

**DOKUZ EYLUL UNIVERSITY GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCES**

**DETERMINATION OF COPPER USING
PORPHYRIN BY CLOUD POINT EXTRACTION**

**by
Ferhat KAPTAN**

**January, 2010
İZMİR**

DETERMINATION OF COPPER USING PORPHYRIN BY CLOUD POINT EXTRACTION

**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of
Dokuz Eylul University
In Partial Fulfillment of
the Requirements for the Degree of Master of Science
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**by
Ferhat KAPTAN**

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İZMİR**

M. Sc. THESIS EXAMINATION RESULT FORM

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DETERMINATION OF COPPER USING PORPHYRIN BY CLOUD POINT EXTRACTION

ABSTRACT

A cloud point extraction procedure was presented for the preconcentration of copper(II) ion in aqueous samples. After complexation by 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (TMPP), copper(II) ion was quantitatively recovered in Triton X-100 after loading to minicolumn containing cotton. 2.5 mL of 25% THF acidified with 1.0 mol L⁻¹ HNO₃ was used as eluent for stripping the surfactant-rich phase prior to its analysis by flame atomic absorption spectrometry (FAAS). The influence of analytical parameters including ligand, Triton X-100 and nitric acid concentration and volume, effect of polar solvent in desorption solution, stirring time, concentration of electrolyte optimized. The effect of the matrix ions on the recovery of copper(II) ions was investigated. The obtained detection limit (4.3 ng mL⁻¹) was sufficiently low for detecting copper in diverse samples. The enrichment factor of 5 with R.S.D. of 2.1% for Cu was achieved. The applicability of the method was confirmed by the analysis of water samples. The interference effects of some anions and cations were also tested

Keywords: Cloud Point Extraction, Preconcentration, copper, 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (TMPP), flame atomic absorption spectrometry

PORFİRİN KULLANILARAK BULUTLANMA NOKTASI EKSTRAKSİYONU İLE BAKIR TAYİNİ

ÖZ

Sulu çözeltideki bakır (II) iyonlarının zenginleştirilmesi için bulutlanma noktası ekstraksiyonu işlemi uygulandı. Cu(II) iyonlarının 5,10,15,20-tetrakis(4-metoksifenil) porfirin (TMPP) ligandı ile Triton X-100 ortamında şelatlandırılıp pamuk ile doldurulmuş minikolona yüklenerek nicel geri kazanımı gerçekleştirildi. Alevli atomik absorpsiyon spektrometresiyle analizi için yüzey aktif madde bakımından zengin fazın geri alma çözeltisi olarak 1 mol L⁻¹ HNO₃ ortamında 2,5 mL % 25 THF (v/v) kullanıldı. Ligand, Triton X-100, HNO₃ derişim ve hacimleri ile karıştırma süresi, geri alım işleminde çözücünün polarlığı ve elektrolit madde derişimi parametrelerinin analitik etkileri optimize edildi. Yabancı iyonların Cu(II) geri kazanımındaki etkileri araştırıldı. Elde edilen gözlenebilme sınırı (4,3 ng mL⁻¹) değişik örneklerdeki Cu(II) geri kazanımları için yeterince düşük bulundu. %2,1 R.S.D. ile Cu iyonlarının 5 kat zenginleştirilmesi gerçekleştirildi. Yöntemin uygulanabilirliği su örnekleri analizi ile doğrulandı. Bazı katyon ve anyonların girişim etkisi de test edildi.

Anahtar Kelimeler: Bulutlanma noktası ekstraksiyonu, zenginleştirme, bakır, 5,10,15,20 tetrakis (4-metoksifenil) porfirin, alevli atomik absorpsiyon spektrometresi

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CHAPTER ONE

INTRODUCTION

1.1 Determination of Trace Metals

Many elements occur in a sample in such minute amounts that, at the beginning of the development of instrumental analytical methods in the 19th century, it was impossible with the existing techniques to determine their concentration quantitatively, although their presence could be detected qualitatively. To indicate such low and barely detectable concentrations the term “trace” was used and the elements were referred to as trace elements. Although nowadays very low concentrations determined accurately and precisely because of improvements to existing and development of new analytical techniques, the term “trace element” is still in use. In general, one speaks of a trace element when the concentration is below $100 \mu\text{g g}^{-1}$. At extremely low concentrations, below 10 ng g^{-1} , one also speaks of “ultra-trace” elements. In spite of their low concentration, these elements may play a very important role in many areas (biochemistry, medicine, environmental, industrial) (Vandecasteele & Block, 1993).

Copper has received considerable attention owing to its uses in metallurgy and chemical industries. Moreover, it is an essential constituent of about thirty enzymes and glycoproteins and is required for the synthesis of hemoglobin and for some biological processes. Also promotes iron absorption from the gastrointestinal system, is involved in the transport of iron from tissues into plasma, helps to maintain myelin in the nervous system, is important in the formation of bone and brain tissues and is necessary for other many important functions. When levels of Cu exceed certain values, however, defense mechanisms to protect against excess Cu are overcome and toxicity results. For this reason, several methods for the determination of copper ion are available for copper ion determination (Shokrollahi, Ghaedi, Gharaghani & Soylak, 2008).

1.2 Preconcentration and Separation

The methods of analytical chemistry can be divided into two large groups: (1) methods of separation and preconcentration of components, (2) methods of determination of components of the material to be analysed.

Separation is a process in which the components constituting the starting mixture are separated from each other. Preconcentration is a technique by which the ratio of concentration (or the amount) of trace components to the concentration (or the amount) of macrocomponent is increased. In separation, the components constituting the mixture may or may not differ in concentration from each other. In preconcentration, the components that have significantly different concentrations are treated.

Numerous methods are used for analytical preconcentration of trace elements. A large majority of them had previously been employed only for separation. Probably evaporation was first utilized for preconcentration; precipitation, extraction, electrochemical and other methods gained recognition at a later stage. Fire assay is one of the oldest methods of preconcentration.

By the nature of the separation methods used, they can be classified into 1) chemical and physico-chemical and 2) physical methods. With the first group may be classed extraction, sorption, precipitation and co-precipitation, partial dissolution of matrix, flotation, volatilization after chemical transformations, chemical transport reactions, fire assay, electrochemical methods and dialysis. The second group may include volatilization, crystallization, freezing out, filtration and gel filtration and ultracentrifugation.

The selection of a preconcentration method is dictated by (i) the practical problem being solved, the nature of the material to be analyzed, trace elements to be determined, the specified metrological parameters of the technique; (ii) the origin and previous history of the material to be analyzed; (iii) the combination of the

selected method of preconcentration and subsequent method of determination of trace elements in a concentrate; (iv) the simplicity, the availability and the duration of the method; (v) the equipment available in the laboratory of the scientist and in those laboratories which will use the method; (vi) the specialization and qualification of the researcher developing the technique and of the analysts of the laboratories where this technique is to be employed; (vii) the need to ensure safe working conditions (Zolotov & Kuz'min, 1990).

1.3 Cloud Point Extraction

Separations and preconcentration based on cloud point extractions are becoming an important and practical application of the use of surfactants in analytical chemistry. Preconcentration steps based on phase separation by cloud point extraction (CPE) (Watanabe, 1992) offer a convenient alternative to more conventional extraction methods. The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap, highly efficient, speedy and of lower toxicity to the environment than those concentration extractions that use organic solvents. The CPE method also provides results comparable to those obtained with other separation techniques. Accordingly, any species that interacts with the micellar system, either directly (generally hydrophobic organic compounds) or after prerequisite derivatization reaction (*i.e.*, metal ions after reaction with a suitable hydrophobic ligand) can be extracted from the initial solution and may also be preconcentrated. (Teo & Chen, 2001)

1.3.1 Micelles in General: Properties, Characteristics and Uses

When a substance has the property of adsorbing on the surface or interfaces of the system and altering to a marked degree the surface or interfacial free energies of those surfaces resulting a decrease in surface tension when it is present at low concentration, it may be called a surfactant, an abbreviation for surface-active agent (Milton, 2004).

In aqueous solution surfactants can aggregate to form colloidal-sized clusters referred to as micelles (normal micelles). For this phenomenon the minimum concentration of surfactant needed to form is called the critical micelle concentration (CMC). Depending upon solution conditions and surfactant type, micelles have a variety of shape ranging from roughly spherical to ellipsoidal (oblate or prolate). The interior region of the normal micelle belongs to the hydrophobic moieties of the surfactant molecules and the outer surface belongs to the hydrated hydrophilic groups along with any bound water molecules. A schematic representation of a typical nonionic surfactant micelle aggregate is shown in Figure 1.1. Micellar shape can be changed, sometimes dramatically, by altering the solution conditions (concentration of surfactant and additives, nature of additive, temperature, etc.) (Quina & Hinze, 1999).

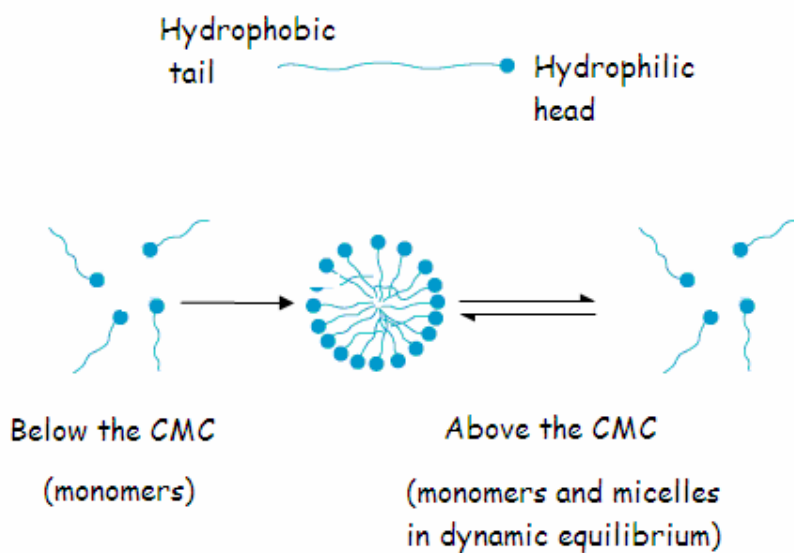
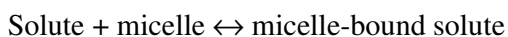


Figure 1.1 The aggregation of monomers to form a normal, aqueous micelle

Surfactants are amphiphilic molecules. Amphiphilic means that the monomer contains both polar and hydrophobic groups. These molecules contain a polar group (head) at the end of a long hydrophobic carbon chain (tail). In contrast to purely polar or non-polar molecules, amphiphilic molecules exhibit unique properties in water. Their polar group forms hydrogen bonds with water molecules, while the

hydrocarbon chains aggregate due to hydrophobic interactions. These properties allow surfactants to be soluble in water. Because of their amphiphilic nature, surfactants are able to solubilize hydrophobic compounds in water.

Solubilization, one of the most important properties of surfactant, is directly related to micelle formation. Both bulk and solvent- soluble and solvent-insoluble species can reversibly interact with and bind to the micellar assembly. Sparingly-soluble or nonwater-soluble materials can be solubilized in water due to their binding to the micelles in solution (Quina & Hinze, 1999).



The most intensely studied and discussed types of microscopically ordered molecular aggregates are the micelles. Micelles are supramolecular structures of colloidal dimensions formed by surfactants molecules that aggregate in a spontaneous way in aqueous solution when critical micelle concentration (CMC) is attained. The CMC of a surfactant is based on several factors including its molecular structure, and experimental conditions such as temperature, ionic strength, counterions, etc. Below the CMC, the surfactant is usually in a nonassociated monomer form. However, when the CMC is reached, the formation process is favored. Micelles are not static structures. An important micelle characteristic is its dynamic equilibrium with the dissolved surfactant monomers, which remain at an approximately constant concentration after reaching the CMC. Micelles are thermodynamically stable and easily reproducible, but they can be destroyed by water dilution when the surfactant concentration becomes lower than its CMC (Bezerra, Arruda & Ferreira, 2005).

The formation of a micelle implies a decrease in the entropy of the system. Such free energy will be used up in its formation. In an aqueous solution, the water molecules attract themselves by hydrogen bonds. Thus, in the dissolution of an ionic or polar substance, the necessary energy to break the hydrogen bonds is compensated by the hydration of dissolved species. However, aliphatic chains of surfactants are

not appreciably hydrated. Then, Van der Waals forces act to their reciprocal attraction by decreasing the contact area among the surfactant molecules and water. Moreover, the hydrophilic head group of surfactants tends to solubilize in water. All these factors become the surfactant molecules agglomeration a spontaneous phenomenon. When the surfactant is dissolved in aqueous solution above its CMC, the hydrophobic group distorts the water liquid structure and this causes increase of free energy. This increase of free energy compensates for the necessary work to create a surface area permitting the micelle formation (Bezerra, Arruda & Ferreira, 2005).

Micelles are able to dissolve chemical species, which can present different polarities and sizes. The solubilization site varies with the nature of the solubilized species and the surfactant. The solubilization sites of micelles can be recognized following ways:

- (a) on micelle surface,
- (b) among the hydrophilic head groups,
- (c) the space among the poly-oxy-ethylene groups (for nonionic surfactants)
- (d) the interface between the head groups and the core, and
- (e) the hydrophobic core.

The charged chemical species are captured in the sites (a), (b), and (c). The amphiphilic species are extracted into the site (d), and the hydrophobic species, in the core (e). The binding site in a micelle more extensively utilized in cloud point extraction (CPE) is the hydrophobic core. Hence, the stable chelate generation is the main step for the majority of developed methods (Bezerra, Arruda & Ferreira, 2005).

Micelle characteristics vary with the nature of the hydrophilic group (Myers, 1988), surfactants are categorized as follows (Bezerra, Arruda & Ferreira, 2005):

1. Anionic: the hydrophilic group carrying a negative charge such as carboxyl (RCOO), sulfonates (RSO₃), or sulfate (ROSO₃). For example; Sodium dodecyl sulfate (SDS), RCOO⁻Na⁺ (soap)

2. Cationic: the hydrophilic group carrying a positive charge, for example, RNH₃⁺ Cl⁻ (salt of a long-chain amine), RN(CH₃)₃⁺ Cl⁻ (quaternary ammonium chloride). This type of surfactants usually contains quaternary nitrogen head groups due to the stability and commercial availability of these materials.

3. Non-ionic: the hydrophilic group has no charge but derives its water solubility from highly polar groups, for example, Polyoxyethylene(23)dodecanol(Brij35) (CH₃(CH₂)₁₁(OCH₂CH₂)₂₃OH). The polar head groups of nonionic micelles generally consist of polyoxyethylene (-OCH₂CH₂O⁻) or polyoxypropylene groups.

4. Zwitterionic: its molecules present both the cationic groups and anionic and, depending of pH, its prevalence the anionic, cationic, or neutral species, for example, Dodecyl dimethyl ammonium butyrate (DAB)(CH₃(CH₂)₁₁N⁺(CH₃)₂).

The nature of the hydrophobic groups may be significantly more varied than for the hydrophilic. Generally they are long-chain hydrocarbon residues (Myers, 1988).

The critical micelle parameters, i.e., CMC, cloud point and aggregation number (the number of surfactant molecules per micelle), for different nonionic and zwitterionic surfactants that have been utilized in cloud point extraction are summarized in Table 1.1 (Quina & Hinze, 1999) .

Table 1.1 Summary of the aqueous solution properties of some of the nonionic and zwitterionic surfactant systems.

Surfactant	CMC, mM	Aggregation Number(N) ^a	Cloud point, °C
Triton X-100 (TX-100)	0.17-0.30	120-140	64–65
Triton X-114 (TX-114)	0.20-0.35	–	23–25
PONPE-7.5	0.085	–	5–20
PONPE-10	0.07-0.085	100	62–65
Igepal CO–630	–	–	48–52
C ₈ E ₃	5.9-7.5	–	10.6
C ₁₀ E ₄	0.6-0.8	30	19-21
Genapol X–80	0.05	–	42
Brij–30(C ₁₂ E ₄)	0.02-0.06	40	2-7
Brij–56(C ₁₆ E ₁₀)	0.0006	–	64–69
octylβ-D Thioglucoside(OTG)	9.0	–	10-20
octylβ-D Glucoside(OG)	20.0-25.0	84	2-20
C ₈ -lecithin	–	500	45
C ₉ -APSO ₄	45.0	–	65

^a aggregation number that is the number of monomers per micelle can vary from 50 to 2000

1.3.2 Micelles in Analytical Chemistry

In analytical chemistry the micellar media application can be described according to two aspects: the first one refers to the exploitation of micellar media properties such as water solubilization of hydrophobic substances, enhanced detection of

spectroanalytical methods by changes of physical and chemical properties of the sample solution, transport and nebulization efficiency improvement, and reactions catalysis. The second aspect refers to the separation and pre-concentration by phase separation phenomenon in the cloud point (Pelizzetti & Pramauro, 1985).

1.3.3 Cloud Point Phenomenon and Its Utilization For Metal Separation and Preconcentration

The cloud point phenomenon occurs when a non-ionic or amphoteric surfactant above its CMC promote the separation of the original solution into two phases when heated at proper temperature. Another cloud point possibility for metal preconcentration is related to anionic surfactants when employed with high acid concentration (Casero, Sicilia, Rubio & Perez-Bendito, 1999).

The cloud point methodology used for metal determinations is relatively simple: a few milliliters of concentrated surfactant solution are added into the aqueous solution (this volume is in the range of tens of hundreds of milliliters). When necessary, a chelating agent solution is dissolved in an organic solvent or directly in water, depending on its solubility. This solution is then heated above its cloud point and the phase separation occurs, which can be accelerated by centrifugation (Figure 2). The discard of bulk aqueous phase after separation of micellar phase is facilitated after an ice bath, because the viscosity of the surfactant-rich phase is increased.

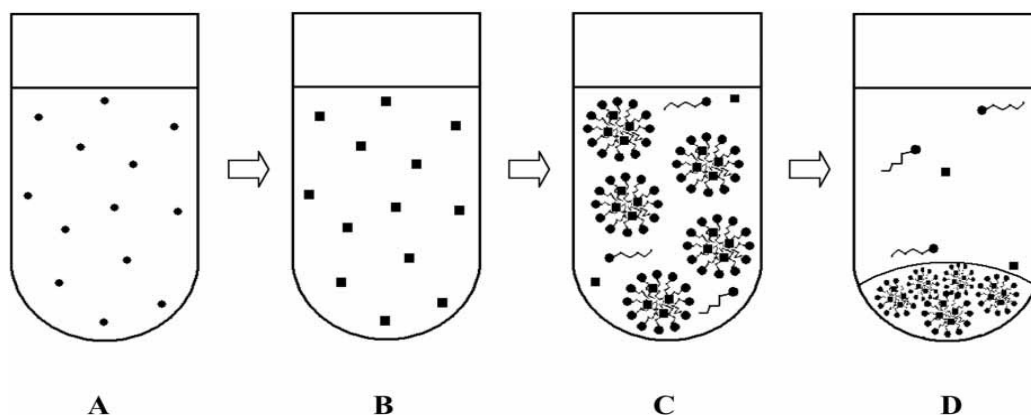


Figure 1.2 Schematic representation of a conventional CPE to metal preconcentration: (A) original solution with metals (circles) in low concentration; (B) metal chelates (squares) formed by addition of complexing reagent in the matrix solution; (C) addition of surfactant in the solution and trapping of metallic chelates into micellar cores; and (D) micellar phase segregate after heating and separation after centrifugation

Before the determination process, it may be necessary to add small amounts of a salting agent (such as alcohol) after the discard of aqueous phase. The objective is to decrease the viscosity of micellar phase and to promote its homogenization in order to improve the manipulation and/or aspiration of the resulting solution. The salting agent content must be a compromise between the ideal viscosity, the metal concentration, and the quantity of organic solvent that some atomizers can support (Stalikas, 2002).

The most experimentally modified condition to attain the cloud point is the temperature. The temperature at which the phase separation (cloud point temperature or only the cloud point) occurs as a function of the surfactant concentration.

1.3.4 Metal Extraction Mechanism

Above the cloud point, surfactant molecules act as an organic solvent in liquid-liquid extraction and the metals being partitioned between the organic and aqueous phases. Thus, an evaluation of the pertinent partition coefficients of ligands and complexes is essential for quantitatively description of the cloud point efficiency.

The CPE efficiency depends of inherent interaction of metallic species with micellar structures. The following factors must be observed: (a) the formation constant of metallic complexes, (b) the kinetics of complexation reaction, and (c) the phase transference of metallic species or chelate into micellar media.

An interesting fact is that there are different metallic chelates distribution constants related to each metallic species extracted. Because of the hydrated nature of the surfactant phase, the distribution coefficients are generally lower than presented by a conventional liquid–liquid extraction. In the last one, the constant distributions are always independent of metallic ions nature. These facts suggest that distribution mechanism is different from that of conventional solvent extraction. In addition, in CPE, the distribution mechanism for chelates is different from the chelating. Chelating reagents are extracted probably due to a specific interaction, such as hydrogen bonding, between functional groups on the chelating reagents and ether oxygen or terminal OH group of non-ionic surfactant and chelates are extracted into the hydrophobic part of aggregated micelles (Tani, Kamidate & Watanabe, 1997; Akita & Takeuchi, 1999).

Another aspect related to the extraction efficiency is the ionic strength. Its increase does not modify the extraction efficiency seriously. However, the salt addition makes the phase separation process easy.

The degree of metal partition from the aqueous solution to the surfactant-rich phase in the CPE can be described by the typical distribution coefficient, D :

$$D = \frac{(C_M)_S}{(C_M)_W}$$

where $(C_M)_S$ is the metal final concentration in the surfactant-rich phase, and $(C_M)_W$ is its concentration in the bulk aqueous phase after the separation process. The extraction factor, E , is given by the following expression:

$$E = D \left(\frac{V_s}{V_w} \right)$$

where V_s/V_w is phase volume ratio, i.e., volume of surfactant-rich phase, V_s , divided by volume of remiscible aqueous phase, V_w , following the phase separation step. The fraction of analyte extracted, P , is given by the expression

$$P = \frac{D}{D + (V_w/V_s)}$$

The percentage of extracted analyte, %p, can be obtained simply by $100p$ (Frankewich & Hinze, 1994). The concentration factor (CF) is an important parameter for evaluating theoretically the extraction capability of micellar system for metal species. Assuming a quantitative transference (recovery) of analyte(s) into the micellar phase, this parameter represents a convenient way to assess the preconcentration process. It can be defined as the ratio between the original matrix sample volume and the surfactant-rich phase volume

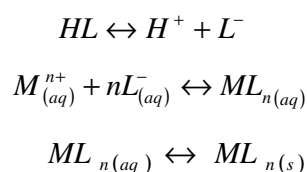
$$CF = \frac{V_{matrix}}{V_s}$$

However, if the mass transference of metal into the micellar phase is not quantitative, it is better represented by the enrichment factor, EF. Thus, EF can be calculated by the ratio of the slopes of the calibration curves with and without preconcentration (Stalikas, 2002).

1.3.5 Factors that Influence the Distribution Coefficient Magnitude in the CPE

Several complexing reagents are used for selective separation of metallic ions by CPE from a buffered aqueous solution. Watanabe and coworkers were the first to study this extraction for cadmium, copper, iron, nickel, and zinc for preconcentration from different ligands. (Watanabe, Kamidate, Kawamorita, Haraguchi & Miyajima,

1987; Saitoh, Kimura, Kamidate, Watanabe & Haraguchi, 1989). Hoshino et al. studied the micellar solubilization equilibrium of some analytical reagents in aqueous solution of non-ionic surfactants (Hoshino, Saitoh, Taketomi, Yotsuyanagi, Watanabe & Tachikawa, 1983). The process involves several equilibrium and chemical species. The equations presented below represent in a simplified way the acid dissociation step of chelating reagent and the formation of a hydrophobic chelate following by its transference into a micellar phase (Silva, Frescura, Aguilera & Curtius, 1998):



where $M_{(w)}^{n+}$ and $L_{(w)}^-$ are the metal and the ligand, respectively, in the aqueous phase, $ML_{n(w)}$ is the complex in the aqueous phase, and $ML_{n(s)}$ is the complex in the surfactant phase. The distribution constant, K_d , the total formation constant, β_n , for the extracted species and the acid dissociation constant of HL, K_a , in aqueous phase are given by equations below:

$$K_d = \frac{[ML_n]_s}{[ML_n]_{aq}} \quad (1)$$

$$B_n = \frac{[ML_n]_{aq}}{[M^{n+}][L^-]^n} \quad (2)$$

$$K_a = \frac{[H^+][L^-]}{[HL]} \quad (3)$$

Combining Eqs. (1)–(3) and rearranging gives

$$\frac{[ML_n]_s}{[M^{n+}]_{aq}} = K_d \beta_n K_a^n [HL]^n [H^+]^{-n} \quad (4)$$

Since the distribution coefficient, D, is given by

$$D = \frac{(C_M)_s}{(C_M)_{aq}} = \frac{[ML_n]}{[M^{n+}]_{aq} + [ML_n]_{aq}} \quad (5)$$

assuming that $[M^{n+}]_w \cdot [ML_n]_w$, and combining Eqs. (4) and (5) results in

$$D = K_d \beta_n K_a^n [HL]^n [H^+]^{-n} \quad (6)$$

Equation (6) shows that the distribution coefficient, D, is influenced by magnitudes of formation constant to specific cations with the complexing, by acid dissociation constant, by the complexing reagent concentration, and by the solution pH. These factors can be used to control the metallic ions extractions and must be evaluated to improve a method (Bezerra, Arruda & Ferreira, 2005).

1.4 Chelating Agents

1.4.1 Porphyrins

The porphyrins are a class of naturally occurring macrocyclic compounds, which play a very important role in the metabolism of living organisms. The porphyrin molecule contains four pyrrole rings linked via methine bridges (Figure 1.2). The porphyrin nucleus is a tetradentate ligand in which the space available for a coordinated metal has a maximum diameter of approximately 3.7 Å (Falk, 1975).

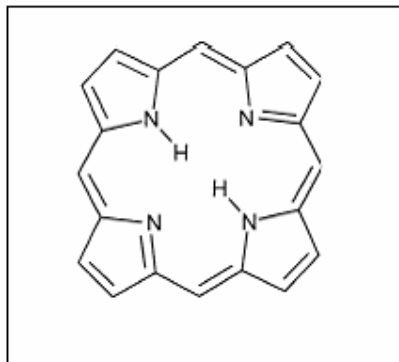


Figure 1.3 Porphyrin macrocyclic system

When coordination occurs, two protons are removed from the pyrrole nitrogen atoms, leaving two negative charges. The porphyrin ring system is very stable and exhibits aromatic character. The porphyrin complexes with transition metal ions are very stable (Figure 1.3).

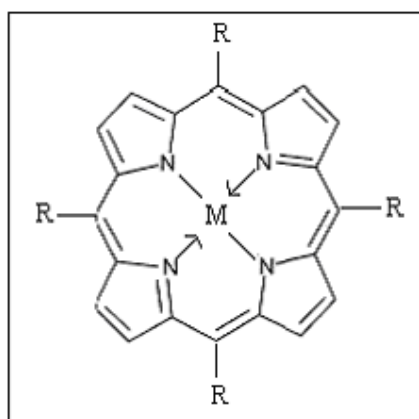


Figure 1.4 Porphyrin-metal complex

Almost all metals form complexes 1:1, although Na, K, Li complexes are 2:1 in which the metal atoms are incorporated slightly below and above the porphyrin macrocycle plane. When divalent metal ions (e.g. Co(II), Ni(II), Cu(II)) are chelated, the resulting tetracoordinate chelate has no residual charge. While Cu(II) and Ni(II) in their porphyrin complexes have generally low affinity for additional ligands, the chelates with Mg(II), Cd(II) and Zn(II) readily combine with one more ligand to form pentacoordinated complexes with square-pyramidal structure. Some

metalloporphyrins (Fe(II), Co(II), Mn(II)) are able to form distorted octahedral with two extra ligand molecules.

Porphyrin metal complexes play an important role in biological activities as for instance iron complex in the haemoproteins, magnesium complexes in the chlorophylls, and a cobalt complex in Vitamin B12. Complexes of many metals with various porphyrins have been extensively studied in order to understand the biosynthetic formation and biological activity of natural compounds. Porphyrin derivatives play a key role in essential biological processes such as photosynthesis, dioxygen transport and storage. From the perspective of coordination chemistry, the porphyrin ligand has turned out to be very versatile, and almost all metals have been combined with porphyrins. Such complexes have been used in a variety of applications as models for biological electron transport, oxygen transport and metalloenzymes.

It is well known that porphyrin is also a high sensitive chromogenic reagent. Porphyrins and their metal chelates generally exhibit characteristic sharp and intensive absorption bands in the visible region. The region from 400 to 500 nm, which is called the Soret band, shows the most intensive absorption and molar absorptivities of the order of 10^5 are often found. The Soret band is widely used for spectrophotometric determination of metalloporphyrins.

Owing to its well known photochemical and redox activity, the porphyrin macrocycle is an attractive building block on which to append additional recognition sites for anion binding. The combination with Lewis acid, such as zinc, complexed in the porphyrin macrocycle cavity, may produce new selective redox active reagents for anions. Indeed various metalloporphyrins have shown a potentiometric response to anions with selectivity sequences solely dependent on the centrally bonded metal (Beer, Drew & Jagessar, 1997; Falk, 1975). The metalloporphyrins have a rich redox chemistry since they have the advantage of including coordination of additional ligands above and below the porphyrin plane.

Due to strong complexing properties and catalytic behaviour of metalloporphyrins, these compounds have found numerous applications in chemical analysis. This review presents applications of porphyrin compounds in spectroscopy, electroanalytical chemistry, flow injection analysis, and chromatography.

1.4.2 Porphyrins for Metal Determination

Separation of many porphyrins and metalloporphyrins by TLC(Lai, Lam & Chan, 1994; Podgorna & Kus, 2000; Podgorna & Dziegielewski, 2006; Saitoh, Kashiwa, Tada, Kiyohara & Suzuki, 1992; Stefaniak, 2007) and HPLC (Hu, Yang, Yin & Yao, 2002; Igarashi, Ide & Takagai, 2000; Itoh, Liu & Komata, 2006) has been described in literature.

In 2004, Yang, Guangyu and Lin determined heavy metal ions in Chinese herbal medicine by microwave digestion and reversed-phase high-performance liquid chromatography. Lead, cadmium, mercury, nickel, copper, zinc, and tin ions in the digested samples were pre-column derivatized with tetra-(4-chlorophenyl)-porphyrin to form the colored chelates which were then enriched by solid phase extraction with C18 cartridge and eluted from the cartridge with tetrahydrofuran (THF). In the original samples the detection limits of lead, cadmium, mercury, nickel, copper, zinc and tin are 4 ng L^{-1} , 3 ng L^{-1} , 6 ng L^{-1} , 5 ng L^{-1} , 2 ng L^{-1} , 6 ng L^{-1} , and 4 ng L^{-1} , respectively (Yang, Guangyu & Lin, 2004).

In 2000, a HPLC procedure has been optimized and applied to porphyrins of environmental samples such as marine sediment without purification using detectors as diode array and/or mass spectrometry, such as etio and octaethylporphyrins and their Vo and Ni compounds by Magi et al. (Magi, Ianni, Rivarolo & Frache, 2000).

In 2002, tetra-(4-bromophenyl)-porphyrin (T₄BPP) as a chelating reagent has been used for the on-line column enrichment and the separation of trace lead, cadmium and mercury ions by reversed-phase high-performance liquid chromatography (RP-HPLC) with photodiode array detector by Hu et al. When the Hg-T₄BPP, Pb-T₄BPP

and Cd-T₄BPP chelates were injected into the injector and sent to the enrichment column with 0.05 mol l⁻¹ of pH 10.0 pyrrolidine-phosphoric acid buffer solution as mobile phase. This method can be applied to the determination (g L⁻¹) level of lead, cadmium and mercury in drinking water (Hu, Yang, Yin & Yao, 2002).

By Yang et al., heavy-metal ions in tobacco and tobacco additive were determined by microwave digestion and reversed-phase high-performance liquid chromatography (RP-HPLC). The lead, cadmium, mercury, nickel, copper, and tin ions in the digested samples were precolumn derivated with tetra-(4-aminophenyl)-porphyrin (T₄-APP) to form color chelates; the Hg-T₄-APP, Cd-T₄-APP, Pb-T₄-APP, Ni-T₄-APP, Cu-T₄-APP and Sn-T₄-APP chelates were then enriched by solid-phase extraction with C₁₈ disks and the retained chelates were eluted from the disks using tetrahydrofuran. The method was applied to the determination of lead, cadmium, mercury, nickel, copper, and tin in tobacco and tobacco additive (Yang, Li, Shi & Wang, 2005).

Porphyrins have been used for metal determination by the techniques of AAS, spectrophotometry and spectrofluorometry.

By Pyrzyńska and Wierzbicki, Amberlite IRA-904 resin modified with tetrakis (*p*-carboxyphenyl) porphyrin (TCPP) was used to pre-concentrate vanadium species. Several parameters, such as sorption capacity of the chelating resin, pH for retention of V(IV) and V(V), volume of sample and eluent, were evaluated. Both vanadium species sorbed on TCPP-modified resin were eluted by use of 2 M nitric acid and determined by atomic absorption spectrometry. The recovery values were >94% and pre-concentration factor of 110 was obtained. The proposed method was examined for reference standard material and river water sample (Pyrzyńska & Wierzbicki, 2004).

By Knap et al., Amberlite IRA-904 resin modified with tetrakis (*p*-carboxyphenyl) porphyrin (TCPP) was used to on-line preconcentration procedure for the determination of manganese using flow-injection approach with flame atomic

absorption spectrometry as a detection method is described. The proposed method is based on the complexation between Mn(II) and 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (TCPP). The sorbed complexes were then eluted with 0.5 ml of 2M HNO₃. A good precision (2.2–3.1% R.S.D. for 50 µg L⁻¹ manganese) and the enrichment factor of 30 were obtained with the detection limit of 12 µg L⁻¹ (Knap, Kilian & Pyrzynska, 2006)

In 2003, the reaction of 5,10,15,20-tetrakis(4-carboxylphenyl)porphyrin with Cd(II), Pb(II), Hg(II) and Zn(II) was studied spectrophotometrically and kinetics, equilibrium constants as well as photodecomposition of complexes were determined by Kilian and Pyrzyn'ska. The detection limit for the recommended procedure was 1.4x10⁻⁹ M (0.9 ng mL⁻¹) and precision in range 20-100 ng mL⁻¹ not exceeds 2.7% RSD (Kilian & Pyrzyn'ska, 2003).

In 2005, an indirect substitution spectrophotometric methodology using porphyrin has been developed for the determination of some metals that do not react with porphyrin directly by Itoh et al. The method is concerned with a multistep reaction system, which consists of 3 complexation reactions that occur in a sequence of EDTA with metal, EDTA with Cu(II) and a cationic porphyrin, *meso*-tetrakis (4-*N*-trimethylaminophenyl)-porphine (ttmapp), with Cu(II) (Itoh, Liu & Komata, 2005).

By Dargiewicz-Nowicka et al., methyl-pyridyl porphyrin and its europium complex were prepared in the monolith gels by sol-gel method. The samples doped with the porphyrins were prepared by tetraethoxysilane hydrolysis and condensation. Their absorption and emission spectroscopic properties in comparison with the spectra of the same compounds in various solvents were investigated. It was seen that fluorescent properties of the europium in sol-gel matrices made this complex useful for some special applications as sensing of molecular oxygen or biomolecules (Dargiewicz-Nowicka, Makarska, Villegas, Legendziewicz & Radzki, 2004).

By Guo et al., a new optical sensor membrane was developed for sensing Hg²⁺ in aqueous solution by using 5,10,15,20-tetraphenylporphyrin as a lipophilized

indicator, which was dissolved in an organically modified sol-gel (Guo, Zhang, Xie, Lin & Chen, 2006).

By Delmarre et al., the influence of different solvents and of the pH on the spectroscopic properties of Sn(IV) tetrapyridyl and tetramethylpyridinium porphyrins were studied. Then these porphyrins were immobilized in sol-gel matrices. The spectroscopic properties of the immobilized porphyrins are dependent on the composition of the matrix and on its polarity (Delmarre, Lemarinier & Bied-Charreton, 1999).

1.5 Cloud Point Extraction For Metal Determination Using Spectroanalytical Techniques

The micellar phase obtained after a CPE presents physical and chemical characteristics that must be considered for the developing an analytical method. The surfactant solutions offer a media that modifies the reaction ratio, equilibrium position, spectral, and analytical parameters (Sanz-Medel, Campa, Gonzalez, & Fernandez-Sanchez, 1999; Pelizzetti & Pramauro, 1985).

1.5.1 Analysis of Micellar Phase by Molecular Spectroscopy

In the presence of surfactant, the chelating agent reacts with metallic species to form chelates, which, in some cases, present outstanding spectral characteristics. Moreover, the surfactant causes an increase in the molecular absorption and fluorescence intensity. The use of surfactant for signal improving without preconcentration is well known in molecular spectrometry. The CPE promotes metal enrichment and, consequently, the sensitivity of chromogenic reaction, and also avoids undesired lateral reactions (Stalikas, 2002).

The CPE was firstly applied in batch mode for spectrophotometric determination of metallic species at trace levels in aqueous samples after a complexing reaction. CPE was studied in the metal quantification by those methods that use fluorescence and phosphorescence techniques.

1.5.2 Micellar Media Applied to Atomic Absorption Spectrometry (AAS)

AAS can tolerate a less efficient separation because it is less prone to interferences. There are few spectral interferences, and these are well documented. Moreover, they are easily avoided by changing the analytical line used in the detection step. Thus, when the CPE is applied to AAS detection, it is possible to attain simple and inexpensive methods that are powerful tools for metal determination and preconcentration.

1.5.2.1 Flame Atomic Absorption Spectrometry (FAAS)

The addition of a diluent into a surfactant-rich phase is often needed to obtain a homogeneous solution with compatible viscosity to attain a good sample introduction into nebulizers. The use of organic solvent in the cold flame increases its sensitivity. This phenomenon is attributed to low superficial tension of organic solvents, which affects the nebulization process as well as increases flame temperature due to the solvent burn (Stalikas, 2002). Table 1.2 presents some CPE methods that have used FAAS as the analytical technique.

Table 1.2. Some CPE applications with FAAS detection

Metal	Sample	Complexing	Agent Surfactant	LOD ($\mu\text{g/L}$)	Reference
Ag	Natural, tap, and wastewaters	Dithizone	Triton X-114	0.56	(Manzoori & Karim-Nezhad, 2003)
Cd	River, sea, and tap waters	TAN	Triton X-114	0.099	(Cheng & Teo, 2001)
Cu	River, tap, waste waters and human blood	DFID	Triton X-114	1.5	(Ahmadi, Khanmohammadi & Tavakoli, 2009)
Cd Pb	Human hairs	DDTP	Triton X-114	0.62 2.86	(Manzoori & Bavili-Tabrizi, 2002)
Ni Cd Pb Cu	Sea Waters	NaDDTC	Triton X-114	0.030 2.1, 0.62 0.27	(Escalearaa, Santellia, Oliveiraa, Carvalhob & Bezerra, 2009)
Cu	Environmental Samples.	4-hydroxy-2-mercapto-6-propylpyrimidine	Triton X-114	1.6	(Shokrollahi, Ghaedi, Gharaghani & Soylak, 2008)
Co	Urine	PAN	Triton X-114	0.38	(Manzoori & Karim-Nezhad, 2003)
Co	Pharmaceutical	5-Br-PADAP	Triton X-100 and SDS	1.1	(Nascentes & Arruda, 2003)
Cr(III) Cr(VI)	River and tap waters	bis-(2-hydroxy-1-naphthaldehyde)	Triton X-100	0.18	(Kiran, Kumar, Prasad, Suvardhan & Janardhanam, 2008)
Co Ni	Waters	Me-BTABr	Triton X-114	0.9 1.1	(Lemos, Silva & Moreira, 2007)
Cu	Rain and drinking waters, human blood and hair	DDTP	Triton X-100	0.94	(Manzoori & Bavili-Tabrizi, 2002)
Cu	Natural waters	Not reported	Non-ionics	0.9	(Giokas, Antelo, Paleologos, Arce & Karayannis, 2002)
Fe	Wines	APDC	Triton X-100 and Triton X-45	20	(Giokas, Paleologos, Tzouara-Karayannis, 2002)
Mg	Waters	APDC	Triton X-114	19	(Giokas, Paleologos, Tzouwara-Karayannis & Karayannis, 2001)
Ni Co	River, sea, tap, and waste waters	1-Nitroso-2-naphthol	PONPE 7,5	1.22 1.09	(Manzoori & Bavili-Tabrizi, 2003)
Cu(II), Co(II) Ni(II)	Biological, Natural and Wastewater	Methyl-2-Pyridylketone Oxime	Triton X-114	1.2 2.1 1.9	(Ghaedi, Shokrollahi, Ahmadi, Rajabi & Soylak, 2008)
Ni	Saline oil-Refinery Effluents	5-Br-PADAP	Triton X-114	0.2	(Bezerra, Conceicao & Ferreira, 2004)

1.5.2.2 Graphite Furnace Atomic Absorption Spectrometry

Organic solvents and surfactants are both compatible with detection by graphite furnace or electrothermal atomization atomic absorption spectrometry (GFAAS or ETAAS) because they stabilize the solution and are completely oxidized before the detection step (Borges, Veiga, Frescura, Welz & Curtius, 2003; Yuan, Jiang, Cai, He & Liu, 2004).

1.5.2.3 Hydride Generation Atomic Absorption Spectrometry (HGAAS)

The analytical sensitivity and selectivity can be improved when using those techniques based on generation of volatile species and atomic detection. Micellar systems in hydride generation can concentrate reagents at molecular levels, modifying the thermodynamic and kinetic of reactions, and facilitating the solubilization of metals and reagents (Campa, Garcia, Va'ldez-Hevia & Temprano, 1995).

1.5.3 Analysis of Micellar Phase by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The adverse effects of organic solvents in plasma are extensively known. Many reports show its influence on several plasma parameters such as excitation conditions, stability, nebulization flux, and dimensions of self-formed plasma (Kreuning & Maessen, 1999; Molineroa, Castilola, Chamorro & Muniozguren, 1997). The presence of surfactant in the plasma may produce all these problems, thus demanding the highest power. A flow injection system is generally used to introduce a small quantity of micellar phase into the plasma in order to avoid an unstable plasma.

1.5.4 Analysis of Micellar Phase by Inductively Coupled Mass Spectrometry (ICP-MS)

The organic solvent or surfactant introduction into the plasma is the most critical step for those methods using CPE and ICP-MS as the analytical technique. In addition, it can affect the stabilization or extinction of the plasma. Organic compounds are a source of carbon atoms, which can recombine to form graphite on relatively cold interface cones and on the ionic lens, obstructing the cone orifices or changing the optimum voltage of the ionic lens. Additionally, carbon polyatomic ions may be formed, mainly with the plasma argon gas, interfering, for example, in the determination of ^{52}Cr (Montaser & Golightly, 1992).

1.6 Aim of This Study

The aim of this study was the determination of copper by flame atomic absorption spectrometry at trace levels after separation and preconcentration using cloud point extraction. In the cloud point extraction process, copper(II) was taken into a hydrophobic complex and then solubilized within the nonionic micelle. In this study, Triton X-100 was used as the nonionic surfactant, and 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin was used as the complexing agent. Phase separation was performed using column packed with cotton.

CHAPTER TWO

EXPERIMENTAL

2.1 Chemical and Reagents

Cu (II) stock solution (1000 mg L⁻¹ Cu): Prepared by dissolving 0.3 g Cu(NO₃)₂·3H₂O (Merck), in 100 mL de-ionized water.

Cu (II) solution (10 mg L⁻¹ Cu): Prepared from Cu (II) stock solution (1000 mg/L) by dilution with deionized water. Working standard solutions were prepared from this solution by appropriate dilution using deionized water.

5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin solution (1x10⁻² mol L⁻¹): Prepared by dissolving 0.73 g of 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (Aldrich) in 100 mL of tetrahydrofuran. Due to its sensitivity to light, this solution was freshly prepared each day and stored in dark.

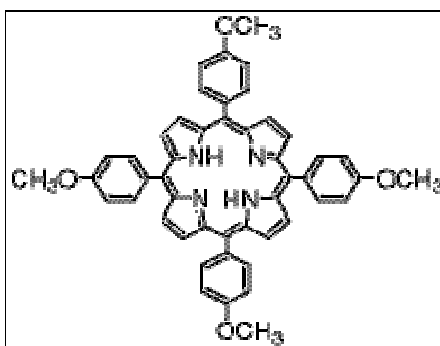


Figure 2.1 Structure of TMPP

Triton X-100 (2.0 % (w/v)): Prepared by diluting 2.0 g of Triton X-100 (Fluka) in 100 mL methanol. Triton X-100 (polyoxyethylene octyl phenyl ether), has been used as nonionic surfactant.

Buffer solution (pH 8): Buffer solution prepared from boric acid, sodium hydroxide and hydrogen chloride was purchased from Merck.

NaCl solution (0.5 mol L⁻¹) : Sodium chloride (Merck) stock solution was prepared as 0.5 mol L⁻¹ in deionized water for phase separation.

All other reagents were of analytical reagent grade. Deionized water obtained from a Millipore water purification system was used for sample and standard preparations. The pipettes and vessels used were kept in 4 mol L⁻¹ nitric acid for at least 24 h and subsequently washed four times with doubly distilled water.

Filtering material: A home-made column packed with suitable filtering material, cotton, was employed to carry out phase separation.

2.2 Instrumentation

2.2.1 Atomic Absorption Spectrometer

Atomic absorption spectrometry is an analytical technique that measures the concentrations of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (mg L⁻¹) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another, higher, energy level. Atomic absorption spectrometry has many uses in different areas of chemistry.

Atoms of different elements absorb characteristic wavelengths of light. Analysing a sample to see if it contains a particular element means using light from that element. An atomic absorption spectrometer (Figure 2.2) needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed (Levinson, 1997).

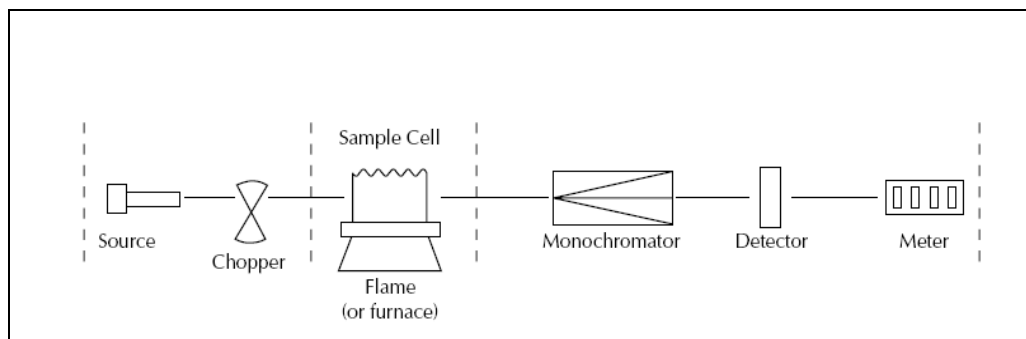


Figure 2.2 Atomic Absorption Spectrometer

2.2.1.1 The Light Source

The common source of light is a hollow cathode lamp. This contains a tungsten anode and a cylindrical hollow cathode made of the element to be determined. These are sealed in a glass tube filled with an inert gas. The ionization of some gas atoms occurs by applying a potential difference of about 300–400 V between the anode and the cathode. These gaseous ions bombard the cathode and eject metal atoms from the cathode in a process called sputtering. Some sputtered atoms are in excited states and emit radiation characteristic of the metal as they fall back to the ground state. A typical atomic absorption instrument holds several lamps each for a different element.

2.2.1.2 The Optical System and Detector

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

2.2.1.3 Atomisation of the Sample

Two systems are commonly used to produce atoms from the sample. Aspiration involves sucking a solution of the sample into a flame; and electrothermal atomisation is where a drop of sample is placed into a graphite tube that is then heated electrically. Some instruments have both atomisation systems but share one set of lamps. Once the appropriate lamp has been selected, it is pointed towards one or other atomisation system.

2.2.1.4 Sample Preparation

Sample preparation is often simple, and the chemical form of the element is usually unimportant. This is because atomisation converts the sample into free atoms irrespective of its initial state. The sample is weighed and made into a solution by suitable dilution. Elements in biological fluids such as urine and blood are often measured simply after a dilution of the original sample.

2.2.1.5 Background Absorption

It is possible that other atoms or molecules apart from those of the element being determined will absorb or scatter some radiation from the light source. These species could include unvaporized solvent droplets, or compounds of the matrix (chemical species, such as anions, that tend to accompany the metals being analyzed) that are not removed completely. This means that there is a background absorption as well as that of the sample.

2.2.1.6 Calibration

A calibration curve is used to determine the unknown concentration of an element in a solution.

The instrument is calibrated using several solutions of known concentrations. A calibration curve is produced which is continually rescaled as more concentrated solutions are used – the more concentrated solutions absorb more radiation up to a certain absorbance. The calibration curve shows the concentration against the amount of radiation absorbed. The sample solution is fed into the instrument and the unknown concentration of the element is then displayed on the calibration curve.

2.2.1.7 Interferences and Matrix Modification

Other chemicals that are present in the sample may affect the atomisation process. For example, in flame atomic absorption, phosphate ions may react with calcium ions to form calcium pyrophosphate. This does not dissociate in the flame and therefore results in a low reading for calcium. This problem is avoided by adding different reagents to the sample that may react with the phosphate to give a more volatile compound that is dissociated easily. Lanthanum nitrate solution is added to samples containing calcium to tie up the phosphate and to allow the calcium to be atomized, making the calcium absorbance independent of the amount of phosphate (Levinson, 1997)

2.2.1.8 Procedure for FAAS

A Perkin Elmer AAnalyst 700 model flame atomic absorption spectrometer (Shelton, USA) operating with air-acetylene flame was used for the analysis. Hollow cathode lamp (HCL) for copper was operated at 30 mA with a spectral bandwidth of 0.7 nm. Instrumental conditions used were those suggested by the manufacturer. Signals were measured as peak height area by using instrument software (WinLab 32). Also the wavelength had conventional value: Cu 324.8 nm.

Concentration range for calibration solutions of copper was selected with respect to linear range of signal versus concentration. Calibration solutions containing from 1.0 to 5.0 mg L⁻¹ for Cu(II), were prepared freshly before use. All of them were prepared in 10 mL volumetric flasks.

2.3 Cloud Point Extraction Procedure

Steps of complex formation and preconcentration of Cu-TMPP complex (Figure 2.3) by cloud point extraction was shown in a flowchart (Figure 2.4). TMPP solution, buffer solution, non-ionic surfactant and electrolyte solution was added to the aqueous solution containing analyte at optimized conditions. The mixture was stirred for 15 min and passed through the collection column, which allowed for the surfactant-rich phase containing the copper chelate to be collected inside the column, while the aqueous phase passed through the column. After loading, the further washing with diluted solution served to remove any sample still present in the lines and in the column. Finally, the retained surfactant-rich phase was eluted with nitric acid in tetrahydrofuran at an optimum flow rate and analyzed by FAAS. All runs were carried out at ambient temperature (15-25 °C).

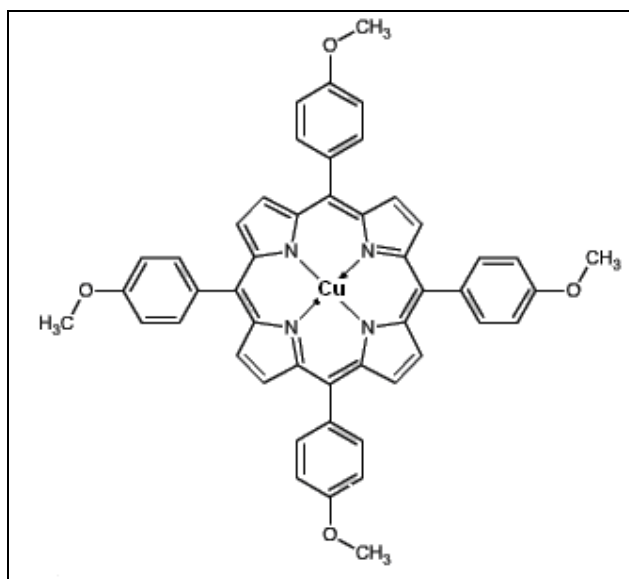


Figure 2. 3 Formula of Cu(II)-TMPP complex

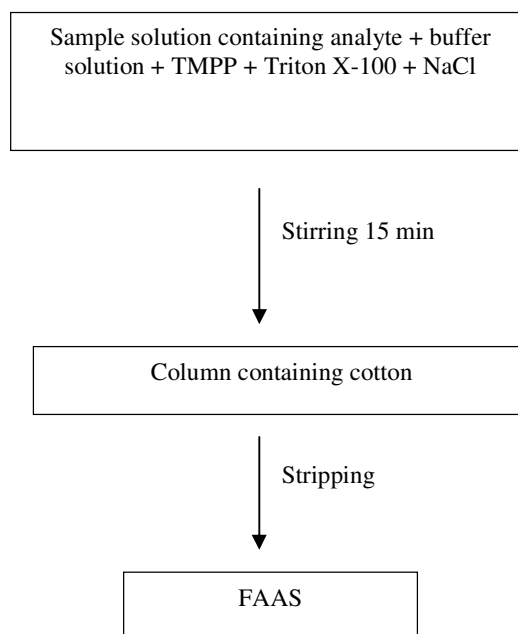


Figure 2.4 Flow chart of cloud point extraction procedure

2.4 Investigation of Optimum Conditions

2.4.1 pH Dependency of Complex Formation

To investigate the effect of pH, a set of solutions was prepared by adding 7.5 mL of 0.60 mg L^{-1} Cu(II), 1.5 mL of buffer solution, 1.5 mL of $1 \times 10^{-3} \text{ mol L}^{-1}$ TMPP, 1.5 mL of %2 (w/v) Triton X-100 and 0.5 mL of 0.125 mol L^{-1} NaCl for final pH of the solution varied in the range of 2 - 10. The final volume of the solutions was taken as 12.5 mL. The mixture was stirred for 15 min. Then the mixture was passed through the collection column and was eluted with 2.5 mL of 1 mol L^{-1} HNO_3 containing 25% (v/v) THF solution. The determination of copper(II) ion in eluate was carried out by FAAS after diluting to necessary ratio.

2.4.2 Stripping of Copper Ions

After preconcentration step, the eluent is employed to transport the metal ion to FAAS. For this, the various mixtures containing nitric acid and tetrahydro furan

(25%, 35%, and 50% (v/v) THF- 0.5, 1.0, and 2.0 mol L⁻¹ of HNO₃) as a volume from 1.0 to 5.0 mL were tested for the quantitative elution of copper ion from minicolumn.

2.4.3 Effect of 5,10,15,20-tetrakis (4-methoxyphenyl) Porphyrin Concentration

In order to find the necessary amount of TMPP solution to be used in the formation of Cu (II)-TMPP complex, several solutions containing 0.36 mg L⁻¹ Cu (II) ion were prepared in which the concentration of TMPP ranging from 0.05, 0.12, 0.2, 0.4, 0.5, 0.6, 0.8 to 1x10⁻³ mol L⁻¹. The pH of solutions was adjusted to pH 8 before adding TMPP. After loading the surfactant-rich phase to column, the complex was eluted with nitric acid in THF solution and absorbance of copper(II) ion was measured using conditions given in Section 2.2.1.6.

2.4.4 Effect of Surfactant Concentration, Triton X-100

To determine the effect of surfactant percentage, a set of solutions was performed. 0.36 mg L⁻¹ Cu (II) was allowed to react with 1.5 mL of 4x10⁻³ M TMPP solution at pH 8 including 0.5 mL 0.125 mol L⁻¹ NaCl in the centrifuge tubes. The final volume of the solutions was 12.5 mL. After the complex formed in the optimized conditions for complex formation Triton X-100 was added to the solution where the surfactant concentration was varied from 0.1 to 24x10⁻²% (w/v) and the contents were mixed. Then 15 minutes was allowed at room temperature for micelles formation and also to entrap analyte to the micelle. After the phase separation using column, the determination of metal ion in eluate was carried out by FAAS.

2.4.5 Effect of Flow Rate

The influence of flow rate of surfactant-rich phase passing through minicolumn has also been investigated. Flow rate values ranging from 1.0 to 10.0 mL min⁻¹ were tested.

2.4.6 Effect of Electrolyte Concentration

For the investigating the influence of electrolyte on performance of cloud point extraction, various experiments were performed by adding different concentration of NaCl in the range of 0.005– 0.028 mol L⁻¹. Other experimental conditions were kept constant.

2.4.7 Effect of co-Existing Ions

In order to see the effect of co-existing ions to the Cu(II)-TMPP complex formation and micellar extraction of Cu(II)-TMPP complex 0.25, 0.36 or 1 mg L⁻¹ Cu (II) was reacted with optimized concentration of TMPP, TritonX-100 and NaCl at pH 8 in the presence of potential co-existing ions such as KNO₃, Ba(NO₃)₂, Ca(NO₃)₂, MgCl₂, Zn(II), Ni(II), Co(II), Cd(II) and Pb(II) to complex formation.

2.5 Real Water Samples

Geothermal water samples were obtained from Manisa, Eagean region in Turkey. Aliquots of 7.5 ml of each sample were subjected to the cloud point extraction and FAAS determination as described in the previous section.

CHAPTER THREE

RESULT

In this study the cloud point extraction of copper with TMPP was carried out and the obtained Cu(II)-TMPP complex was determined by flame atomic absorption spectrometry. Throughout the study, the parameters affecting the complex formation in aqueous media, the cloud point extraction, the absorption measurement for copper were discussed, the presence of co-existing ions effects were controlled and the figures of merit for the determination of copper with this proposed method were measured.

3.1 Effect of pH

The effect of the sample pH on the copper response was investigated within the range of 2 – 10. As can be seen in Figure 3.1 the optimum pH 8 for complex formation provides the highest extraction efficiency for the cloud point extraction. Therefore all cloud point extractions were carried out at pH 8 through out this study. The decrease in absorbances at pH > 8.5 is probably due to the precipitation of copper ions in the form of hydroxide and at pH < 7.0 may be due to competition from hydronium ion toward ions for complexation with TMPP or decomposition of complex at pH values smaller than 8, which led to the decrease in absorbances. At lower pH values, the formation of complexes is not quantitative, and at higher pH values, the hydrolysis of cations may occur.

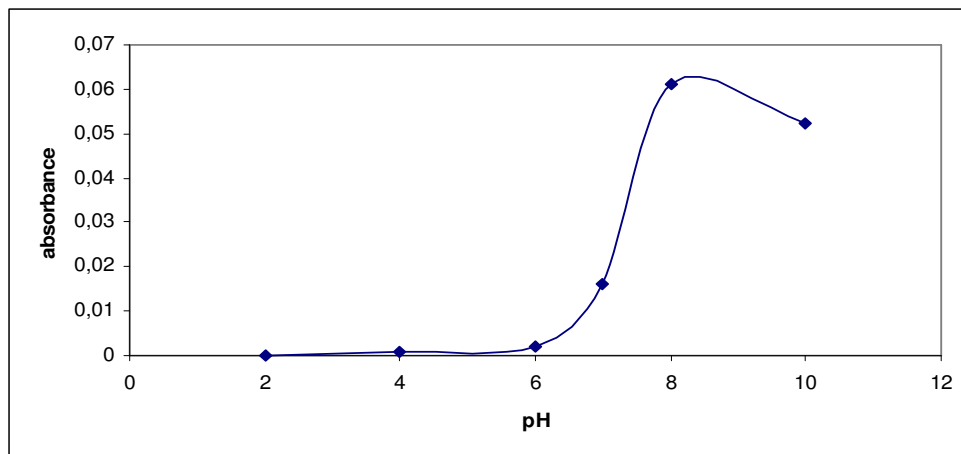


Figure 3.1 Effect of pH on the formation of Cu(II)-TMPP complex. Conditions: 12.5mL sample solution, $0.36 \mu\text{g mL}^{-1}$ Cu(II) ion, 1.5 mL of $1 \times 10^{-3} \text{ mol L}^{-1}$ TMPP, 1.5 mL of 2% (w/v) Triton X-100, 0.5 mL of 0.125 mol L^{-1} NaCl.

3.2 Stripping of Copper Ion

After the preconcentration step, an appropriate solvent should be used to eluate the copper-containing surfactant aggregated within the column. This eluent is also employed to transport the metal ions to FAAS hence the selection of proper eluent is an essential factor determining the analytical signal of copper. Desorption of the retained copper from the cotton column has been investigated. In order to elute the analytes containing micelles from the minicolumns 1 mL of nitric acid solutions at concentrations of 0.5, 1 and 2 mol L^{-1} in 25, 35 and 50% THF were tested as stripping agent (Figure 3.2). The highest analytical signal was observed with 1 mol L^{-1} HNO_3 in 25% THF. Then the volume of stripping solution was tested. As shown from the Figure 3.2, 2.5 mL of this solution was the best one.

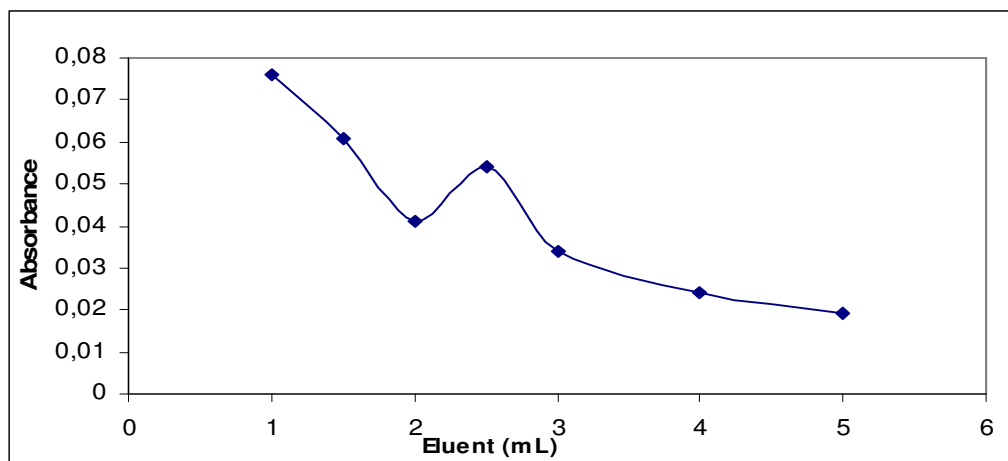


Figure 3.2 Effect of volume of stripping solvent, 1 M HNO₃, 25% THF. Conditions: 12.5mL sample solution, 0.36 μg mL⁻¹ Cu(II) ion , pH 8, 1.5 mL of 1x10⁻³ mol L⁻¹ TMPP, 1.5 mL of 2% (w/v) Triton X-100, 0.5 mL of 0.125 mol L⁻¹ NaCl.

3.3 Effect of 5,10,15,20-Tetrakis (4-Methoxyphenyl) Porphyrin Concentration

In general, the concentration of a chelating reagent has a remarkable influence on the extraction efficiency. The effect of the concentration of the chelating reagent on analytical response was studied and the concentration of TMPP was evaluated over the range 0.05 to 1 x10⁻³ mol L⁻¹. The extraction efficiency was examined and the results are presented in Figure 3.3 was seen that the maximum signal was obtained at 0.6x10⁻³ mol L⁻¹ TMPP. Therefore, TMPP concentration of 0.6x10⁻³ mol L⁻¹ was chosen as the chelating reagent for this study.

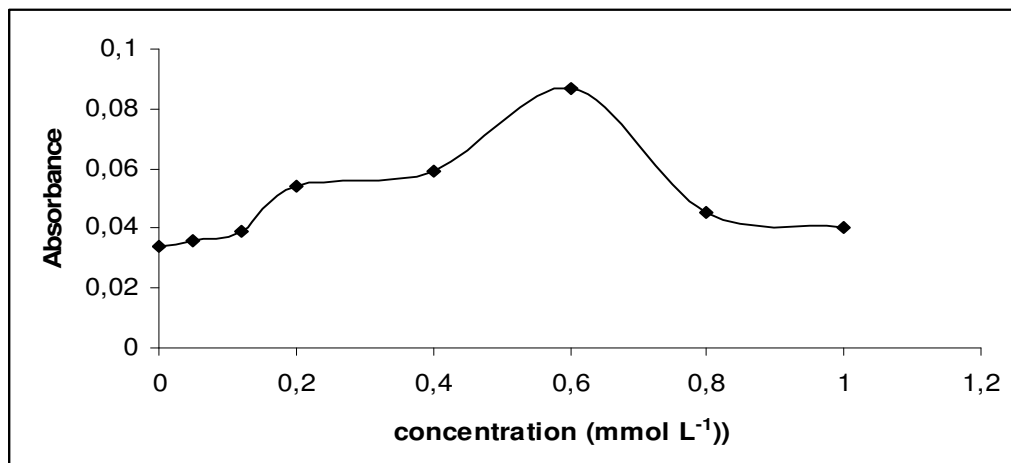


Figure 3.3 Effect of concentration of TMPP. Conditions: 12.5mL sample solution, $0.36 \mu\text{g mL}^{-1}$ Cu(II) ion at pH 8, different concentrations of TMPP, 1.5 mL of 2% (w/v) Triton X-100, 0.5 mL of 0.125 mol L^{-1} NaCl.

3.4 Effect of Surfactant Concentration

The preconcentration efficiency was evaluated using Triton X-100 concentrations ranging from 0.001% to 0.24% (w/v). The results are demonstrated in Figure 3.4. The highest copper(II) ion recovery was obtained with 0.01% (w/v) Triton X-100. This result might be related to the presence of the high amount of surfactant, resulting in an increase in the volume of the surfactant-rich phase. In addition, the viscosity of the surfactant-rich phase increases, leading to poor sensitivity due to assemblies that were inadequate to quantitatively entrap the hydrophobic complex. A surfactant concentration of 0.01% (w/v) for Triton X-100 was selected for all further works.

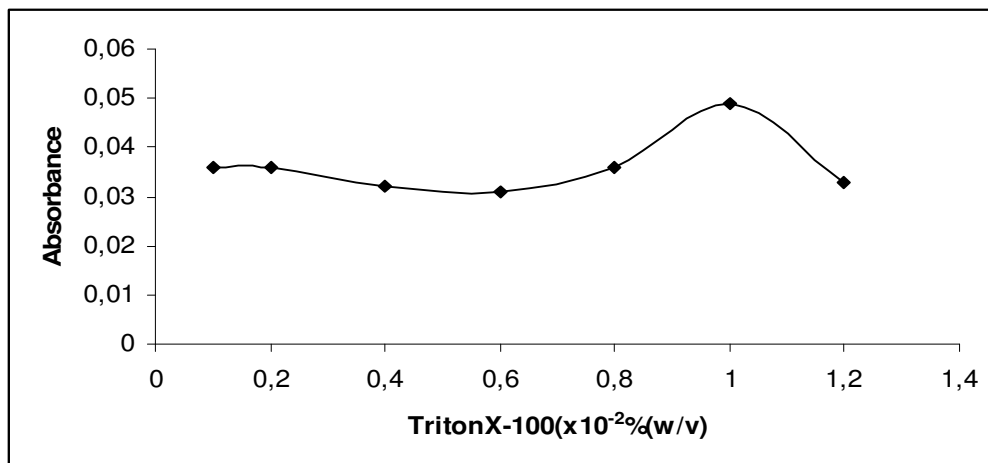


Figure 3.4 Effect of amount of Triton X-114 on the recoveries of Cu (II) ion. Conditions: 12.5mL sample solution, $0.36 \mu\text{g mL}^{-1}$ Cu(II) ion at pH 8, $0.6 \times 10^{-3} \text{ mol L}^{-1}$ of TMPP, different percentage of Triton X-100, 0.5 mL of 0.125 mol L^{-1} NaCl.

3.5 Effect of Flow Rate

The influence of flow rate of surfactant-rich phase passing through column has also been investigated. Flow rate values ranging from 1 to 10 mL min^{-1} were tested (Figure 3.5). The absorbance signals decreased significantly at flow rates higher than 1 mL min^{-1} . The most favorable collecting conditions were achieved when using flow rate as 1 mL min^{-1} . The flow rate of surfactant-rich phase was fixed at 1 mL min^{-1} . The same flow rate was also used for elution step.

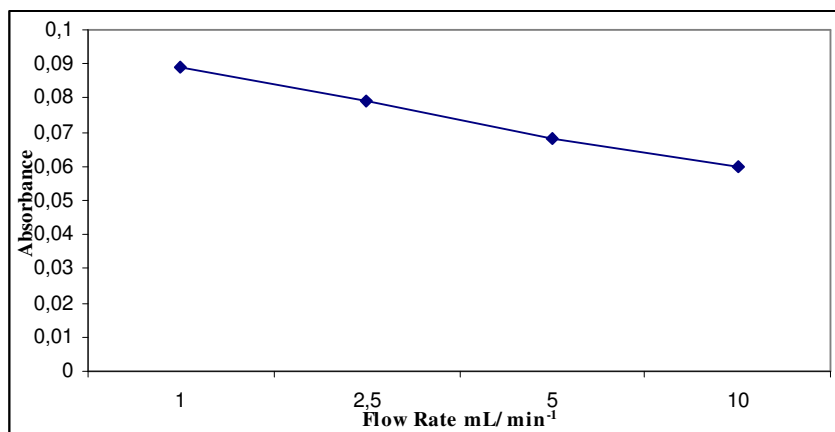


Figure 3.5 Effect of flow rate on minicolumn

3.6 Effect of NaCl Concentration

Studies on the effects of some additives, such as anionic and non-ionic surfactants and electrolytes, as NaCl, KNO₃ and MgCl₂, on the cloud point behavior of non-ionic surfactants have been reported (Nascentes & Arruda, 2003; Gu & Galera-Go´mez, 1995; Komaromy-Hiller, Calkins & Wandruszka 1996). It was observed that the presence of electrolytes decreases the cloud point (salting-out effect), resulting in a more efficient extraction. The lower cloud point is attributed to electrolytes promoting dehydration of the poly(oxyethylene) chains (Armstrong, Chowdhry, Snowden & Leharne, 1998). According to Hiller et al. (Komaromy-Hiller, Calkins, & Wandruszka 1996) the salting-out phenomenon is directly related to desorption of ions to the hydrophilic parts of the micelles, increasing inter-attraction between micelles and consequently leading to the precipitation of surfactant molecules. Based on this discussion, NaCl was investigated as electrolyte in the concentration range from 0.005 to 0.028 mol L⁻¹ with the goal of obtaining the cloud point without heating (Figure 3.6). From Fig. 3.6 the cloud point of Triton X-100 decreases as the concentration of NaCl solution increases. At the same time, the time of back extraction in which the turbid micelle solution became clarified increased. The reason was probably that the inorganic electrolyte made hydrogen bonds with micelles, fracturing and separating hydrophobic groups from the water phase. The maximum absorbance was obtained in the presence of NaCl at concentrations near to 0.02 mol L⁻¹ for the analyte, thus this concentration was selected for subsequent experiments.

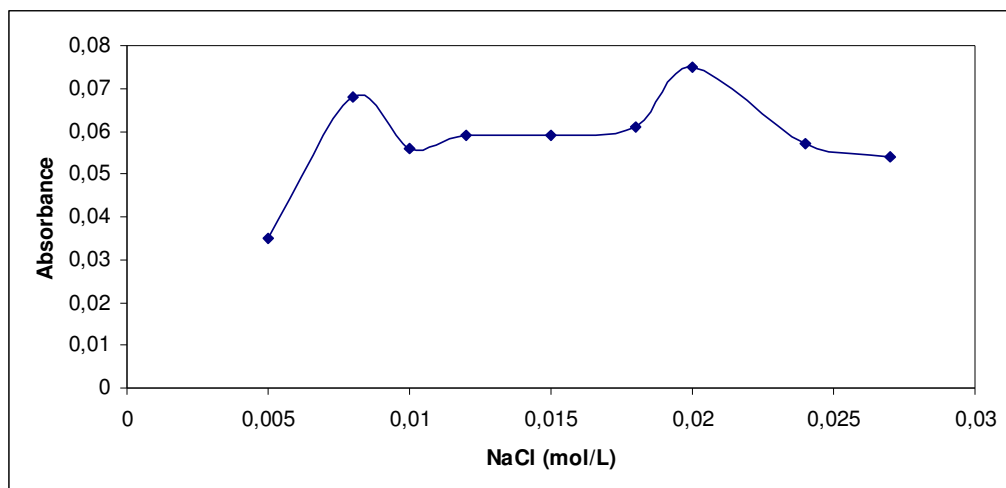


Figure 3.6 The effect of concentration of NaCl. Conditions: 12.5mL sample solution, $0.36 \mu\text{g mL}^{-1}$ Cu(II) ion at pH 8, $0.6 \times 10^{-3} \text{ mol L}^{-1}$ of TMPP, 0.01%(w/v) Triton X-100, different concentration of NaCl.

3.7 Effect of co-Existing Ions

To evaluate the interference of co-existing ions on the determination of copper, the solutions of Cu(II) and a single and multiple coexisting ions were prepared. Various kinds of ions were added to the aqueous solution containing $0.25 \mu\text{g mL}^{-1}$ Cu(II). Copper was determined by the on-line CPE proposed system. A relative error of less than 5% was considered to be within the range of experimental error. NaCl interference was studied without considering the salt amount added as electrolyte. As shown in Table 3.1, the presence of tested species caused no appreciable interferences in the determination of copper. The presences of large amounts of alkali and alkaline earth metals have no significant effect on the preconcentration of copper. It was found that the chelating agent, TMPP was selective for copper ion. In a competitive conditions, TMPP may react with Co(II), Ni(II), Cd(II) and Pb(II) ions. According to the results, the complexation order of studied ions with TMPP by the proposed cloud point extraction procedure might be Cu(II) > Ni(II) > Co(II) > Cd(II) > Pb(II) > Zn(II).

Table 3.1 Tolerance limits of co-existing ions in the determination of 250 $\mu\text{g mL}^{-1}$ copper.

Ions	Added as	Copper to interferent ratio
K^+ , NO_3^-	KNO_3	1:5000
Mg^{2+} , Ca^{2+} , Ba^{2+}	MgCl_2 , $\text{Ca}(\text{NO}_3)_2$, $\text{Ba}(\text{NO}_3)_2$	1:2000
$\text{Cd}(\text{II})$, $\text{Ni}(\text{II})$, $\text{Co}(\text{II})$, $\text{Zn}(\text{II})$, $\text{Pb}(\text{II})$		1:4

3.8 Analytical Features

Calibration curves were obtained by the preconcentration of 12.5 mL of a sample solution in the presence of 0.01% (w/v) Triton X-100 in medium at pH 8. The samples were introduced into the flame by conventional aspiration following the eluting surfactant rich phase from column with 2.5 mL of 1.0 mol L^{-1} HNO_3 in 25% THF solution. In this case, linear relationships between the absorbance measured and the concentration of metal placed in solution were obtained. Table 3.2 gives the summary of analytical features of the method. The relative standard deviation (R.S.D.) obtained for five replicates determination of 360 ng mL^{-1} of copper was 2.1 % and limit of detection (LOD) calculated, as three times the standard deviation of the blank signals was 4.3 ng mL^{-1} . Enrichment factor was calculated as the ratio of the slopes of the linear section in calibration graphs for preconcentration and direct aspiration, respectively. Consumptive index (CI) was also calculated. This parameter is defined as the volume of the sample consumed to achieve a unit of EF (Fang, 1993). It was calculated by the ratio of the sample volume, in milliliters, and EF. The calibration graph for copper is linear up to 500 ng mL^{-1} .

Table 3.2. Analytical characteristics of the method

Parameter	Analytical feature
Linear range, ng mL ⁻¹	50-500
Limit of detection, ng mL ⁻¹	4.3
Sample volume, mL	12.5
Enrichment factor	5
Consumptive index, mL	2.5
Precision (Cu, 360 ng mL ⁻¹ , n:5)RSD%	2.1

3. 9 Application of The Method

Proposed method was applied to the determination of copper(II) in geological water samples. Accuracy of the proposed method was proved by spike-recovery test. Aliquots of 7.0 ml of different samples were spiked with two different concentrations of copper at 178 and 357 ng mL⁻¹ (100 and 200 ng mL⁻¹ when diluted to 12.5 mL) and recovery experiments were conducted as well for these samples. The results are summarized in Table 3.3. A good agreement was obtained between the added and measured analyte amounts. The percentage recovery (R) was determined by using the equation: $R = \{100(C_m - C_o)/m\}$. Where C_m is a value of metal in a spiked sample, C_o is a value of metal in a sample and m is the amount of metal spiked (Lemos, Baliza, Carvalho et al. 2008). The recovery values calculated for the added standards were always higher than 96%, thus confirming the accuracy of the procedure and its independence from the matrix effects.

Table 3.3 Results obtained for copper determination in geothermal water samples (n:3)

Sample	Added (ng mL ⁻¹)	Found (ng mL ⁻¹)	Recovery (%)
1 st	0	67.9±0.9	
	178	246.4±1.6	100
	357	414.3±1.8	97
2 nd	0	71.4±0.5	
	178	250.0±1.6	100
	357	428.6±2.3	100
3 rd	0	100.0±0.4	
	178	282.1±1.2	102
	357	453.6±1.8	99
4 th	0	55.4±0.6	
	178	228.6±1.3	97
	357	417.9±2.2	102

CHAPTER FOUR

CONCLUSION

Cloud point extraction is a separation and preconcentration procedure that has been extensively used for trace metal determination in different analytical matrices. This technique offers several advantages including experimental convenience, low cost, environmental safety. These aspects include it in a set of analytical methods in agreement with the “green chemistry” principles.

In this work, the cloud point extraction as an alternative method has been proposed for the preconcentration of copper as a prior step. For that the copper (II) complex (5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin, TMPP-Cu) formed was extracted by Triton X-100. The cloud-point was induced at the optimized conditions: pH 8.0, concentration of TMPP 0.6×10^{-3} mol L⁻¹, 0.01 % TritonX-100 (w/v), stirring time 15 min and concentration of NaCl 0.02 mol L⁻¹. The quantitative measurements were carried out by a flame atomic absorption spectrometry. Using minicolumn in collection of surfactant rich phase instead of batch cloud point extraction method causes higher speed of analysis and decreasing the risks of contamination and analyte loss in this procedure. In contrast to some other preconcentration techniques, no organic solvent is employed; therefore, the environmental pollution is limited to a small amount of surfactant.

The method has been successfully applied to the determination of copper in the geothermal water samples.

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