Spectrophotometric Determination of The Complexation of Heavy Metal Ion with Organic Reagent

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Abstract. A simple, accurate, sensitive, and rapid spectrophotometric method have been developed for the direct estimation of copper (II) using 3-(4-Antipyriyl azo)-1-Nitroso-2-naphthol (APANN) as a new reagent. The methods are based on complexation reaction between copper (II) and APANN at pH 8.5. The complex has a maximum absorption at 430.5 nm. Various factors to yield maximum absorption and sensitivity have been studied and optimized. The calibration graph is linear over the concentration ranges of (0.1–2.5 μg. mL−1) with apparent molar absorptivity values of 1.83 × 10^4 L. mol⁻¹. cm⁻¹. The stoichiometry between copper (II) and APANN was found as 1:2. The method developed was successfully applied to the analysis of tea leaves samples without any preliminary concentration or separation. The effect of interferences such as cations, anions was also studied.

Key Word: Heavy Metal Ion, Spectrophotometric Studies, 3-(4-Antipyriyl azo)-1-Nitroso-2-naphthol.

1. Introduction

Determination of Copper is important, particularly from a biological and industrial point of view. Nowadays the industrial production of copper takes the second place after aluminum one. This is due to the application of copper in the production of cables, electrical conductive installations, heat exchangers, etc. It is the essential component of brass, bronze, copper-nickel and other alloys, which are characterized by excellent corrosion resistance in air, high electrical conductivity, ductility and sufficient strength. Copper is an essential micro-nutritional substance in all living organisms. Human beings, animals and plants need copper for growth and healthy development. Newborn infants are bearers of 5–10 times more copper than adults, as a life insurance for growth, immunity, etc. Similar to other essential metals, both excess and deficiency of Copper in the body can cause serious diseases, such as Wilson disease and Alzheimer’s disease. Therefore, many methods to estimate copper (II) ion are available, including Differential pulse polarography, atomic absorption spectrometry, Inductively Coupled plasma- Atomic Emission (ICP-AES). These methods are fast and accurate for the determination of copper (II) ion in biological and environmental samples, but they often require expensive apparatus or complicated processes. Currently, in order to satisfy the requirements of environmental management and environmental risk assessment, it is necessary to develop sensitive, fast, reproducible, simple, low-cost, and accurate analytical methods for the
determination of copper. In the present study, we created a reliable, direct, and rapid spectroscopic method for investigation of 3-(4-Antipyryl azo)-1-Nitroso-2-naphthol interaction with copper (II) ion and selecting the optimal conditions for the chelate complex compound creation.

2. Experimental

2.1. Equipment

The pH was determined with a model Hanna, HI9811-5 pH – meter with combined glass calomel electrode. Shimadzu UV – visible – 1700 double beam spectrophotometer using 1 cm quartz cells was used for spectrophotometric measurements. Conductivity was measured in DMSO \(\left(10^{-3}\right)\) solution with an Alpha digital conductivity model-800.

2.2. Standard copper (II) solution

A stock solution of copper (100 mg mL\(^{-1}\)) was prepared by dissolving an accurately weighed amount of Copper chloride dihydrate (0.0537g) in 200 mL of distilled water, other working standard of Cu (II) solutions were prepared by simple dilution of the appropriate volume of the standard Cu (II) solution (100 µg . mL\(^{-1}\)) with distilled water.

2.3. 3-(4-Antipyryl azo)-1-Nitroso-2-naphthol (APANN) solution

3-(4-Antipyryl azo)-1-Nitroso-2-naphthol (APANN) was synthesized and recrystallized as reported in previous study \(^{(10)}\). The stock solution (0.001 M) of APANN was prepared by dissolving 0.0969g of this reagent in a 250 mL of ethanol. The working solutions of APANN were prepared by diluting the stock solution aliquot in ethanol. The structural formula of the reagent is:

Figure 1: structure of APANN

2.4. Preparation of Copper (II) complex

The complex was prepared by stoichiometric amount from ligand in 100 mL of ethanol then added drop wise with stirring to a stoichiometric amount 2:1 for copper salt in 50 ml distilled water. The mixture was stirred at room temperature for 5 min. The pH of solution was adjusted to 8.5 then left for 24 hr. The solid product thus formed off, washed with distilled water, and recrystallized from ethanol.
2.5. Solutions of foreign ions (100 µg.mL⁻¹)

Various metal ion solutions were prepared by dissolving an appropriate amount of their salts in distilled water. All of the chemicals used were of analytical grades. Double distilled water was used throughout the work.

2.6. Recommended procedure for determination of Cu(II) by using APANN

Transfer increasing volumes of Cu(II) working solution 10 ppm to cover the range of calibration curve into a series of 10 mL calibrated flask and add 2.5 mL of 1mM of (APANN) solution and pH was adjusted to 8.5 with dilute hydrochloric acid and sodium hydroxide. The complex formed was solubilized in water and diluted up to 10 mL in a standard flask. The absorbance of the coloured complex was measured at 430.5 nm against reagent blank prepared in similar manner but containing no copper ion. The color of the complex is stable for 24 hrs.

3. Results and Discussion

3.1. Spectrophotometric studies:

3.1.1. Absorption spectra

The absorption spectra of the Cu(II) - APANN complex show a maximum absorption at 430.5 nm. Hence 430.5nm was chosen as the wavelength for the determination. Absorption spectra of APANN, and its Copper complex is shown in the Figure 2.

![Absorption spectra of [Cu (II) + APANN] treated as described under procedure and against reagent solution as blank.](image)

**Figure 2**: Absorption spectra of [Cu (II) + APANN] treated as described under procedure and against reagent solution as blank.
3.1.2. Infrared spectra

The FT-IR spectra of the free ligand and the prepared complex have been compared, and the data was listed in Table 1. The IR spectra of the ligand exhibited broad band at 3425 cm$^{-1}$, which was assigned to the stretching vibration of naphtholic OH group$^{(11)}$, which was shifted to lower frequency with low intensity at 3402 cm$^{-1}$ in the spectra of metal complex, indicating de-protonation of the OH group upon binding with metal ion. The IR spectrum of the ligand appeared band at 1650 cm$^{-1}$ which was assigned to the stretching band of $\nu$(C=O) of pyrazole azo. This band is shifted to higher with low intensity at 1681 cm$^{-1}$ frequency value upon complexation. Band characteristic of the azo bridge vibration at 1535 cm$^{-1}$ shifted to lower and higher frequency with change in shape in spectra of complex, which is an indication of the bonding of this group in the coordination with the metal ion$^{(12,13)}$. The bonding of oxygen to the metal ion is provided by the occurrence of band at 486 cm$^{-1}$ as the result of $\nu$(M-O)$^{(14,15)}$. In addition, the FTIR spectra of the complex show new band at 439 cm$^{-1}$, assigned to $\nu$(M-N) stretching band$^{(16,17)}$.

![Figure 3: F.T.I.R Spectrum of APANN](image-url)
Figure 4: F.T.I.R Spectrum of Cu-complex

Table 1: Selected FT.IR data of (APANN) and it’s complex with Cu (II)

| Compound     | v (OH) | v (C-H) | v (N=N) | v (C=O) | v (M-O) | v (M-N) |
|--------------|--------|---------|---------|---------|---------|---------|
| APANN        | 3425 m | 3062 m  | 1535 m  | 1650 s  | –       | –       |
| Cu(APANN)₂   | 3402 b | 3062 m  | 1458 m  | 1681 m  | 486 w   | 439 w   |

S: sharp ; m: medium ; w: weak; b: broad

3.2. Optimum conditions for Complex Formation

There are many parameters affecting on the absorption intensity of the formed products were studied and the reaction conditions were optimized.

3.2.1. Effect of pH

The effect of pH on the absorbance of Cu(II)-APANN complex compound was investigated over the range (5–11). The pH of metal complex solutions was adjusted using dilute solutions (0.05M) NaOH and (0.05 M) HCl and the absorbance–pH curve for metal ion measured at λmax is plotted in Figure 5. The optimum pH range for complex formation lies between pH 7–9.5. So, further investigations were carried out at pH 8.5.
Various volumes of 1 mM reagent solution were added to the sample solution containing fixed concentration of Copper (II) at optimum pH values. 2.5 mL of 1 mM (APANN) solution was sufficient and gave minimum blank value and was consider to be optimum for the concentrating range. Therefore 2.5 mL of 1 mM of APANN was chosen in all subsequent experiment Figure.6.

The effect of reaction time on the stability of complex was showed in Figureure 7. Constant maximum absorbance was obtained just after dilution to volume, and remained unaltered for 24 h.
3.2.4. Effect of temperature

Temperature effect on the absorbance of the Cu(II)– complex was studied between 10°C and 70°C. The maximum absorbance was between 20°C and 40°C. Above 40°C, absorbance decreases gradually with increasing temperature until it reaches 70 °C. This may be explained by the dissociation of the complex. The results are shown in Figure 8.

Figure 7: Effect of time on complex of Cu[APANN]₂

Figure 8: Effect of temperatures on complex Cu[APANN]₂
3.3. Calibration graph

The system obeyed Beer’s law over the concentration range (0.1 – 2.5 µg mL\(^{-1}\)) of copper(II) in Figure 9. The molar absorptivity and sandell’s sensitivity are given in Table 2.

![Calibration graph of Cu(II)-complex](image)

**Figure 9:** Calibration graph of Cu(II)-complex

3.4. Composition of the complex

Job’s method \(^{18}\) of continuous variation and the mole ratio \(^{19}\) method were applied to suggestion the stoichiometric composition of the complex, results illustrated in Figures 10 and 11. A Cu(II)-APANN (1 : 2) complex was indicated by both methods.

![Job's method for Cu[APANN]_2 complex](image)

**Figure 10:** Job’s method for Cu[APANN]\(_2\) complex
Figure 11: mole-ratio method for Cu[APANN]₂ complex

Table 2: Spectral data for the determination of Copper ion using APANN as spectrophotometric reagent

| Parameter                              | Cu (II) – complex |
|----------------------------------------|-------------------|
| Maximum Absorption peak (nm)           | 430.5             |
| Beer’s law range (ppm)                 | (0.1–2.5)         |
| Molar absorptivity (L. mol⁻¹. cm⁻¹)    | 1.83 ×10⁴         |
| Sandell’s sensitivity μg . cm⁻²         | 0.00349           |
| Stability constant (L. mol⁻¹)           | 8.237 ×10⁸        |
| pH range                               | (7 – 9.5)         |
| Composition (Metal :Ligand)            | 1:2               |

3.5. Conductivity measurements

The molar conductivity of the complex is shown in Table 3. Measurements were carried out in ethanol and DMSO solvents for complex concentration of 10⁻³ M, at 25°C. Low values for molar conductance indicate that the complex is non electrolyte by nature, and that there are no free anions outside the coordination sphere.²⁰,²¹
Table 3. Conductivity values of complex

| Complex      | Molar conductivity, S·mole⁻¹·cm² DMSO | Molar conductivity, S·mole⁻¹·cm² Ethanol |
|--------------|--------------------------------------|----------------------------------------|
| Cu[APANN]₂   | 15.3                                 | 14.2                                   |

So that according to the above results we supposed structural formula of the complex as showed in Figure 12.

![Figure 12: structure of Complex](image)

3.6 Interferences

The effects of various foreign ions on the absorbance of the experimental solution containing Cu(II) were studied under optimum conditions. Various amounts of foreign ions were prepared, and the procedure for the determination of Cu(II) was followed. The metal ion can be determined in the presence of a 8 or more fold excess of cation and anion. In the experiment, a certain amount of standard Cu(II) solution, coexisting ion solution and masking agent (or absence of masking agent) were added. It is found that all the studied ions interfere seriously. However, their interferences are masked efficiently by addition 1.0 mL of 0.1 M of (KCl, Thiourea, Na₂HPO₄).
Table 4: Influence of interfering cations and anions on the relative error (E, %) of 10 μg/mL of copper(II)

| foreign ions | E%  | foreign ions | E%  |
|--------------|-----|--------------|-----|
| Zn^{2+}     | 31.42 | Pd^{2+}     | 34.9 |
| Mn^{2+}     | 21.27 | S_{2}O_{8}^{2-} | 1.4 |
| Co^{2+}     | 29.63 | Br^{-}      | 0.2 |
| Cd^{2+}     | 12.1  | SO_{4}^{2-}  | 0.1 |
| Ni^{2+}     | 17.4  | Cl^{-}      | 0.4 |
| Fe^{3+}     | 25.3  | CO_{3}^{2-}  | 1.3 |
| Hg^{2+}     | 15.34 | F^{-}       | 0.2 |

3.7. Precision and Accuracy

To check the accuracy and precision of the proposed analytical method, APANN is determined at two different concentrations with six replications. The results are shown in Table 5, which indicate good accuracy and precision.

Table 5: Accuracy and precision values of complex

| Amount taken of Cu (II) p.p.m | R.S.D % | Error% | Re. % | Detection limit ppm. |
|-------------------------------|---------|--------|-------|----------------------|
| 0.5                           | 0.97    | 0.6    | 99.4  | 0.0149               |
| 1                             | 0.41    | 0.3    | 99.7  | 0.0122               |

** For six determinations

3.8. Applications

3.8.1. Determination of Copper (II) in tea samples (22,23)

1 g amount of tea sample was accurately weighed and placed in a porcelain crucible and dried at 250 °C for half an hour and incinerated in a furnace at 800-850 °C until the sample turned into ash. After incineration was complete, a 2 mL of water were added to wet it. The residue was dissolved in 10 mL of hydrochloride acid solution (1:1, v/v). The filtrate was transferred into a 25 mL volumetric flask and then diluted with distilled water to the mark of the volumetric flask as test solution. An appropriate amount of the above-stated test solution was taken and placed in a 10 mL volumetric flask for copper determination according to the general procedure. Then, recovery determination experiments were made and atomic absorption spectrometry was used for contrast determination. The above analytical results are given in Table 6.

Table 6 Cu(II) levels (g.g^{-1}) in tea samples

| sample               | Amount found by our Spectrophotometric method, g.g^{-1} | Amount found by atomic absorption method, g.g^{-1} |
|----------------------|----------------------------------------------------------|--------------------------------------------------|
| 1-Lipton clear green tea | 6.25                                                      | 5.8                                              |
| 2-Lord black tea     | 8.7                                                      | 7.14                                             |
4. Conclusion

In this study it was shown that 3-(4-Antipyriyl azo)-1-Nitroso-2-naphthol could be used as a reagent for the Spectrophotometric determination of copper (II). The method described in this paper is successfully applied to the determination of trace amounts of Copper in tea leaf samples and in synthetic mixtures.

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