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College of Pharmacy

Investigation of Toxic Metals in some medicinal plants After Cloud-Point Extraction

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بسم الله الرحمن الرحيم

﴿ يُؤتِي الْحِكْمَةَ مَنْ يَشَاءُ وَمَن يُؤتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا وَمَا يَذَّكَّرُ إِلَّا أُولُوا الْأَلْبَابِ ﴾

صدق الله العلي العظيم

سورة البقرة الاية [٢٦٩]

Acknowledgment

First of all, thank God for helping us in performing this work. Wewould like to introduce our deepest thanks to our supervisor Dr. Azhar A. Ghali Al-Adilee ,for her guidance and kindness throughout the study.

We would like to express our sincere gratitude to our families for their support and help to perform this study in the best way.

DEDICATIONS

To my mother

To my father

To my sisters

To my brothers

Summary:

In the present study, a reagent 2-[(6 – Methyl – 2 – Benzothiazolyl) azo] – 4 - Chlorophenol (6-MeBTAClP) was used for the cloud point preconcentration of Copper and Nickel from in some medicinal plants and sample of drug . The method was based on the complexation of Copper and Nickel with (6-MeBTAClP) in the presence of Triton X-114 at pH 4 and 5, respectively using with suitable buffer (Acetate buffer). Optimum experimental conditions were investigated with respect to a standard solution of the same matrix. The proposed procedure was applied to the analysis in some medicinal plants.

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Chapter one

Introduction

1.1 General Introduction

The use of plant and plant products in treatment of diseases is as old as mankind. Herbal medicines being natural are still preferred to contemporary synthetic medicines by a major section of the world. According to the World Health Organization (WHO), 80% of the world population relies on traditional or herbal medicines for their primary health care needs[(1)] .Plants are sensitive to environmental conditions and they accumulate heavy metals in their harvestable parts [(2)]. The possibility that the toxic heavy metals can be transmitted to humans and animals through the use of herbs grown in polluted areas is a major concern for traditional and herbal medicine .Due to this reason, WHO advocates that herbs and herbal products should not be used without qualitative and quantitative analysis of heavy metals contents therefore interest in the development of analytical techniques for determination of the heavy metals (atomic weights: 63.5g mol^{-1}) such as Copper and Nickel [(3)].

1.2 The Copper

Copper is essential for the survival of plants and animals. Animal and human studies have shown that copper is involved in the function of several enzymes. The essentially of copper for humans was first shown during the 1960s in malnourished children from Peru. These children had an anemia refractory to iron therapy, neutropenia, and bone abnormalities that were responsive to copper supplementation. Further studies confirmed these findings and established that copper was required for infant growth, host defense mechanisms, bone strength, red and white cell maturation, iron transport, cholesterol metabolism, myocardial contractility, glucose metabolism, and brain development. Major alterations in mental development are also observed in Menkes syndrome, which is a genetic

syndrome in which alterations in copper absorption and transport Copper to early death.[4]

1.2.1 Copper plays many important roles in maintaining a healthy body and some of its benefits include

1.2.1.1 Arthritis

The health benefits of copper relate to its anti-inflammatory actions that assist in reducing the symptoms of arthritis. The consumer market is also flooded with copper bracelets as well as other accessories for Cuing this condition. Copper can also work as a home remedy for arthritis; store water in a copper container overnight to accumulate copper traces. These are beneficial in strengthening the muscular system, so drink the water when you wake in the morning. You will feel energized and active for the day, because your metabolism will have a good source of copper for its daily processes.

1.2.1.2.Proper Growth

Copper is essential for normal growth and health. Thus, it is very important to include this mineral in balanced levels in your regular diets. It is also helpful in the protection of the skeletal, nervous and cardiovascular systems. If you suffer from a copper deficiency, the normal and healthy growth of organs and tissues, as well as their proper oxygenation from an ample red blood cell concentration, would be impossible.

1.2.2. Copper deficiencies

Copper deficiencies are seen in many third world countries and are reflected in the number of birth and growth defects in children of those nations. Pigmentation of Hair and Eyes: Copper is a vital component of the natural dark pigment, melanin, which imparts coloration to the skin, hair, and eyes. Melanin can be produced by melanocytes only in the presence of the cuproenzyme called tyrosinase, which is derived from copper. Intake of copper supplements also helps in protecting against graying of the hair. So while it is often overlooked as an antioxidant mineral, it does protect the integrity of those cells and keeps you looking young! It also maintains the color of your eyes and is

essential, along with zinc, to keep your eyes beautifully colored into your old age.

Copper deficiency is rare, but can occur in people who are severely undernourished or who have chronic diarrhea. Disorders that impair nutrient absorption, such as Crohn's disease, can also Copper to copper deficiency, as can high dietary intakes of iron or zinc. Signs of deficiency include bleeding under the skin, damaged blood vessels, hair loss, pale skin, and an enlarged heart. Symptoms include fatigue and, because copper plays a role in immunity, imbalances can make you more susceptible to infections.[5]

1.2.3. Copper toxicity

Copper cause toxicity through transition of metal and is capable of generating oxidative stress. Effectson epithelia and mucous membranes are irritative and corrosive in nature. Redoxreactions contribute to the majority of systemic copper toxicity, including renal and hepatic damage with hemolysis.[6]

1.2.4.The people who are at risk of copper toxicity

1. People who live near or work at copper-producing facilities, such as mines, smelters, or refining facilities
2. People with copper pipes or who are drinking from / cooking with copperlined vessels
3. Vineyard workers exposed to copper sulfate and hydrated lime Exposure to algaecides, herbicides, wood preservatives, pyrotechnics, ceramic glazes, electrical wiring, welding, or brazing with copper alloys[6] .

1.3. The Nickel

Nickel is an important constituent of several steel alloys. Its determination is required to control both raw material and industrial products.1 In addition it is readily encountered in the environment, which enters from land-based resources, such as oxidic and sulfide ores or industrial sources, such as spent catalysts, alloy scrap, sludge, dust, and wastewater.

Nickel is widely used in electroplating, the manufacture of Ni-Cd batteries, rods for arc welding, pigments of paints, ceramic, surgical and dental prostheses, magnetic tapes and computer components and nickel catalysts. Nickel enters

waters from dissolution of industrial processes and waste disposal [1]. Nickel was thought to be essential for plants and some domestic animals [7], but not considered to be a metal of biological importance until 1975, when Zerner discovered that urease was a nickel enzyme [8]. Nickel is essential constituent in plant urease.
Jack beans and soybeans generally contain high concentration of nickel [1]. Compared with other transition metals, nickel is moderately toxic element, and still at low concentration produces a general toxic effect on the human organism, causing nasopharynx and lung diseases, malignant tumors and dermatological discase [9].

Nickel-containing sewage is harmful after being ingress into water. This fact explained the importance of the monitoring of nickel concentration in natural and waste water samples. Flame and graphite furnace atomic absorption spectrometry and spectrophotometric methods provides accurate and rapid determination of nickel in natural and waste waters [10]. However, very frequently a direct determination cannot be applied due to low concentration of analyte or matrix interferences.

Nickel constitutes the threat for humans. Based on animal studies are set permitted nickel content in products for humans (for example, indicators TUIL, TDI). TUIL is a Tolerable Upper Intake Levels for vitamins and minerals. This indicator said what is a pose which is no risk for health for a daily nutrient intake. The highest concentration of nickel is in cocoa (8.2-12 mg/kg), next in soya beans (4.7-5.9 mg/kg) and oatmeal (0.33-4.8 mg/kg). All nickel in human tissues should not exceed 0.5 mg, in human blood – 1-5 μg/L. Above this value, nickel is accumulated in lungs, adrenals and other organs [11,12].

TDI – Tolerable Daily Intake – is the another indicator. Its value is similar to TUIL. This indicator is controlled by EFSA – European Food Safe Authority According to EFSA, daily intake should be lower than 500 μg [13,14].

1.3.1 The effect of nickel levels on human body

The low concentration of nickel is required for proper functioning of living organisms. Nickel is normally present in human tissues. However, the exposition to high concentrations of Ni may lead to significant increase of this element in human body. In general, the health effect of nickel is depend on the route of exposure (inhalation, oral or dermal) and physicochemical form of this element [15, 16].

Contributions of Ni to the body can differ. Body exposition to the Ni in the air and in drinking water is usually less important than its dietary intake. Nickel shows its neurotoxic and carcinogenic properties[17].

A high concentration of nickel, like for example the oral doses in weight of 73 mg elemental Ni results nausea, headache, cough, diarrhea, decreased pulse.
Nickel supplied to the body from food is poorly absorbed and eliminated. A particular threat is skin contact and inhalation. Nickel which is inhaled with air is accumulated and damages the mucous membranes [18], [19].

The chemical form of nickel affects its fate in human body. For instance, the water-soluble forms of this element are more readily absorbed than others. Nickel is easily absorbed in the ionic form. However, even less soluble compounds of this element can be phagocytized. Nickel is transported and distributed throughout the body in the form of its complex compounds. It is usually bound to proteins or amino acids. The changes in human body functions are noticed when nickel interacts with other elements, like zinc, magnesium and calcium [17]. These connections can suppress or modify the toxic or cancerogenic effects of nickel [8].

Nickel deficiency causes a reduction in the level of hemoglobin in the blood, fat accumulation in the liver and skin changes. The most important function of this element is to activate dehydrogenases [9].
The influence of nickel in the form of its nanoparticles to the human is related to increasing concentration of ROS. There are studies with indicate that cells exposed to the presence of nickel nanoparticles increased their activity of caspase-3, which suggests increase the risk of apoptosis of these cells. Particularly vulnerable organ to the nickel nanoparticles are the lungs [15]. Moreover, the research indicated that nickel nanoparticles show their genotoxicity and impair the mechanism of degradation of lipids in the liver and
can interfere ion transport in mitochondria [16]. However, the studies on nickel nanoparticles effect on humans are still conducted.

1.3.2 Nickel Toxicity:

the most common disorder following nickel exposure is Nickel dermatitis which are two types

1. Primary – an eczematous allergic reaction following direct contact. Erythematous papules and vesicles may progress to lichenification (Type IV hypersensitivity).
2. Secondary – widespread rash caused by ingestion, transfusion, inhalation, or implanted medical devices orthodontic appliance.

1.3.2.1 toxicity symptoms

nickel compound that is most commonly causes acute, generalized nickel toxicity is Nickel carbonyl a highly volatile liquid compound used in nickel refining and petroleum processin. It has two metabolites:

1. Carbon monoxide
2. Elemental nickel

Initial symptoms after inhalational exposure often produce nonspecific respiratory complaints, including airway irritation, cough, and dyspnea. Nausea, weakness, and headache may develop, as may chemical pneumonitis. Severe symptoms of inhalational or ingestional exposure include myocarditis, ARDS, and cerebral edem [20].

1.3.2.2 The effects of chronic nickel exposure include

Chronic airway irritation and mucosal atrophy may occur along with the development of reactive airway disease and pulmonary fibrosis. Nickel is listed as an IARC Group 1 carcinogen and is associated with nasal and pulmonary carcinogenesis with long-term exposure.

1.4. Cloud point extraction (CPE)

Clouding behavior of micellar solutions is widely exploited as CPE for the extraction and preconcentration of various metal ions, organic and inorganic industrial pollutants, pesticides and proteins. The typical cloud point methodology used for extraction of metal ions is given in Fig. 1. Cloud point extraction (CPE), as an effective separation/pre- concentration technique, first studied by Watanabe and co- workers in the early 1980s, has been demonstrated to have the distinct merits of low cost, simplicity, speed, lower toxicity to the environment than extractions that use organic solvents and a high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and high concentration factors. On the other hand, simple combination with spectral, atomic absorption, chromatographic, and electro- chemical analyses allows using cloud-point extraction for high-sensitive and convenient analytical methods [21,22].

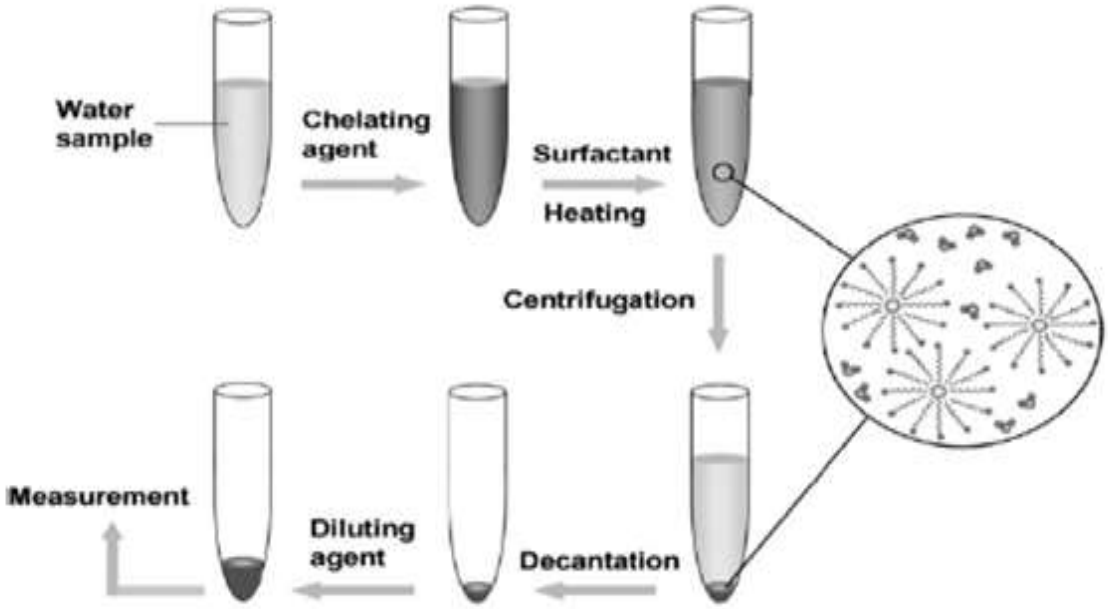

Fig. 1.1 Schematic representation of a conventional CPE to metal preconcentration.

Chapter Two

Experimental

2.1 Instrumentation

2.1.1 UV-Vis Spectrophotometeric Measurements

A Shimadzu 1650 PC model double-beam UV-Vis spectrophotometer (Japan). While absorbance measurements in the optimization study and detection of metals were done with spectrophotometer Sunny UV-7804C (China) Single beam, C-T type, 1200L/mm grating .

2.1.2 Other Supplementary Equipment Used in This Work Were:

A- Water bath WB 710 model (OPTIMA, china).

B- A Microprocessor pH meter 211 model (Triup International Corp, Italy) pH –Meter.

2.2 Chemicals

All chemicals used in this study were presented in Table (2-1) and used directly without any further purification: deionized water was used throughout the experimental work .

Substance	Company	Molecular weight(g/mol)	Purity
Absolute Ethanol	(GCC, England)	46.07	99%
Copper Nitrate	Aldrich	187.55	Analar
Nickel Chloride	Aldrich	129.59	Analar
Triton X-114 CAS#: 9002-93-1	Acros Organics, New Jersey, USA	558.75	100%

. Table 2-1 Chemicals used

2.3 Cloud-Point Extraction for Copper and Nickel

2.3.1 Preparation of Standard Solutions

2.3.1.1 Cu(II) Stock Solution(1000 µg mL^{-1})

This solution was prepared by dissolving 0.1598 g of $Cu(NO_3)_2$ in 100 mL deionized water .The working standard solutions were prepared freshly by appropriate diluting of the stock standard solution.

2.3.1.2 Ni(II) Stock Solution(1000 µg mL^{-1})

This solution was prepared by dissolving 0.1630 g of $NiCl_2$ in 100 mL deionized water. The working standard solutions were prepared freshly by appropriate diluting of the stock standard solution.

2.3.1.3 (10% v/v) Triton X-114

This solution was prepared by taking 10 mL of Triton X-114 in deionized water and diluting to the mark in a 100 mL volumetric flask.

2.3.1.4 Preparation of Buffer Solutions

Acetate buffer solutions were prepared with different pH by dissolving 0.7708 g (0.01 M) ammonium acetate in deionized water and then adjusting the pH by adding different volume from concentrated acetic acid or concentrated ammonia and complete the volume to 1 L with water.

2.3.2 General Procedure for CPE for Cu and Ni Determination

To an aliquot of 2.5 mL of a solution containing known amount Copper (II) or Nickel(II) standard or sample solution, 0.2 mL (for Cu) or 0.3 mL (for Ni) of 5 x 10^{-4} mol L^{-1} (6-MeBTAClP) reagent solution, 1 mL of acetate buffer solution

(pH = 4.0 for Cu or 5.0 for Ni) , 0.5 (for Cu) or 0.4 mL(for Ni) of Triton X-114 (10%) were mixed in a 5-mL standard flask and diluted to mark with distal water. The contents of the flask were transferred into a 10 mL centrifuging tube and the phase separation was induced by heating the contents in a water bath at 60 ºC for 20 min. Separation of the phases was accelerated by centrifuging at 4000 rpm for 20 min. Without cooling, the surfactant-rich phases became viscous. Then, the aqueous phase was separated by using a syringe. Subsequently, 3 mL of ethanol was added to the surfactant-rich phase in order to decrease its viscosity and make the final volume feasible to transfer into the optical cell of 10-mm for the measurement of Ni and Cu spectrophotometrically at 610 and 640 nm respectively against a reagent blank prepared under similar conditions without metal ion .

2.3.3 Optimization Parameters for CPE of Cu and Ni Determination (Classical Optimization)

All of the parameters in the CPE method such as; pH, reagent concentration(C_R) , percentage of surfactant(TX), reaction temperature(T) and incubation time (Et) for the formation of complex and extraction processes were optimized to improve the sensitivity and detection limit for the determination of each ion.

2.3.3.1 Effect of pH

In a separated 5-mL volumetric flasks , aliquots of 2.5 mL of a solution containing 5 mg mL^{-1} Cu (II) or 4.7 mg mL^{-1} Ni(II) , 0.2 mL (for Cu) or 0.3 mL (for Ni) of 5 x 10^{-4} M (6-MeBTAClP) , 0.5 mL (for Cu) or 0.4 mL (for Ni) of 10 % (v/v) Triton X-114 were mixed and dilute to the mark with water after adding varying amount of acetate buffer in the range of pH 3-9. The content of the flask was transferred into a 10 mL centrifuging tube and subjected to general procedure for CPE . The absorbance was recorded for each metal ion at their specific λ_{max} and the results are shown in Figure 3-10 .

2.3.3.2 Effect of (6-MeBTAClP) Concentration

In a separated 5-mL volumetric flasks , aliquots of 2.5 mL of a solution containing 50 mg mL^{-1} Cu (II) or 4.7 mg mL^{-1} Ni(II), varying amounts (0.1-0.5) mL of $5x10^{-4}$ mol L^{-1} (6-MeBTAClP) , 0.5 mL or 0.4 mL of 10% Triton X-114 at optimum pH were mixed and subjected to CPE procedure. The absorbance was recorded and the results are shown in Figure 3- 6 .

2.3.3.3 Effect of Triton X-114 Concentration

Different volumes of 10% (v/v) Triton X-114 ranging from 0.1-1 mL were used in this study keeping other conditions constant were the same as described in 2.5.4.1for all experimentales at optimum pH . The absorbance of each solution including different concentrations of surfactant were measured and the results are shown in Figure 3- 7 .

2.3.3.4 Effect of Equilibrium Temperature and the Incubation Time

The effects of the equilibrium temperature and the incubation time were examined at optimized other conditions . The temperature was varied from 30 ^{o}C to 80 ^{o}C in a search of optimum value and the results are shown in Figure 3- 8, 3-9 .

2.3.4 Preparation of Calibration Curve

Seven standard solutions were prepared by pipetting (0.125 -2.5) mL of 200 mg mL^{-1} of standard Copper solution or (0.106 -2.5) mL of 15 mg mL^{-1} Nickel standard solution into 5 mL volumetric flasks , then 0.2 mL (for Cu) or 0.3 mL (for Ni) of 5.0×10^{-4} mol L^{-1} (6-MeBTAClP) reagent solution, 1 mL of acetate buffer solution (pH = 4.0 for Cu or 5.0 for Ni) , 0.5 (for Cu) or 0.4 mL(for Ni) of Triton X-114 (10%) were added to each flask and diluted to mark

with water . These standard solutions are corresponding to (5-100 mg mL^{-1}) for Copper and (0.32-7.5 mg mL^{-1}) of Nickel. The content of each flask was transferred into a 10 mL centrifuging tube and subjected to the general CPE procedure and an aliquot was transferred into the optical cell of 10-mm for the measurement of each metal ion spectrophotometrically at 550 and 524 nm for Ni and Cu respectively against a reagent blank prepared under similar conditions without metal ion. The standard calibration graph was constructed by plotting absorbance signals versus each metal concentration from which the concentration of Cu(II) or Ni(III) ions in sample solution was determined by regression(Tables 3-34 and 3-36) . The represntitative statistical results for the analysis of Cu(II) and Ni(II) by combined CPE- spectrophotometry was shown in **Tables 3-12 and 3-19**.

2.4 Preparation of Plant Samples [23,24]

0.1 g of the medicinal plant was treated with 5 mL of concentrated HNO_3 and H2SO4 mixture (5 + 2 v/v) for30 min at 160°C in oven , it was then neutralized with 2 mL of 2 mol/L sodium hydroxide, the digest diluted to the mark in a 250-mL calibrated flask and this solution was used as a sample solution.

Chapter Three

Results and Discussion

3.1 Identification of the Prepared Complexes

The UV-Vis spectrum of an ethanolic solution of the of 2-[(6-Methyl -2-benzothiazoly azo)]-4-chlorophenol (1 $x10^{-4}$ M) shown absorption maximum at 203 and 275 nm due to the ($\pi \rightarrow \pi^*$) , at 325 nm due to ($n \rightarrow \pi^*$) and 430 nm where the azo compound displays mainly a broad band in the visible region as shown in Figure 3-1.

The spectra of Cu (II) and Ni(II) complexes show the absorption maxima of 610 and 630 nm with molar absorptivities (ε) of 2 $x10^4$ L mol^{-1} cm^{-1} and 1 x 10^4 L mol^{-1} cm^{-1} obtained respectively, while the ligand 2-[(6-Methyl -2-benzothiazoly azo)]-4-chlorophenol gave the absorption maxima of 430 nm as also depicted in Figure 3-1.

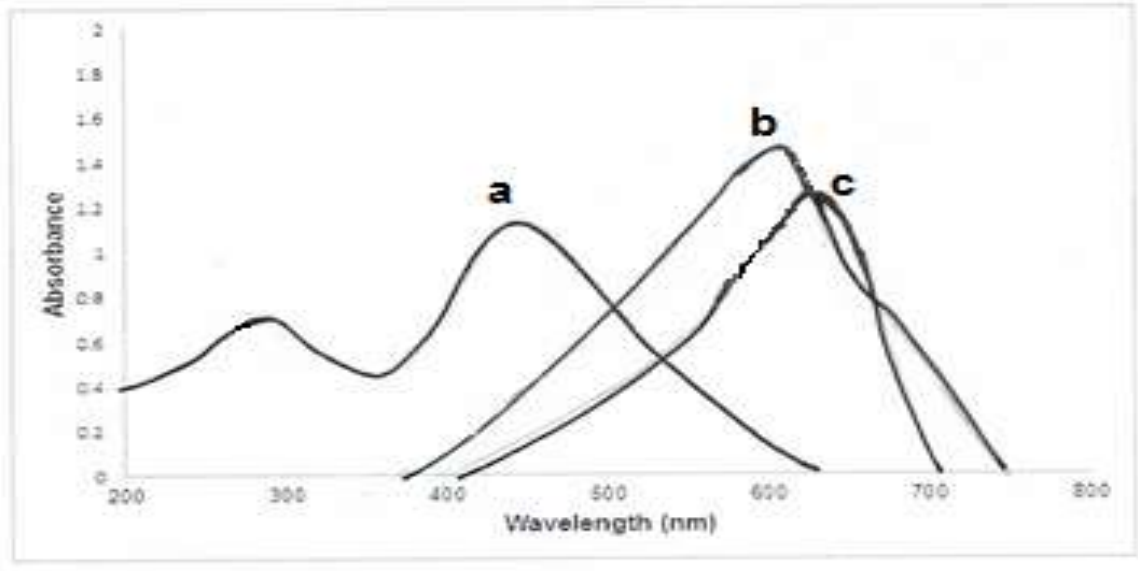

Figure 3-1 Absorption spectra (a) Reagent 2-[(6-Methyl -2-benzothiazoly azo)]-4-chlorophenol = 5 x 10^{-4} M (b) Cu(II)- 2-[(6-Methyl -2-benzothiazoly azo)]-4-chlorophenol complex . (c) Ni(II) - 2-[(6-Methyl -2-benzothiazoly azo)]-4-chlorophenol complex.

3. 2 IR Spectra

The IR spectrum of 2-[(6-Methyl -2-benzothiazoly azo)]-4-chlorophenol was obtained with the compound prepared as (KBr disk) by using a Shimadzu FT-IR spectrophotometer series 8400S. Figure 3-2 and Table 3-1 summarize the IR data of the synthesized (6-MeBTAClP)[25].

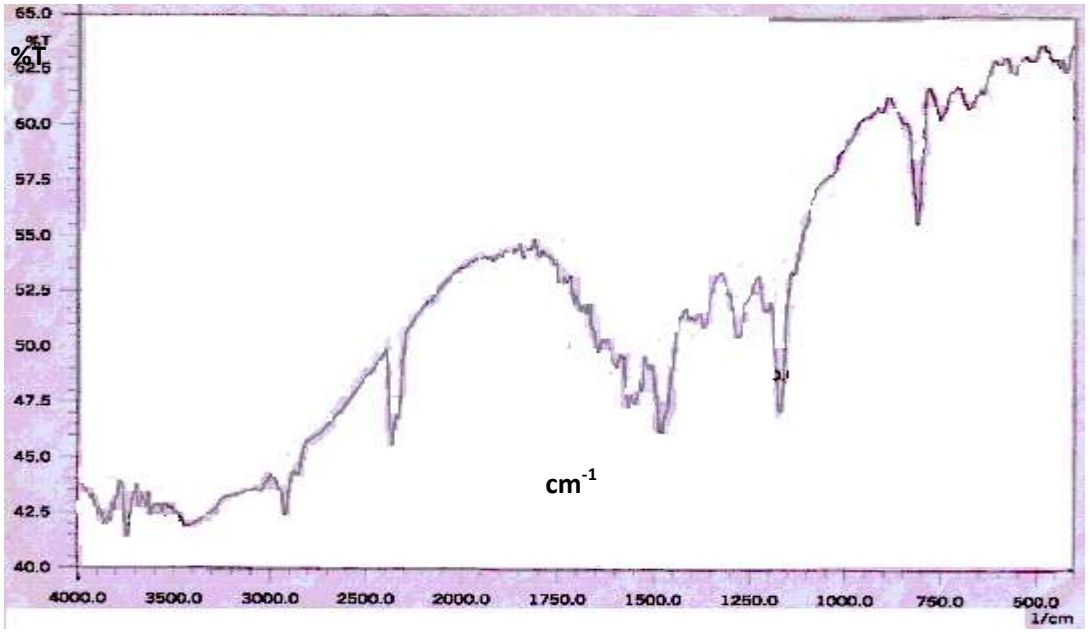

Figure 3-2 IR spectrum (KBr) of (6-MeBTAClP)

Wave number (Cm^{-1})	Groups
٣٢٠٠-٣٧٥٠	υ O-H,N-H
٢٨٥٦	υ C-H Aliphatic
٢٩٢٢	υ C-H Aromatic
١٧٠٠	υ C=N
١٤٨٣	υ N=N
١٥١٠	υ C=C
١١٧٣	υ C-S
٨١٦	υ C-Cl
١٢٨٠	υ C-O Phenolic

Table 3-1: The characteristic I.R Spectra of (6-MeBTAClP)

The infrared spectra of the complexes have shown some other new bands which appeared in the range of 501-509 cm^{-1} are due to the ν(M- O) and ν(M- N) respectively [26] .

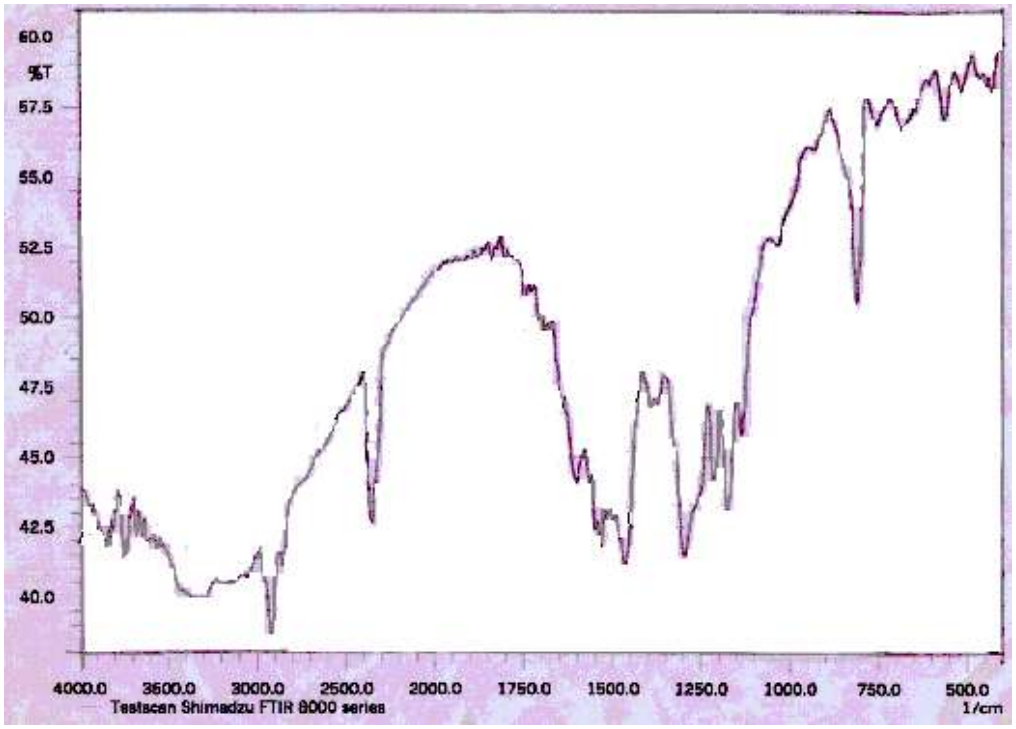

Figure 3-3 IR spectrum (KBr) of Cu - (6-MeBTAClP)complex.

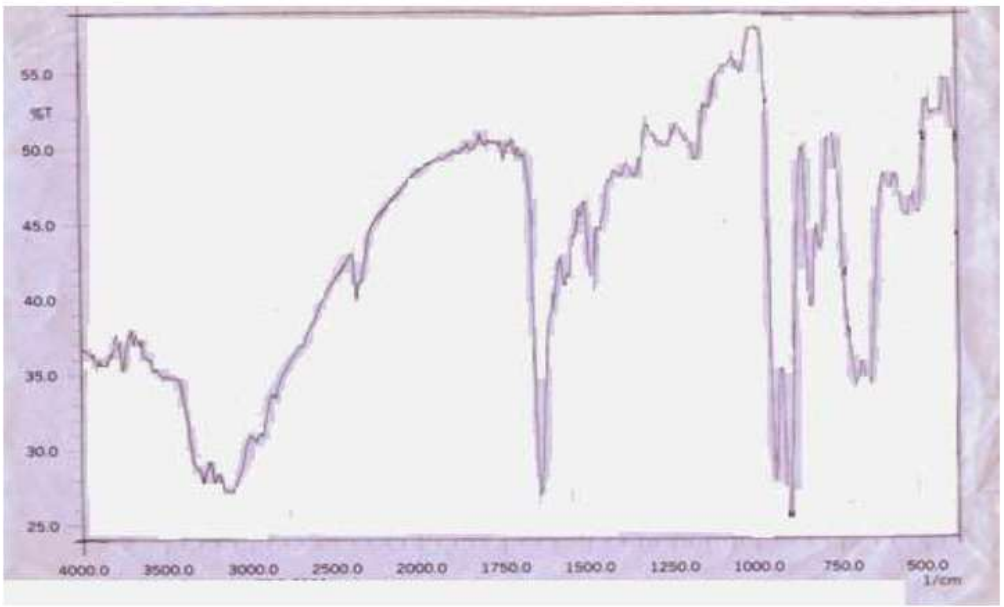

Figure 3-4 IR spectrum (KBr) of Ni -(6-MeBTAClP)complex .

Wave number (Cm^{-1})	Groups
٣٢٠٠-٣٧٥٠	υ O-H,N-H
٢٨٥٤	υ C-H Aliphatic
٢٩٢٠	υ C-H Aromatic
١٦٨٥	υ C=N
١٤٦٣	υ N=N
١٥١٠	υ C=C
١١٧٥	υ C-S
٨١٤	υ C-Cl
١٢٩٤	υ C-O Phenolic
410	υ N-M
420-500	υ O-M

Table 3-2: The characteristic I.R Spectra of M(II)- (6-MeBTAClP) complex
M= Cu or Ni

3.3 Cloud-point Extraction and Determination of Copper and Nickle with (6-MeBTAClP) (Optimization of CPE Procedure)

The effects of several experimental parameters which impact the CPE efficiency were carried out by classical optimization .

3.3.1 Effect of pH

The effect of pH on formation of the M(II)-(6-MeBTAClP) complexes in Triton X-114 medium was determined by recording their absorbance signals at λ_{max} ,over the range of 3-9 ,using different pH acetate buffer solutions. The results are shown in Figure 3-5. As can be seen in Figure 3-5, the absorbance first increased with increasing pH and reached a maximum at pH 4.0 and 5.0 for Cu(II) and Ni(II) complexes ,respectively. The absorbance gradually decreased because of partial dissociation of the complexes at higher pH, which may result in incomplete extraction of both complexes. Therefore, pH 4.0 and 5.0 were selected as the

optimum pH's for complete formation of Cu(II) and Ni(II) complexes respectively .

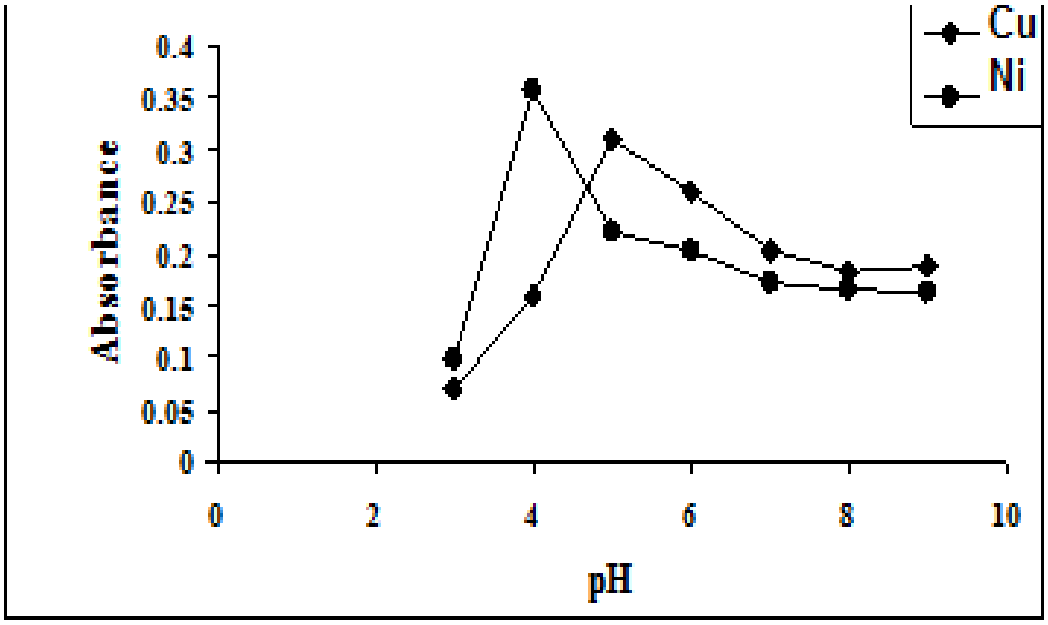

Figure 3-5 Effect of pH on the formation of (6-MeBTAClP)- M(II) complexes formed with Cu (II) and Ni (II)).

3.3.2 Effect of Reagent Concentration

The effect of the (6-MeBTAClP) concentration was investigated by measuring the absorbance signal according to the general CPE procedure of solution containing Cu or Ni , and various amounts of the (6-MeBTAClP) (0.1-0.5 mL) of $5x10^{-4}$ mol L^{-1}. In both cases (Cu(II) or Ni(II)), the analytical responses increase rapidly as the concentration of (6-MeBTAClP) increases, and decrease slightly with further increase in the chelating agent (Fig.3-6).

Consequently, 0.2 or 0.3 mL of $5x10^{-4}$ mol L^{-1} of (6-MeBTAClP) was chosen as optimum for Cu(II) or Ni(II). The slight difference in the contact of

chelating agent with Cu(II) or Ni(II) may be attributed to differences in the stability constants of complexes formation in the micellar medium[27] .

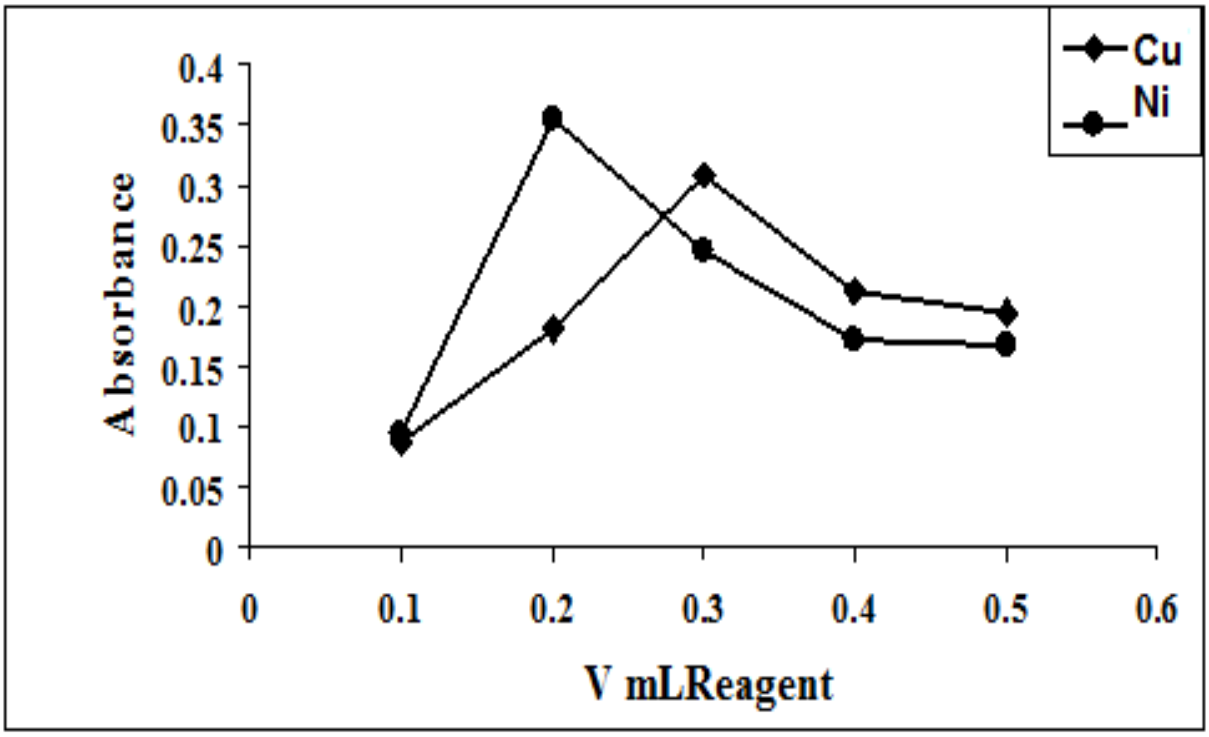

Figure 3-6 Effect of (6-MeBTAClP) concentration on the determination of Cu(II) and Ni(II) .

3.3.3 Effect of Triton X-114 Concentration

Figure 3-7 depicts the effect of variation of Triton X-114 amount on the absorbance signal for the determination of Cu (II) and Ni(II) ion. Different volumes of Triton X-114 (10% v/v) ranging from 0.1- 1 mL were used in this study at previously optimum conditions. As shown in Figure 3-7 , the absorbance for both ions increased by increasing the Triton X-114 concentration up to 0.5 and 0.4 mL of 10% (v/v) for Cu(II) and Ni(II) respectively , and then suddenly decreased at higher amounts. Therefore, 0.5 and 0.4 mL of 10% (v/v) Triton X-114 was used as the optimum concentration for Copper and Nickle respectively .

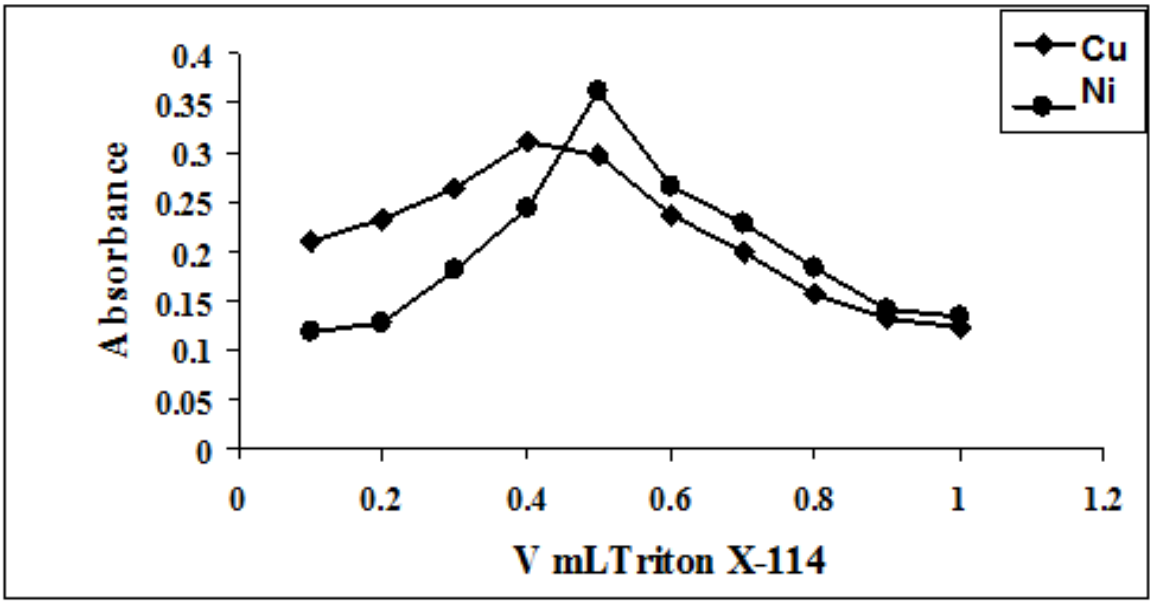

Figure 3- 7 Effect of TritonX-114 concentration on the analytical signal.

3.3.4 Effect of Equilibration Temperature and Incubation Time

The effects of the equilibrium temperature and the incubation time were examined due to their importance for the reaction completion and efficient separation of the phases, which reflect certainly the magnitude of preconcentration factor of an analyte. Consequently, a study was carried out to choose the range of temperature that enhances higher absorbance signals for Cu (II) and Ni (II) ions. The temperature was varied from 30 ^{o}C to 80 ^{o}C in a search of optimum value. It can be seen from Figure 3-8 that the highest absorbance signals were achieved when the temperature is 60 ^{o}C.

It was also observed that the incubation time of 20 min is sufficient for the maximum absorbance of both ions (Figure 3-9). Thus, the temperature of 60^{o}C for

20 min was selected to fulfill efficient separation conditions. The effect of centrifugation rate and time also was investigated on extraction efficiency. A centrifuge time of 20 min at 4000 rpm was selected for the entire procedure as being optimum and beyond this time no confirmation was observed for improving extraction efficiency.

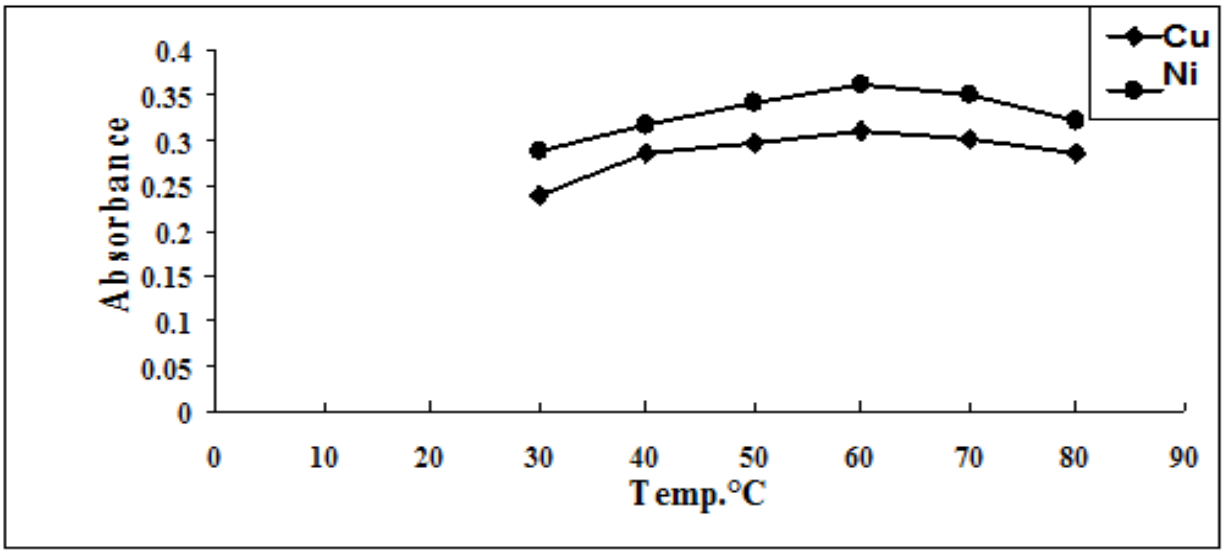

Figure 3- 8 Effect of temperature on the absorbance for Cu(II) and Ni(II) complexes

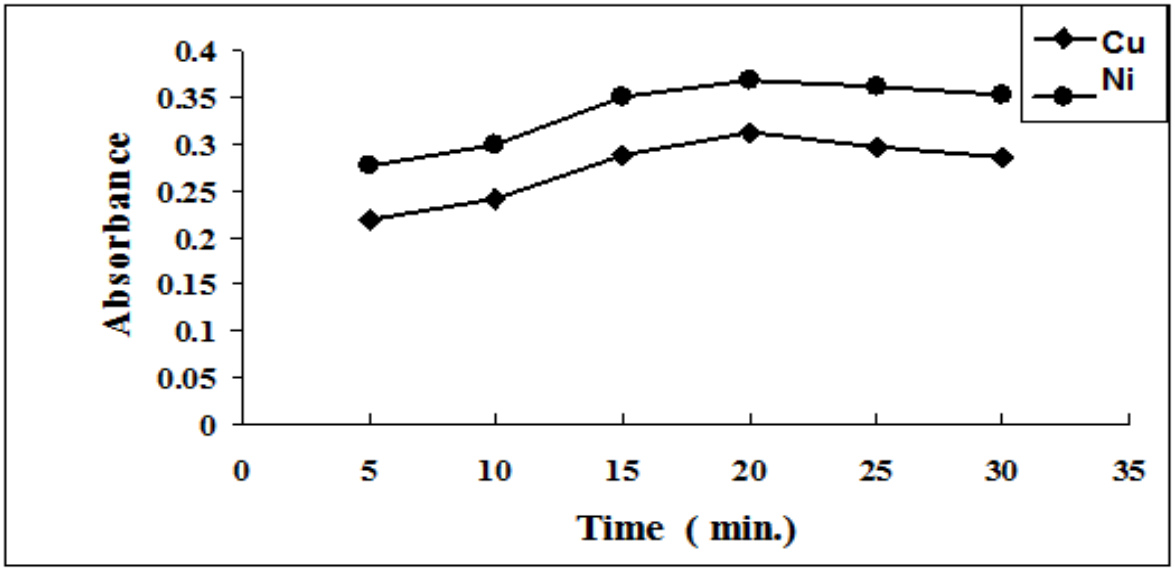

Figure 3-9 Effect of the incubation time on the absorbance for Cu(II) and Ni(II) complexes.

3.4 Calibration Curve for Copper

Under the optimized conditions established by CPE procedure for the determination of Cu(II) , linear calibration graph was established by plotting absorbance versus concentration of Cu(II) ion . Figure 3-10 represents the calibration Curve.

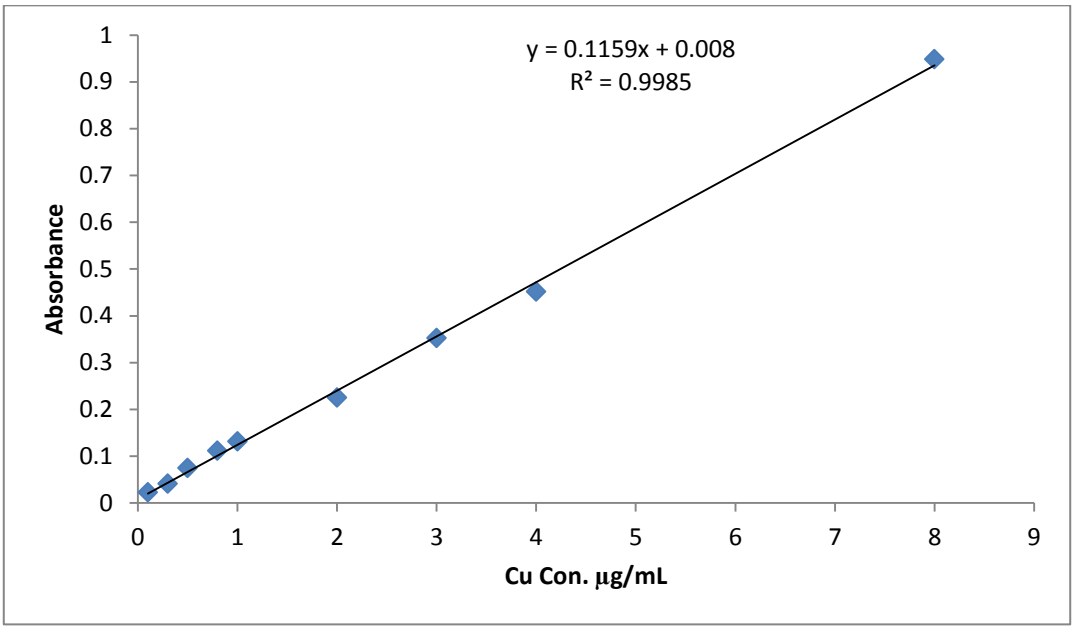

Figure 3-10 Calibration graph for Cu.

3.5 Calibration Curve for Nickle

Linear calibration graph through the origin was obtained which obeyed Beers law over the range (0.05 – 8.0) µg.ml-1 0f Cd^{2+} .The average molar absorptivity was found to be (1.23 x 10^{+4}) L. mol^{-1}. cm^{-1} .

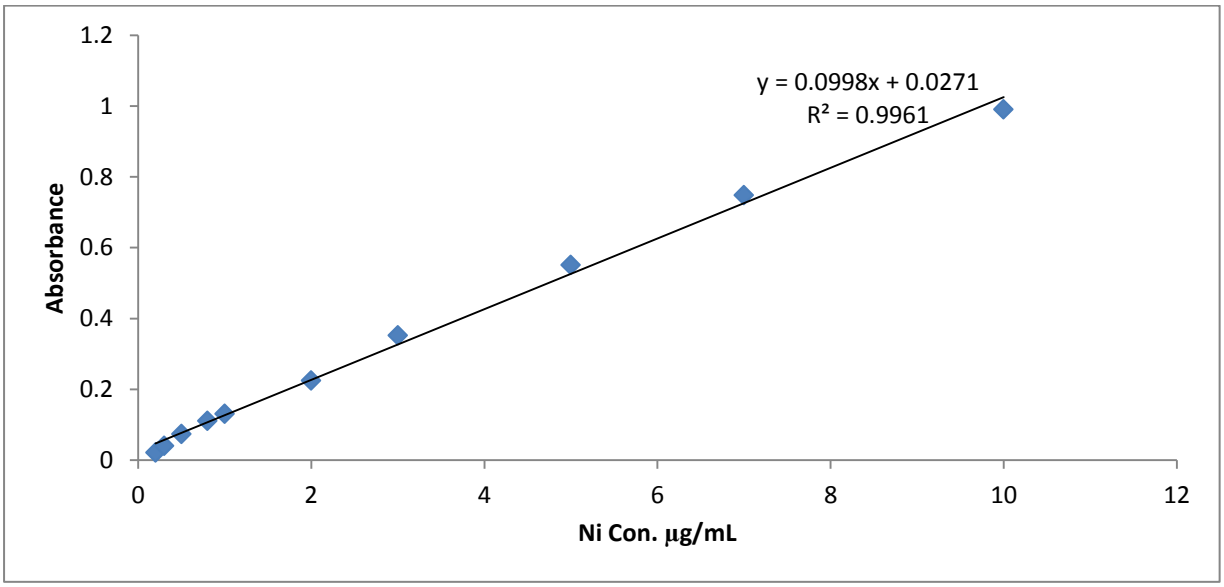

Figure 3-11 Calibration Curve for Nickle.

3.6 Determination of Cu(II) and Ni(II) in some medicinal plants

The developed method was applied to the copper and nickel determination in five medicinal plants samples purchased from local markets and one drug. The results of the combined CPE-Spectrophotometry were showed in Table 3-3.

Scientific name	Common name	Part used	Concentration of Cu (µg g-1)	Concentration of Ni (µg g-1)
Spinacia oleracea L	spinach	leaves	0.52	0.33
Coriandrum sativam L	coriander	leaves	0.60	0.41
Menthe spicata L	Spearmint	leaves	0.57	0.45
Cinnamomum zeylanicum	cinnamon	Bark	0.44	0.39
Syzygium aromaticum	clove	Flower bud	0.49	0.36

Table 3-3: Spectrophotometric determination of Cu (II) and Ni (II) in some medicinal plants samples using CPE

The results in Table 3-3 revealed that mean values obtained of Cu in all the selected samples were considerably below the permissible criteria i.e < 0.3 μg g^{-1} and ranged from 0.067 to 0.288 μg g^{-1} according to the norms established by the Codex Alimentarius Commission of the Food Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations[28]. Whereas Ni concentration exceeded the permissible level for the most selected medicinal plants according to Codex Alimentarius Commission norms which should be at 0.03 μg g-1. However, Ni concentration in most samples (Table 3-3) gave below the permissible levels according to European Union Standards 40 i.e.<. 0.1 μg g^{-1} and ranged from 0.02 to 0.098 μg g^{-1}[29.30].

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