

# Separation of Copper I and II Iron II and III in Composite Aqueous Matrix by Competitive Chelation Using Disc Electrophoresis Technique

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**Summary:** A new, simple and rapid Disc electrophoresis method has been developed in our laboratory for determination of different oxidation states of Iron and copper singly as well as in mixture form through the specially prepared glass tubes of polyacrylamide gel using glycine buffer at pH 4 and 3.5. For quantitative analysis the intensity of electrophoretically separated colored bands of respective metal ions in the tubes were measured using Thin layer chromatographic scanner CS-910. The developed plates were scanned separately in UV, Vis and fluorescence regions on pre-determined wavelengths simultaneously integration and profile mode. It has been observed that, very sharp Disc bands with particular colour of both cations with different oxidation state were obtained with approximately ten micron thickness. The detection limits has been achieved in the range of ng/ $\mu$ l level.

## Introduction

The electrophoresis technique has been widely used in separation of amino acids, proteins and other organic molecules [1,2]. However, its use has not received much attention for analysis of metal ions in aqueous matrix due to availability of other techniques. Electrophoresis studies of metal ions and their corresponding complexes have been made mostly on paper or thin layer plates [3]. In fact, very little attention has been given to gel electrophoresis techniques for the study of metal ions and their corresponding complexes.

Literature review indicated that, Iron II and III mixture were separated simultaneously on paper chromatography, electrophoresis in 0.005 M sulfuric acid and by colorimetry using 1,10 phenanthroline reagent for coordination [4-7].

Cu (I) and anionic complex of Cu (II) were separated on Whatman filter paper using matrix of EDTA, NaF and HCl as electrolyte. Dithioamide was used as a color developing reagent [8].

In present paper emphasis has been laid to develop a rapid and simple disc electrophoretic

technique for the separation of different oxidation states of Iron and Copper, using glycine and acetic acid buffer at pH 4.0 and 3.5. The glycine have been used in this study, with dual purpose of serving as electrolyte and complexing agent. However it forms colored complexes with metal-ions. Glycine is a dipolar ion forms salts (zwitter ion) with metals, and with heavy metals it forms chelates [9].

## Results and Discussion

By developing this technique, the separation of glycine complexes of Iron II and III, copper I and II has been investigated.

After optimization of conditions for resolution of coloured bands in glass tubes, the spectral analysis to identify and quantify the presence of various metal ions was accomplished using TLC Scanner at corresponding maxima of the individual components of the matrix. A linear graphs of each cation standards was drawn by fitting least square method. The graphs are given in figure 1.

The mixture of Iron III and II copper II and I were studied in 10% polyacrylamide gel using glycine

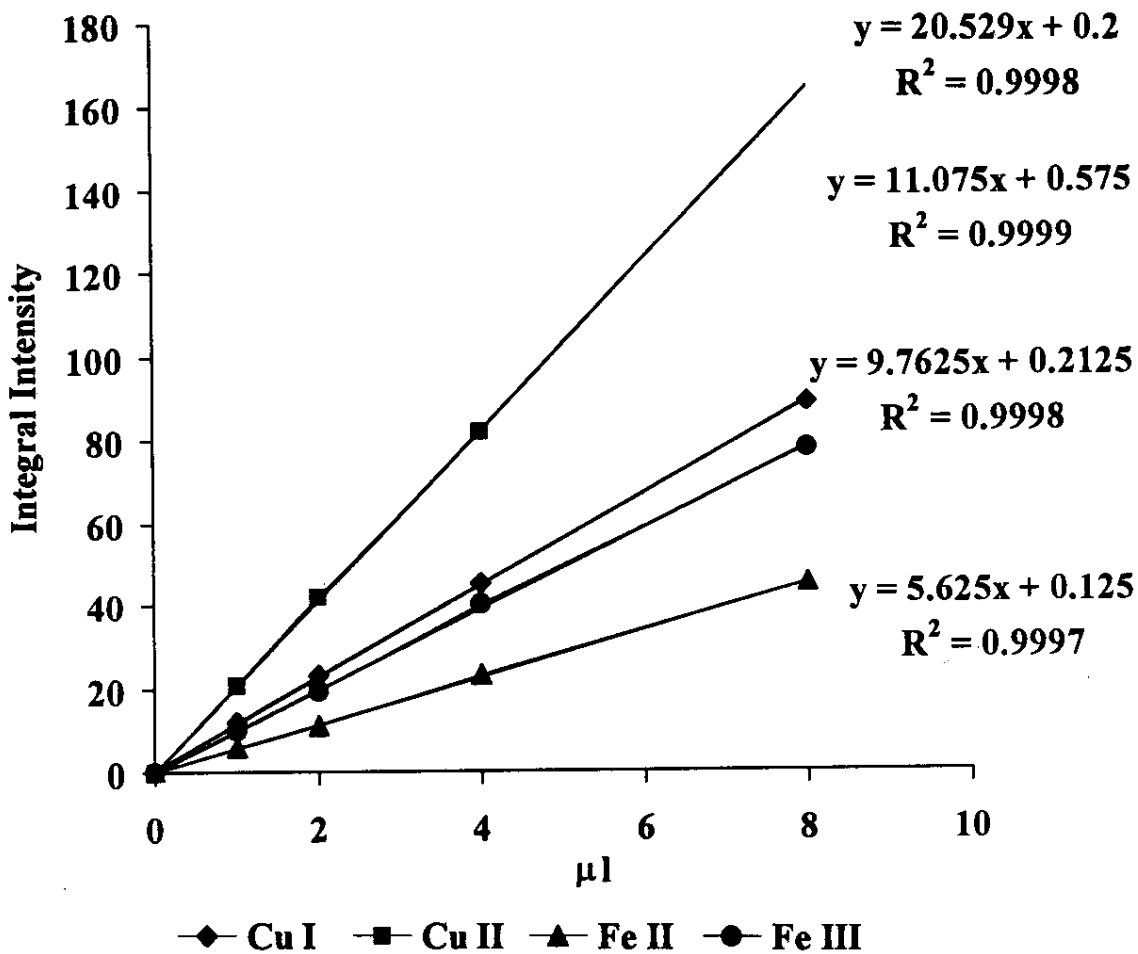


Fig. 1: TLC study of different cations glycinate complexes

Statistical linear graph of metal ions plotted Conc. Vs absorbance of different cations glycinate complex using TLC scanner CS-910

Experimental parameters

Mode = transmission

Chart range = 5 mV

Integral = scanning

Concen. 10 ppm for each metal ions

( $\mu$ l)	Cu I	Cu II	Fe II	Fe III
0	0	0	0	0
1	12	20.5	6	10
2	23	42	11	19.5
4	45	82	23	40
8	89		45	78

buffer (pH 3.5) at different time intervals. The results are mentioned in table 1. The rate of migration of Fe II has been observed low as compared to Fe III ion. The color of Fe II band has been seen light brown while the dark brown for Fe III. The rate of migration of Cu I was found faster as compared to Cu II.

Table 1: Migration distance of the metal Glycinates  
 Experimental parameters: Gel matrix 10% Polyacrylamide Gel (pH 8.9)  
 Electrode Buffer = pH 3.5  
 Tube length = 8.4 cm x 0.4 (id)  
 Current = 55mA  
 10  $\mu$ l of mixture of 1 ppm of each cations

Complexes	Migration period (min.)	Mean Distance (cm)
Fe (III) glycinate	15	1.5 $\pm$ 0.1
	30	2.5 $\pm$ 0.1
	45	3.8 $\pm$ 0.1
Fe (II) glycinate	15	0.8 $\pm$ 0.05
	30	1.2 $\pm$ 0.1
	45	1.2 $\pm$ 0.1
Cu (II) glycinate	15	2.0 $\pm$ 0.3
	30	3.3 $\pm$ 0.4
	45	4.5 $\pm$ 0.4
Cu (I) glycinate	15	2.8 $\pm$ 0.2
	30	4.0 $\pm$ 0.4
	45	6.2 $\pm$ 0.4

However, in case 7.5 % acrylamide gel the separation of mixture of same metal ions were studied using glycine buffer at (pH 4). The tubes were used longer with length of 13.0 cm x 0.5 (id) as compared to those were used for 10% gel system in order to investigate the effects on migration system. It has been observed that, the rate of migration of all metal ions were higher as compared to distance traveled in 10 % poly acrylamide gel system. Thus the pore size of gel greatly effect on the rate of migration. The analysis of mixture of both cations in different combination and in different ratio the response of individual complex was nearly the same as those of standard run singly. Results are in table 2.

Table 2: Migration distance of the metal glycinate  
Experimental parameters: Gel matrix 7.5% Polyacrylamide, Gel (pH 7.5), Electrode Buffer = pH 3.5, Current = 90 mA,  
Tube length = 13 cm x 0.5 (id), 10  $\mu$ l of mixture of 1ppm of each cations

Complexes	Migration period (min.)	Mean distance (cm)	Color of complexes
Fe (III) glycinate	15	1.5 $\pm$ 0.1	Dark Brown
	30	2.3 $\pm$ 0.1	
	45	3.8 $\pm$ 0.1	
Fe (II) glycinate	30	1.0 $\pm$ 0.1	Light Brown
	45	1.8 $\pm$ 0.1	
Cu (II) glycinate	30	3.2 $\pm$ 0.3	Blue
	45	6.8 $\pm$ 0.4	
Cu (I) glycinate	30	5.8 $\pm$ 0.2	Pink
	45	8.5 $\pm$ 0.4	

The experimental data obtained in table 3 shows that by using the metal cations with different anions, the migration distance was not differ and does not effect on separation sequences.

Table 3: Evaluation of the metal ion salts  
Experimental parameters: Gel matrix 10% Polyacrylamide Gel (pH 8.9), Electrode Buffer = pH 3.5, Tube length = 8.4cm x 0.4 (id), Current = 55 mV

Salts used	Migration period (min.)	Mean distance (cm)	% Impurity
1(a) Fe Cl <sub>3</sub> . 6H <sub>2</sub> O	45	Fe (II) 1.2	2.8
	45	Fe (III) 3.8	
(b) After oxidation with HNO <sub>3</sub>	45	Fe (III) 3.8 $\pm$ 0.2	
2. Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	45	Fe (III) 3.9 $\pm$ 0.2	3.2
	45	Cu (II) 4.8 $\pm$ 0.3	
3. (a) CuSO <sub>4</sub> . 5H <sub>2</sub> O	45	Cu (I) 6.5 $\pm$ 0.2	3.2
	45	Cu (II) 4.8 $\pm$ 0.2	
(b) After oxidation with HNO <sub>3</sub>	45	Cu (II) 4.8 $\pm$ 0.2	
4. Cu(CH <sub>3</sub> COO) <sub>2</sub> . H <sub>2</sub> O	45	Cu (II) 4.8 $\pm$ 0.2	2.6
	45	Cu (I) 6.5 $\pm$ 0.3	

The separation pattern of Iron and copper salts indicates the appearance of additional bands. However, these salts were further oxidized with concentrated HNO<sub>3</sub> and than spotted, the bands due to Fe (II) and Cu (I) were disappeared. The % impurity of these ion was calculated by statistical standard graph of each ion. The detection limits was determined by changing the different parameters of Instrument and recorder were found individually for each metal ions in range of 1 ng/ $\mu$ l, 16 ng/ $\mu$ l, 0.16 ng/ $\mu$ l and 0.16 ng/ $\mu$ l for Iron III, II, copper II and I respectively.

### Experimental

All the chemicals and reagents were used of analytical reagent grade.

- Glycine buffer pH 3.5: Dissolved 28 g glycine in small amount of water added 36 ml 96% acetic acid diluted up to 1 liter in deionized water.
- Glycine buffer pH 4.0: Dissolved 28 g glycine in small amount of water added 3.06 ml 96% acetic acid diluted up to 1 liter in deionized water.
- Trisbuffer(pH8.9): Dissolved 36.3 g of tris (hydroxymethyl)-aminomethane in 48ml of 1M HCl. Added 0.46ml of N, 1M, N', N-tetramethyl ethylene diamine (TEMED) and volume made up to 100ml.
- Tris buffer (pH 7.5) dissolved 6.85 g of tris (hydroxymethyl)-aminomethane in 4.8 ml of 1M HCl: Added 0.46ml of N, N, N', N-tetramethyl ethylene diamine (TEMED) and volume made up to 100 ml.
- Acrylamide solution: 25 g and 30 g of acrylamide and 0.4 g to 0.8g of N, N' methyl-bis acrylamid was dissolve in 100 ml water in separate flasks and stored as stock gel solution.
- Ammonium per sulphate solution used 0.14 and 0.28 g salt /100 ml separately.
- Prepared 1000 ppm of Fe<sup>+2</sup>, Fe<sup>+3</sup>, Cu<sup>+2</sup>, and Cu<sup>+</sup> solution from different salts as stock standard solutions. Working standard of 1 ppm for each metal ion was prepared from stock standard solutions freshly.

### Preparation of Gel Tubes for Disc Electrophoresis

Two types of polyacrylamide gel systems 10%, 7.5% have been used for the separation of metal ions. Glass tubes of different dimensions were used

8.4 cm x 0.4 (id) for 10% gel and 13 cm x 0.5 (id) for 7.5% gel.

7.5% Gel (pH 7.5) was prepared by mixing reagents, 1ml of tris buffer (pH 7.5), 2 ml of 30% acrylamide solution, 4 ml of 0.14% ammonium per sulphate solution and 1 ml of distilled water.

10% Gel (pH 8.9) 2ml of tris buffer (pH 8.9), 6.4 ml of 25% acrylamide solution, 4 ml of 0.28% ammonium per sulphate and 3.6 ml of deionized water. Polymerization takes 15 to 30 minutes.

#### *Preparation of Metal Glycinates, and Determination of Their Absorption Maxima.*

1 ml of 1000 ppm of metal ion solutions were prepared by 1 mg/ml were mixed with glycine buffer solution in the ratio of 1:2 for divalent ion and in the ratio of 1:3 for trivalent ions and diluted with distilled water.

Absorption of each metal complex was measured using Pye Unicam SP-500 UV-Visible spectrophotometer.

An indigenous disc electrophoretic setup has been made in the glass tubes containing polyacryl amide gel positioned vertically in the chambers constructed in our laboratory as devised by Davis [10]. Chamber is made of Perspex sheets, consisting of upper and lower reservoirs. The lids were fitted with platinum wire acting as electrodes. After the preparation of gel tubes, 1 to 8  $\mu$ l of 1 ppm of Iron II and III copper I and II ions were spotted separately as well as in mixture form using microsyringe on the top of separate tubes.

The spotted gel tubes were inserted into the rubber plugs fitted in the holes of the upper vessel of the electrophoretic chamber.

So that all the tubes could stick out about 1 cm above the plug. The tubes passing down through the holes of lid of lower electrophoretic chamber were dipped evenly in the buffer in lower and upper compartment. The electrode discs fitted with lids were dipped in the buffer and connected to D.C. power supply for different time periods and experimental voltage and current adjusted as per experiments.

The experiments were also repeated with the same cation using different anions. For each cation

with different oxidation state was run separately to maximize the condition and obtained detection limits of each cations.

Quantitative analysis of Iron III and II copper I and II was made after their electrophoretic study individually as well as in composite mixture of ions with different concentration. The resolution of colored band in glass tubes for blank and samples were run individually and in mixture.

The  $\lambda_{max}$  of each metal ion was confirmed and mentioned in table 4.

Table 4: Determination of absorption maxima of complexes using Pye Unicam SP 500, UV-Vis Spectrophotometer and TLC Scanner CS - 910  
Glycine-metal complexes: Fe (III), Fe (II), Cu (II), Cu (I) Conc. of metal ion solution = 1mg/l  
pH of solution = 4

Complexes	UV-SP - 500	TLC scanner CS - 910
	$\lambda_{max}$ nm	$\lambda_{max}$ nm
Fe (III) glycinate	530	530
Fe (II) glycinate	510	500
Cu (II) glycinate	635	625
Cu (I) glycinate	738	725

Polyacryl amide gel does not only serve as the supporting medium, but it participates in the separation process is called sieving effect.

#### Conclusions

The present experimental data can be advantageously exploited further to quantify the presence of various oxidation states of same metal ion or its compounds or salts. Ordinarily, such data is not obtainable by other analytical techniques in single step experiments except using electrochemical or surface chemistry. Although the reported techniques are highly sophisticated, costly and time consuming as compared to this technique.

#### References

1. A. D. Mary, *Anal. Chem.*, **33** (12), 1752 (1961)
2. H.H Strain and J.F. Binder, *Anal. Chem.*, **33** (4) 527 (1961).
3. D. F. Harold and R. J. Brame, *Anal. Chem.* **34**(1), 170, (1962).
4. W. E. Lingren and R. J. Marson, *Anal. Chem.* **40**, 1585 (1968).
5. M.F. Khan, T. G. Kazi and G. H. Kazi Paper presented at Eastern Analytical Symposium (EAS) at Sommersit, N.J, USA (1990).
6. L. Ornstein, *Ann. N. Y. Acad. Sci.* **121**,

- 321,(1964).
7. G. H. Kazi and T. Iqbal *J. Chem. Soc. Pak*, **2**, 45 (1980).
  8. H. G. Mukherjee, M. Pattanyek, *Anal. Abst.* **13B29** (1), 3 (1975).
  9. P.G Jesse and Milton Wintz, *Chemistry of amino acids*. Vol. I, p.569, John Wiley and Sons, New York. (1961).
  10. B.J Davis, *Ann. N. Y. Acad. Sci.*, **121**, 404 (1964)