Simultaneous Spectrophotometric Estimation of Copper(II) and Iron(III) by using 3, 4 dihydroxy benzaldehyde-1- (3 chloro- 2-Quinoxalinyl) hydrazine (3, 4-DHBCQH) in presence of Neutral Tween-80 Micelles

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Abstract: 3, 4 dihydroxy benzaldehyde-1- (3 Chloro- 2-Quinoxalinyl) hydrazine (3, 4-DHBCQH) has been synthesized, characterized and employed as a simple simultaneous second order derivative spectrophotometric method for the analysis of a three-component system containing Copper(II) and Iron(III) without separation. The reagent (DHBCQH) reacts with Copper(II), and Iron(III) at pH 6.5, forming soluble orange red coloured species. Copper and Iron present in the mixture are simultaneously determined without solving the simultaneous equations by measuring the second derivative amplitudes at 430 nm and 420 nm, respectively. Further, the derivative amplitudes obey Beer’s law at 430 nm and 420 nm for Cu(II), and Fe(III) in the range 30.0 – 110 ng/ml and 25.0 – 95 ng/ml, respectively. A large number of additional ions did not interfere with the method. The simultaneous method can be applied for the determination of copper(II) and Iron(III) in grape leaf sample.

Keywords: Spectrophotometric Determination, Iron, Copper, 3,4-DHBCQH, Surfactant Tween-80.

I. INTRODUCTION

Copper is a widely distributed metal in nature, and is an essential metal required by almost all living organisms in some of their biological activities. Deficiency of copper may lead to certain physiological disorders in both plants and animals, but at higher concentrations it works essentially as a pollutant. Iron is one of the most important transition element in living systems, being vital to both plants and animals.

The stunted growth of the former is well known in soils, which arc either themselves deficient in iron or in which high alkalinity renders the iron too insoluble to be accessible to the plants. Iron was the first minor element to be recognized as being essential to human being and was used in the treatment of anaemia. The unique characteristics of micellar media promoted their widespread use in separation science and in chemical analysis. These include just their use to enhance one of the phenomenal phase is enhancing analyte/analytical reagents in water. This eliminates the need for harsh solvent/water miscible organic co-solvents for solubilization. The enhanced extinction coefficient of coloured species increases the sensitivity (microgram to sub microgram through nanogram) and batho/hipso chromic shift of spectral profile results in selectivity of analytes.

Hydrazides and hydrazones found extensive applications in pharmaceutical preparations, analytical chemistry for detection/determination of low concentration of metal ions and intermediate/starting molecules in the preparation/synthesis of high impact chemical moieties.

Hydrazones are the condensation moieties of hydrazides/hydrazines with aldehydes/ketones with astounding chelating characteristics with metal ions in solution/solid phases. The multifaceted explorative research studies opened new vistas in analytical chemistry and biochemical interactions in_vitro/in_vivo/in_silico.
The results from chemical biology perspective shows hydrazones are prospective molecules in the pipeline of drug acceptance for treatment of mental disorders, iron overload [1], spasimolytic activity, hypertension, malignant neoplasms, leukaemia/sarcoma/carcinoma[2]. These molecules have also been in use as insecticides, rodenticides, herbicides, regulator of plant growth and sterilizers for house flies [3].

The industrial applications encompass dying, photography and polymers arenas [4, 5]. The research findings on the chemical, biological and industrial implications are extensive. The review papers and recent typical reviews on hydrazones are summarized [6-14]. In this research article we describe our results on the simultaneous determination of Cu (II) and Fe (III) in acidic medium using second order derivative spectrophotometry.

II. EXPERIMENTAL

A. Synthesis of Ligand

Synthesis and Characterization of 3, 4 dihydroxy benzaldehyde-1-(3 chloro-2-Quinoxalinyll) hydrazone (3, 4-DHBCQH).

This compound was prepared in four steps

1) Step 1: Quinoxaline-2,3-dione (or) 2,3-diol

O-phenylene diamine (SD fine) 5.4 g was dissolved in 50.0 ml HCl(4.0N) and refluxed with oxalic acid (6.3 g) (SD. Fine) for 2 hours. On cooling, gray coloured needles were observed, which are separated by filtration and washed with water. It was dissolved in NaOH solution, heated, treated with charcoal, filtered and the filtrate acidified with concentrated HCl to get pure compound. The yield was 90% and melting point was 3600C.

2) Step 2: 2, 3-dichloroQuinoxaline (II)

A mixture of POCl3 (SD fine) 81.0 ml and DMF (SD fine) 2.0 ml was added slowly with stirring in a round bottom flask containing 8.1. g of (I). The contents were refluxed, for 2hours at 1058C. It was cooled to room temperature and poured in to crushed ice. The compound separated was filtered with water and dried. The yield was 85% and melting point was 1508C.

3) Step 3: 3-chloro – 2- hydrazineQuinoxaline (III):

To stirred solution of 2,3-dichloroQuinoxaline( 9.9g, 0.05m) (II) in Methyl alcohol- (60.0ml) containing triethylamine ( 5.05g,7.0m, 0.05m ) a solution of hydrazine hydrate (80%, 3.125g, 30 ml, 0.05 m) in Methyl alcohol (30.0 ml) (Merck) was added at room temperature. The reaction mixture was stirred at room temperature for 3hours. The separate product was filtered, washed with water and dried in desiccators over anhydrous CaCl2 to obtain 3-chloro-2-hydrazinequinoxaline.The yield was 75% and melting point was 1808C.

4) Step 4: 3,4-dihydroxybenzaldehyde-1-(3-chloro-2-Quinoxalinyll) hydrazone (3, 4- DHBCQH)

To a stirred solution of 3, 4-dihydroxybenzaldehyde (3.175 g, 0.05 m) (Aldrich) in DMF (30.0 ml) at room temperature, the hydrazine derivative (1.945g, 0.01 m) (III) was added as solid in small lots, on completion of which a clear solution was obtained. The mixture was refluxed a further period of 2 hours. When a bulk orange yellow solid separated out, it was filtered, washed with water and recrystallised from CHCl3- hexane. The yield was 55% and melting point was 2718C. The reactions for above steps explained in Figure-1.
B. Preparation of Solutions
Buffer solutions were prepared using HCl, CH3COOH and Sodium acetate in acidic medium and NH4OH, NH4Cl in basic medium. 
(iii) Preparation of metal solutions and reagent solution
3,4-dihydroxybenzaldehyde-1-(3-chloro-2-Quinoxalinyl)hydrazone (3,4DHBCQH): 0.0783 g of recrystallised sample of the reagent 3,4-dihydroxybenzaldehyde-1-(3-chloro-2-Quinoxalinyl) hydrazone was dissolved in DMF in 25 ml volumetric flask to obtain the stock solution (1 x 10-2 mol dm-3), and it was suitably diluted to get the required concentration. Fresh reagent solution is prepared as and when required.
Copper sulphate: Requisite quantity of CuSO4.5 H2O is dissolved in double distilled water and made up to the mark in a 100 ml volumetric flask to get (1 x 10-2 mol dm-3) stock solution of Cu(II). The resulting solution is standardized with hypo solution.
Ferric chloride: Requisite quantity of FeCl3.6 H2O was dissolved in double distilled water containing few drops of concentrated HCl and made up to the mark in a 100 ml volumetric flask to obtain (1 x 10-2 mol dm-3). The resulting solution was standardized with EDTA solution.

C. Zero Order Spectrums of Copper(II) and Iron(III)
A Solution containing 1ml 1x10-6 mol dm-3 copper sulphate and 1ml of 1x10-6mol dm-3 Ferric sulphate are taken in a 10ml volumetric flask. 5ml of a buffer solution of (pH 6.5)and 1.0ml of 1%(v/w) Tween-80 was added. The contents of the flask are made up to the mark with distilled water. The solution is shaken well for uniform concentration. A blank solution is prepared on the same lines but without containing the metal ions.

D. Simultaneous Determination of Cu (II) and Ni (II) with Second Order Derivative Spectrophotometry
A Solution containing 1ml 1x10-6 mol dm-3 copper sulphate and 1ml of 1x10-6mol dm-3 Ferric sulphate are taken in a 10ml volumetric flask. 5ml of a buffer solution of (pH 6.5),1ml 1x10-4mol dm-3DHBCQH reagent and 1.0ml of 1%(v/w) Tween-80 was added. The contents of the flask are made up to the mark with distilled water. The solution is shaken well for uniform concentration. A blank solution is prepared on the same lines but without containing the metal ions.
III. RESULTS AND DISCUSSION

Studies carried out with various metal ions with the present reagent revealed that 4-HBTS gives a green colored solution with Cu (II) and pale green colored solution with Ni (II). The authors have carried out the effect of PH on the color reaction in presence of Cu (II) as well as Ni (II). The color development is maximum in the PH range 5 – 6 with both the metal ions taken individually. In view of this a solution of PH 6 is fixed for further investigations. Studies relating to the effect of metal ion concentration, reagent concentration, time, organic solvent are carried out to establish the optimum conditions for maximum color development. The order of addition of various components of the reaction system has no influence on the color reaction. It is also observed that a minimum of 10 times excess of the reagent is essential for the complete formation of color either with individual metal ion (or) an admixture. The color is quite stable for one hour; hence the absorbance measurements can be made from various solutions even after one hour. Job’s continuous variation method and mole ratio method are performed to determine the composition and stability constants of the complexes. Both the metal ions form 1:2 (M:L) complexes with the reagent. The stability constants are 5.93x10^10 and 2.76x10^11 for copper and nickel respectively.

A. Zero Order Spectra

The zero order spectra of Cu (II) (Figure 2a) and Fe (III) with the reagent (Figure 2b) against the reagent blank are recorded in the wavelength range 400 to 600 nm and are shown in Figure 2(a&b). An analysis of the figure reveals that the Cu(II) and Fe(III) complexes showed maximum absorbance at 375 and 360 nm respectively. An attempt to make simultaneous determination of Cu (II) and Ni (II) did not produce fruitful results. It is because of the small difference in the λmax values. The zero order spectrum of the mixture of Cu(II) and Fe(III) is recorded against the reagent blank and is shown in Figure 3. The resolution of Cu (II) and Fe(III) is not possible and the resolution becomes more difficult as the concentration of metal ions are increased.

B. Second order Derivative Spectrophotometry

The spectrum for a solution consisting of Cu (II) and Fe(III) as well as the reagent in acidic medium of PH 6.5 is recorded against the reagent blank and shown in Figure 4. An examination of the figure suggests that there are two peaks corresponding to the two metal ions at 430 and 435 nm respectively. Individual second order derivative spectra are recorded for both the metal ions under the same conditions. The results revealed that the peak and valley at 430 and 410 nm correspond to Cu(II) and 420 and 415 nm respectively. Experiments pertaining to the effect of copper(II) concentration and iron(III) concentration on respective peak and valley amplitudes are carried out. The simultaneous determination of the metal ions was carried out using zero crossing measurement method. Graphs are also plotted taking the sum of peak and valley amplitudes for both the metal ions individually. Straight line plots are obtained even in this case. Copper(II) and Iron(III) are bivalent transition metal ions and show similar chemical behavior. As a result, the simultaneous determination poses problems. Prior separation before determination is usually carried out in many cases. In the present method no prior separation is required. The method is simple, rapid, selective and applicable over a wide range of concentrations.

IV. INTERFERENCE

Interference of various metal ions usually associated with copper(II) and iron(III) and various anions is investigated. The relevant data is shown in Table 1. It is seen from the table that Mn (II) and Sn (II) seriously interfere while the other metals can be tolerated at least to certain extent. Most of the anions do not show much influence.

| Ion added  | Tolerance limit(µg/ml) | Ion added  | Tolerance limit(µg/ml) |
|------------|------------------------|------------|------------------------|
| Cu(II)     | Fe(III)                | Cu(II)     | Fe(III)                |
| Iodate     | 753                    | 572        | Mn(II)                 | 12                     |
| Tartarate  | 1136                   | 854        | Cd(II)                 | 125                    |
| Bromide    | 648                    | 618        | Al(III)                | 21                     |
| Chloride   | 436                    | 417        | Mo(VI)                 | 30                     |
| Thiocyanate| 658                    | 454        | V(V)                   | 42                     |
| Nitrate    | 471                    | 410        | Sn(II)                 | 10                     |

Table 1: Interference of foreign ions in the determination of 63.5ng/ml of Cu (II) and 55.84ng/ml of Fe(III).
Table 2: Simultaneous determination of Cu (II) and Fe (III) in grape sample

| Sample     | Certified value(APARI) | Amount found* | Recovery |
|------------|------------------------|---------------|----------|
| Grape leaf | Cu(II) | Fe(III) | Cu(II) | Fe(III) | Cu(II) | Fe(III) |
|            | 55.40  | 33.70  | 55.30  | 33.42  | 99.8   | 99.6   |

*Average five determinations

V. CONCLUSIONS

Simultaneous determination of Cu (II) and Fe (III) in acidic medium using second order derivative spectrophotometry with DHBCQH as a reagent is carried out. Both metal ions can be determined Coer(II) and Iron(III) in the ranges 30 to 110 ng/ml and 25 to 95 ng/ml using this method. The compositions and stability constants of the complexes are 1:2 (for both) $5.93 \times 10^{12}$ (Cu(II)) and $2.76 \times 10^{11}$ (Fe(III)). The method has been applied for the determination of copper (II) and Iron(III) in grape sample.

Figure 2: Zero order spectrum of M(II) solution: 2(a): Cu(II) and 2(b): Fe(III)

![Figure 2](image)

Figure 3: Zero order spectrum of mixture of Cu(II) + Fe(III)

![Figure 3](image)
REFERENCES

[1] Becker, E. & Richardson, D.R., (1999) J. Lab. Clin. Med, 134(5), 510-21.
[2] Richard, D.R., et al, Blood, (1995) 86, 4295.
[3] BuzykIn,B.I., Bystrykh,N.N., Bulgakova,A.P., & Kitaev,Yr.P., Otkriitya. Lzobret., Prom Obraztsy, Tovaryne Znaki., (1978) Chem. Abstr., 88, 132038.
[4] Bernhardt,P.V., Caldwell,L.M.,T.B . Chaston,T.B., Chin,P., Richardson, D.R., (2003) J. Biol Inorg Chem, 8(8), 866-80.
[5] Luede,H., (1978) chem. Abstr., 88, 9892.
[6] Kölmel,D.K., & Kool,E.T., (2017) “Oximes and hydrazones in bioconjugation mechanism and catalysis” Chemical Reviews, 117(15), 10358-10376.
[7] Chen,P., & May,J.A., (2016) “The usefulness of hydrazine derivatives for mass spectrometric analysis of carbohydrates” Mass Spectrometry Reviews, 32(5), 366-385.
[8] Su,X., & Aprahamian,J., (2014) “Hydrazone-based switches, metallo-assemblies and sensors” Chemical Society Reviews, 43(6), 1963-1981.
[9] Shao.Z., & Zhang.H., (2012) “N-tosylhydrazones: Versatile reagents for metal- catalyzed and metal-free cross-coupling reactions.” Chemical Society Reviews, 41(2), 560-572.
[10] Lakshmi Narayana,S., Young,S.K., Sung,B.O., Varada Reddy,A., (2012) “Review on Analytical and Biological applications of Hydrazones and their Metal Complexes” E-Journal of Chemistry, 9(3), 1288-13
