An-Najah National University

Faculty of Graduate Studies

Spectrophotometric determination of some metal ions via complex formation with carboxylated tris(2-aminoethyl)amine chelating agent

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Dedication

I dedicate this thesis to all who stood by me and supported my steps in any way and means…

My greatest thanks and gratitude to Allah first of all, then to my family, specially my father and mother for their endless encouragement and advice, my sisters and brother …

To my uncles, aunts, grandmothers and grandfathers, who encouraged me to go ahead and never give up...

To my companions who bear me through all the difficulties I went through, who always push me forward, …

To all of you and to those whom I didn't mention, my gratitude and appreciation…

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الاقرار

انا الموقع ادناه مقدم الرسالة التي تحمل عنوان:

Spectrophotometric determination of some metal ions via complex formation with carboxylated tris(2-aminoethyl)amine chelating agent

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية اخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name:	**اسم الطالب:**
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List of Abbreviations

Abbreviation	Full Name
TAHA	Tris(2-aminoethyl)aminehexaacetic acid
APCAs	Aminopolycarboxylic acid
$\mathbf{X_{ligand}}$	The mole fraction of ligand
$\mathbf{X_{metal}}$	The mole fraction of metal
$\mathbf{A_1}$	The absorbance of solution containing the other absorbing substance
$\mathbf{A_{corrected}}$	The corrected absorbance
$\mathbf{A_{measured}}$	The measured Absorbance
$\mathbf{V_{initial}}$	The initial volume of solution
$\mathbf{V_{titrant}}$	The volume of added titrant
$\mathbf{A'_{measured}}$	The measured absorbance corrected for dilution
$\mathbf{K_{formation}}$	The formation constant
$\mathbf{A_a}$	The absorbance at break point
$\mathbf{A_b}$	The actual absorbance
ε	The molar absorptivity
LOD	Limit of detection
$\mathbf{S_{analyte}}$	The analyte’s signal
$\mathbf{S_{blank}}$	The average blank signal
$\boldsymbol{\sigma}_{\mathbf{blank}}$	The standard deviation of the blank signal
Z	A factor of the desired confidence level
$\mathbf{C_{analyte}}$	The analyte’s concentration
LOQ	Limit of quantitation
b	Cell path length

Spectrophotometric determination of some metal ions via complex formation with carboxylated tris(2-aminoethyl)amine chelating agent

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Abstract

An aminopolycarboxylic acid chelating agent tris(2-aminoethyl)aminehexaacetic acid (TAHA) was prepared and characterized. The TAHA was prepared from tris(2-aminoethyl)amine and potassium chloroacetate by nucleophilic substitution reaction. FT-IR spectroscopy was used to ensure the formation and elucidate the purity of the potassium salt and the acid form of TAHA.

TAHA forms stable complexes with copper(II) ions and some other metal ions at pH 10. The complexation behavior was studied by spectrophotometry at the complex maximum wavelength.

Mixtures of Cu(II) and other metal ions (M^{2+}; Ca^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}) were titrated with TAHA at pH 10. $[Cu(NH_3)_4]^{2+}$ complex is more stable than $[M(NH_3)_4]^{2+}$. Thus, at the beginning, TAHA replaces ammonia in $[M(NH_3)_4]^{2+}$ complex until completion. Then it exchanges ammonia from $[Cu(NH_3)_4]^{2+}$ to form Cu(II)–TAHA complex. As expected, TAHA does not form complexes with alkali metal ions. The stoichiometry of ligand - to - metal was found to be 1:1 for the metal ions studied.

The stability constant of Cu(II)–TAHA complex was determined to be 1.86×10^{5} by using the continuous variation method. The complex showed a good stability for an extended period of time.

Beer’s low was obeyed over the concentration range 3.0×10^{-4} M – 1.2×10^{-2} M for Cu(II) solution. The results of the quantitative determination of Cu(II) gave LOD and LOQ values of 7.285×10^{-6} M and 2.428×10^{-5} M respectively. The relative standard deviation for five replicate samples was found to be 1.088% and 4.804% for 1.2×10^{-2} M and 3.0×10^{-4} M respectively.

Chapter One

Introduction

1.1 Background:

The detection of metal ions, especially transition metals both in solution and in solid materials is of increased concern, as a result of increased environmental pollution worldwide. Metals play an important role for biological activities of all living organisms in a proper balance. Transition metals deficiency causes many clinical disorders and physiological abnormalities in the human body. For example, iron is vital for normal brain and nerve functions. Copper is important for the human body to maintain a healthy lifestyle [1, 2].

While the required levels for animals and the human body are very low (trace amount), and much lower than the levels of heavy metals present in foods. Some metal ions may cause serious health problems and toxicity to humans, animals as well as to plants [3].

Free metal ions cause challenging problems in many industrial processes. They may catalyze the decomposition of organic compounds, or form insoluble metal salt precipitates. Aminopolycarboxylic acid chelating agents interact with metal ions, and restrict them from playing their normal role in unwanted chemical reactions [4].

Spectroscopic methods of analysis introduce a good choice for detecting metal ions. These methods provide the advantages of simplicity, low cost, time and effort saving, besides the ease with which the data can be manipulated and analyzed.

Researchers were able to resolve many industrial and biological problems with the aid of complexation of metal ions. This technique can be used for recovering metal ions from various sources. Complexation concepts provided an attracting area of work and opened new technological pathways [5].

1.2 Aminopolycarboxylic acid chelating agents

Chelating agents are important for complexing divalent and trivalent metal ions from aqueous solution. These agents are generally classified into two categories; aminopolycarboxylates (APCAs) and polyphosphonates [6]. Aminopolycarboxylic acid compounds are recognized by having one or more amino groups and at least two carboxyl groups [7].

Aminopolycarboxylates have interesting chemical properties; their complexes with metal ions are highly soluble in water with high stability constants.

Stability of APCAs complexes is attributed to two reasons; the first is the formation of one or more stable heterocyclic rings. This aspect is called chelate effect.The second reason is the presence of basic secondary or tertiary amino groups that give large negative charge to the chelating agent, and take part in the high stability of complexes [4].

Examples of aminopolycarboxylates are ethylenediaminetetraacetic acid (EDTA), iminodiacetic acid, N-(2-hydroxyethyl) ethylenediaminetriacetic acid, nitrilotriacetic acid, diethylenetriaminepentaacetic acid and ethylenediamine N, N' bis(2-hydroxyphenyl glycine). These compounds have been named in expressions of the free acids [7, 8].

1.3 Preparation of Aminopolycarboxylic acid compounds

Aminopolycarboxylic acid compounds can be prepared by the reaction between ammonia, or mono-, di- or polyamines with halogenated carboxylic acids, such as; chloroacetic, iodoacetic, bromooacetic, chloropropionic and substituted chloroacetic and chloropropicnic acids [7, 8].

Another method that can be used for the preparation of aminopolycarboxylates is the two - step singer method. The first step is the reaction of certain amines with hydrocyanic acid and formaldehyde in acidic media. In the second step, the product separated from the system is washed and treated with sodium hydroxide. In this method the pure salt results [9- 12].

A third method is the single - step synthesis, which is performed by the addition of certain amines, sodium cyanide and formaldehyde in alkaline medium. The sodium salt produced is then acidified with sulfuric acid [9- 12].

Many aminopolycaroxylates such as, DTPA (diethylenetriaminepentaacetic acid), IDA (iminodiacetic acid), HEDTA (ethylenediamine hydroxybutyric acid), NTA (nitrilotriacetic acid), ADA (two amino acid acetate), are produced in the same way [9, 10].

1.4 Traditional chelating agents including, EDTA and NTA

Many chelating agents have been used in colorimetric or titrimetric methods to induce analytical signals after complex formation with metal ions. Such as, ethylenediamine tetraacetic acid (EDTA) [13].
One of the first APCAs chelating agents is nitrilotriacetic acid (NTA) which was prepared according to Heintz method in 1862 [4, 6].

The Quadridentate chelating ligand (NTA) comprise four donor atoms and it forms 1:1 complexes with metal ions by making three chelate rings with metal ions [4].

EDTA is a hexadentate ligand, that ideally forms octahedral complexes with metal ions in a 1:1 stiochiometric ratio [4, 13]. Important aspect for EDTA include; i) It can be obtained as a primary standard so it does not need further standardization. ii) Rapid complex formation in water with high solubility. iii) An equivalence point is facilely reached in complexometric titrations [14].

1.5 Applications for APCAs

APCAs are widely used in a large number of industrial processes and products because of their metal sequestering capacity [4]:

1) To prevent metal salt from precipitation.

2) To hinder metal ion catalysis of unwanted chemical reactions, such as decomposition of organic compounds.

3) To keep metal ions in the solution.

4) To remove metal ions from systems.

Aminopolycarboxylic acids are used in many industrial applications, such as pulp and paper production, agrochemicals, industrial cleaning, water treatment, photographic industry, textile industry, metalworking, food industry, and in blood banks [6, 15, and 16].

In this study, tris(2-aminoethyl)aminehexaacetic acid was chosen as a ligand, because it is similar to EDTA and it is expected to have the following properties:

1. High solubility in water.
2. High complex stability with large formation constants.
3. High selectivity.
4. Rapid complex formation.
5. Intense absorption bands in the infrared region because of carboxylic acid functional groups, which are strong infrared absorbers [13].

Tris(2-aminoethyl)aminehexaacetic acid is expected to form more stable complexes than EDTA due to higher number of chelation sites [17].

The structure of tris(2-aminoethyl)aminehexaacetic acid is shown in figure 1.1:

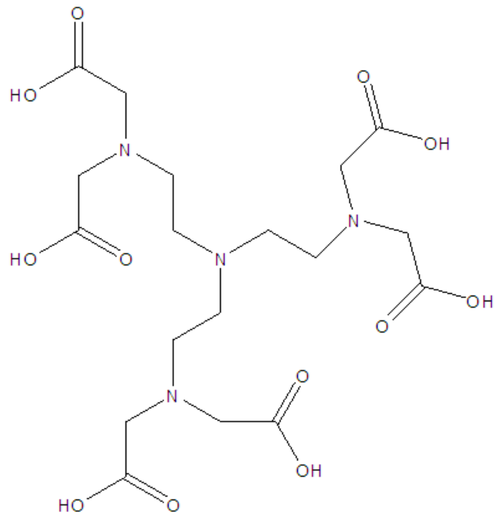

Figure 1.1: Tris(2-aminoethyl)aminehexaacetic acid structure.

According to the IUPAC system, tris(2-aminoethyl)aminehexaacetic acid is named as: 2, 2', 2'', 2''', 2'''', 2''''' - (2, 2', 2'' - nitrilotris(ethane - 2,1 diyl) tris (azanetriyl)) hexaacetic acid.

Tris(2-aminoethyl)aminehexaacetic acid (TAHA) like other APCAs is important in science and for medical proposes, in both diagnostic and therapeutic applications [18].

TAHA and its metal complexes can be characterized by several techniques, such as potentiometry, NMR [17], FT-IR, spectrophtometry, mass spectrometry, X-ray spectroscopy and elemental analysis.

1.6 Heavy metal complexes with APCAs

Complexation reactions are very common in nature and in human body [15]. Optical methods applied for detection of heavy metals benefit from the high affinity of oxygen, nitrogen and sulfur donor atoms towards these ions [19].

According to the Hard Soft Acid Bases (HSAB) principles, Ag^+, Cu^+, Cd^{2+}, Hg^{2+} and Pb^{2+} are classified as soft lewis acids. They bind favorably to ligands containing sulfur atom; and the borderline acids Ni^{2+}, Cu^{2+}, Co^{2+} and Zn^{2+} prefer binding to nitrogen atom [19].

Aminopolycarboxylates chelating agents such as ethylenediaminedisuccinic acid (EDDS), iminodisuccinic acid (ISA), N-bis[2-(1,2-dicarboxyethoxy)-ethyl]glycine (BCA5), N-bis[2-(1,2-dicarboxyethoxy)ethyl]aspartic acid (BCA6), N-tris[(1,2-dicarboxy-ethoxy)ethyl]amine (TCA6), and N-bis[2-(1,2-dicarboxyethoxy)ethyl]methylglycine (MBCA5), were reacted with copper (II). Other transition metals and alkaline earth metals form stable complexes with these ligands. The protonation and complex formation equilibria of these ligands with metal ions were studied in aqueous solution by potentiometric titration [20].

1.7 Selectivity in complexometric titrations

In complexometric titrations, increasing selectivity of metal – ligand complexes can be achieved through controlling the pH, using an auxiliary complexing or masking agent, proper titrant, and controlling differential reaction rates.

Selectivity of APCAs in complexometric titrations can be enhanced by proper control of pH using a suitable buffer solution as an auxiliary complexing agent. Some metals can be titrated at basic media with high pH value like, Ba, Cr, Mg, Ca, at pH 11. While at pH 4 – 7 metals like Fe, Co, Mn, Pb, Zn, Ni, Cd, Al, and Cu can be titrated.

Calcium can be titrated at pH 10 in the presence of interference such as Zn or Cd by using KCN as a masking agent. In the presence of ammonia buffer, $Cd(NH_3)_2$ forms less stable complex than $Zn(NH_3)_2$, so only Cd^{2+} ions are titrated [14].

1.8 Determination of stoichiometry and stability

Spectrophotometric methods are valuable in studying complex stoichiometry and in determining equilibrium constants [21].

A spectroscopic method is usually more applicable than usual chemical methods. An important reason is that chemical methods are destructive [22].

Many techniques like potentiometric titration, conductometry, and spectrophotometry, are known for the determination of stability constants. Spectrophotometric methods have the advantage of sensitivity in addition to being appropriate for determination of stability constants in solution under different experimental conditions.

Overlapping of spectra of different chemical species present in the equilibrium is an important problem. It is difficult or even impossible by classical methods to find stability constants. The obtained results may also have large uncertainties. However, chemometric method resolve this problem [23].

UV – visible spectrophotometry provides an easy way to determine stability constants of complexes, depending on some well known methods. These are continuous – variation method, mole – ratio method, and slope – ratio method. These methods are used widely because they rely on simple experimental measurements and straightforward theories [21].

1.8.1 Continuous variation method

This is the most common method for determining complex formula. In this method, a series of solutions are prepared with varied number of moles of ligand and metal ion, while keeping the total number of moles constant.

The mole fraction of ligand (X_{ligand}) and the mole fraction of metal (X_{metal}) are used to express the relative amount of each in each solution.

Absorbance of solutions at complex maximum wavelength, is plotted versus X_{ligand}. This gives two linear segments that intersect at a point that represents the stochiometric amount.

Curve curvature at the region of maximum absorbance gives an idea about complex dissociation. Extrapolation of the two segments gives the right ligand to metal ratio.

If more than one species absorbs at the selected maximum wavelength of the complex, absorbance must be corrected to give right complex formula [24].

The corrected absorbance may be written as in Equation (1.1) [25]:

$$A_{corrected} = A_{measured} - \frac{(\text{Volume metal (mL)} - \text{Volume TAHA (mL)})}{\text{Volume metal (mL)} + \text{Volume ligand (mL)}} \times A_1 \qquad (1.1)$$

Where A_1 is the absorbance of solution containing the other absorbing substance.

Some precautions are important when applying the continuous variation method; firstly, a single metal – ligand complex is formed. If more than one complex results and absorbs at the maximum wavelength, suitable correction is required. Secondly, the complex must obey Beer’s law upon the concentration range used. Thirdly, buffer solution should be used to control pH because complex formation is greatly affected by pH. Fourthly, when the complex stability constant is small, a plot of absorbance versus the mole fraction of ligand may exhibit a significant curvature so it is difficult to use extrapolation to determine the complex formula [24].

1.8.2 Mole ratio method

In this method, a series of solutions are prepared, with the molar concentration of the metal is held constant and the molar concentration of the ligand is increased. The absorbance is measured at the complex maximum wavelength. The absorbance is then plotted against mole ratio of ligand to metal.

If more than one species absorbs at the selected maximum wavelength of the complex, absorbance must be corrected to give right complex formula [24].

The corrected absorbance may be written as in Equation (1.2) [25]:

$$A_{corrected} = A_{measured} - \frac{(\text{Volume metal (mL) - Volume TAHA (mL)})}{\text{Volume metal (mL)}} \times A_1 \qquad (1.2)$$

Where A_1 is the absorbance of solution containing the other absorbing substance.

1.8.3 Slope ratio method

This method is widely applicable for studying the stoichiometry of weak complexes. Herein, complex formation can be forced to be completed by using a large excess of the metal or ligand [26].

In this method two series of solutions are prepared, the first series contains constant amount of metal ion and a various amount of ligand. The total concentration of metal must be much larger than the total concentration of ligand. The complex is expressed as (MxLy).

If absorbance is monitored at a wavelength where only MxLy absorbs, then $A = \varepsilon\, b\, [MxLy] = \varepsilon\, b\, C_L/y$.

Where; x and y are the moles of metal and ligand respectively in the complex. b is the cell path length (cm).

A plot of absorbance versus ligand concentration will be linear, giving a slope equal to:

$$Slope\ ligand = \frac{\varepsilon b}{y} \qquad (1.3)$$

The second series consists of constant concentration of ligand that is much larger than the total variable concentration of metal.

A plot of absorbance versus metal concentration will be linear, giving a slope equal to:

$$Slope\ metal = \frac{\varepsilon b}{x} \qquad (1.4)$$

The ratio of the two slopes gives the mole ratio of the ligand to the metal in the complex [24, 26].

1.9 Photometric Titrations

Photometric titrations have useful applications in acid – base titrations and complexometric titrations. They are extensively used in organic analysis.

There are some basic requirements for a successful photometric titration. Such as; i) The absence of an interfering species. ii) The molar absorbtivity of the complex need to be high and show sharp change with an acceptable titrant volume. iii) Correction must be made to compensate for dilution, or when more than one complex is formed at the measuring wavelength [14].

1.9.1 Correction method:

A modified correction method was applied to solve the problem that different absorbing species are formed during titration. These species absorb at the studied wavelength with a strong overlap.

The modified correction method combines the second correction method (Equation 1.2) [25] with the absorbance dilution correction. The modified method was used in this study for spectrophotometric titrations.

The second correction method (Equation 1.2) was used previously for mole ratio and continous variation methods to follow only one absorbing species [25]. Herein, modification of the correction method was made according to the behavior of metal(II)-TAHA and other metal-buffer complexes present in the titration mixture. This correction was used for the titration of a single metal as well as for metal mixtures with TAHA ligand at pH 10.

For spectrophotometric titrations, the measured absorbance was initially corrected for dilution by multiplying absorbance with the dilution factor:

$$\text{Dilution factor} = \frac{(V_{\text{initial}} + V_{\text{titrant}})}{V_{\text{initial}}} \qquad (1.5)$$

Where; V_{initial}: initial volume of solution, and V_{titrant}: volume of added titrant.

Absorbance was corrected according to the modified method as Equation (1.6):

$$A_{\text{corrected}} = A'_{\text{measured}} - \frac{(\text{Volume metal (mL)} - \text{Volume TAHA (mL)})}{\text{Volume metal (mL)}} \times A_1 \qquad (1.6)$$

Where; A'_{measured} is the measured absorbance corrected for dilution, A_1 is the absorbance of solution containing the other absorbing substance.

Specific description for the modification will be discussed later in chapter three. The titration curve using the modified correction method gives more clear results with sharper end points.

1.10 Objectives

The main objectives of this work are:

- Preparation and characterization of aminopolycarboxylic acid chelating agent (TAHA).
- Studying the complexation behavior of some metal ions, especially copper(II) ions with the synthesized ligand spectrophotometrically.
- Determining the formula and stability constant of the copper(II) complex.

1.11 Hypothesis

This work involves some important aspects like:

- TAHA is expected to form stable complexes with some heavy metals such as Cu^{2+}, Co^{2+}, Cd^{2+}, and Ni^{2+}. This constitutes a basis for the determination of these metals either by classical visual titrimetry or by spectrophotometry.

- In the case of mixtures of Cu(II) and other metal ions (Ca^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}), TAHA is hypothesized to replace ammonia in $[M(NH_3)_4]^{2+}$ complex until completion. Then it exchanges ammonia from $[Cu(NH_3)_4]^{2+}$ to form Cu(II)–TAHA complex.

- A modified correction method can be used to resolve absorbance-overlapping problem that arose from the fact that more than one complex form and absorb at the studied wavelength.

- TAHA and its metal complexes are hypothesized to be stable for an extended period of time.

1.12 Novelty of this work

The novel aspect of this work includes:

- Spectrophotometric determination of some heavy metals by complex formation with free TAHA for the first time.

- Studying the complexation nature of various divalent metal ions with free TAHA by spectrophotmetry for the first time.

- Applying a modified correction method to solve the problem that more than one complex are formed in the reaction mixture and absorb at the studied wavelength with a strong overlap for the first time.

Chapter Two

Experimental

2.1 Reagents

Tris(2-aminoethyl)amine, Chloroacetic acid and $CdCl_2.2H_2O$ (Sigma - Aldrech). KOH, NaCl, NaOH, $CuSO_4.5H_2O$, CH_2Cl_2, KCl, NH_4OH and NH_4Cl (Frutarom). $CoSO_4.7H_2O$, $NiSO_4.6H_2O$, and $CaCl_2$ anhydride (Riedel). HCl (Merck). Ethanol (Sun farm). Methanol (Chem.Samuel). EDTA disodium salt dihydrate (Alfa Aesar).

All chemicals were analytical grade.

All solutions were prepared with distilled water.

Absorbance was measured against ammonia buffer as a blank solution.

2.2 Apparatus

Shimadzu (UV-3101DC) UV-VIS-NIR scanning spectrometer was used for absorption measurements. Quartz cuvettes (1-cm bath length) were used. JENWAY (3510) pH Meter was used to measure pH. ATR- Fourier transform infrared spectrophotmeter (Necolet Is5 - Id3) was used to obtain IR-Spectra. Heidolph, LABOROTA 4000 - efficient, rotary evaporator.

2.3 Procedure

2.3.1 Solutions

The following aqueous solutions were prepared and used:

1. Ammonia buffer solution (pH 10).
2. Aqueous solutions of different concentrations of metal ions.

3. Different concentrations of tris(2-aminoethyl)aminehexaacetic acid.

2.3.2 Synthesis of TAHA ligand

Potassium chloroacetate was prepared by the addition of dried chloroacetic acid (5.67g, 0.06 mol) to KOH solution (11.2 mL, 5.3 M).

Then tris(2-aminoethyl)amine (1.5 mL, 0.01 mol) was added and the mixture was heated to 90 - 100^{o}C.

The pH was adjusted to 10, and maintained throughout the reaction, by the addition of KOH solution and reflux for 10 minutes.

This last step was repeated until no consumption of the base was observed [8].

2.3.3 Acidification of TAHA:

The above solution was chilled at 0°C in a freezer for 24 hours. The precipitated potassium chloride was filtered and discarded. The filtrate was acidified to a pH of 3 - 4 with formic acid, cooled in an ice bath with checking the acidity every 15 minutes. Ethanol (equal volume) was added, and the lower aqueous layer was separated and dried under reduced pressure at 70^{o}C [8].

A small amount of the product was run on FT-IR spectrophotometer. The results showed that acidified tris(2-aminoethyl)aminehexaacetic acid was formed with no by - products.

2.3.4 Absorption spectra

2.3.4.1 Absorption spectra of $[M(NH_3)_4]^{2+}$ complexes:

To determine wavelength of the maximum absorbance of $[M(NH_3)_4]^{2+}$ complexes (M^{2+}; Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Ca^{2+}), 0.1 mmol of M^{2+} solution were mixed with excess ammonia buffer. The solution was scanned in UV-visible region.

2.3.4.2 Absorption spectra of M(II)-TAHA complexes:

To determine wavelength of the maximum absorbance of M(II)-TAHA complexes (M^{2+}; Cu^{2+}, Co^{2+} , Ni^{2+}, Cd^{2+}, Ca^{2+}), 0.1 mmol of M^{2+} solution were mixed with ammonia buffer and excess TAHA solution was added to the mixture. The solution was scanned in UV-visible region.

2.3.5 Spectrophotometric titrations

2.3.5.1 Metal - ligand titration:

A 10.0 mL aliquot of a 0.010 M of the required metal solution (Cu^{2+}, Co^{2+}, Ni^{2+}) was placed in an Erlenmeyer flask. Then 20.0 mL of ammonia buffer solution (pH 10) were added. The resulting solution was titrated with 0.010 M TAHA ligand. Absorbance was measured at the maximum wavelength of the complex formed as a function of ligand volume.

2.3.5.2 Metal mixture - ligand titration:

A 5.0 mL aliquots of 0.020 M copper(II) ion and 5.0 mL of 0.020 M solution of another metal (Ca^{2+}, Na^{+}, Co^{2+}, Ni^{2+}, Cd^{2+}) were placed in an Erlenmeyer flask. Then 20.0 mL of ammonia buffer solution (pH 10) were added. The resulting solution was titrated with 0.010 M TAHA ligand. Absorbance was measured at 736 nm the maximum wavelength of Cu(II)–TAHA complex as a function of titrant volume.

2.3.6 Determination of stoichiometry and stability constant of Cu(II)–TAHA complex by continuous variation method:

Various volumes of 0.025 M Cu(II) solution (0, 1, 2, 3, 4, 5, and 6 mL) were pippetted into seven 25.0 mL volumetric flasks. A 10.0 mL aliquot of ammonia buffer solution was added to each flask. Then different volumes of 0.025 M TAHA (6, 5, 4, 3, 2, 1, and 0 mL) were added respectively to the flasks which were then diluted to volume with distilled water. Absorbance was measured for each flask at the maximum wavelength of copper(II)–TAHA complex at pH 10. One of the above solutions was kept for several weeks, and the absorbance was measured at 736 nm the maximum wavelength of the complex at different time intervals.

2.3.7 Calibration curve

Different volumes of a standard Cu(II) solution were pippetted into seven 25.0 mL volumetric flasks and 10.0 mL buffer solution were added to each flask. A certain volume of standard TAHA solution was added to each flask. The volumes were then diluted to the mark with distilled water. The volumes and concentrations of Cu(II) solutions are shown in Table 2.1.

Table 2.1: Data for the calibration curve of Cu(II).

Solution No.	Cu^{2+} volume (mL)	TAHA volume (mL)	[Cu(II)] initial (M)	[TAHA] initial (M)	[Cu(II)] final (M)	Absorbance at 736 nm
1	0.300	0.30	0.025 M	0.025 M	3.00×10^{-4} M	0.0313
2	0.500	0.50	0.025 M	0.025 M	5.00×10^{-4} M	0.0494
3	1.00	1.00	0.025 M	0.025 M	1.00×10^{-3} M	0.0963
4	3.00	3.00	0.025 M	0.025 M	3.00×10^{-3} M	0.286
5	1.00	1.00	0.100 M	0.100 M	4.00×10^{-3} M	0.396
6	2.00	2.00	0.100 M	0.100 M	8.00×10^{-3} M	0.758
7	3.00	3.00	0.100 M	0.100 M	12.0×10^{-3} M	1.151

Absorbance was measured for these solutions at 736 nm the maximum wavelength of Cu(II)–TAHA complex against ammonia buffer as blank solution.

A plot of absorbance versus copper(II) concentration at the maximum wavelength was made.

2.3.8 Precision

Two sets of solutions with Cu(II) concentrations of 1.2×10^{-2} M and 3.0×10^{-4} M respectively were prepared, with 5 identical solutions in each set. Absorbance was measured at 736 nm for each of them.

Absorbance was also measured ten times for ammonia buffer solutions (pH 10) at 736 nm the complex maximum wavelength.

Chapter Three

Results and Discussion

3.1 Preparation of TAHA:

TAHA was prepared from tris(2-aminoethyl)amine and potassium chloroacetate by a nucleophilic substitution reaction. Heat was used to drive the reaction.

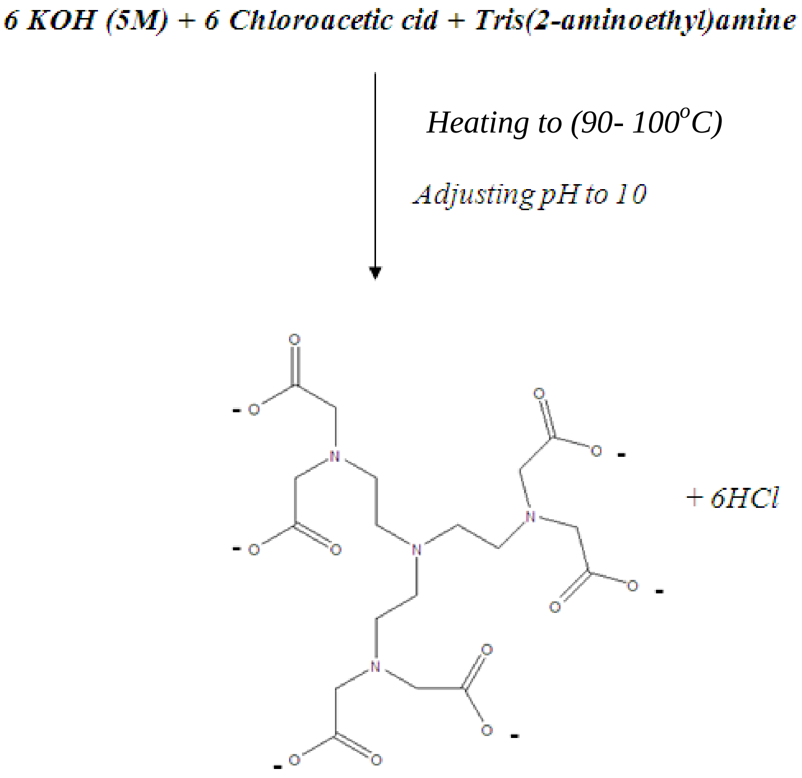

Figure 3.1: Synthesis of deprotonated Tris(2-aminoethyl)aminehexacetic acid.

Potassium hydroxide was used to neutralize chloroacetic acid instead of sodium hydroxide. Potassium chloride has roughly constant solubility over a wide range of temperatures that makes solubility significantly decreases with temperature decreasing as compared to sodium chloride. Another reason is that potassium formate is soluble in alcohol.

Acidification of TAHA was made with formic acid that was added to TAHA potassium salt to pH 3-4.

Water soluble alcohol such as ethanol was added to purify TAHA from potassium salt, after separation into two layers the lower aqueous layer was treated more than once with ethanol to increase the amount of product purified **[8].**

3.2 Identification of TAHA:

3.2.1 FT- IR spectra for TAHA potassium salt:

Formation of carboxylated compound on tris(2-aminoethyl)amine was confirmed by the appearance of the carbonyl group peaks at 1572 cm^{-1} and 1393 cm^{-1} as a result of asymmetric and symmetric stretching, respectively.

The C–O stretching vibration for the carboxylated group appeared at 1317 cm^{-1}.

The presence of stretching vibration at 2832 cm^{-1} is an indication of the presence of CH_2 groups.

The C–N stretching band for tertiary amines is expected to be found between 1250 and 1000 cm^{-1} [27]. The C–N stretching vibration appeared at 1076 cm^{-1}.

Some important peaks of tris(2-aminoethyl)amine disappeared, as an indication of reaction completeness, e.g., the N-H bending out of plane peak at 832 cm^{-1}.

The C-Cl peak of chloroacetic acid at about 635 cm^{-1} disappeared in the TAHA chelating agent, Figure 3.2.

3.2.2 FT- IR spectra for protonated TAHA:

The pH of TAHA was lowered using formic acid to pH 3. The protonation of carboxylate group is noticed by the shift in the stretching frequency of COO^- group in the TAHA potassium salt from 1570 to 1705 cm^{-1}.

The C–O stretching vibration for the carboxylic group appeared at about 1300 cm^{-1}, Figure 3.3.

3.2.3 Copper(II)–TAHA complex IR spectra:

Changes in the vibrational modes are expected because of complex formation, especially for the carboxyl group bands [27].

The asymmetric stretching frequency of the COO^- group in the TAHA potassium salt was 1572 cm^{-1} shifted to 1587 cm^{-1} when the complex is formed, Figure 3.4-a.

The Cu–N stretching vibration band appeared at 458 cm^{-1}. While the Cu–O stretching vibration band appeared at 410 cm^{-1}, Figure 3.4-b.

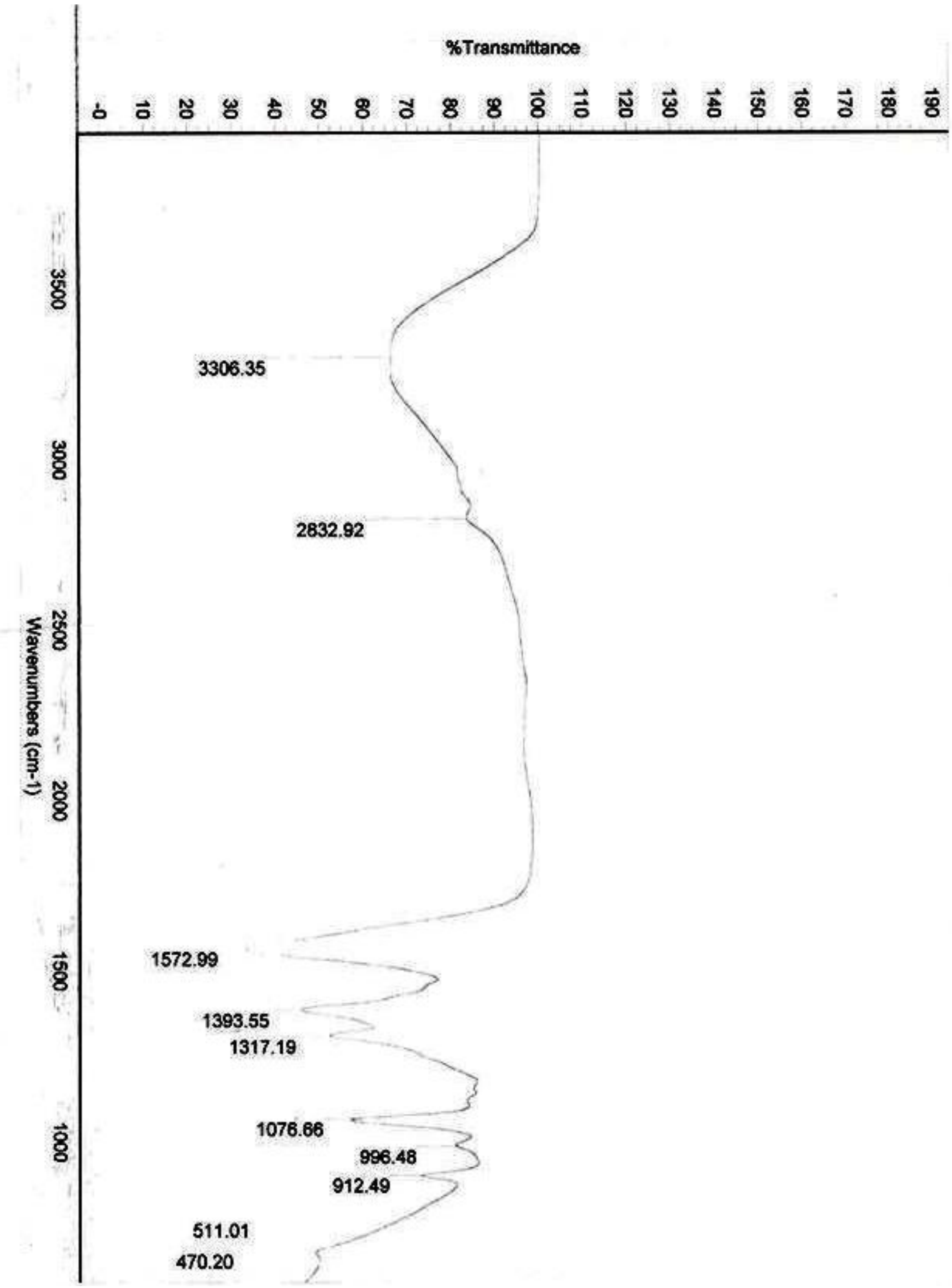

Figure 3.2: IR spectrum of Tris(2-aminoethyl)aminehexaacetate (3500- 450 cm^{-1})

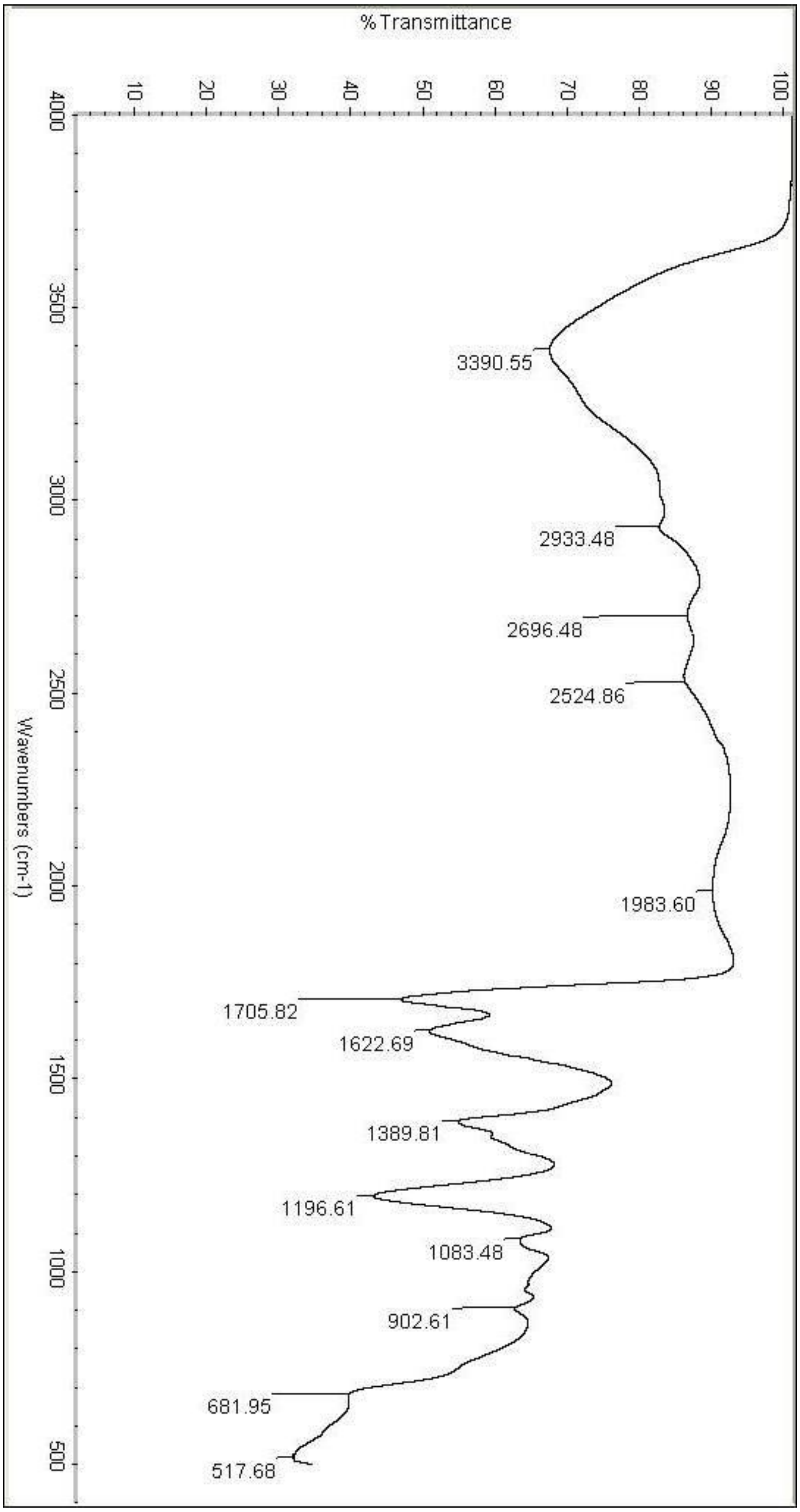

Figure 3.3: IR spectrum of protonated Tris(2-aminoethyl)aminehexaacetic acid**.**

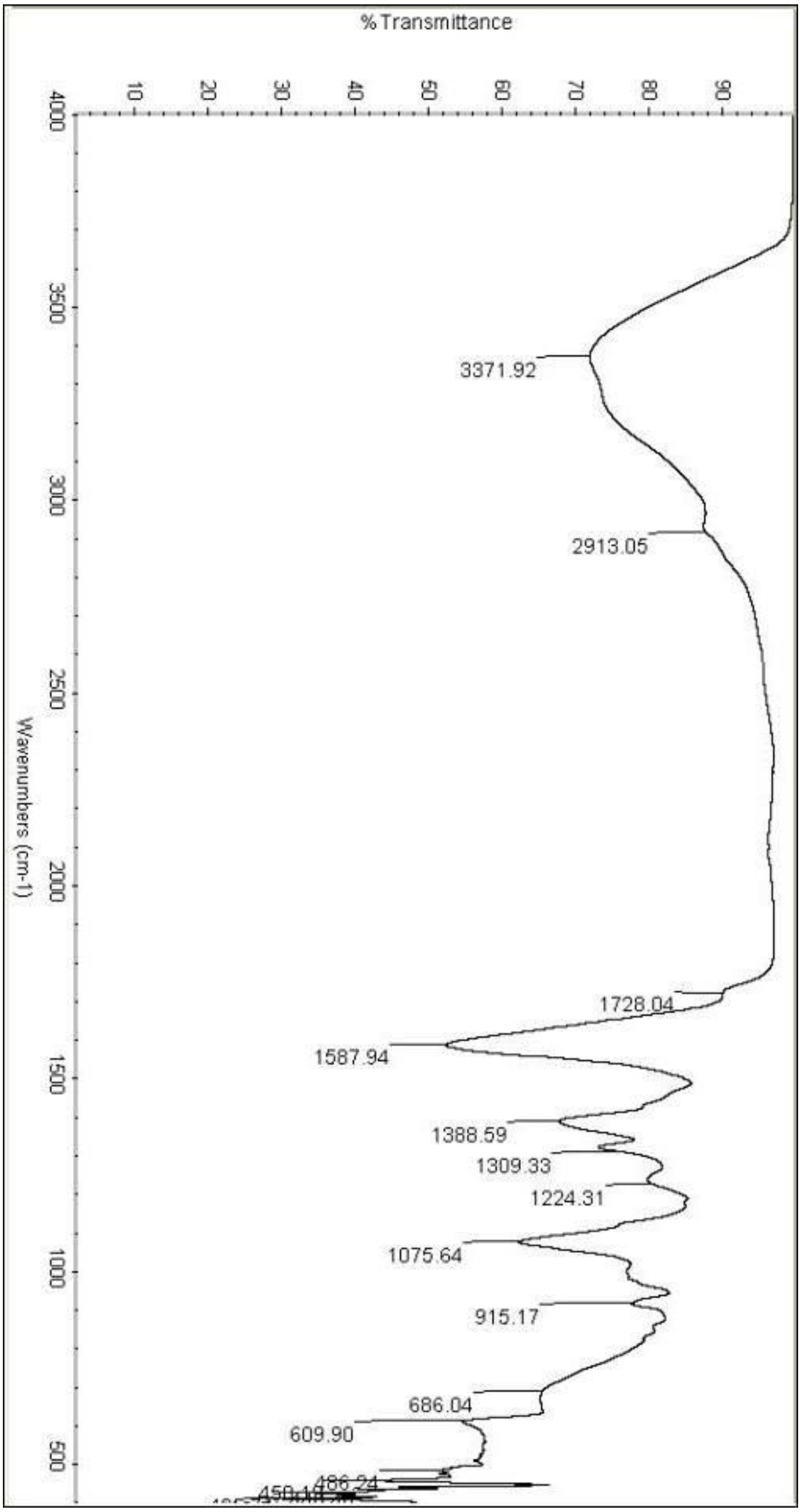

Figure 3.4-a: IR spectra of Copper(II)–TAHA complex (4000- 500 cm^{-1})

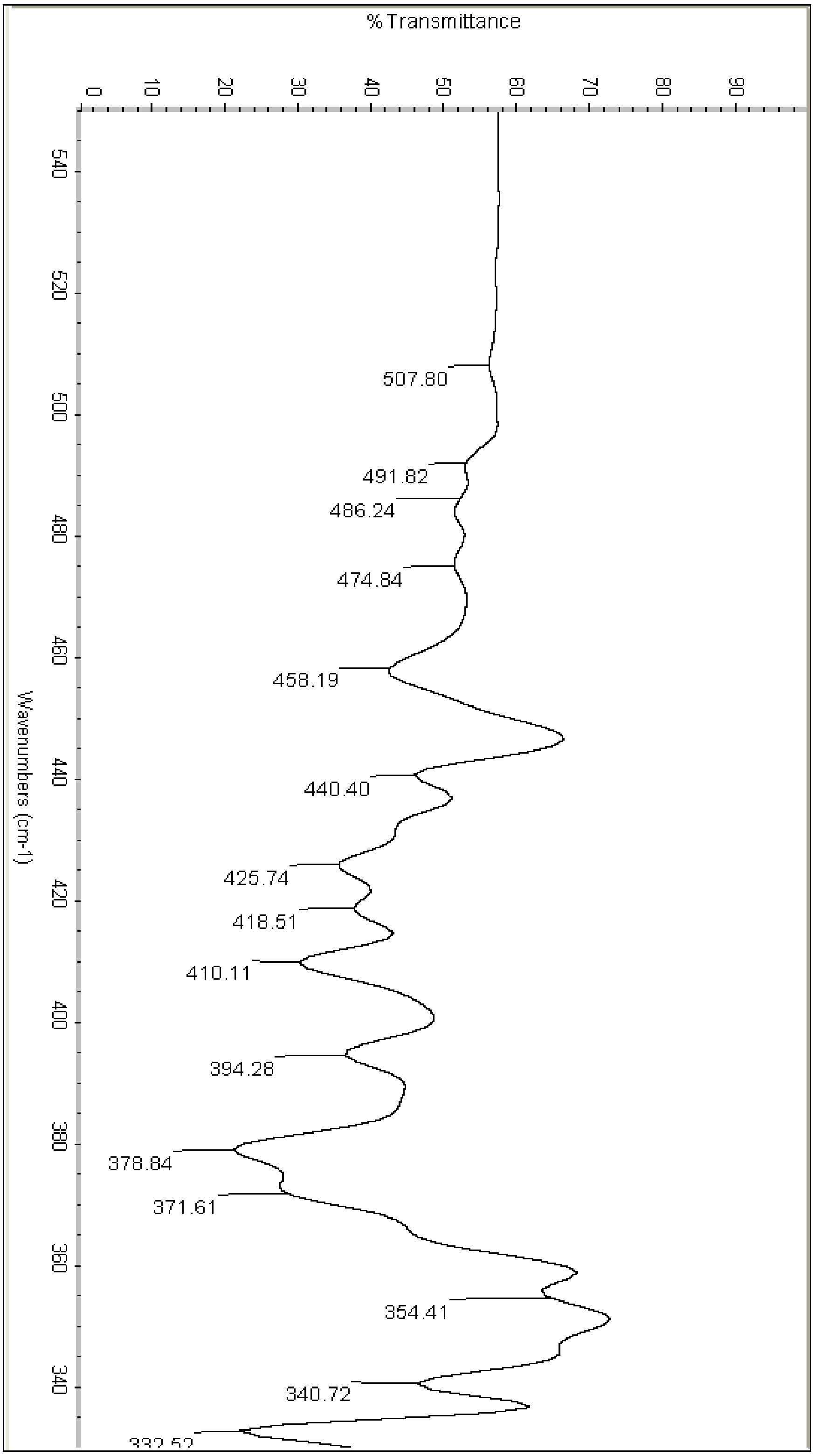

Figure 3.4-b: IR spectra of Copper(II)–TAHA complex (550- 330 cm^{-1})

3.3 Absorption spectra:

3.3.1 Absorption spectra of $[M(NH_3)_4]^{2+}$ complexes:

To determine the wavelength of maximum absorption of $[M(NH_3)_4]^{2+}$ complexes (M^{2+}; Cu^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Cd^{2+}), the solution of the metal ion and ammonia buffer was scanned in the UV-visible region. The wavelengths of maximum absorption were determined to be 641.5 nm and 309 nm for $[Cu(NH_3)_4]^{2+}$ and $[Co(NH_3)_4]^{2+}$ complexes respectively. The $[Ni(NH_3)_4]^{2+}$ complex had two maxima at 360 nm and 557 nm, as seen in Figure 3.5.

$[Ca(NH_3)_4]^{2+}$and $[Cd(NH_3)_4]^{2+}$don't absorb within the studied rang.

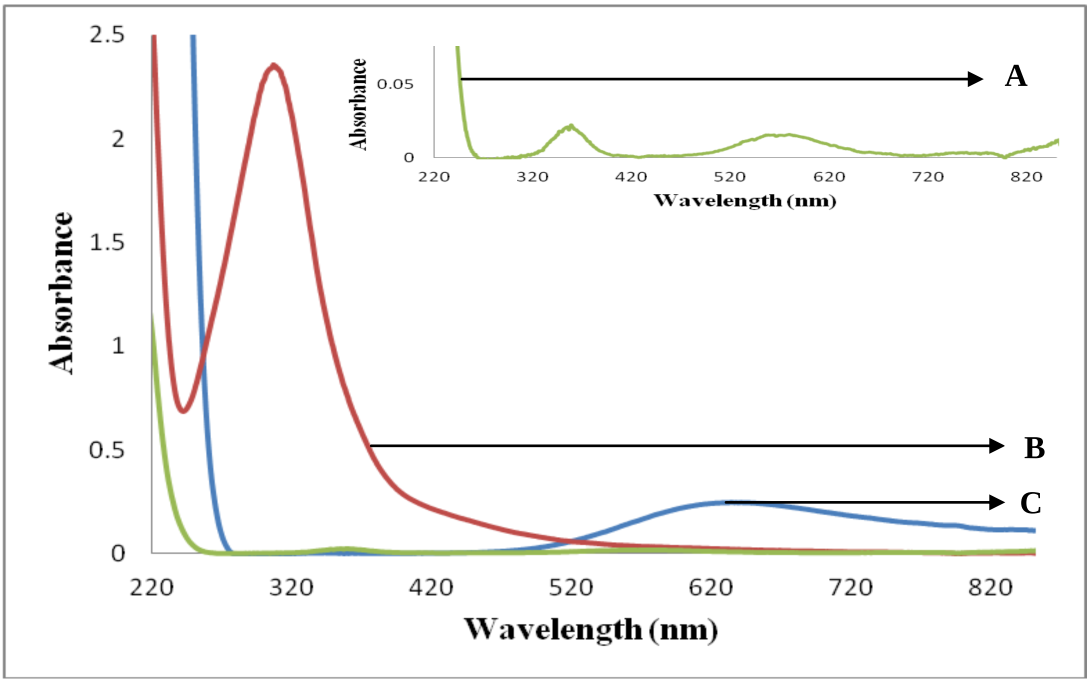

Figure 3.5: Absorption spectra of $[M(NH_3)_4]^{2+}$ complexes at pH 10; A) Ni^{2+} (10 mL, 0.01 M). B) Co^{2+} (10 mL, 0.01 M). C) Cu^{2+} (10 mL, 0.01 M).

3.3.2 Absorption spectra of M(II)-TAHA complexes:

The wavelength of maximum absorption of M(II)-TAHA complexes (M^{2+}; Cu^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Cd^{2+}) was determined from the absorption spectra shown in Figure 3.6.

The maximum wavelength of Cu(II)-TAHA complex was found to be 736 nm. A small shift was observed in wavelength upon the formation of Co(II)-TAHA complex with strong overlap with the $[Co(NH_3)_4]^{2+}$ maxima. Thus, the decrease in absorbance of $[Co(NH_3)_4]^{2+}$ was followed during the titration.

Ni(II)-TAHA complex has almost the same color as $[Ni(NH_3)_4]^{2+}$ complex. Hence, no shift in wavelength was observed as Ni(II)-TAHA complex was being formed. Absorbance was measured at 557 nm during the titration.

Ca(II)-TAHA and Cd(II)-TAHA don’t absorb within the studied rang.

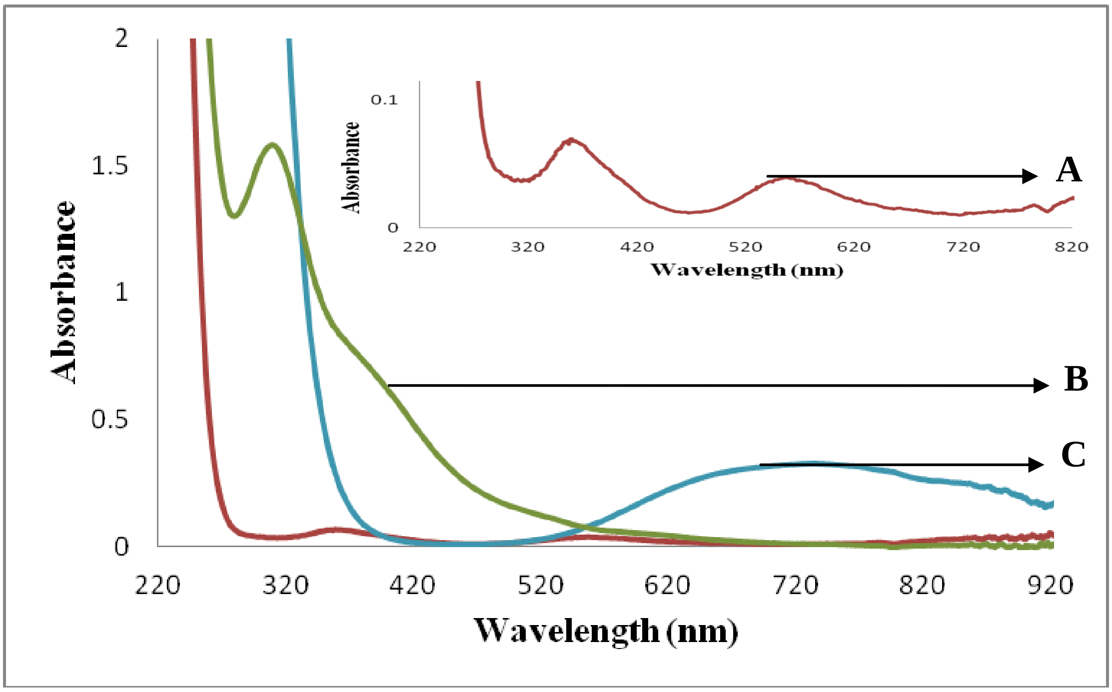

Figure 3.6: Absorption spectra of various M(II)-TAHA complexes at pH 10; (10 mL of 0.01 M M^{2+} plus 10 mL of 0.01 M TAHA). A) Ni^{2+}. B) Co^{2+}. C) Cu^{2+}.

3.4 Spectrophotometric titrations

3.4.1 Titration of a single metal ion:

In the titration of copper(II) ions, absorbance was measured at 736 nm the maximum wavelength of copper(II)–TAHA complex.

The end point was identified by monitoring the increase in absorbance until it reached a constant value. The volume at the end point indicated a 1:1 metal – to – ligand mole ratio, Figure (3.7).

Two different ligands (ammonia and TAHA) are present in the titration mixture, so most of the copper ions will bind to the one that forms the more stable complex.

At the beginning, Cu(II) forms $[Cu(NH_3)_4]^{2+}$ complex with ammonia (dark blue) at a maximum wavelength of 641.5 nm. Absorption increases with addition of titrant, where $[Cu(NH_3)_4]^{2+}$ complex decomposes in favor of the formation of copper(II)-TAHA complex. A maximum absorption value was reached at the equivalence point. After that, absorption remained constant.

Titration curve for corrected absorbance versus ligand volume is shown in Figure 3.7. Correction is very important to get straight line and a reliable intersection at the equivalence point. Without correction, the graph will bend toward the x- axis and will give an incorrect end point [28].

Absorbance correction was performed by two ways; in the first method, the measured absorbance was multiplied by the dilution factor (Equation 1.5):

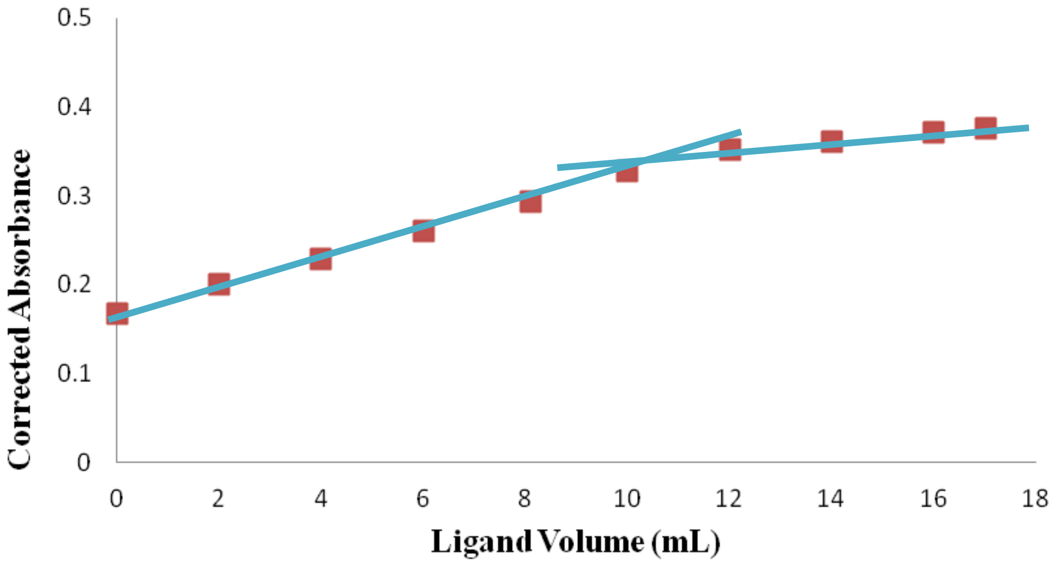

Figure 3.7: Spectrophotometric titration curve for the titration of 10 mL of 0.01 M Cu^{2+} with a 0.01 M TAHA. (pH = 10, λ = 736 nm).

A second correction method was used because more than one complex were formed ($[Cu(NH_3)_4]^{2+}$ and Cu(II)–TAHA). Both have a blue color and absorb at 736 nm. Thus, a modified correction method was used to follow the absorption of Cu(II)-TAHA complex only (Equation 1.6), Figure 3.8.

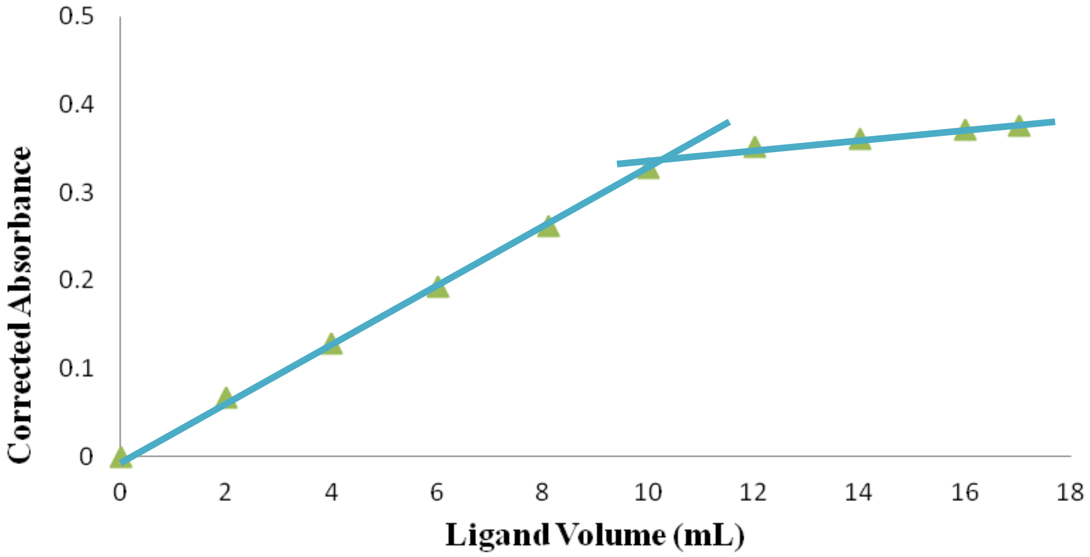

Figure 3.8: Spectrophotometric titration curve for the titration of 10 mL of 0.01 M Cu^{2+} with 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected by the modified correction method.

When excess amount of ligand is added, no correction is needed; because there is no excess Cu^{2+} to complex ammonia [25].

Titration of cobalt(II) with TAHA was also studied. Absorbance was measured at 309 nm, the maximum wavelength of $[Co(NH_3)_4]^{2+}$ complex. This wavelength was used because a small shift was observed upon the formation of Co(II)-TAHA complex with strong overlap. Thus, the decrease in absorbance of $[Co(NH_3)_4]^{2+}$ was followed during the titration.

Titration curve for corrected absorbance versus ligand volume is shown in Figure 3.9. Absorbance correction was performed by the first method, the measured absorbance was multiplied by the dilution factor (Equation 1.5).

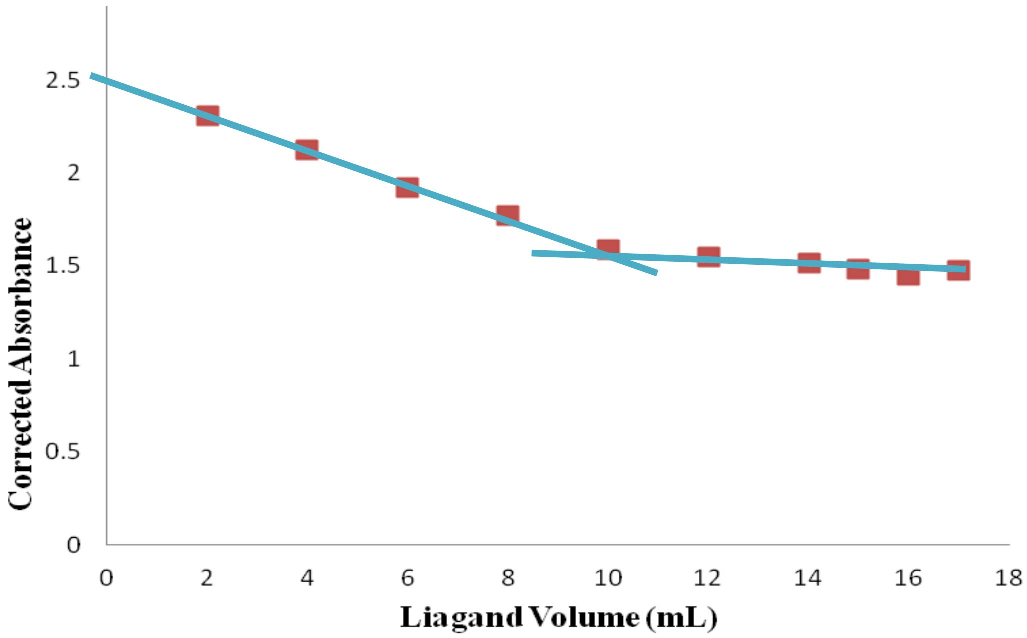

Figure 3.9: Spectrophotometric titration curve for the titration of 10 mL of 0.01 M Co^{2+} with a 0.01 M TAHA. (pH = 10, λ = 309 nm). Absorbance was corrected for dilution.

Titration curve for the titration of Co(II) with TAHA is shown in Figure 3.10. The absorbance was corrected according to the modified method (Equation 1.6).

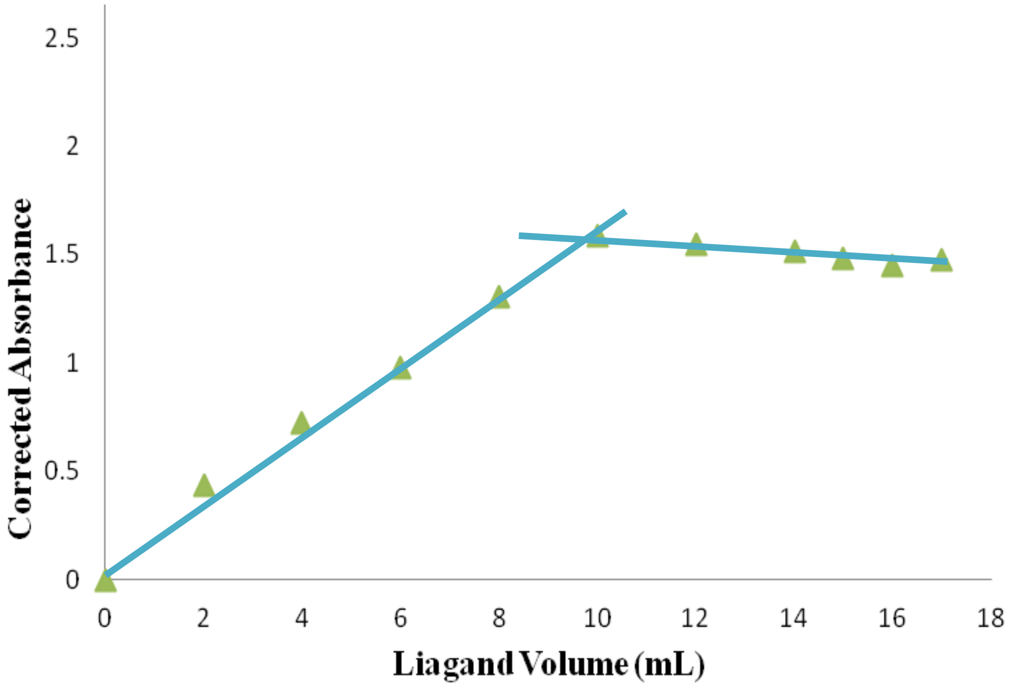

Figure 3.10: Spectrophotometric titration curve for the titration of 10 mL of 0.01 M Co^{2+} with a 0.01 M TAHA. (pH = 10, λ = 309 nm). Absorbance was corrected by the modified correction method.

A titration curve was constructed for the titration of nickel(II). Ni(II)–TAHA complex has two absorption maxima; 360 nm and 557 nm. Absorbance was measured at the later wavelength.

Titration curve for the corrected absorbance versus ligand volume is shown in Figure 3.11. Correction was performed by the first method, the measured absorbance was multiplied by the dilution factor (Equation 1.5).

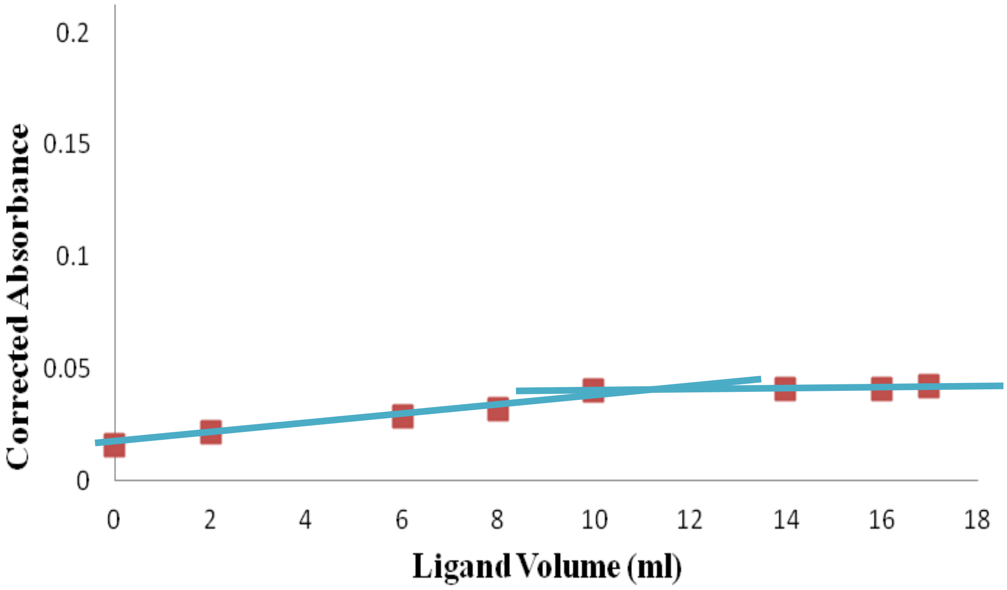

Figure 3.11: Spectrophotometric titration curve for the titration of 10 mL of 0.01M Ni^{2+} with a 0.01M TAHA. (pH = 10, λ = 557 nm). Absorbance was corrected for dilution.

The titration curve for the titration of Ni(II) with TAHA is shown in Figure 3.12. Absorbance was corrected according to the modified correction method (Equation 1.6).

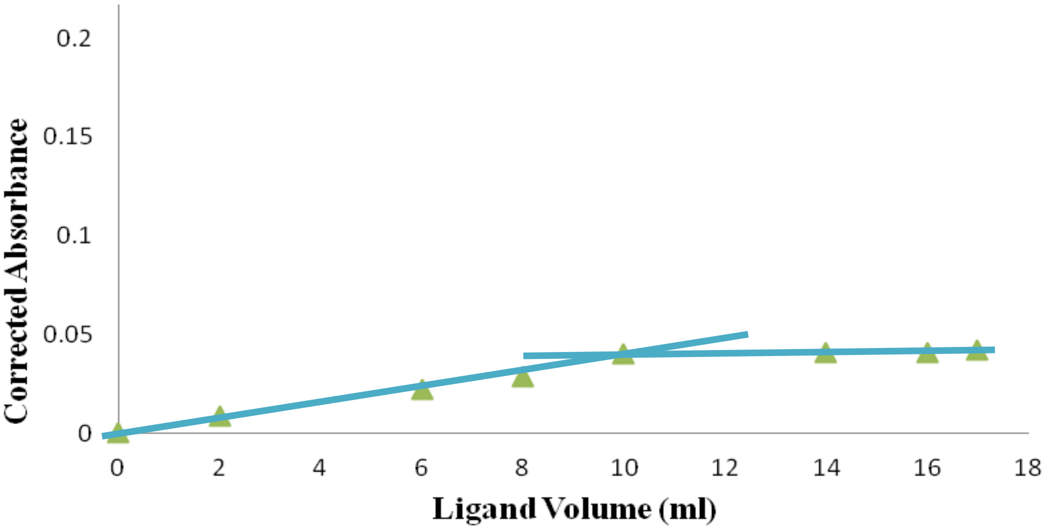

Figure 3.12: Spectrophotometric titration curve for the titration of 10 mL of 0.01 M Ni^{2+} with a 0.01 M TAHA. (pH = 10, λ = 557 nm). Absorbance was corrected by the modified correction method**.**

3.4.2 Titration of a mixture of metal ions:

Titration was carried out for several Cu(II)–M^{n+} mixtures, where M^{n+} represents; Ca^{2+}, Na^{+}, Co^{2+}, Ni^{2+}, or Cd^{2+}.

3.4.2.1 Cu^{2+}– Ca^{2+} mixture:

Ammonia acts as a secondary ligand, with $[Cu(NH_3)_4]^{2+}$ complex being more stable than $[Ca(NH_3)_4]^{2+}$. As TAHA is a stronger ligand than ammonia, it will complex Ca^{2+} at first by replacing ammonia. When Ca^{2+} had completely finished, TAHA will start complexation of Cu^{2+}.

$[Cu(NH_3)_4]^{2+}$ has a dark blue color with a maximum wavelength at 641.5 nm, whereas, Cu(II)–TAHA complex has a blue color with a maximum wavelength at 736 nm.

The other solute particles such as calcium or TAHA either alone or as a complex involved during the titration are all either non – absorbing or very slightly absorbing at the maximum wavelength of 736 nm.

As seen in the titration curve of Cu^{2+}– Ca^{2+} mixture with TAHA, three straight lines were obtained, figure 3.13. Two intersections corresponding to two equivalence points were produced. The first for Ca(II) (10 mL), and the second for Cu(II) (20 mL). Beyond the second equivalence point, there was no further increase in absorbance because TAHA does not absorb at this wavelength.

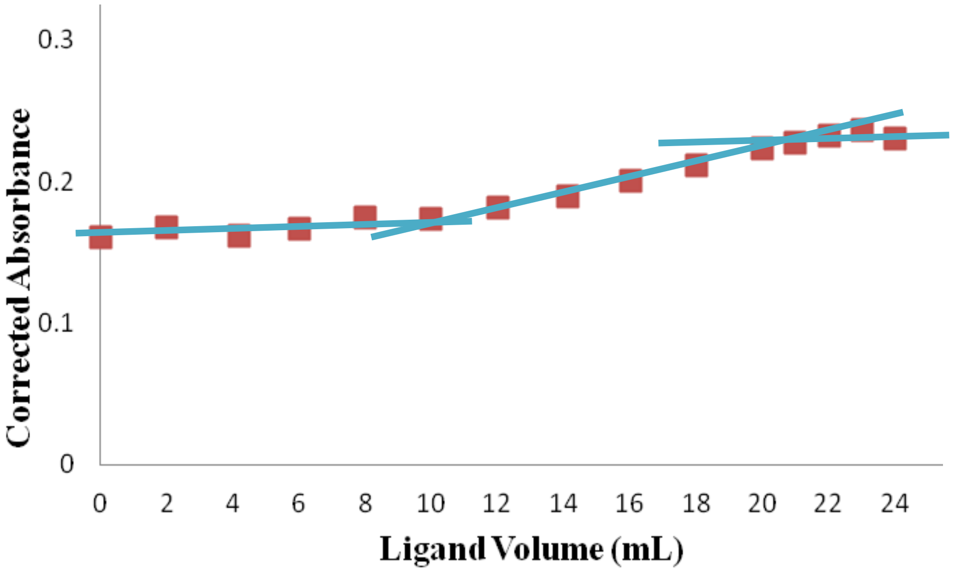

Figure 3.13: Spectrophotometric titration of Cu^{2+}- Ca^{2+} mixture (10 mL of 0.01 M each) with 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected for dilution.

The modified correction method was applied to avoid overlapping of absorbance that occurs during the formation of Ca(II)–TAHA complex, and the formation of Cu(II)–TAHA complex at 736 nm.

This correction keeps only one absorbing species and eliminates the other. firstly, $[Ca(NH_3)_4]^{2+}$ (colorless) decomposed to form Ca(II)–TAHA complex (colorless) as ligand is added. Since the original solution has a blue color due to $[Cu(NH_3)_4]^{2+}$, this causes an overlap in absorbance reading.

So for the first 10 mL of ligand, absorbance was corrected according to Equation (1.6) as follows:

$$A_{corrected} = A'_{measured} - \frac{(10\ \text{mL}\ Cu^{2+} - 0\ \text{mL ligand})}{10\ \text{mL}\ Cu^{2+}} \times A_1$$

A_1: absorbance for Cu^{2+} solution in ammonia buffer.

Secondly, as $[Cu(NH_3)_4]^{2+}$ decomposes to form Cu(II)–TAHA complex, the two complexes absorbs strongly at 736 nm. Thus, the modified correction method was used to follow the absorption of Cu(II)–TAHA complex only.

Absorbance was corrected according to Equation (1.6) as follow:

$$A_{corrected} = A'_{measured} - \frac{(10\ \text{mL}\ Cu^{2+} - (1\ \text{to}\ 10)\ \text{mL ligand})}{10\ \text{mL}\ Cu^{2+}} \times A_1$$

A_1: absorbance for Cu^{2+} solution in ammonia buffer.

When excess amount of ligand is added, no further correction is needed; because there is no excess Cu^{2+} to complex ammonia buffer. The titration curve for the same mixture using the modified correction method gives more clear results with sharper end points as shown in Figure 3.14.

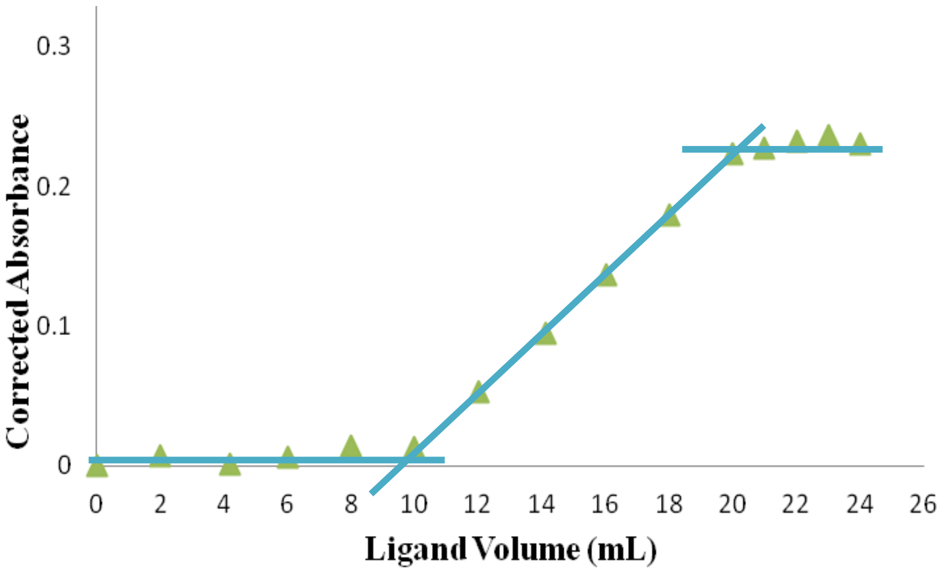

Figure 3.14: Spectrophotometric titration curve for the titration of Cu^{2+}- Ca^{2+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected by the modified correction method.

3.4.2.2 Cu^{2+} and Na^{+} mixture:

Alkali metal ions do not form complexes at all [29], alkali metals are poor complexing agents because they are weak oxidizing agents, they act as strong reducing agents [30]. Thus, Na^{+} will not form a complex with TAHA, and TAHA will make just one stable complex with Cu^{2+}.

The other solute particles such as Na^{+} or TAHA involved during the titration are either non absorbing or very slightly absorbing with respect to wavelength maxima of 736 nm.

In the titration curve for Cu^{2+} and Na^{+} mixture, two straight lines were obtained with one intersection corresponding to equivalence point (10 mL). After that there is no further increase in absorbance, Figure 3.15.

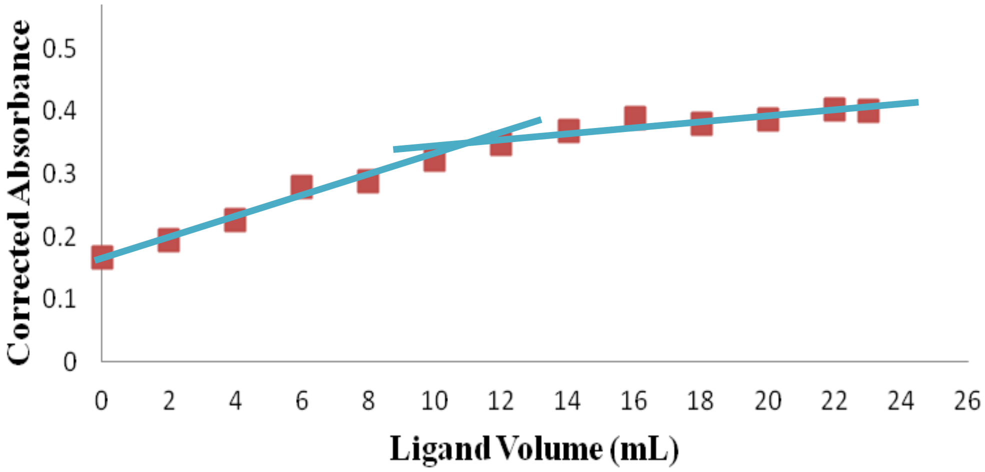

Figure 3.15: Spectrophotometric titration curve for the titration of Cu^{2+} - Na^{+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected for dilution**.**

The modified correction method (Equation 1.6) was applied for the two complexes; $[Cu(NH_3)_4]^{2+}$ and Cu(II)–TAHA , because both absorb at 736 nm, Figure 3.16.

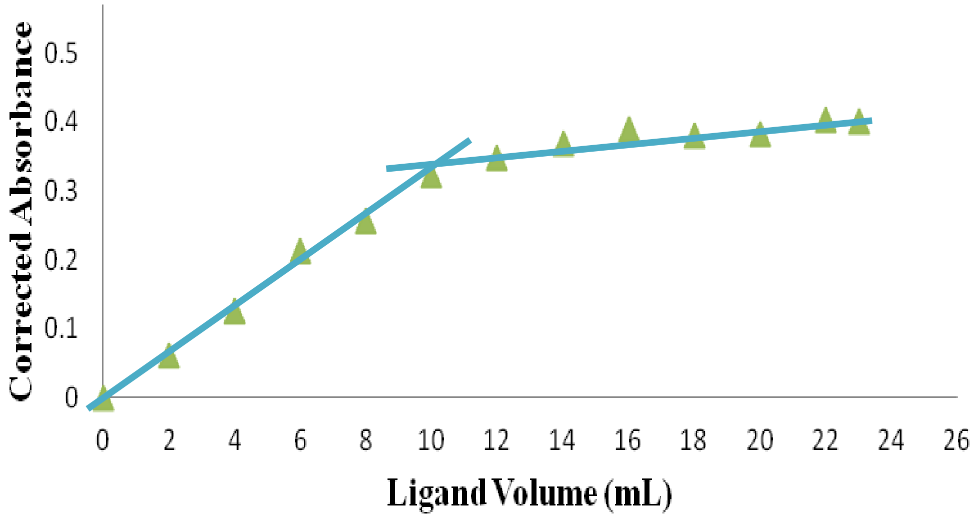

Figure 3.16: Spectrophotometric titration curve for the titration of Cu^{2+}- Na^{+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected by the modified correction method.

3.4.2.3 $Cu^{2+}$and Co^{2+} mixture:

Ammonia forms complexes with both of Cu^{2+} and Co^{2+} metal ions as a secondary ligand. However, $[Cu(NH_3)_4]^{2+}$ is more stable than $[Co(NH_3)_4]^{2+}$.

So TAHA reacts initially with Co^{2+} by replacing ammonia. When Co^{2+} had completely finished, TAHA starts to react with Cu^{2+}.

Cu^{2+} and Co^{2+} mixture in ammonia buffer has a dark green color with two maxima at 309 nm and 736 nm, Figure 3.17.

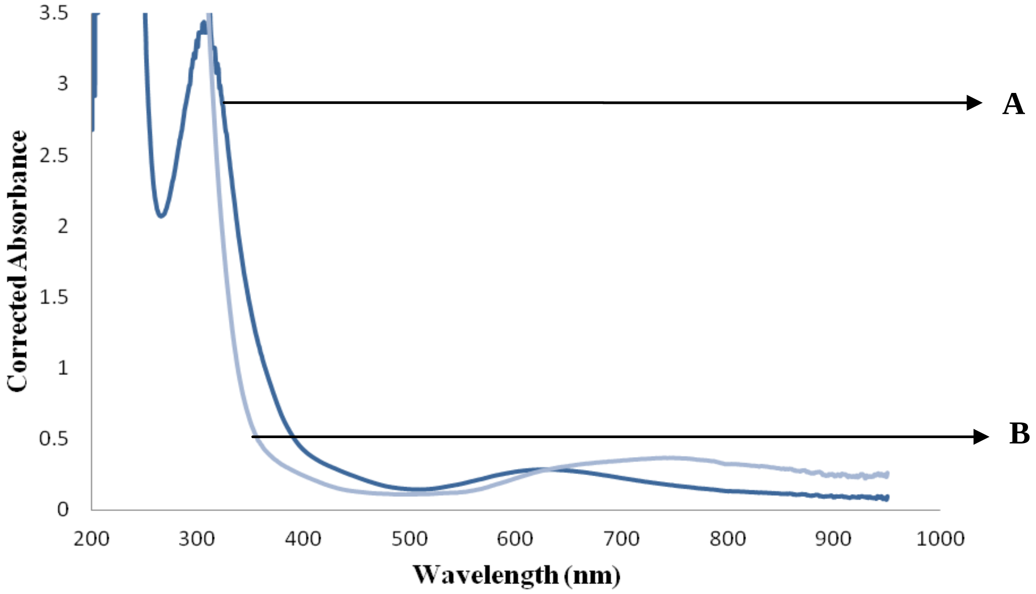

Figure 3.17: Absorption spectra at pH 10. A) Cu^{2+}- Co^{2+} mixture (10 mL of 0.01 M each). B) A plus 20 mL of a 0.01 M TAHA. Absorbance was corrected for dilution.

The titration curve for the titration of the mixture is shown in Figure 3.18.

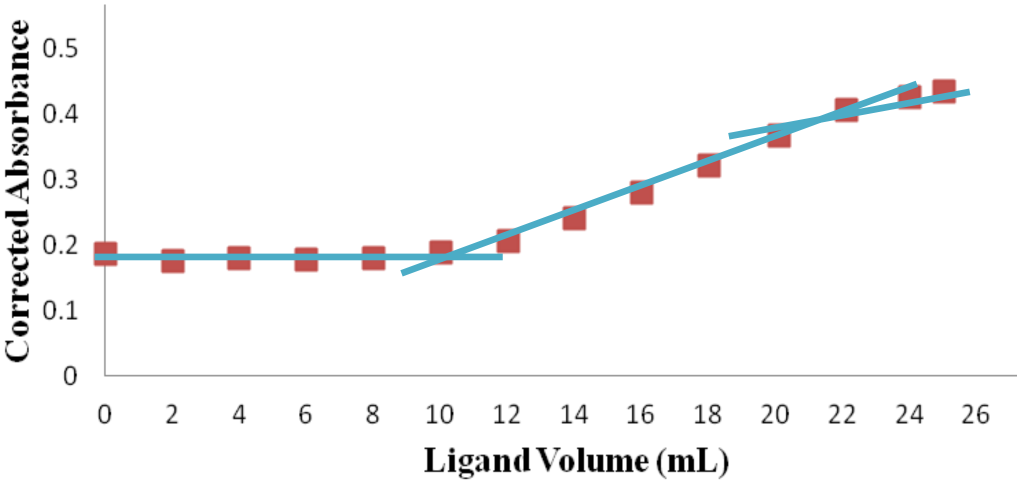

Figure 3.18: Spectrophotometric titration curve for the titration of Cu^{2+}- Co^{2+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, $\lambda = 736$ nm). Absorbance was corrected for dilution.

The titration curve for this same solution using the modified correction method is shown in Figure 3.19.

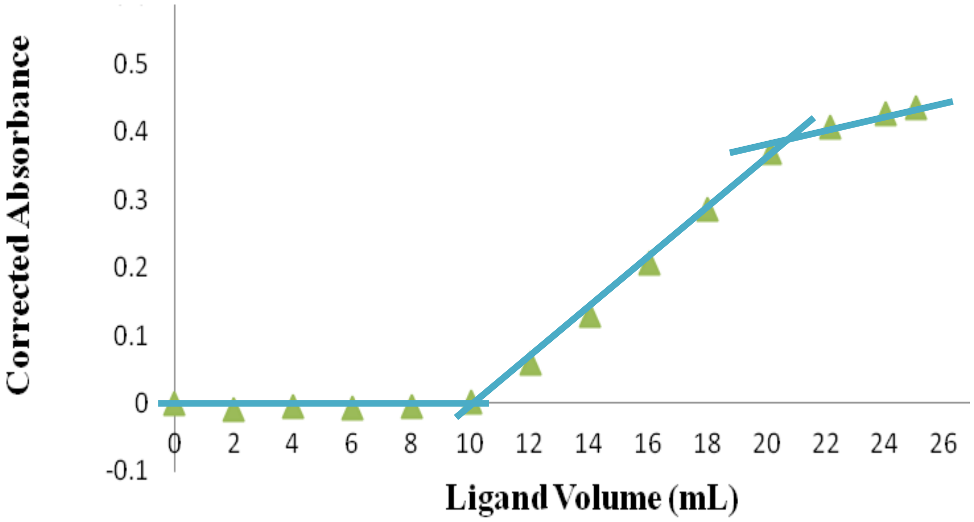

Figure 3.19: Spectrophotometric titration curve for the titration of Cu^{2+}- Co^{2+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected by the modified correction method.

3.4.2.4 $Cu^{2+}$and Ni^{2+} mixture:

$[Cu(NH_3)_4]^{2+}$ is a more stable complex than $[Ni(NH_3)_4]^{2+}$. Therefore, TAHA reacts completely with Ni^{2+}, and then starts to complex Cu^{2+}.

$[Ni(NH_3)_4]^{2+}$ has two peaks at 360 nm, and 557 nm. The later overlaps with $[Cu(NH_3)_4]^{2+}$ peak at 641.5 nm giving one larger peak (Figure 3.20).

A plot of the corrected absorbance versus wavelength shows the behavior of the mixture during titration. TAHA reacts initially with Ni^{2+} after decomposing $[Ni(NH_3)_4]^{2+}$ complex.

When all Ni^{2+} has consumed, TAHA starts to complex Cu^{2+}. As more TAHA is added, it exchanges ammonia to form Cu(II)–TAHA complex, and shifts to higher wavelengths, Figure 3.20.

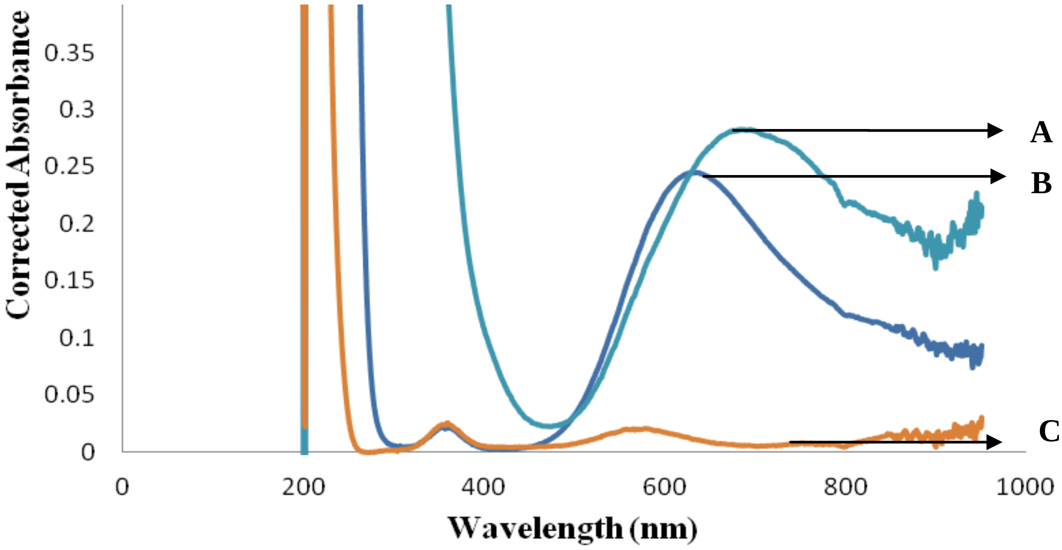

Figure 3.20: Absorption spectra at pH 10. A) Cu^{2+}- Ni^{2+} mixture (10 mL of 0.01 M each) plus 20 mL of a 0.01 M TAHA. B) Cu^{2+}- Ni^{2+} mixture (10 mL of 0.01 M each). C) Ni^{2+} (10 mL of 0.01M). Absorbance was corrected for dilution.

Titration curves for the titration of Cu^{2+}– Ni^{2+} mixture using the first and the modified correction methods are shown in Figures 3.21 and 3.22 respectively.

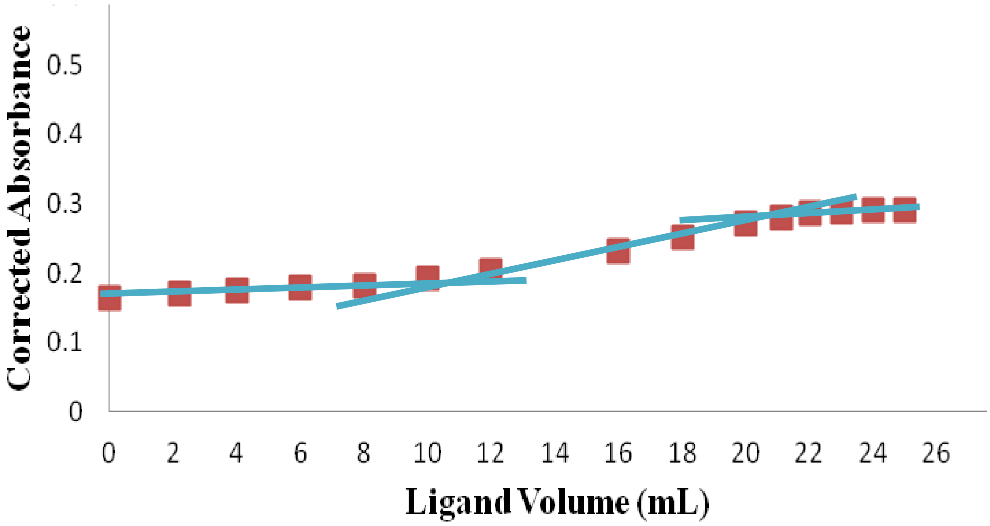

Figure 3.21: Spectrophotometric titration curve for the titration of Cu^{2+}- Ni^{2+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected for dilution.

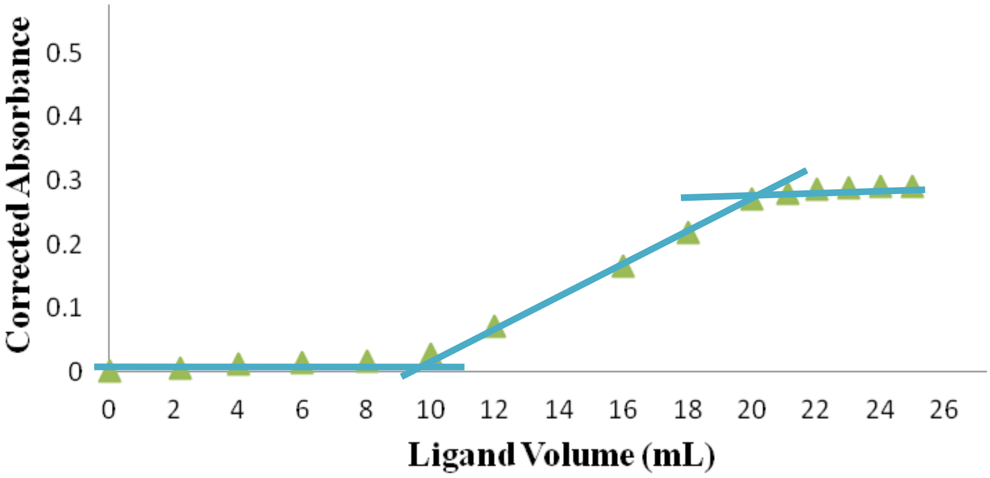

Figure 3.22: Spectrophotometric titration curve for the titration of Cu^{2+}- Ni^{2+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected by the modified correction method**.**

3.4.2.5 $Cu^{2+}$and Cd^{2+} mixture:

$[Cu(NH_3)_4]^{2+}$ is a more stable complex than $[Cd(NH_3)_4]^{2+}$. Therefore, TAHA reacts completely with Cd^{2+}, and then starts to complex Cu^{2+}.

A plot of the corrected absorbance versus wavelength, shows the behavior of the mixture during titration. TAHA reacts initially with Cd^{2+} on the expense of $[Cd(NH_3)_4]^{2+}$ complex at 736 nm.

When all Cd^{2+} has been consumed, TAHA starts to form a complex with Cu^{2+}. As more TAHA is added, TAHA exchanges ammonia to form Cu(II)–TAHA complex, and shifts to higher wavelengths.

Titration curves for the titration of $Cu^{2+}$and Cd^{2+} mixture using the first and the modified correction methods are shown in Figures 3.23 and 3.24 respectively.

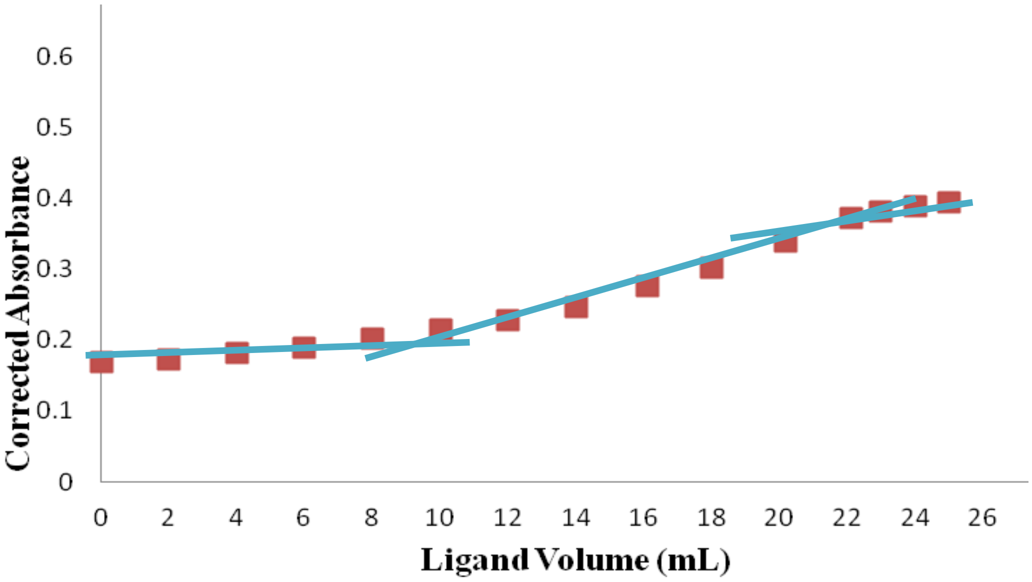

Figure 3.23: Spectrophotometric titration curve for the titration of Cu^{2+}- Cd^{2+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected for dilution.

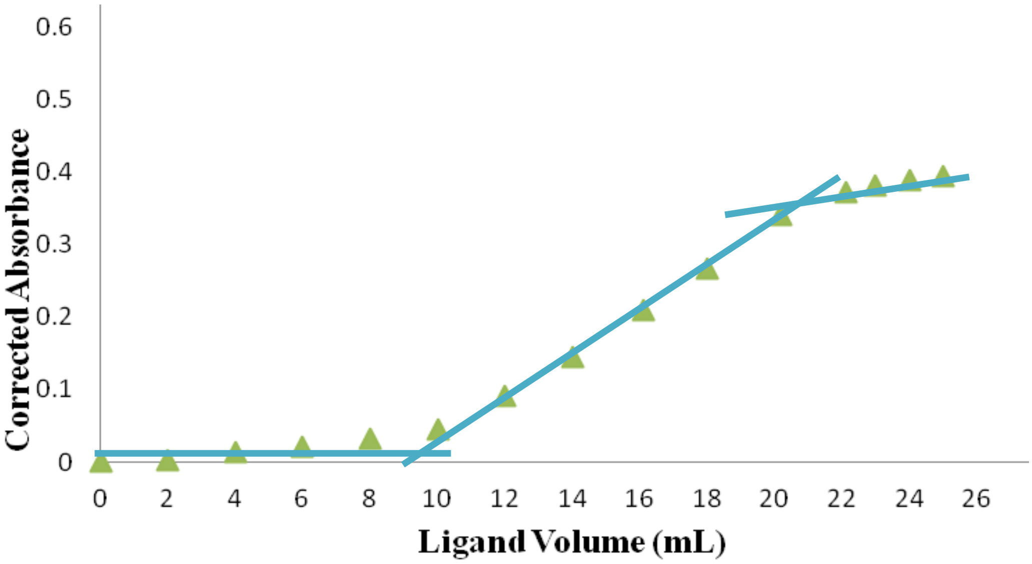

Figure 3.24: Spectrophotometric titration curve for the titration of Cu^{2+}- Cd^{2+} mixture (10 mL of 0.01 M each) with a 0.01 M TAHA. (pH = 10, λ = 736 nm). Absorbance was corrected by the modified correction method.

3.5 The formula and stability of Cu(II)–TAHA complex:

The continuous variation method was used for the determination of the formula and the stability constant of Cu(II)–TAHA complex. A series of solutions were prepared, with constant total moles of metal and ligand. The results are tabulated in Table 3.1.

The absorbance was measured for each solution at 736 nm the wavelength of Cu(II)–TAHA complex maximum absorption.

Table 3.1: Data for Cu(II)–TAHA complex (continuous variation method)

Solution No.	**Cu^{2+} volume (mL) (0.025 M)**	**TAHA volume (mL) (0.025 M)**	**X_{Cu2+}**	**X_{TAHA}**	**$[TAHA]_{final}$**	**$[Cu^{2+}]_{final}$**
1	0	6	0	1.000	6×10^{-3}M	0
2	1	5	0.166	0.833	5×10^{-3}M	1×10^{-3}M
3	2	4	0.333	0.666	4×10^{-3}M	2×10^{-3}M
4	3	3	0.500	0.500	3×10^{-3}M	3×10^{-3}M
5	4	2	0.666	0.333	2×10^{-3}M	4×10^{-3}M
6	5	1	0.833	0.1666	1×10^{-3}M	5×10^{-3}M
7	6	0	1.000	0	0	6×10^{-3}M

The measured absorbances must be corrected to give the absorbances due to the Cu(II)–TAHA alone. This correction should be made for solutions 5, 6, and 7.

Two ways can be used to correct the absorbance [24]:

$$A_{corrected} = A_{measured} - \varepsilon_M b C_M - \varepsilon_L b C_L \qquad (3.1)$$

Or as Equation (1.1):

$$A_{corrected} = A_{measured} - \frac{(\text{Volume metal (mL)} - \text{Volume TAHA (mL)})}{\text{Volume metal (mL)} + \text{Volume ligand (mL)}} \times A_1 \qquad (1.1)$$

where $A_{measared}$ is the absorbance measured, A_1 is the absorbance of solution #7 where only Cu^{2+} and buffer are present in the flask. $A_{corrected}$ is the corrected absorbance. No correction needs to be made for solutions in which sufficient TAHA has been added to convert all the Cu^{2+} to complex [25]. (this correction method was used in this work).

Corrected absorbance was plotted versus the mole fraction of Cu^{2+} to find complex formula and formation constant.

By extrapolating the two lines, they intersect at a point opposite to mole fraction of Cu^{2+}, that can be used to find the formula using the Equation (3.2) [24]:

$$Y = \frac{X_{Cu2+}}{(1 - X_{Cu2+})} \qquad (3.2)$$

The formation constant can be determined from the Equation (3.3) [1]:

$$K_{formation} = \frac{[A_b / A_a]}{[1 - A_b/A_a] \times [C_{ligand} - C_{metal} \times A_b/A_a]} \qquad (3.3)$$

Where A_a = absorbance at break point, A_b = actual absorbance, C_{metal} = concentration of metal, and C_{ligand} = concentration of ligand.

In solution, copper(II) aqua complex has λ_{max} = 807 nm. Water molecules can be replaced by ammonia to form a complex with maximum wavelength at 641 nm. TAHA is a stronger ligand than both (water and ammonia), ammonia can be easily replaced by TAHA to form more stable complex with Cu^{2+} at λ_{max} = 736 nm. TAHA has no absorption peak within the measured range.

$[Cu(NH_3)_4]^{2+}$ complex absorption peak overlaps with Cu(II)–TAHA complex absorption peak. However, the absorbance correction method solves this problem.

The absorbance was measured for each solution at 736 nm. The corrected absorbance was plotted versus the mole fraction of Cu^{2+}, Figure 3.25.

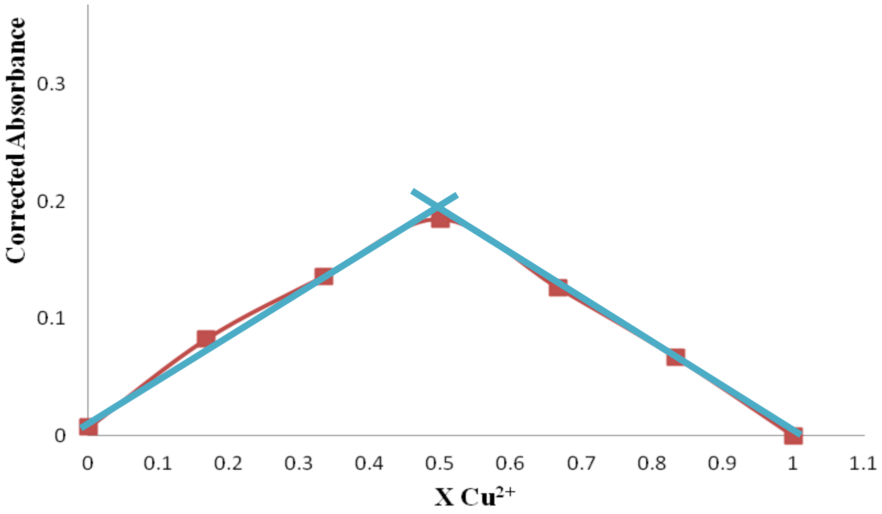

Figure 3.25: Curve for the continuous variation method, for Cu(II)–TAHA Complex at pH 10, λ = 736nm.

Extrapolating the two lines to find the maximum absorbance. The two lines intersect at a point that correspond to a mole fraction of Cu^{2+}= 0.5

Thus, the stoichiometry of Cu(II)–TAHA complex is 1:1.

By substituting the following values in Equation (3.3):

A_a= 0.193, A_b= 0.185, C_{metal}= 3×10^{-3}M, and C_{ligand} = 3×10^{-3}M.

$K_{formation}$ was calculated to be 1.86×10^5.

3.6 Effect of time on complex stability:

A series of solutions of Cu(II)–TAHA complex were prepared, and one of them was kept for a month at room temperature.

The Cu(II)–TAHA complex was stable for an extended period of time, with no significant change, Figure 3.26.

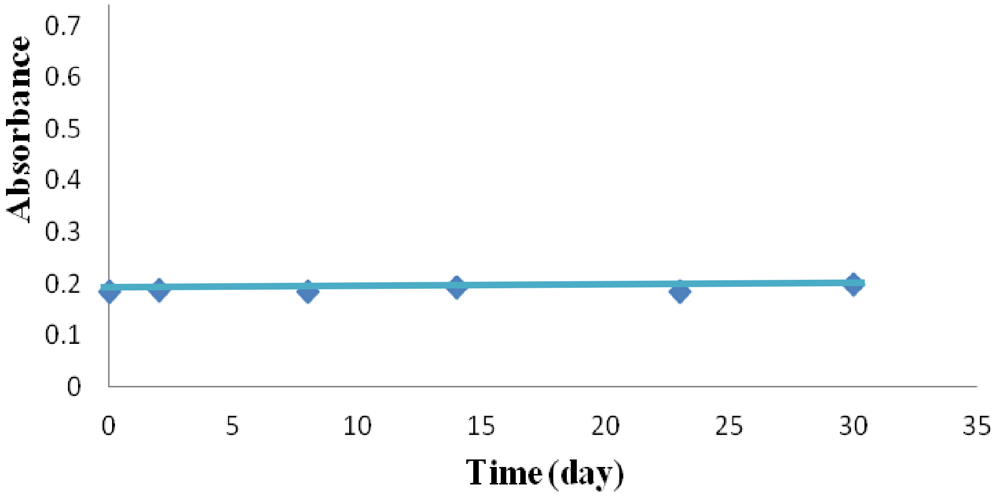

Figure 3.26: Effect of time on Cu(II)–TAHA complex stability, $[Cu^{2+}]$= [TAHA]= 3×10^{-3} M, at pH 10, λ = 736nm**.**

3.7 Calibration Curve:

A calibration curve was constructed by plotting the absorbance (measured at 736 nm) versus copper(II) concentration. The curve is shown in Figure 3.27. A linear relationship was obtained over the studied concentration range.

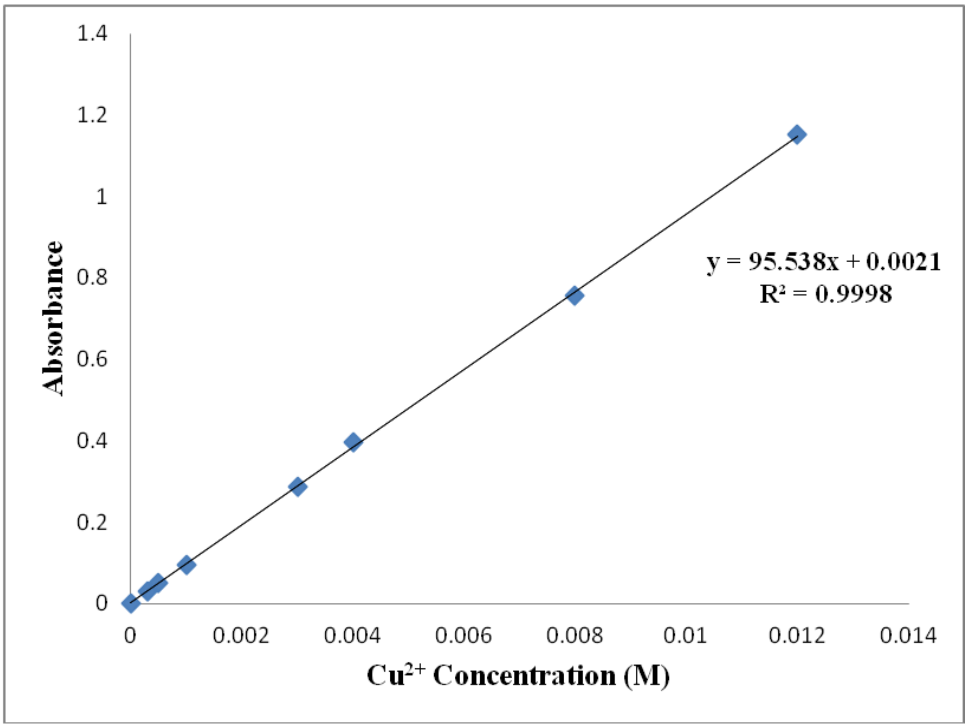

Figure 3.27: Calibration curve for Cu(II) at pH 10, λ = 736 nm.

The molar absorptivity, ε was found to be 95.538 L.mol $^{-1}$. cm^{-1}.

Beer's low was obeyed over the concentration range 3.0×10^{-4} M – 1.2×10^{-2} M for Cu(II) solution

3.8 Performance characteristics:

3.8.1 Limit of detection (LOD):

Instrumental error applies also to blank measurement, which is the instrument response when no analyte is found in the sample. For analyst it is important to know when analyte signal is acceptable or not. In analytical chemistry, it must be at least three times greater than the blank signal [31, 32].

The analyte's signal ($S_{analyte}$) at the detection limit is given by Equation (3.4):

$$S_{analyte} = S_{blank} + z\sigma_{blank} \qquad (3.4)$$

where S_{blank} is the average blank signal, σ_{blank} is the standard deviation of the blank signal, z is a factor of the desired confidence level, is set to 3 at 95% confidence level [31, 32].

The analyte's concentration ($C_{analyte}$) at the detection limit is given by Equation (3.5) [24]:

$$C_{analyte} = \frac{(S_{analyte} - S_{blank})}{Slope} \qquad (3.5)$$

The standard deviation of the blank was determined by measuring the absorbance of the blank (at 736 nm) ten times.

The detection limit of the method was calculated to be 7.285×10^{-6} M.

3.8.2 Limit of Quantitation (LOQ):

LOQ defined as the lowest analyte concentration in a sample that can be determined with acceptable value of accuracy and precision [32].

The analyte's signal ($S_{analyte}$) at the quantitation limit is given by Equation (3.6) [31]:

$$S_{analyte} = S_{blank} + 10\sigma_{blank} \qquad (3.6)$$

where S_{blank} is the average blank signal, σ_{blank} is the standard deviation of the blank signal [31, 32].

The analyte's concentration ($C_{analyte}$) at the quantitation limit is given by Equation (3.7) [32]:

$$C_{analyte} = \frac{(10\sigma_{blank})}{Slope} \qquad (3.7)$$

The quantitation limit of the method was calculated to be 2.428×10^{-5} M.

3.8.3 Precision:

The precision of the method was calculated for two sets of Cu(II) concentrations; 1.2×10^{-2} M and 3.0×10^{-4} M. Absorbances were measured at 736 nm for each one.

The results are shown in Table 3.2 below:

Table (3.2): Absorbances for two sets of concentrations for Cu(II) at 736nm.

Sample	Absorbance (1.2×10^{-2} M)	Absorbance (3.0×10^{-4} M)
1	0.7579	0.033
2	0.7566	0.0308
3	0.7569	0.0294
4	0.7645	0.0308
5	0.7756	0.0294

The relative standard deviations were 1.088% and 4.804% for 1.2×10^{-2} M and 3.0×10^{-4} M respectively.

Conclusion

In this work, an aminopolycarboxylic acid chelating agent (TAHA) was prepared and characterized. TAHA formed stable complexes with copper(II) ions and some other metal ions. The complexation behavior was studied by spectrophotometry at Cu(II)–TAHA complex maximum wavelength.

The formula of Cu(II)–TAHA complex was determined by the continuous variation method. The stoichiometry was found to be (1:1) ligand to metal mole ratio.

Stability constants for Cu(II)–TAHA complex was calculated using continuous variation method. $K_{formation}$ was calculated to be 1.86×10^5.

TAHA and its Copper(II) complex were found to be stable for an extended period of time.

This work has some novel aspects such as, studying the complexation nature of various divalent metal ions with TAHA by spectrophotmetry. A modified correction method was applied to solve a problem that arose from the fact that more than one complex are formed in the reaction mixture, and absorb at the studied wavelength with a strong overlap.

Suggestions for further work

The following recommendations are suggested for future work:

- Studying TAHA complexation behavior with Metal(II) ion in real samples.
- Supporting TAHA chelating agent on some solid supports such as silica gel, to prepare an optical sensor and to study it is characteristics by spectrophotometry.
- Characterizing TAHA chelating agent and its complex with Cu(II) by NMR and X – ray to give a more clear picture about complex formation.
- Studying TAHA chelating agent degradation in soil and water waste.

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جامعة النجاح الوطنية
كلية الدراسات العليا

تحديد أيونات بعض العناصر بواسطة التحليل الطيفي عبر تشكيل معقد مع كاربوكسيليتد ترس(2-امينوايثل)أمين

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس- فلسطين.

2014

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الملخص

في هذا العمل تم تحضير Tris(2-aminoethyl)aminehexaacetic acid (TAHA) وتشخيصه. ملح البوتاسيوم للمركب المحضر تم تحضيره من خلال تفاعل الاستبدال النيوكليوفيلي بين Tris(2-aminoethyl)amine وملح البوتاسيوم لحمض الايثانويك.

تم استخدام مطياف الأشعة تحت الحمراء (FT- IR Spectroscopy) من أجل التأكد من اكتمال التفاعل والتأكد من نقاوة النواتج لكل من ملح البوتاسيوم والحمض.

TAHA يكوّن معقدات ثابتة مع أيونات النحاس(II) وبعض أيونات المعادن الأخرى التي تم دراستها على درجة حموضة قاعدية تقريبا 10. طبيعة تكون المعقد تم دراستها من خلال التحليل الطيفي على الطول الموجي الخاص بالمعقد.

خليط من النحاس(II) وبعض أيونات المعادن الأخرى مثل (Ca^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}) تم معايرتها مع TAHA على درجة حموضة تساوي تقريباً 10. معقد $[Cu(NH_3)_4]^{2+}$ أكثر ثبات من المعقد الذي تشكله باقي الايونات مع الامونيا. لذلك تم استبدال الامونيا في معقدات الايونات الأخرى أولاً. بعد ذلك تم تكوين معقد النحاس(II) مع TAHA. كما هو متوقع لم يكوّن TAHA معقد مع العناصر الفلزية القلوية. وجد أن نسبة مولات TAHA – للمعدن في المعقد الناتج هي (1:1) للايونات التي تم دراستها.

تم حساب ثابت تكوين معقد النحاس(II) مع TAHA باستخدام طريقة التغير المستمر (Continuous Variation Method). كما وجد أن TAHA و معقد النحاس(II) ثابتين لمدة طويلة.

قانون Beer ينطبق على التراكيز المستخدمة من النحاس(II) (3.0×10^{-4} M - 1.2×10^{-2} M). الدراسات الكمية لأيونات النحاس(II) مثل LOD و LOQ وجد أنها تساوي 7.285×10^{-6} M و 2.428×10^{-5} M على التوالي. تم حساب الانحراف المعياري النسبي (RSD) لخمس عينات للتراكيز: 1.2×10^{-2} M و 3.0×10^{-4} M من أيونات النحاس(II) ووجد أنها تساوي 1.088% و 4.804% على التوالي.