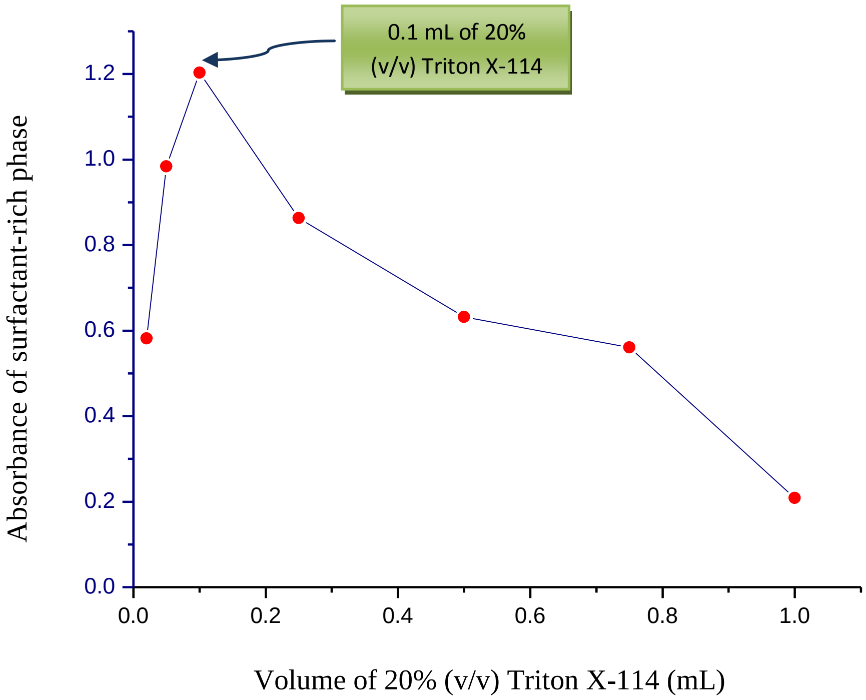


Figure 3.9 The effect of Triton X-114 on extraction of iron ion by CPE method. At pH 4.2, 10mL of 2 µg.mL^{-1} Fe (III), 0.5 mL of 1% (w/v) Benzidine.

The effect of the type of surfactant on Cu (II)-SAO complex was studied by using different types of surfactant such as Triton X-100, Triton X-114 and sodium dodecyl sulfate (SDS). In Figure 3.10 shows the effect on the absorbance of Cu (II)-SAO complex value which was increases with the Triton X-114 surfactant, thereby the effect of Triton X-114 surfactant concentration on the performance of the developed method was studied. A series of volume (0.05 to 1.00) mL of 20% v/v of Triton X-114 was examined. The result of this test illustrated in Figure 3.11. The analytical signal of Cu (II)-SAO- complex was increased with an increase of the Triton X-114 amount.

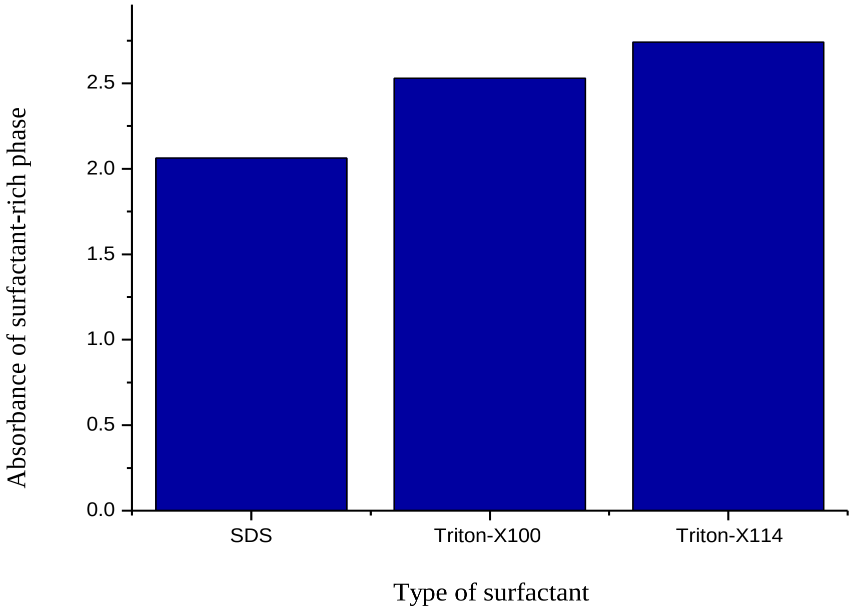


Figure 3.10 Effect of type of surfactant on Cu (II)-SAO complex

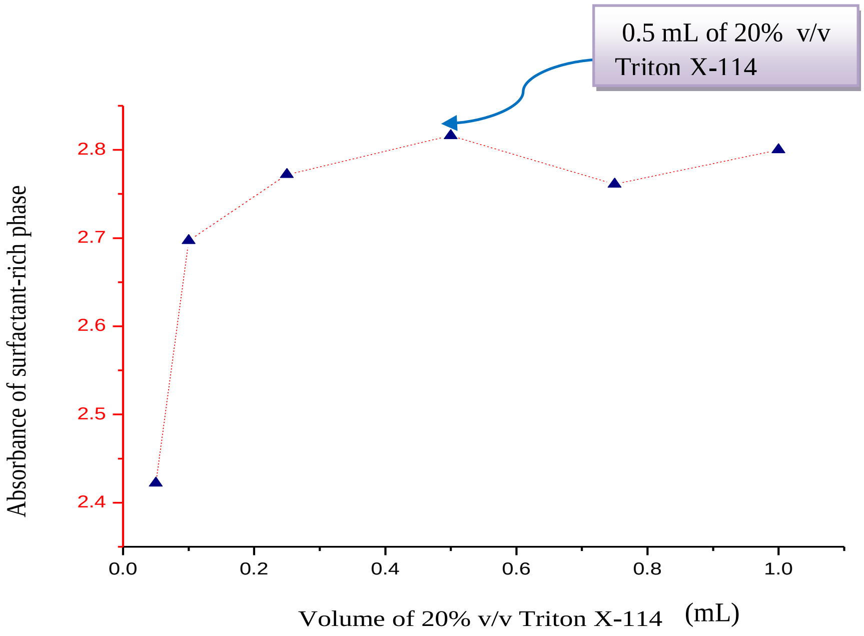


Figure 3.11 Effect of the amount of Triton X-114 surfactant on Cu (II)-SAO complex

3.2.5 Effect of Equilibrium Temperature

The cloud point extraction efficiency is strongly affected by the temperature. The effect of the equilibrium temperature was examined due to their importance for the reaction completion and efficient of separation of phases [149]. The water bath temperature was varied from (50 to 85) °C, to study the effect of increasing temperature on the increasing of the clouding of surfactant phase for iron(III) complex so that can be seen the highest absorbance signals when the temperature at 70 ℃ in Figure 3.12. Due to the increasing of the equilibrium temperature that leads to decrease a surfactant-rich phase because of decreasing the amount of water in this phase, so the cloud point extraction efficiency increased [150].

In addition, the effect of temperature increasing on the surfactant rich phase of the Cu (II) complex separation was down by varying the temperature of water bath from (50 to 85) ℃ . All the results were tabulated in Table 3.4. That can be noted the absorbance signals increased in an increase of temperature to arrive at 75 ℃ .

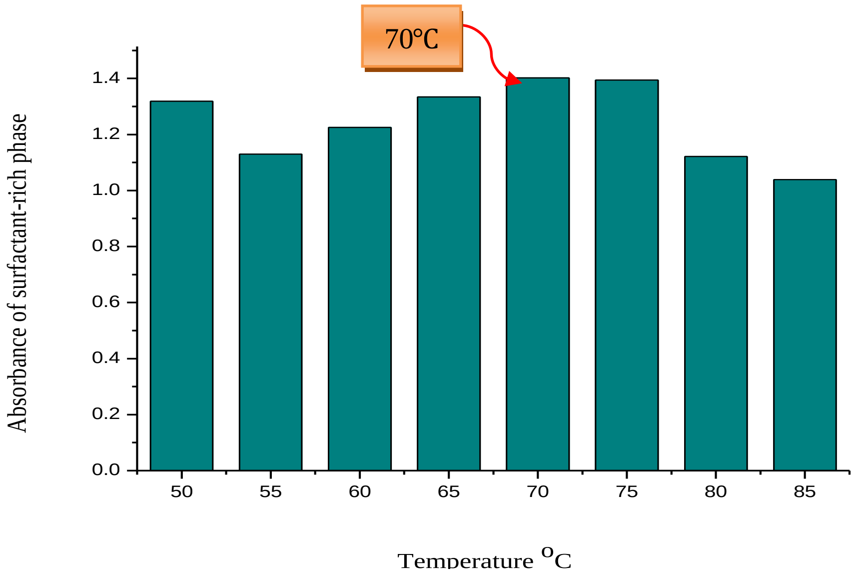


Figure 3.12 Effect of equilibrium temperature on the CPE of iron (III). At pH 4.2, 10mL of 2 µg.mL^{-1} Fe (III), 0.5 mL of 1% (w/v) Benzidine, 0.1 mL of 20% (v/v) Triton X-114

Table 3.4 Effect of equilibrium temperature on the Cu (II)-SAO complex. At pH 4.2, 10mL of 10 µg.mL^{-1} Cu (II), 0.1 mL of 1% (w/v) SAO.

Temp.℃	Ao	Aaq	As	D	E%
50	1.792	0.386	2.710	7.047	87.57
55	1.792	0.246	2.711	11.063	91.71
60	1.792	0.079	2.614	331.29	99.69
65	1.792	0.128	2.661	20.804	95.41
70	1.792	0.047	2.721	58.020	98.30
75	1.792	0.001	2.727	2753.3	99.96
80	1.792	0.045	2.677	59.615	98.35
85	1.792	0.011	2.707	246.24	99.59

3.2.6 Incubation Time Effect

In order to achieve easy phase separation and efficient pre-concentration in cloud point extraction processes, it was desirable to employ the shortest incubation time. The effect of incubation time at water bath to heat the cloud solution was investigated in the ranges (1 to 25) min. for the iron complex separation. The results were demonstrated 5 min as the best incubation time and it was chosen for further experiments to optimize conditions of the extraction method.

The effect of incubation time on the analytical signal of the extracted copper (II) complex was studied by varying the range of time from (5 to 25) min. The results were desired to employ in the incubation time of 10 min, which was chosen for further experiments. The results of the incubation time effect are demonstrated in the Figures 3.13 and 3.14, that show the iron and copper complexes respectively.

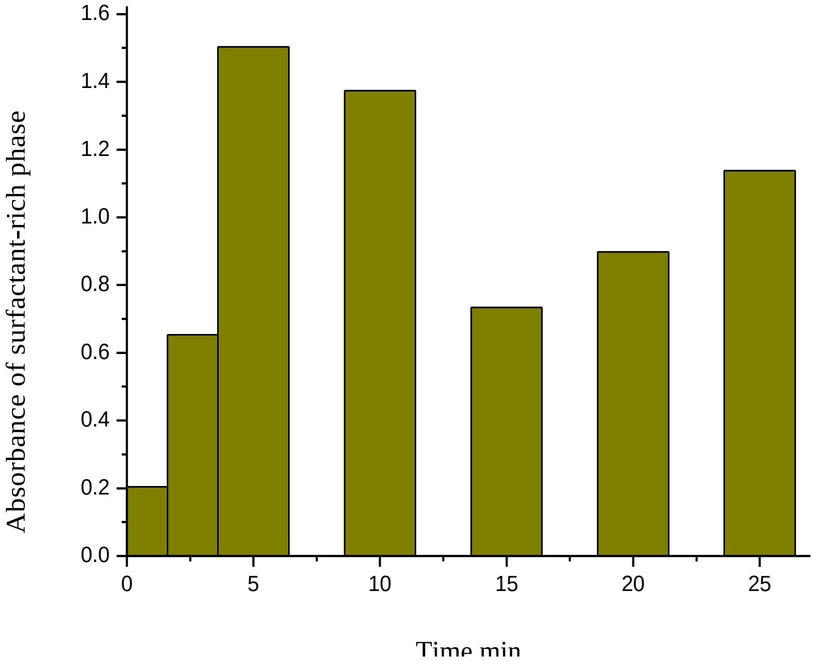


Figure 3.13 Effect of incubation time on the Fe (III)-Benzidine complex extracted. At pH 4.2, 10mL of 2 μg.mL^{-1} Fe (III), 0.5 mL of 1% (w/v) Benzidine, 0.1 mL of 20% (v/v) Triton X-114, 70 ℃

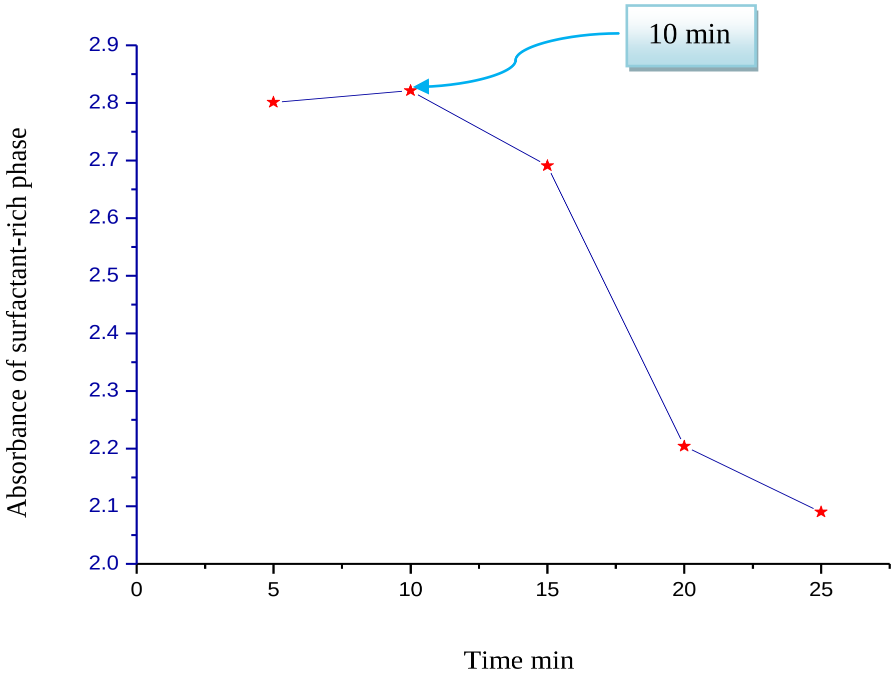


Figure 3.14 Effect of incubation time on the Cu (II)-SAO- complex. At pH 4.2, 10mL of 10 μg.mL^{-1} Cu (II), 0.1 mL of 1% (w/v) SAO, 0.5 mL of 20% (v/v) Triton X-114, 75 ℃

3.2.7 Salt out Effect

In cloud point extraction addition of salt to sample solution enhances for phase separation and increasing the mass transfer of analyte from aqueous phase to surfactant - rich phase. The effect of salts on CPE was studied by adding 0.5 mL of 15% w/v of NaCl, KCl, Na_2CO_3 and Na_2SO_4 to the solution of Fe (III) and the solution of the Cu (II) and was subjected to the complete procedure. The results obtained demonstrated that an increasing in the absorbance value was found in the presence of some salts. This is because that the salts water molecules will pull and destroy the hydration shell of metal ions leads to increase in the bonding between the reagent and the metal ions. On the other hand, the salting-out effect of electrolytes, their presence decreases the cloud point temperature and stimulates dehydration of the ethoxy groups on the outer surface on the micelles [151]. Table 3.5 and 3.6 show the effect of salt out on the Fe (III) –benzidine and Cu (II)-SAO complexes respectively.

Table 3.5 Effect of salts out on the Fe (III)-Benzidine complex formation. At pH 4.2, 10mL of 2 µg.mL^{-1} Fe (III), At optimum coonndit

Salts 0.5 mL of 15% (w/v)	As	Recovery %	E_{re}%
Without salts	1.127		
NaCl	0.442	39.219	60.780
KCl	0.401	35.581	64.418
Na_2CO_3	0.404	35.847	64.152
Na_2SO_4	2.103	186.601	86.601

The values of Ao and Aaq equal approach to zero

Table 3.6 Effect of salts out on the Cu (II)-SAO complex, At pH 4.2, 10 mL of 10 µg.mL^{-1}, At optimum conditions

Type of added salt	Ao	Aaq	As	*Recovery %	*E_{re}%
without salt	1.692	0.001	1.769		
NaCl	1.743	0.1012	2.473	139.79	39.79
KCl	1.761	0.0103	2.349	132.78	32.78
Na_2CO_3	1.801	0.686	2.304	130.02	30.24
Na_2SO_4	1.695	0.242	2.740	154.88	54.88

*** Re.%= (Measured Value/ Actual Value)*100, * Ere% : Relative error percentage=**

(absolute error / **Actual Value)*100, absolute error = Actual Value - Measured Value**

3.2.8 Interference Study

The effects of foreign ions on the extraction of Fe (III) and Cu (II) ions were studied, the cations that may react with the analytical reagents (Benzidine, SAO). That may react with analytes and decrease the extraction efficiency. To perform this study. 100 µg. mL^{-1} of interfering ions were added to solutions of 2 µg. mL^{-1} of Fe (III) and 2 µg. mL^{-1} of Cu (II) and were subjected to the complete procedure. The results obtained demonstrate that the presence of large amounts of species commonly present in water samples have significant effect on the cloud point extraction efficiency. Table 3.7 tabulated the effect of interference on the extraction of the Fe (III)-benzidine and Cu (II)-SAO complexes.

Table (3-7) Divers ions effect on the absorbance of Fe(III)-Benzidine and Cu(II)-SAO complexs complex by UV-Vis-CPE spectrophotometry

Interferent	Composition divers ions compound	Interferent conc. / metal conc. µg.mL-1	As of Fe(III)	As of Cu(II)	Fe (III)-Benzidine complex		Cu (II)-SAO complex	
					Recovery %	E_{re}%	Recovery %	E_{re}%
Cu(II)	$Cu(NO_3)_2.3H_2O$	50	1.803	---	77.984	22.015	---	---
Fe(III)	$FeCl_3$	50	---	2.925	---	---	557.14	457.10
Co(II)	$Co(NO_3)_2$	50	1.921	2.810	83.088	16.911	535.20	435.23
Ni(II)	$Ni(NO_3)_2$	50	1.642	2.512	71.020	28.979	478.47	378.47
Cr(III)	$Cr(NO_3)_3$	50	0.972	0.622	42.041	57.958	118.47	18.47
Al(III)	$Al (NO_3)_3.9H_2O$	50	0.721	0.212	31.185	68.814	40.38	59.61
Pb(II)	$Pb(NO3)_2$	50	1.721	0.538	74.437	25.562	102.47	2.476
Zn(II)	$ZnCl_2$	50	1.812	1.214	78.373	21.626	231.23	131.23

Without addition of interferent with Fe(III) have absorbance 2.312, 2 µg.mL^{-1}
Without addition of interferent with Cu(II) have absorbance 0.525, 10 µg.mL^{-1}

3.3 Stoichiometry of the Complexes

The stoichiometry of the Fe (III)-benzidine and Cu (II)-SAO complexes were determined by using two methods.

3.3.1 Job's Method

In the Job method, solutions of $1x10^{-3}$ M reagent (benzidine) and $1x10^{-3}$ M iron (III) solution were prepared and mixed in continuous variation then, diluted to 10 mL of deionized water after that measuring the solution by UV-Vis Spectrophotometer at 425 nm. The stoichiometry of the (Fe: Benzidine) complex determined by Job's method and found to be 2.33:1.0. Figure 3.15 shows the Job's plot of absorbance at 425 nm versus the mole fraction of Fe (III) ion.

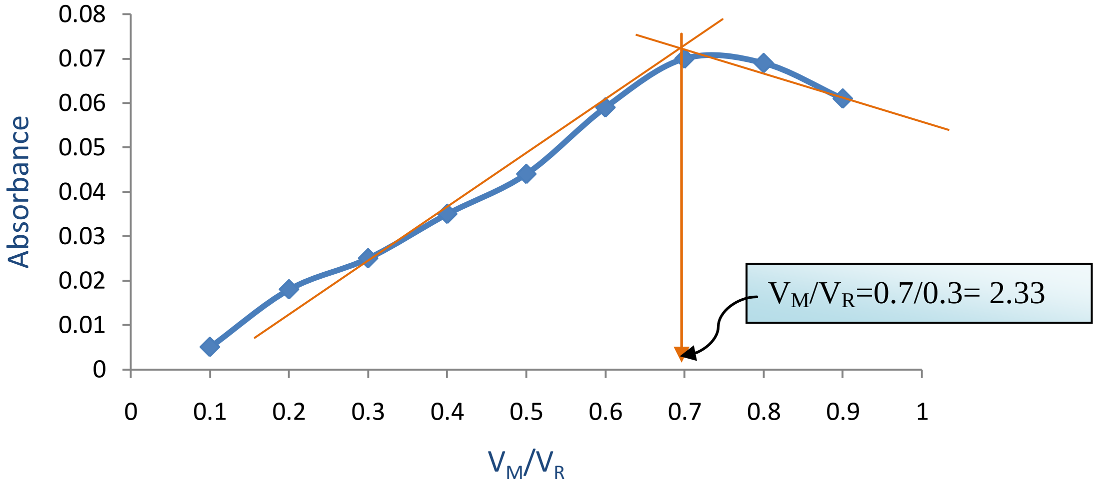


Figure 3.15 The Job's plot of absorbance at 425 nm (V_M: Volume of Fe (III) (mL), VR: Volume of benzidine reagent (mL)

By continuous variation of volume $1x10^{-3}$ M from each of the Cu (II) and SAO reagent solutions have been studied to determine the stoichiometry of Cu (II)-SAO complex. The stoichiometry of the (Cu : SAO) complex determined by Job's method was found to be (1.0:1.0). Figure 3.16 shows the Job's plot of absorbance at 380 nm versus the mole fraction of Cu (II) ion.

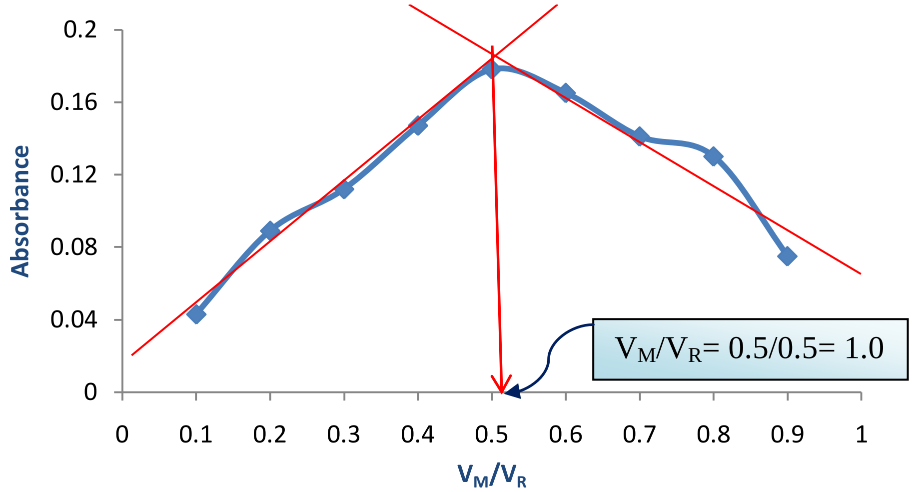


Figure 3.16 The Job's plot of absorbance at 380 nm (V_M: Volume of Cu (II) (mL), VR: Volume of SAO reagent (mL)

3.3.2 Mole Ratio

There are two ways that can be followed in determining the mole ratio. In the mole ratio method a solution of $1x10^{-3}$ M reagent (Benzidine) and Iron (III) solution were prepared, the volume of Iron (III) solution was fixed and a change volume of reagent (Benzidine) or vice versa, then diluted to 10 mL of deionized water after this measurement the solution by UV-Vis Spectrophotometer at 425 nm. The stoichiometry of the complex determined by Mole ratio method was found to be (2.33: 1.00) Figure 3.17 shows mole ratio plot of Benzidine- Fe (III) complex.

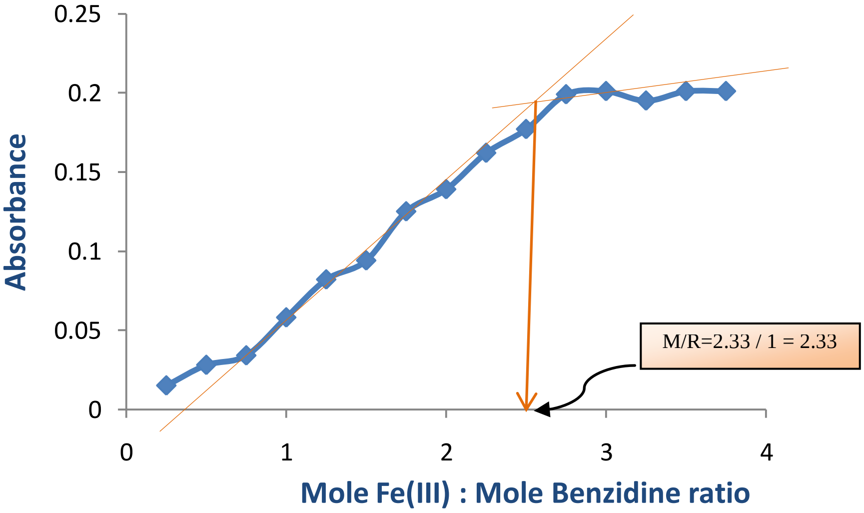


Figure 3.17 The molar-ratio plots of Fe (III)-Benzidine complex, R: mole of benzidine reagent, M: mole of metal Fe (III)

By using the mole ratio method, at constant volume of 1x10^{-3} M Cu (II) and varying volume of 1x10^{-3} M SAO reagent. The absorbance of Cu (II)-SAO-complex was measured at λmax 380 nm. Figure 3.18 observed at 1.0:1.0 mole ratios of SAO and Cu (II).

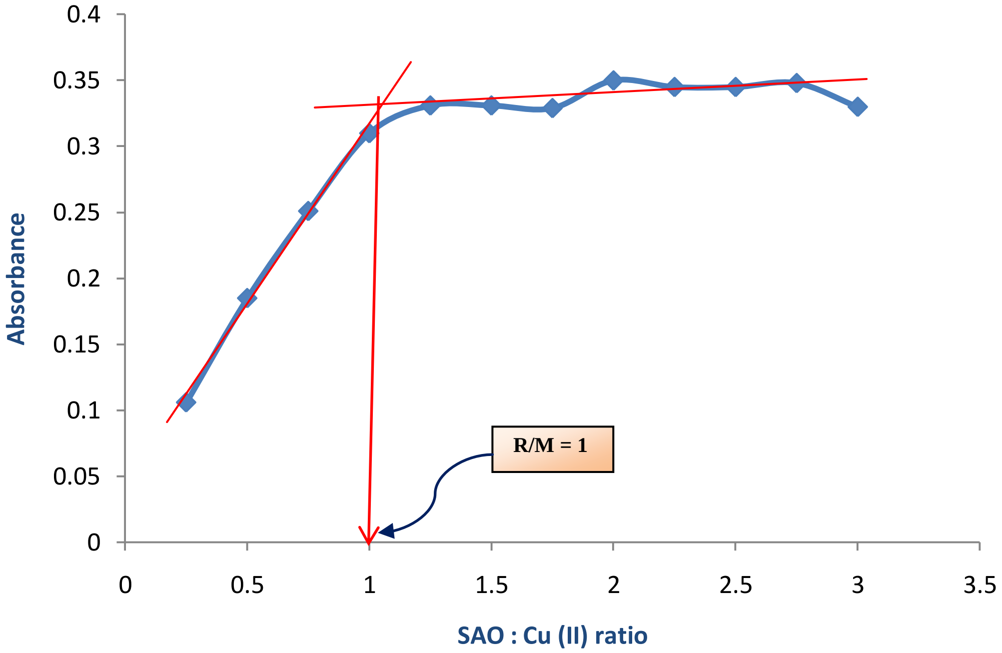


Figure 3.18 The molar-ratio plots of Cu (II)-SAO complex. R: mole of SAO reagent, M: mole of metal Cu (II)

3.4 Scattering Curve

3.4.1 Calibration Curve of Fe (III) and Cu (II) by UV-Vis -CPE

In order to test the linearity of the method under the optimized conditions established by CPE procedure for the Fe (III) and the Cu (II) ions, the calibration curve was established by plotting the absorbance versus concentration of Fe (III) ion Figure 3.19. A series of Fe (III) solutions (0.10 -7.00) μg.mL^{-1} were prepared. Table 3.8 expains the using range of concentrations of Fe (III) μg. mL^{-1} by using UV-Vis-CPE method to determine Fe (III) ion.

Table 3.8 Summary of the calibration graph results of Fe (III) by UV-Vis -CPE method

Conc. of Fe(III) μg mL^{-1}	Abs. y_i	Mean $\bar{y}$	*SD σ_{n-1}	*RSD%	*C.I $\bar{y}\pm t_{0.05/2}\frac{\sigma_{n-1}}{\sqrt{n}}$
0.25	0.242 , 0.212 , 0.223	0.223	0.0151	6.8057	0.223±0.037
0.5	0.204 , 0.317 , 0.321	0.280	0.0664	23.6670	0.280±0.164
1.0	0.700 , 0.675 , 0.694	0.689	0.0130	1.8923	0.689±0.0324
2.0	1.299 , 1.068 , 1.265	1.210	0.1247	10.3015	1.210±0.309
3.0	1.294 , 1.595 , 1.566	1.485	0.1660	11.1814	1.485±0.412

***C.I: confidence interval, *RSD: relative standard deviation= (SD/ ȳ)*100,**

***SD: standard deviation=** $\sqrt{\frac{\sum(yi-\bar{y})^2}{n-1}}$ **, Critical value of** $t_{0.05/2\ at(\ N=3)}$**=4.303**

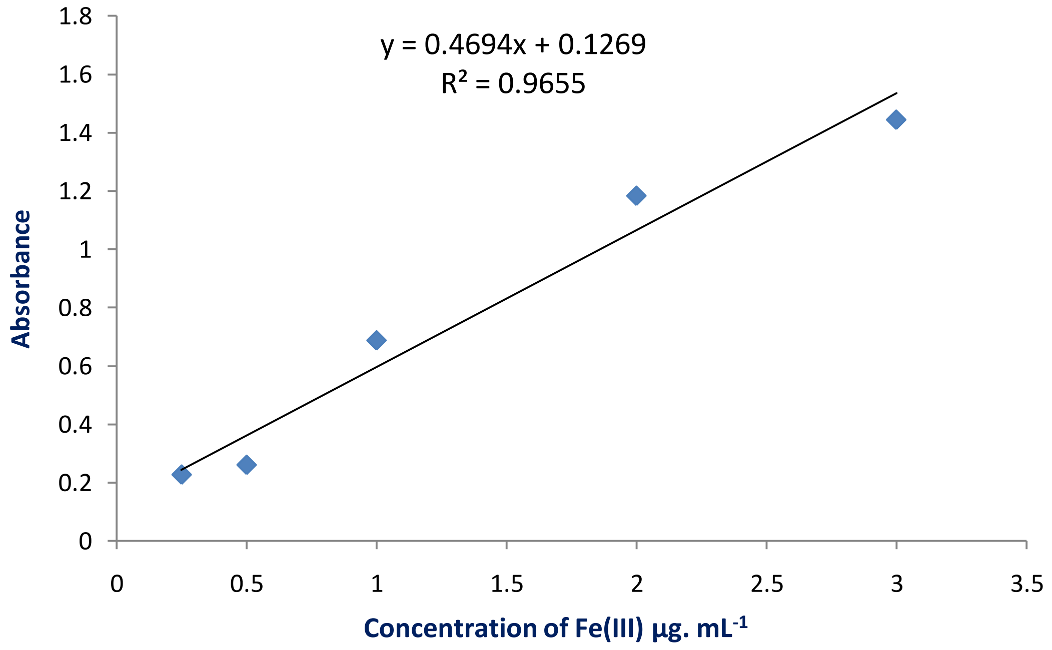


Figure 3.19 Calibration graph for Fe (III) by UV-Vis spectrophotometric method coupling with CPE

Under the optimum experimental conditions, the calibration curve of absorbance versus concentration of Cu (II) solutions was constructed by collecting the analytical signals of different concentrations of Cu (II) standard solutions. The linearity curve of Cu (II) ion was made from (0.50 -

16.00) µg.mL^{-1}. Table 3.9 and Figure 3.20 show it the resulting of scattering curve for determination of Cu (II) ion. Table 3.10 tabulated the results of the linear regression analysis for the scatter curves of iron (III) and copper (II) ions.

Table 3.9 Summary of results for Calibration graph for Cu (II) by using UV-Vis spectrophotometry- CPE

Conc. of Cu(II) µg. mL^{-1}	Abs. y_i	Mean $\bar{y}$	SD σ_{n-1}	RSD%	C.I $\bar{y} \pm t_{0.05/2}\frac{\sigma_{n-1}}{\sqrt{n}}$
0.5	0.092 , 0.020, 0.091	0.067	0.041284	61.010	0.067±0.102
1	0.143, 0.120, 0.21	0.157	0.046758	29.656	0.157±0.116
2	0.321 , 0.279, 0.273	0.291	0.026153	8.9874	0.291±0.064
4	0.569 , 0.56 , 0.561	0.563	0.004933	0.8756	0.563±0.012
6	1.018 , 0.972, 0.700	0.896	0.171864	19.1670	0.896±0.426
8	1.470, 1.040, 1.218	1.242	0.216059	17.3866	1.242±0.536
10	1.782 , 1.619 , 1.670	1.690	0.083381	4.9327	1.690±0.206
12	1.966, 1.895, 2.100	1.987	0.104101	5.2391	1.987±0.258
16	2.598 , 2.508 , 2.399	2.501	0.099651	3.9833	2.501±0.247

C.I: confidence interval, RSD: relative standard deviation, SD: standard deviation
Critical value of t0.05/2 at (n=3) = 4.303

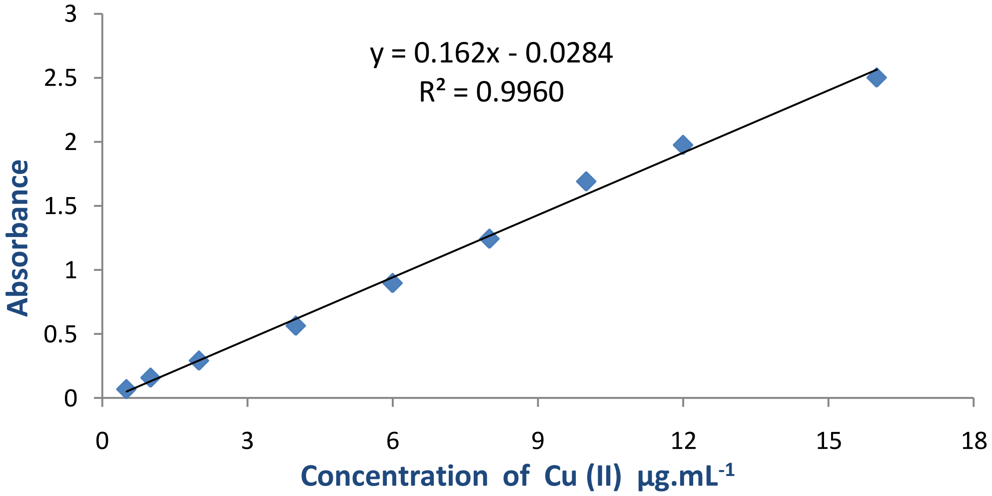


Figure 3.20 Calibration curve for Cu (II) - SAO complex by UV-Vis spectrophotometric method coupling with CPE

Table 3.10 Results for linear regression analysis of iron (III) and copper (II) by using UV-Vis-CPE technique

	Range of conc. $\mu g.\ mL^{-1}$	**Linear regression equation**	**r r^2 $r^2\%$**	**t_{table} at 95% confidence level , n-2**	**Calculated t-value $t_{cal}=(\lvert r\rvert\sqrt{n}-2)/(\sqrt{1-r^2})$**
Fe(III) n=5	0.25 – 3.00	Y=0.4694 X + 0.1269	0.9825 0.9650 96.50	3.182 <<< 9.163	
Cu(II) n=9	0.50 – 16.00	Y=0.162 X – 0.0284	0.9979 0.996 99.60	2.365 <<< 41.74	

n= number of concentration in calibration curve, r: Correlation coefficient, r^2: coefficient of determination, $r^2\%$: relative coefficient of determination

3.4.1.1 Limit of Detection LOD

A method's detection limit is the smallest amount or concentration of analyte that can be detected with statistical confidence. The International Union of Pure and Applied Chemistry (IUPAC) define the detection limit as the smallest concentration or absolute amount of analyte that has a signal significantly larger than the signal arising from a reagent blank [152]**.** The LOD value (XL) is given by equation (3-1).

$$XL = X_{bl} + KS_{bl} \ \ldots\ldots\ldots\ldots\ 3.1$$

Where X_{bl} is the mean of the blank measures, S_{bl} is the standard deviation (SD) of the blank measures and K is a numerical factor chosen according to the confidence interval [153]. The limit of detection can be calculated by using two methods. Gradual dilution of low concentration in the calibration graph or based on the numerical value of slope. The results are tabulated in Table 3.11.

Table 3.11 Limit of detection for Fe (III) and Cu (II) by UV-VIS-CPE technique

	Theoretical based on the value of slope $X=3S_b/slope$	**Theoretical based on the linear equation $X=X_b + 3S_B$**	**Practically based on gradual dilution for the minimum concentration**
Fe(III)	0.060	0.053	0.050 $\mu g.\ mL^{-1}$
Cu(II)	0. 180	0.103	0.100 $\mu g.\ mL^{-1}$

3.4.1.2 Precision Study

3.4.1.2.1 Repeatability

The test of precise of UV-Vis-CPE method for iron (III) and copper (II) ions determination was applied by calculation of the relative standard deviation (RSD %) values for the results of the repeatability test. The concentrations 0.5 and 2.0 µg. mL^{-1} of Fe (III) while 4.0 and 8.0 µg. mL^{-1} of Cu (II) were used. The values of the relative standard deviation (RSD %) for Fe (III) and Cu (II) ions are tabulated in Table 3.12. The percentage relative standard deviation less than 5% can be achieved using this method.

Table 3.12 Repeatability of Fe (III) and Cu (II) complexes at optimum parameters

	No. of repeating	**Conc. of metal µg. mL^{-1}**	**Abs.**	**Mean ± SD**	**RSD%**
Fe(III)	6	0.5	0.252 , 0.355 , 0.295 , 0.332 ,0.300 , 0.313	0.308 ± 0.035	11.360
	6	2	0.396 , 0.401 , 0.411 , 0.429 , 0.399 , 0.399	0.406 ± 0.012	2.955
Cu(II)	6	4	0.500 , 0.520 , 0.509, 0.560 , 0.561 , 0.542	0.532 ± 0.026	4.887
	5	8	1.260 , 1.246 , 1.292 , 1.101 , 1.218	1.223 ± 0.073	5.968

3.4.2 Calibration Curve of Fe (III) and Cu (II) using FAAS–CPE

3.4.2.1 Introduction of Atomic Absorption Spectrometry

Measurement of trace element concentrations is very important. So, many studies are interested in determining the total amounts of these elements in different samples, so it requires instruments and techniques with high efficiency and sensitivity to determine the low concentration of these elements [154]. Atomic absorption spectrometry (AAS) is the most widely used technique to measure the concentrations of the metals. The occurrence of atomic absorption was noted by Wollaston and Fraunhofer and explained by Kirchoff and Bunsen in the nineteenth century [1]. The basis of the atomic absorption technique is the absorption of light by atoms. These atoms have many levels of energy. Most of these atoms are in a stable state. When these atoms are absorbed into light, they will be stimulated and transferred to higher levels. Flame FAAS follows an exponential relationship between the intensity I of transmitted light and the absorption path length b (Lambert's law), as shown below [153].

$$I = I^{o}exp\,(-Kvb) \qquad \text{--------------------}\ 3.2$$

Where I^{o} is the intensity of the incident light beam and K, is the absorption coefficient at the frequency v. In quantitative spectroscopy, absorbance A is defined by

$$A = log\,(I^{o}/I) \qquad \text{-------------------------}\ 3.3$$

Absorbance is so important because it is directly proportional to the concentration, c, of the light absorbing species in the sample (Beer-Lambert law):

$$A = \varepsilon cb \qquad \text{---------------------------------}3.4$$

A simple schematic of a typical FAAS instrument is show in Figure 3.2 [155].

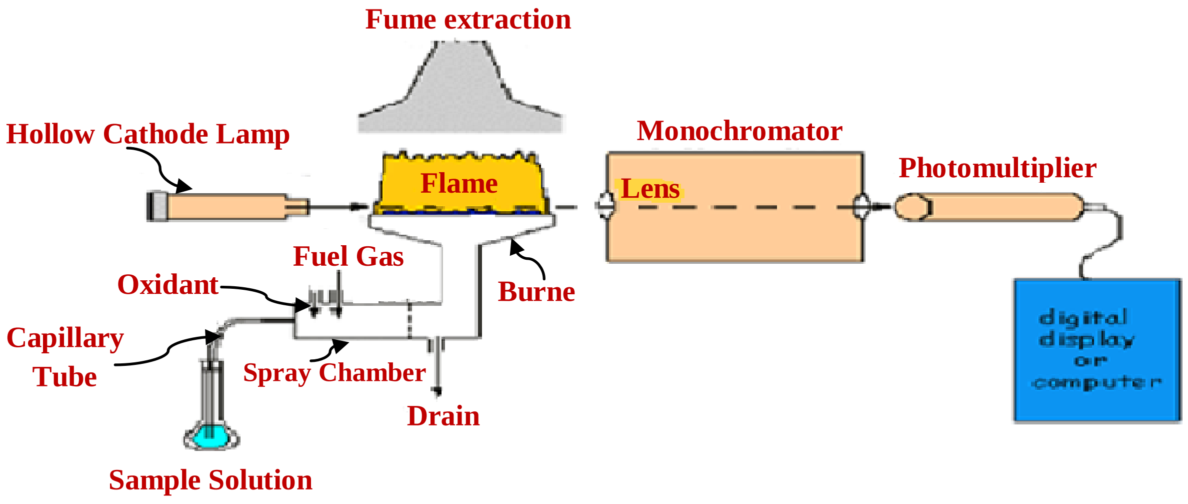


Figure 3.21 Schematic diagram of flame atomic absorption instrument

3.4.2.2 Linearity Curve of FAAS

Prior the measure the series of standards iron and copper solutions in the flame atomic absorption instrument, it was adjusted according to the catalogue were listed in section 2.2.2. The series of the standard solution were varied from (0.0 to 5.0) µg. mL^{-1} for each metal iron copper solutions and to determine in FAAS technique. Figures 3.22A and B illustrated linearity curves of Fe (III) and Cu (II) analysis respectively.

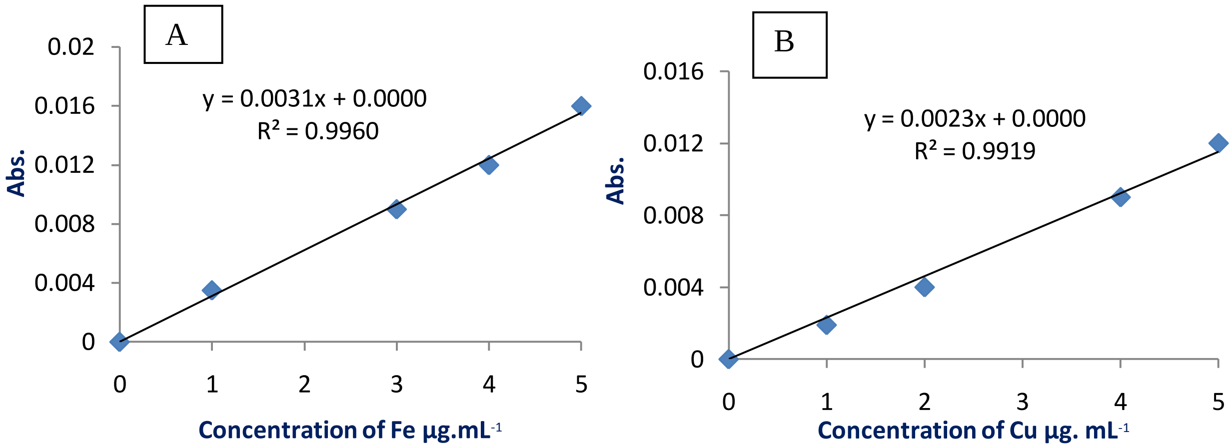


Figure 3.22 Calibration graphs for determination of metals by FAAS. (A) Iron, (B) Copper

3.5 Application

3.5.1 Trace Element Measurement in Urine Sample

After the digestion urine samples as described in 2.5 section, the pre-concentration and extraction steps were applied by using cloud point extraction procedures on the urine samples and the trace iron and copper the concentration were estimated by using the UV-Vis spectrophotometry techniques and FAAS methods.

Iron (III) ion was measured by spectrophotometric method at λmax 425 nm and a 0.5 mL of 1% w/v ascorbic acid was used as masking for Cr(III), a 0.5 mL of 1% w/v glycine as masking for Ni(II) ion [156] and a 0.5 mL of 1% w/v EDTA as masking for Al(III) [156, 157] . At the same condition of CPE method for urine samples the iron (III) was determined by FAAS method. The results of iron (III) ion concentration were described in Appendix A1and A2, whereas. Copper (II) ion was estimated using the UV-Vis spectrophotometric method at λmax 380 nm after using a 1mL of 10% w/v NaF (F^-) as masking agent for Co(II), Ni(II), Fe(III) and Al(III) [156-158] . At the optimum condition of CPE to determine Cu (II) in urine sample, it is measured by FAAS method where the results were described in Appendix B1 and B2.

3.5.2 Statistical Treatment of Data

Various statistical software packages are available which can utilized to do statistical analysis such as Microsoft Excel 2010, Originpro 8.1portable and Chem. Sketch (freeware). All of these have been tried to apply to our data of interest.

3.5.2.1 Outlier test

3.5.2.1.1 Grubbs test

Grubbs test was used for outliers; the international organization for standardization (ISO) recommended test for outliers is Grubbs' test. This test compares the deviation of the suspect value from the sample mean with the standard deviation of the sample. The suspect value is the value that is the furthest away from the mean. In order to use Grubbs' test for an outlier, that is to test H_o: all measurements come from the same population, the statistic G_{exp} is calculated [159, 160].

$$G_{exp} = \left| x_{out} - \bar{x} \right| / s \qquad \text{-------------------3.5}$$

Where $\boldsymbol{X_{out}}$ is the potential outlier, $\boldsymbol{\bar{x}}$ is samples mean and $\boldsymbol{S}$ is samples standard deviation. The results of Cu (II) and Fe (III) concentration obey the Grubbs test where the number of outliers of results are illustrated in Table 3.13 and are labeled in Appendix A and B.

Table 3.13 Results of Grubbs-test applied on the Fe (III) and Cu (II) ions concentrations

No. of sample		**Readings excluded**			***C.F**	**Range of concentration μg. mL^{-1}**		
		UV-Vis -CPE	**FAAS - CPE**	**Direct FAAS**		**UV-Vis - CPE**	**FAAS - CPE**	**Direct FAAS**
Fe(III)	44	7	7	--	22.5	0.007-0.151	0.017-0.208	--
Cu(II)	44	0	--	0	22.5	0.052-0.382	--	0.088-1.15

***C.F= concentration factor= (volume of matrix / volume of organic layer)**

This test was applied to 44 samples as shown in Tables 3.14 and 3.15. Because of this test, some samples were neglected due to not consistent with the practical result, and other samples were lost due to damage during the work. There are many tests for outliers such as Dixon’s test (sometimes called the Q-test) but cannot be applied because the number of samples is large [152]. As described in Appendix A1, A2, B1 and B2. The bar graphs 3.23 and 3.24 represent the iron and copper concentrations measuring by the UV-Vis-CPE and FAAS methods.

Table 3.14 Results of iron (III) concentration in urine samples by different techniques

Occupation	**Method**	**No. of sample**	**Mean(ȳ) ±SD**	**C.I** $\bar{y} \pm t_{0.05/2}\frac{\sigma_{n-1}}{\sqrt{n}}$
Oil refineries	UV-Vis -CPE	14	0.0618±0.0145	0.0618 ± 0.0083
	AAS - CPE	14	0.0539±0.0300	0.0539±0.0173
Welding	UV-Vis -CPE	12	0.0616±0.0247	0.0616±0.0157
	AAS - CPE	12	0.0481±0.0237	0.0481±0.0150
Casting alloys	UV-Vis -CPE	3	0.0704±0.0281	0.0704±0.0695
	AAS - CPE	3	0.0364±0.0090	0.0364±0.0220
Terminals to provide fuel	UV-Vis -CPE	5	0.0562±0.0340	0.0562±0.0422
	AAS - CPE	5	0.0406±0.0203	0.0406±0.0252
Dyeing	UV-Vis -CPE	3	0.0849±0.0111	0.0849±0.0276
	AAS - CPE	3	0.0659±0.0334	0.0659±0.0829
Total	UV-Vis -CPE	37	0.0635±0.0219	0.0635±0.0073
	AAS - CPE	37	0.0512±0.0261	0.0512±0.0087

C.I: confidence interval, SD: standard deviation, Mean ȳ: Mean of concentration μg. mL^{-1}

Critical value of t0.05/2 at n=3(4.303), at n=5 (2.776), at n=12(2.201), at n=14(2.160), at n=37(2.028)

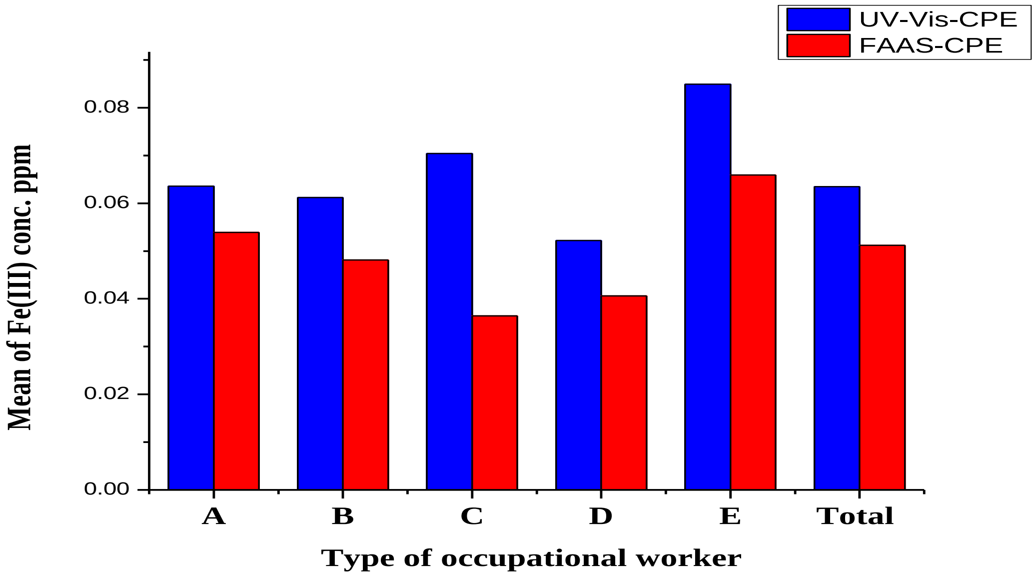


Figure 3.23 Diagram a of the iron (III) concentration in urine samples by UV-Vis-CPE and FAAS –CPE .Where is A: Oil refineries, B: Welding, C: Casting alloys, D: Terminals to provide fuel and E: Dyeing.

Table 3.15 Results of copper (II) concentration in urine samples by different techniques

Occupation	**Method**	**No. of sample**	**Mean(ȳ) ±SD**	**C.I** $\bar{y} \pm t_{0.05/2} \frac{\sigma_{n-1}}{\sqrt{n}}$
Oil refineries	UV-Vis -CPE	16	0.1838±0.0830	0.1838±0.0442
	Direct AAS	16	0.1757±0.0661	0.1757±0.0366
Welding	UV-Vis - CPE	12	0.1767±0.0790	0.1767±0.0501
	Direct AAS	12	0.2116±0.0780	0.2116±0.0495
Casting alloys	UV-Vis - CPE	5	0.1994±0.1381	0.1994±0.1714
	Direct AAS	5	0.1936±0.0367	0.1936±0.0580
Terminals to provide fuel	UV-Vis -CPE	6	0.1766±0.0715	0.1766±0.0750
	Direct AAS	6	0.1765±0.1055	0.1765±0.1107
Dyeing	UV-Vis -CPE	5	0.1883±0.0615	0.1883±0.0764
	Direct AAS	5	0.1498±0.0546	0.1498±0.0670
Total	UV-Vis - CPE	44	0.1832±0.0813	0.1832±0.0055
	Direct AAS	44	0.1847±0.0718	0.1847±0.0223

C.I: confidence interval, SD: standard deviation, Mean ȳ: Mean of concentration µg. mL^{-1}, Critical value of t0.05/2 at n=5 (2.776), at n=6(2.571), at n=12(2.201), at n=16(2.131), at n=44(2.012)

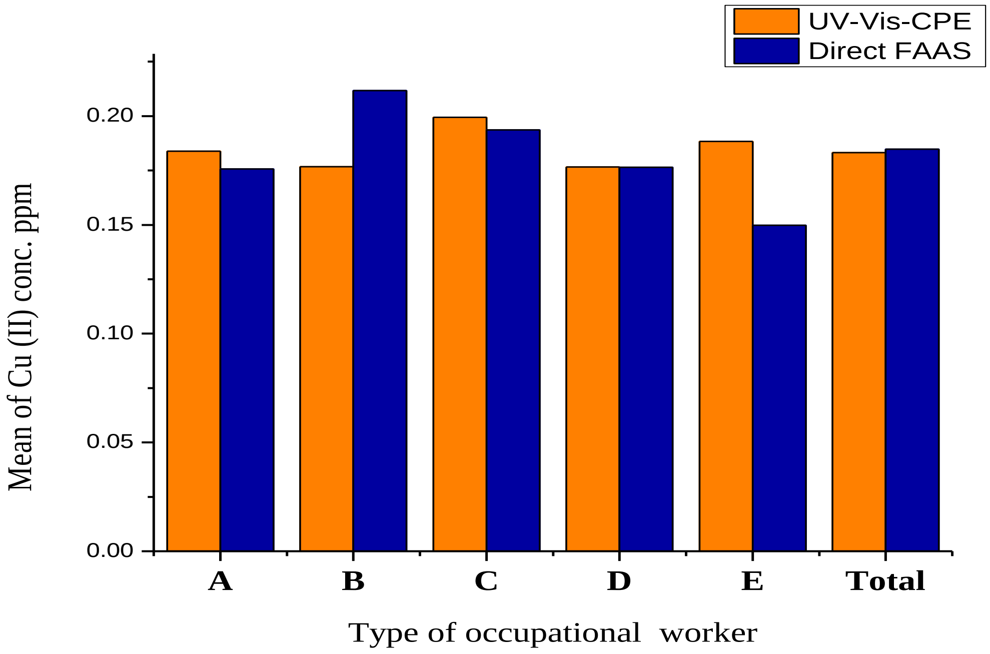


Figure 3.24 Diagram a of the copper (II) concentration in urine samples by UV-Vis-CPE and direct FAAS. Where is A: Oil refineries, B: Welding, C: Casting alloys, D: Terminals to provide fuel, E: Dyeing.

3.5.2.2 Necessity of Method Comparisons

The comparison methods are performed on a regular basis in clinical chemistry laboratories to evaluate the agreement between two analytical methods. It is also useful to resort to method comparison in routine work either to troubleshoot and analytical issue or to check any agreement between the two analyzer. The significance tests described so far are used for comparing means and hence for detecting systematic errors. In many cases it is also important to compare the standard deviations, i.e. the random errors of two sets of data. Figure 3.25 describs the flow chart for statistical testes [152, 159].

The hypothesis can be estimated as follow:

Null hypothesis: There is no significant difference between the mean obtained in all measured data i.e

$$H_o : \mu_o = \mu_o = \bar{X} \text{ .... } 3.6$$

Alternative hypothesis: there is a significant difference between the means of UV-Vis-CPE and FAAS-CPE i.e. Alternative

$$A : \mu_o \neq \mu_o \neq \bar{X} \ \ldots\ldots\ldots\ldots\ldots\ 3.7$$

At a significant level of $\alpha = 0.05$ (confidence of 95%) any value < 0.05 will reject Null hypothesis in favour of Alternative hypothesis any value of significant > 0.05 will accept Null hypothesis and reject the alternative hypothesis [159].

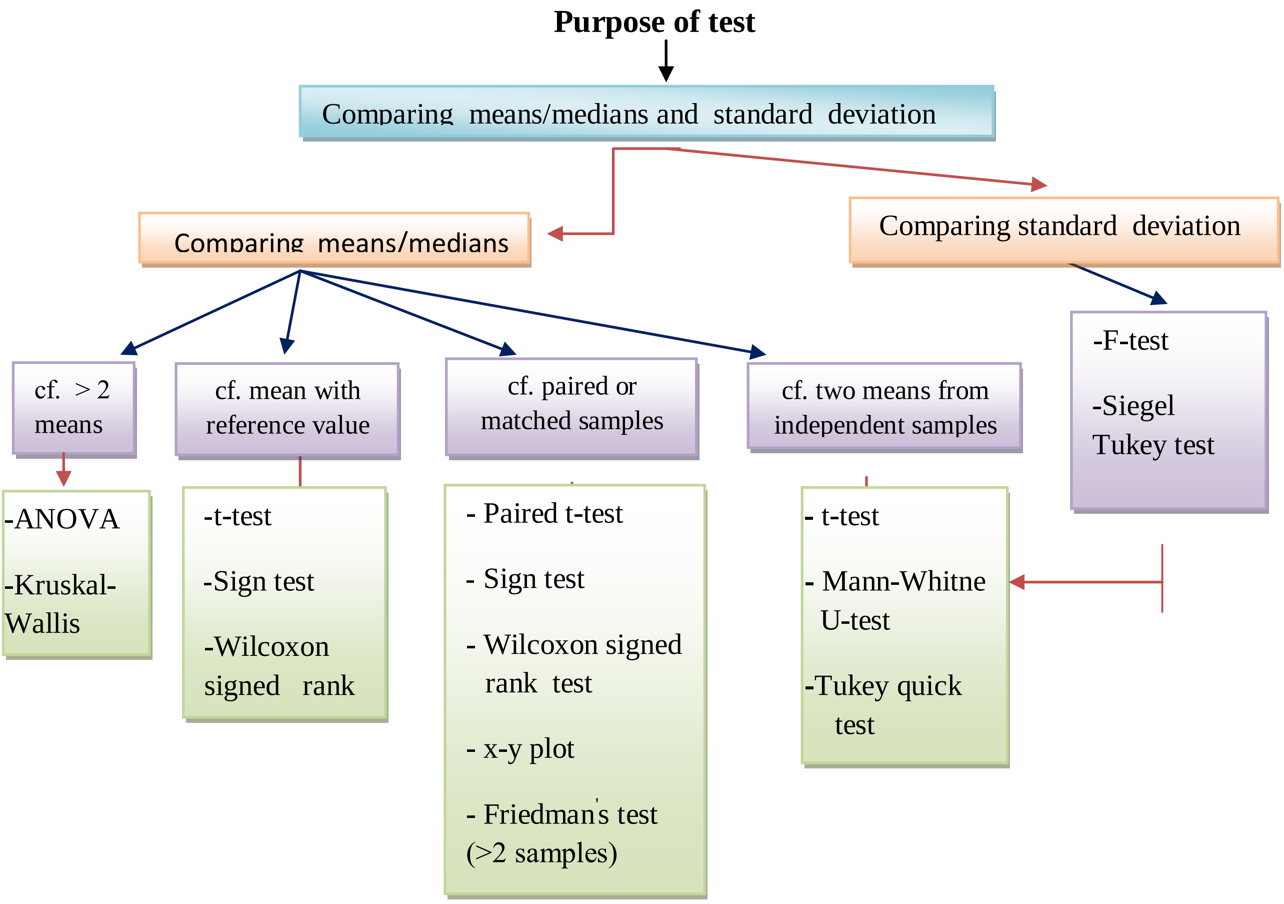


Figure 3.25 Flow chart for statistical testes

3.5.2.2.1 F-test

F-test can be applied to test the equality of two population variances. The F-test can also be used to know whether there is any homogeneity between the two independent estimates of the population variance. The F-test can be used to compare the variances for two samples [152]. Figure 3.26 explains the Flow chart for F-test and multi way of t-test can be used depending on the F-test results [161].

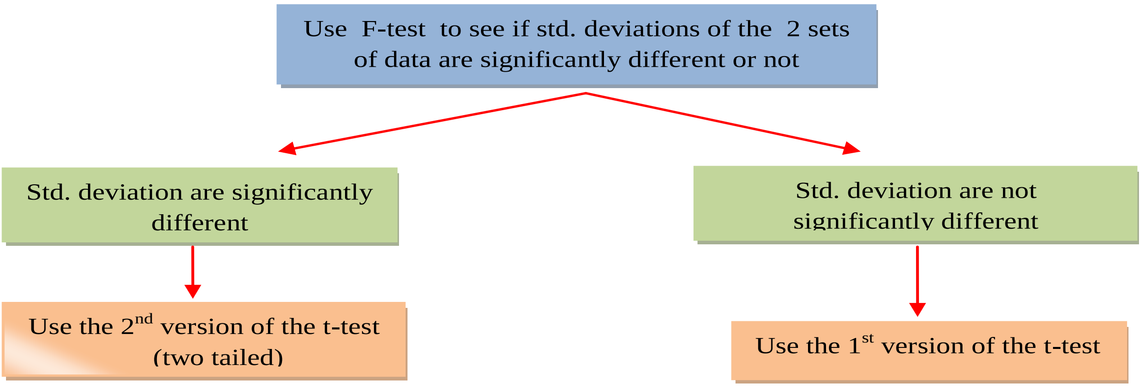


Figure 3.26 Flow chart for F-test and multi way t-test

Table 3.16 Application F-test on the Fe (III) concentration in samples to comparison between the results by two methods (UV-Vis-CPE and AAS-CPE)

occupation	**Type of measurement**	**d.f =n-1**	**SD n-1**	**Varience σ2**	**F-calculate $F=\frac{\sigma^2}{\sigma^2}$**	**F-critical value**	**P- value**
Oil refineries	UV-Vis -CPE	13	0.0145	0.000210	4.2857	2.6900	0.0066
	AAS - CPE	13	0.0300	0.000900			
Welding	UV-Vis -CPE	11	0.0247	0.000610	1.0873	2.8000	0.4460
	AAS - CPE	11	0.0237	0.000561			
Casting alloys	UV-Vis -CPE	2	0.0281	0.000789	9.7482	19.000	0.09303
	AAS - CPE	2	0.0090	0.000081			
Terminals to provide fuel	UV-Vis -CPE	4	0.0340	0.001156	2.8058	6.3900	0.1708
	AAS-CPE	4	0.0203	0.000412			
Dyeing	UV-Vis -CPE	2	0.0111	0.000123	9.0541	19.000	0.0994
	AAS - CPE	2	0.0334	0.001115			
Total	UV-Vis -CPE	36	0.0219	0.000479	1.4203	2.1100	0.14869
	AAS - CPE	36	0.0261	0.000681			

P-value < 0.05 sig. difference, p-value > 0.05 non sig. difference , *Comparison with the results of literature review for healthy of urine samples

All the results of F-test for the Fe (III) and Cu (II) concentrations on the samples are listed in Tables 3.16 and 3.17 respectively. That can be seen, almost the p-value is a non significant different between the measuring of iron and copper concentrations by two methods for the varies occupation types. Also for overall the result of iron and copper measuring by the different method, on the other hand, can use the UV-Vis -CPE methods as alternative methods of the FAAS methods to determine the iron and copper in urine sample.

Table 3.17 Application F-test on the Cu(II) concentration in samples to comparison between the results by two methods (UV-Vis-CPE and AAS-CPE)

Occupation	**Type of measurement**	**d.f =n-1**	**SD σn-1**	**Varience σ^2**	**F-calculate F= $\frac{\sigma^2}{\sigma^2}$**	**F-critical value**	**P-value**
Oil refineries	UV-Vis -CPE	15	0.0830	0.00688	1.5767	2.5400	0.1941
	Direct AAS	15	0.0661	0.00436			
Welding	UV-Vis -CPE	11	0.0790	0.00621	1.0258	2.8000	0.4840
	Direct AAS	11	0.0780	0.00604			
Casting alloys	UV-Vis -CPE	4	0.1381	0.01907	14.1597	6.3900	0.0125
	Direct AAS	4	0.0367	0.00134			
Terminals to provide fuel	UV-Vis -CPE	5	0.0715	0.00511	2.1771	4.3900	0.2066
	Direct AAS	5	0.1055	0.01113			
Dyeing	UV-Vis -CPE	4	0.0615	0.00378	1.2687	6.3900	0.4117
	Direct AAS	4	0.0546	0.00298			
Total	UV-Vis -CPE	43	0.0813	0.00660	1.2817	1.6900	0.2101
	Direct AAS	43	0.0718	0.00515			

P-value < 0.05 sig. difference, p-value > 0.05 non sig. difference

***Comparison with the results of literature review for healthy of urine samples**

3.5.2.2.2 Student t-Test

Although, the F-Test was applied, the student t-test was used to compare between the results of the two methods for a certain that are significance different or non significance between the concentration of the iron and copper element in occupational workers urine samples with the true value the concentration of these element in urine depending on the WHO values and literature review value. Student t-test is testing a sample mean, $\bar{y}$, obtained in an experimental measurement against a population mean, μ, from a normal distribution is carried out on the basis of a Gaussian or t test. In analytics, this is applied to compare an experimental mean with a true value. In practice, the population standard deviation, σ, is usually unknown, and the comparison of a sample mean with the true mean must be based on Student's *t* test [160]. Through the results obtained by the t-test application, there was a significant difference between the concentration of iron in occupational workers urine samples with the true value the concentration of this element in urine according to the World Health Organization (WHO) or the values published in literature research and at the same way for the copper. That can be indicated by an increase of the concentration of these elements in the urine occupation samples compared with the concentration of iron and copper in control values. That often caused by a several reasons such as not taking enough precautions for safety and security for the works, as, not wearing a protective clothing during work and the workers are exposed to various chemicals, radiation and a vapors. All this leads to an accumulation of these elements in the human body. Table 3.18 shows the results of one way of student t-test for iron (III) and copper (II) concentration.

Table 3.18 the results of t-test(one tail) to comparison the concentration of Fe (III) and Cu (II) in urine samples with the literature value

Occupation	Method	Fe(III)			Cu(II)		
		d.f n-1	t-calculated $t = \bar{y} - \mu \frac{\sqrt{n}}{\sigma_{n-1}}$	P-Value	d.f n-1	t-calculated $t = \bar{y} - \mu \frac{\sqrt{n}}{\sigma_{n-1}}$	P-Value
Oil refineries	UV-Vis – CPE	13	14.6802	0.00001	15	8.1909	0.00001
	FAAS	13	6.1103	0.000019	15	9.8059	0.00001
Welding	UV-Vis – CPE	11	7.9517	0.00001	11	7.3840	0.00001
	FAAS	11	6.3141	0.000029	11	9.0317	0.00001
Casting alloys	UV-Vis – CPE	2	4.0372	0.02811	4	3.0944	0.01820
	FAAS	2	6.062	0.01307	4	11.290	0.000175
Terminals to provide fuel	UV-Vis – CPE	4	3.3737	0.01397	5	5.7645	0.00110
	FAAS	4	3.9322	0.00853	5	3.9047	0.00567
Dyeing	UV-Vis – CPE	2	12.482	0.00317	4	6.5451	0.00140
	FAAS	2	3.1632	0.04354	4	5.7947	0.00220
Total	UV-Vis – CPE	36	16.274	0.00001	43	14.269	0.00001
	FAAS	36	10.789	0.00001	43	16.298	0.00001

True value of iron μ = 4.9 ng. mL , True value of copper μ = 8.3 ng. mL[67].
$t_{0.05\ (n-1)}$, n=3(4.303), n=4(3.182), n=5(2.776), n=6(2. 571), n=11(2.228), n=12(2.201), n=14(2.160), n=15(2.145), n=16(2.131), n=37(2.028), n=44(2.012).
***Comparison with the results of literature review for healthy of urine samples**

3.5.2.2.3 One-Way ANOVA Test

One-way ANOVA is then used to separate and approximate the diverse sources of variation, rather than to test whether several sample means differ significantly [159]. Table 3.19 explains the results one-way ANOVA test applied for comparison between different groups to determine of Cu (II) and Fe (III) by using UV-Vis spectrophotometer and FAAS, where the results for P-value showed that there is a significant difference between the concentration of iron (III) in varies groups of occupation samples by using UV-Vis spectrophotometry and FAAS. While there is non significant difference between these groups for the copper (II) concentration in the same way of determination.

Table 3.19 One-way ANOVA test application of comparison between the concentration of Fe(III) and Cu(II) in varies types of occupation workers groups

Metal	**Group**	**Conc. µg.mL^{-1}**		**d.f$_{between}$** **d.f$_{within}$** **d.f$_{total}$**	**SS$_{betwen}$** **SS$_{within}$** **SS$_{total}$**	**MS$_{between}$** **MS$_{within}$**	$F = \frac{MS_{between}}{MS_{within}}$	**P-value**
		UV-Vis-CPE	**FAAS**					
Fe(III)	Group A	0.0618	0.0539	1 8 9	0.0008 0.0010 0.0019	0.0008 0.0001	6.20	0.037
	Group B	0.0616	0.0481					
	Group C	0.0704	0.0364					
	Group D	0.0562	0.0406					
	Group E	0.0849	0.0659					
Cu(II)	Group A	0.18388	0.1757	1 8 9	0.00003 0.00247 0.00250	0.00003 0.0003	0.099	0.760
	Group B	0.1767	0.2116					
	Group C	0.19942	0.1936					
	Group D	0.1766	0.1765					
	Group E	0.18831	0.1498					

p-value < 0.05 sig. difference , p-value > 0.05 non sig. difference, A: Oil refineries, B: Welding, C: Casting alloys, D: Terminals to provide fuel and E: Dyeing

On the other hand, Table 3.20 indicates the comparison between determination of Fe (III) and Cu (II) by using UV-Vis spectrophotometry and FAAS methods. Depending on the p-value, it was found that there is no significant difference in the estimation of trace elements using both methods. It was also found that when using UV-Vis spectrophotometry coupled with the cloud point extraction has increased the efficiency, accuracy and sensitivity of this technique and can use this method as an alternative technique for FAAS to estimate trace elements.

Table 3.20 One-way ANOVA test application of comparison between the concentration of Fe(III) and Cu(II) in UV-Vis-CPE and FAAS

Metal	Method	Source	SS	df	MS	F	p-value
Fe(III)	UV-Vis	Between-treatments	0.0019	4	0.0005	0.91865	0.465543
		Within-treatments	0.0158	31	0.0005		
		Total	0.0177	35			
	FAAS	Between-treatments	0.0023	4	0.0006	0.81545	0.525091
		Within-treatments	0.0218	31	0.0007		
		Total	0.024	35			
Cu(II)	UV-Vis	Between-treatments	0.0022	4	0.0006	0.0745	0.98950
		Within-treatments	0.2893	39	0.0074		
		Total	0.2915	43			
	FAAS	Between-treatments	0.0168	4	0.0042	0.8210	0.5197
		Within-treatments	0.1999	39	0.0051		
		Total	0.2167	43			

p-value < 0.05 sig. difference , p-value > 0.05 non sig. difference.

SS: sum of sequres between qroup, df: dgree of freedom, MS: mean sequre

3.6 Effect of Age factor Estimation

Histogram of iron (III) and copper (II) concentration in urine sample by two methods (UV-Vis-CPE and FAAS-CPE) with age of sample was represented. That can be seen the result of Table (3-21) and Figure (3-27), the concentration of iron (III) remained steady between 20 and 40 years, and suddenly dropped to 45 years after that the concentration of iron went up to 55years old. That was due to the lack or poor absorption of iron by the body as a result of changes in metabolic processes as a result of progressing age,

leading to increasing concentration of Fe (III) in urine [144].

Table (3-21) histogram of Fe (III) concentration in urine samples with age

Age (years)	Mean of concentration (μg.mL^{-1}) for Fe (III) by UV-Vis	Mean of concentration (μg.mL^{-1}) for Fe (III) by FAAS
20-25	0.05217	0.03993
25-30	0.07576	0.04333
30-35	0.05729	0.06696
35-40	0.06818	0.06635
40-45	0.04100	0.03869
45-50	0.05350	0.04629
50-55	0.08684	0.06365
55-60	0.05798	0.04513

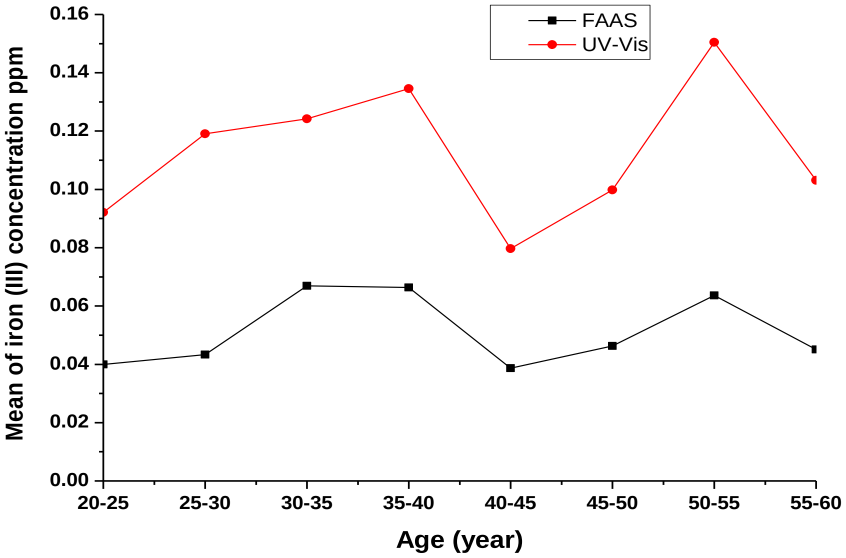


Figure 3.27 effect of age parameter on the concentration of iron (III) in urine samples

Whereas the effect of increasing age of samples with the copper (II) concentration in urine represents in Table (3-22) and Figure (3-28), the results are almost plateau straight line with an increase of age of samples.

Table (3-22) Age effect on the concentration of Cu (II) in occupation workers urine samples with age

Age (years)	Mean of concentration (µg.mL^{-1}) for Cu (II) by UV-Vis	Mean of concentration (µg.mL^{-1}) for Cu (II) by Direct FAAS
20-25	0.168177	0.18171
25-30	0.18008	0.18972
30-35	0.14312	0.13568
35-40	0.17438	0.23018
40-45	0.17933	0.15388
45-50	0.2019	0.20737
50-55	0.1978	0.16002
55-60	0.21064	0.16632

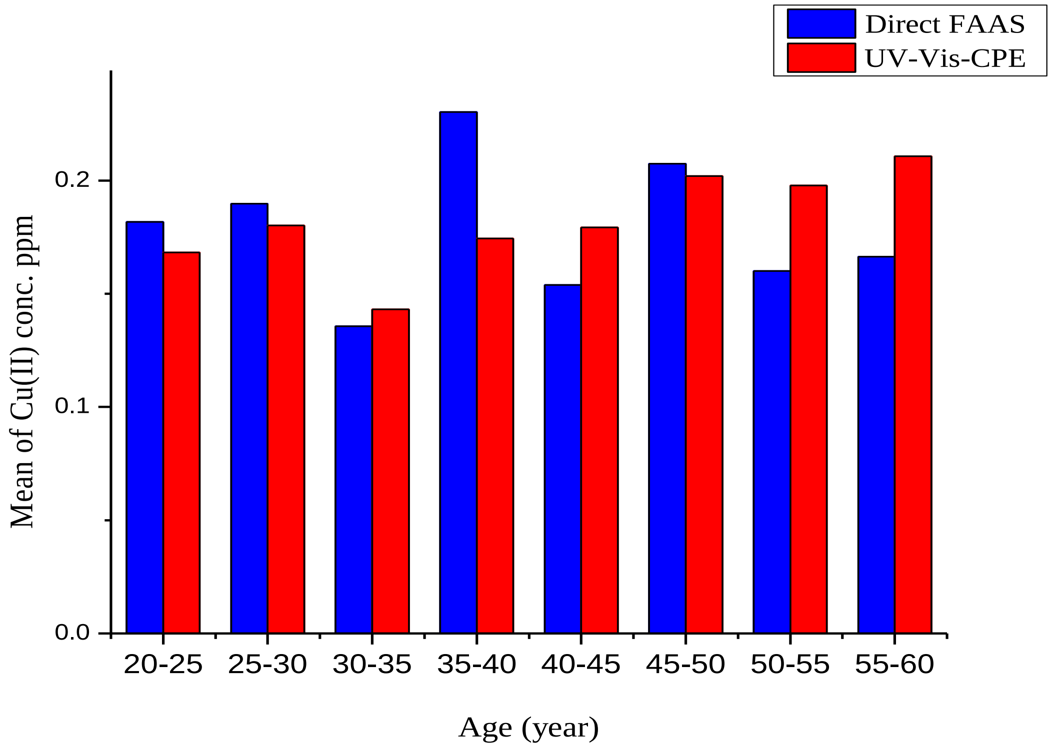


Figure (3-28) histogram of copper (II) concentration with the age of occupation workers samples

CHAPTER FOUR

CONCLUSIONS & FUTURE WORKS

4.1 conclusions

Speciation analysis is a difficult task for analytical chemists for it is important to identify trace elements which affect the human life, because some of these elements are toxic and it is present in different samples, such as food, environmental, biological and water samples. This research has many important conclusions, including:

Found a suitable new, easy and inexpensive method and environmentally friendly to extraction and pre-concentration low concentrations of trace elements which depend on the cloud point extraction method. Therefore, in recent years, the cloud point extraction method has been widely used to determine low concentrations of trace elements in different samples as one of the pre-concentration methods due to several characterized for this method including, simplicity, short extraction time, low cost, good efficiency concentration, inexpensive, environmentally friendly and compatible to the principles of green chemistry, reduces the consume of the solvent, lower toxicity and safety.

Therefore this research included extracting and determined for iron (III) and copper (II) elements in urine samples by using 4, 4-biphenyldiamine (Benzidine) and Salicylaldoxime (SAO) as reagents for iron and copper respectively, to form complexes with the help of micellar solutions by using Triton X-114 as nonionic surfactant under optimum conditions and then it is measured by using UV-Vis spectrophotometry and FAAS techniques.

Because these techniques coupling with the cloud point extraction method, these methods are more accurate in results and efficiency to extract and determine the trace elements in different samples without resorting to expensive and complex techniques such as: HPLC-ICP-MS, ICP-OES, ETAAS and other techniques.

Using the Cloud Point extraction method requires adjusting the optimum conditions which have a significant impact on the efficiency and success of this method. So, the most important conditions studied in this work are: order addition effect, pH effect, influences the concentration of reagent, type and concentration of surfactants effect, equilibrium temperature effect, time of incubation effect, salt out effect and interferences of ions effect. Therefore, by adjusting these conditions getting good results which are indicated in detail in Chapter III.

4.2 Future works

Through this study and from the results obtained the various future works can be studied such as:

1. It is possible to estimate the number of other ultra trace elements in the urine by the cloud point extraction method and use different analytical methods such as inductively coupled plasma (ICP), fluorescence , etc.

2. Prepare a number of new organic reagents and use them as analytical reagents in order to separate a number of elements in the same way.

3. Test other types of surfactants for the extraction such as Triton X-100, SDS, CTAB etc.

4. The analytical methods presented in this study can be used to estimate copper and iron in the different biological and natural samples.

5. Possible of use other methods of pre-concentration such as Liquid-Liquid Extraction, Solid Phase Extraction and Co-precipitation method to estimate and extraction of iron and copper in the urine samples

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APPENDIX

Appendix A

A1- iron concentration in urine sample by using UV-Vis -CPE

ID	**Gender**	**Age(year)**	**Occupation**	**Duration of work (year)**	**Abs. of Fe (III) by UV- Vis spectrophotometer**	**Conc.of Fe (III) µg. mL^{-1} in urine samples by UV-Vis**
H001	Male	55	Oil refineries	35	0.980	0.065
H002	Male	37	Oil refineries	12	1.130	0.077
H003	Male	28	Oil refineries	7	1.019	0.068
H004	Male	26	Welding	2	1.179	0.081
H005	Male	27	Welding	6	1.233	0.085
H006	Male	50	Welding	25	1.261	0.087
H007	Male	23	Casting alloys	8		
H008	Male	22	Casting alloys	7	1.439	0.101
H009	Male	60	Casting alloys	20	0.715	0.045
H010	Male	49	Casting alloys	35		
H011	Male	28	Casting alloys	2	0.981	0.066
H012	Male	29	Welding	15	1.012	0.068

H013	Male	53	dyeing	10	1.109	0.075
H014	Male	45	Welding	20	0.813	0.053
H015	Male	32	dyeing	9		
H016	Male	25	dyeing	11	2.101	0.151
H017	Male	27	Oil refineries	10	1.009	0.068
H018	Male	26	dyeing	2	1.200	0.082
H019	Male	35	dyeing	20	1.393	0.097
H020	Male	45	Oil refineries	4	1.112	0.076
H021	Male	24	Welding	3	0.224	0.007
H022	Male	22	Welding	8	0.579	0.035
H023	Male	35	Welding	10	0.656	0.041
H024	Male	42	Oil refineries	20		
H025	Male	35	Welding	25	1.109	0.075
H026	Male	25	Welding	10	1.270	0.088
H027	Male	38	Welding	2	0.820	0.053
H028	Male	55	Oil refineries	12	0.954	0.063

H029	Male	47	Terminals to provide fuel	11	0.813	0.053
H030	Male	45	Oil refineries	10	0.791	0.051
H031	Male	38	Oil refineries	12	1.102	0.075
H032	Male	48	Oil refineries	3	0.611	0.037
H033	Male	41	Oil refineries	11	0.511	0.029
H034	Male	34	Terminals to provide fuel	2	0.814	0.053
H035	Male	55	Terminals to provide fuel	35		
H036	Male	53	Oil refineries	11	1.113	0.076
H037	Male	22	Oil refineries	2	0.110	-0.001
H038	Male	28	Oil refineries	8	0.987	0.066
H039	Male	43	Oil refineries	15	0.813	0.053
H040	Male	33	Oil refineries	12	0.934	0.062
H041	Male	52	Terminals to provide fuel	15	1.554	0.109
H042	Male	41	Terminals to provide fuel	9	0.313	0.014
H043	Male	40	Welding	15	1.009	0.068
H044	Male	48	Terminals to provide fuel	24	0.813	0.053

🟩 **Samples were lost due to damage during work.** 🟥 **Sample was neglected because applied G-test.** 🟨 **Sample was deleted because it is illogical.**

A2- iron concentration in urine samples by FAAS-CPE

ID	Gender	Age (year)	Type of occupational worker	Duration of work (year)	Absorbance of Fe (III) by FAAS	Concentration of Fe (III) µg. mL^{-1} in urine samples by FAAS
H001	Male	55	Oil refineries	35	0.004	0.052
H002	Male	37	Oil refineries	12	0.002	0.104
H003	Male	28	Oil refineries	7	0.009	0.023
H004	Male	26	Welding	2	0.007	0.030
H005	Male	27	Welding	6	0.003	0.070
H006	Male	50	Welding	25	0.003	0.070
H007	Male	23	Casting alloys	8		
H008	Male	22	Casting alloys	7	0.008	0.026
H009	Male	60	Casting alloys	20	0.005	0.042
H010	Male	49	Casting alloys	35		
H011	Male	28	Casting alloys	2	0.005	0.042
H012	Male	29	Welding	15	0.004	0.052
H013	Male	53	dyeing	10	0.001	0.208

H014	Male	45	Welding	20	0.006	0.035
H015	Male	32	dyeing	9		
H016	Male	25	dyeing	11	0.004	0.052
H017	Male	27	Oil refineries	10	0.004	0.052
H018	Male	26	dyeing	2	0.005	0.042
H019	Male	35	dyeing	20	0.002	0.104
H020	Male	45	Oil refineries	4	0.003	0.070
H021	Male	24	Welding	3	0.004	0.052
H022	Male	22	Welding	8	0.005	0.042
H023	Male	35	Welding	10	0.002	0.104
H024	Male	42	Oil refineries	20		
H025	Male	35	Welding	25	0.010	0.021
H026	Male	25	Welding	10	0.005	0.042
H027	Male	38	Welding	2	0.005	0.042
H028	Male	55	Oil refineries	12	0.005	0.042
H029	Male	47	Terminals to provide fuel	11	0.004	0.052

H030	Male	45	Oil refineries	10	0.006	0.035
H031	Male	38	Oil refineries	12	0.009	0.023
H032	Male	48	Oil refineries	3	0.004	0.052
H033	Male	41	Oil refineries	11	0.007	0.030
H034	Male	34	Terminals to provide fuel	2	0.007	0.030
H035	Male	55	Terminals to provide fuel	35		
H036	Male	53	Oil refineries	11	0.002	0.104
H037	Male	22	Oil refineries	2	0.001	0.208
H038	Male	28	Oil refineries	8	0.007	0.030
H039	Male	43	Oil refineries	15	0.006	0.035
H040	Male	33	Oil refineries	12	0.002	0.104
H041	Male	52	Terminals to provide fuel	15	0.012	0.017
H042	Male	41	Terminals to provide fuel	9	0.003	0.069
H043	Male	40	Welding	15	0.01	0.021
H044	Male	48	Terminals to provide fuel	24	0.006	0.035

🟩 Samples were lost due to damage during work. 🟥 Samples were neglected because applied G-test

Appendix B

B1- copper concentration in urine sample by using UV-Vis-CPE

ID	Gender	Age(year)	Type of occupational worker	Duration of work (year)	Absorbance of Cu (II) by UV-Vis spectrophotometer	Concentration of Cu (II) µg. L^{-1} in urine samples by UV-Vis
H001	Male	55	Oil refineries	35	1.691	0.382
H002	Male	37	Oil refineries	12	0.482	0.113
H003	Male	28	Oil refineries	7	0.712	0.165
H004	Male	26	Welding	2	0.522	0.122
H005	Male	27	Welding	6	1.344	0.305
H006	Male	50	Welding	25	0.321	0.078
H007	Male	23	Casting alloys	8	0.731	0.169
H008	Male	22	Casting alloys	7	1.445	0.327
H009	Male	60	Casting alloys	20	0.257	0.063
H010	Male	49	Casting alloys	35	1.591	0.360
H011	Male	28	Casting alloys	2	0.321	0.078
H012	Male	29	Welding	15	0.599	0.139
H013	Male	53	dyeing	10	0.986	0.225
H014	Male	45	Welding	20	1.224	0.278
H015	Male	32	dyeing	9	0.381	0.091
H016	Male	25	dyeing	11	0.871	0.200
H017	Male	27	Oil refineries	10	0.699	0.162

H018	Male	26	dyeing	2	1.103	0.251
H019	Male	35	dyeing	20	0.754	0.174
H020	Male	45	Oil refineries	4	0.941	0.215
H021	Male	24	Welding	3	0.844	0.194
H022	Male	22	Welding	8	0.21	0.053
H023	Male	35	Welding	10	0.866	0.199
H024	Male	42	Oil refineries	20	0.212	0.053
H025	Male	35	Welding	25	0.484	0.114
H026	Male	25	Welding	10	0.925	0.212
H027	Male	38	Welding	2	0.735	0.170
H028	Male	55	Oil refineries	12	1.119	0.255
H029	Male	47	Terminals to provide fuel	11	0.235	0.059
H030	Male	45	Oil refineries	10	0.645	0.150
H031	Male	38	Oil refineries	12	1.217	0.277
H032	Male	48	Oil refineries	3	0.715	0.165

H033	Male	41	Oil refineries	11	0.438	0.104
H034	Male	34	Terminals to provide fuel	2	0.841	0.193
H035	Male	55	Terminals to provide fuel	35	0.611	0.142
H036	Male	53	Oil refineries	11	0.942	0.216
H037	Male	22	Oil refineries	2	0.412	0.098
H038	Male	28	Oil refineries	8	0.724	0.167
H039	Male	43	Oil refineries	15	1.211	0.275
H040	Male	33	Oil refineries	12	0.625	0.145
H041	Male	52	Terminals to provide fuel	15	1.198	0.273
H042	Male	41	Terminals to provide fuel	9	0.902	0.207
H043	Male	40	Welding	15	1.13	0.257
H044	Male	48	Terminals to provide fuel	24	0.811	0.187

B2- copper concentration in urine samples by FAAS direct

ID	Gender	Age(year)	Type of occupational worker	Duration of work (year)	Absorbance of Cu (II) direct by FAAS	Concentration of Cu (II) µg. L^{-1} in urine samples direct by FAAS
H001	Male	55	Oil refineries	35	0.014	0.164
H002	Male	37	Oil refineries	12	0.006	0.383
H003	Male	28	Oil refineries	7	0.013	0.177
H004	Male	26	Welding	2	0.019	0.121
H005	Male	27	Welding	6	0.013	0.177
H006	Male	50	Welding	25	0.018	0.128
H007	Male	23	Casting alloys	8	0.016	0.144
H008	Male	22	Casting alloys	7	0.012	0.192
H009	Male	60	Casting alloys	20	0.011	0.209
H010	Male	49	Casting alloys	35	0.012	0.192
H011	Male	28	Casting alloys	2	0.01	0.230
H012	Male	29	Welding	15	0.008	0.288
H013	Male	53	dyeing	10	0.013	0.177
H014	Male	45	Welding	20	0.008	0.288
H015	Male	32	dyeing	9	0.02	0.115
H016	Male	25	dyeing	11	0.025	0.092
H017	Male	27	Oil refineries	10	0.016	0.144
H018	Male	26	dyeing	2	0.01	0.230
H019	Male	35	dyeing	20	0.017	0.135

H020	Male	45	Oil refineries	4	0.026	0.088
H021	Male	24	Welding	3	0.014	0.164
H022	Male	22	Welding	8	0.009	0.256
H023	Male	35	Welding	10	0.016	0.144
H024	Male	42	Oil refineries	20	0.013	0.177
H025	Male	35	Welding	25	0.012	0.192
H026	Male	25	Welding	10	0.011	0.209
H027	Male	38	Welding	2	0.006	0.383
H028	Male	55	Oil refineries	12	0.013	0.177
H029	Male	47	Terminals to provide fuel	11	0.006	0.383
H030	Male	45	Oil refineries	10	0.013	0.177
H031	Male	38	Oil refineries	12	0.016	0.144
H032	Male	48	Oil refineries	3	0.016	0.144
H033	Male	41	Oil refineries	11	0.012	0.192
H034	Male	34	Terminals to provide fuel	2	0.014	0.164
H035	Male	55	Terminals to provide fuel	35	0.02	0.115
H036	Male	53	Oil refineries	11	0.012	0.192
H037	Male	22	Oil refineries	2	0.015	0.153

H038	Male	28	Oil refineries	8	0.01	0.230
H039	Male	43	Oil refineries	15	0.016	0.144
H040	Male	33	Oil refineries	12	0.018	0.128
H041	Male	52	Terminals to provide fuel	15	0.016	0.144
H042	Male	41	Terminals to provide fuel	9	0.026	0.088
H043	Male	40	Welding	15	0.012	0.192
H044	Male	48	Terminals to provide fuel	24	0.014	0.164

Appendix C

الاستمارة الخاصة بمعلومات النماذج

الأسم الثلاثي / ....

العمر / ....سنة

الجنس / ذكر ☐ ...... أنثى ☐

السكن / ....

نوع العمل / ....

مدة العمل / ....سنة

مكان العمل / ....

كمية النموذج / ....مليلتر

تأريخ أخذ العينة / / / ٢

المـلاحظات :

الخلاصة :

تضمنت الرسالة على أربعة فصول رئيسية متعلقة باستخلاص بعض العناصر النزرة مثل الحديد والنحاس في عينات الادرار للعمال الحرفين في مدينة كربلاء-العراق باستخدام طريقة الاستخلاص بنقطة الغيمة ومن ثم تقدير هذه العناصر بتقنيتي مطيافية الأشعة المرئية و الفوق البنفسجية, و جهاز الامتصاص الذري اللهبي . حيث تضمن **الفصل الاول** مقدمة للمحاور الرئيسية لمضمون عنوان البحث . اما **الفصل الثاني** فتضمن وصف كامل للمواد الكيميائية وتحضيراتها, وأهم الادوات التي تم أستخدامها وذلك بتثبيت جميع المعلومات الخاصة بها, بالاضافة الى ذلك فقد تم التطرق الى خطوات العمل الاساسية وطريقة العمل المتبعة للوصول الى نقطة الغيمة من أجل الاستخلاص, ومن ثم التقدير للعناصر النزرة بالاضافة الى النمذجة, و جمع عينات الادرار, و وصف خطوات عملية الهضم لها بشكل مفصل. أظهر **الفصل الثالث** أهم النتائج التي تم الحصول عليها من عملية التقدير الكمي للعناصر النزرة في عينات الادرار للعمال الحرفيين, وبعدها تم مناقشة هذه النتائج بشكل علمي. فقد تم استخلاص و تقدير عنصر الحديد بتكوين معقد اخضر اللون بتفاعله مع الكاشف العضوي ثنائي فنيل داي امين والمعروف بالبنزدين عند طول موجي 425 نانو متر, في حين ان عنصر النحاس قد تفاعل مع الكاشف العضوي سالسلديهايد اوكزايم او سالسالدواكزايم لتكوين معقد اخضر فاتح اللون ذو طول موجي 380 نانو متر وتمت هذه الدراسة عند الدالة الحامضية 4.20 لكلا العنصرين وباستخدام Triton X-114 كأحد انواع عوامل الشد السطحي ومن ثم استخلاص هذه المعقدات لعنصري الحديد والنحاس وتقديرها بتقنيات ملائمة بعد تثبيت اهم الظروف المؤثرة على كفاءة عملية الاستخلاص بنقطة الغيمة ومن اهمها : تسلسل الاضافات, و الدالة الحامضية لوسط التفاعل, و تركيز الكواشف العضوية, و انواع عوامل الشد السطحي, و تركيز عوامل الشد السطحي, و درجة الحرارة اللازمة للوصول الى نقطة الغيمة, و زمن الاحتضان, و تأثير اضافة الاملاح, و تأثير المتداخلات الايونية, و دراسة تركيب المعقدات الناتجة. وبعد

ضبط هذه الظروف تم الحصول على منحنيات المعايرة لكلا العنصرين حيث كان مدى الخطية لمنحني المعايرة من 0.25 – 3.00 مايكروغرام . مل $^{-١}$ و 0.50 – 16.00 مايكروغرام . مل $^{-١}$ و قيمة معامل الارتباط r هي 0.9825 و 0.9979 اما حد الكشف فكان 0.05 مايكروغرام . مل$^{-١}$ و 0.10, مايكروغرام . مل$^{-١}$ وقيمة الانحراف القياسي النسبي هي 2.955 % و 4.887% لعنصري الحديد والنحاس على التوالي. كما تم تطبيق تلك الطرائق لاستخلاص و تقدير الحديد والنحاس في 44 عينة من ادرار بعض العمال في المجال الصناعي,كما خضعت النتائج المستحصلة لتراكيز عنصري الحديد والنحاس باستخدام تقنيتي مطيافية الأشعة المرئية و الفوق البنفسجية و جهاز الامتصاص الذري اللهبي الى المعالجات الاحصائية و بالاخص الى اختبارات F-test و t-test و One way ANOVA Test لتبيان وجود فرق جوهري بين النتائج المستحصلة من التحليل والقيم المنشورة في الادبيات و البحوث العالمية, ومن جهة اخرى لمعرفة فيما اذا كان هنالك فرق جوهري في التقدير لتلك العناصر في نماذج الادرار باستخدام تقنيات مختلفة مقترنة مع طريقة الاستخلاص بنقطة الغيمه.

لقد تبين من خلال النتائج الاحصائية وجود فرق جوهري في تراكيز العناصر النزرة الحديد والنحاس المقدرة مع القيم المنشورة في البحوث العالمية. كما تبيين من خلال النتائج الاحصائية للاختبارات المعنوية بعدم وجود فرق جوهري للتقدير بتقنيتي مطيافية الأشعة المرئية و الفوق البنفسجية و جهاز الامتصاص الذري اللهبي و لكلا العنصرين مما يعني امكانية استخدام طريقة مطيافية الأشعة المرئية و الفوق البنفسجية المقترنة بطريقة الاستخلاص بنقطة الغيمة كطريقة بديلة لتقدير عنصري الحديد و النحاس في النماذج البايوجية بدلا من طريقة الامتصاص الذري اللهبي. كما اتضح انه باقتران تقنية مطيافية الأشعة المرئية و الفوق البنفسجية مع الاستخلاص بنقطة الغيمة قد زاد من دقة وكفاءة وحساسية هذه الطريقة في القياس.

الاهداء

إلهي لا يطيب الليل إلا بشكرك ولا يطيب النهار إلى بطاعتك .. ولا تطيب اللحظات إلا بذكرك .. ولا تطيب الآخرة إلا بعفوك .. ولا تطيب الجنة إلا برؤيتك .... "لمرضاة الله جل جلاله"

إلى من بلغ الرسالة وأدى الأمانة ...... نبي الرحمة وهادي الامة وأهل بيته الاطهار صلوات الله عليهم أجمعين

إلى أستاذي و قدوتي الذي بذل كل ما بوسعه من نصيحة و توجيه وانار لي في الحياة دربا . . . . أ . م . د . أحمد فاضل خضير

إلى من اوصى الله بهم . . . . الوالدين العزيزين

إلى اخوتي واخواتي . . . . أخلاصا ومودة

إلى زوجتي وأولادي . . . . وفاءا وحبا

إلى كل من ساعدني وساندني وتمنى لي النجاح . . . .

((أهدي ثمرة جهدي المتواضع هذا))

مؤيد

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء / كلية العلوم
قسم الكيمياء

التقدير و التركيز المسبق للحديد والنحاس لعينات من أدرار العمال الحرفيين باستخدام الاستخلاص بنقطة الغيمة

رسالة مقدمة الى
مجلس كلية العلوم – جامعة كربلاء
كجزء من متطلبات نيل درجة الماجستير في الكيمياء التحليلية

من قبل
مؤيد خضير حسن الأسدي
بكالوريوس علوم كيمياء / جامعة بابل (٢٠٠٢)

باشراف

أ. م. د. أحمد فاضل خضير

١٤٣٩ هـ ٢٠١٧ م