Separation and identification of heavy metal ions by thin layer chromatography on silica gel-G

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ABSTRACT

Thin layer chromatographic method has been developed for the separation of metal ions such as Cr (VI), Cr (III), Ni (II), Co (II), Cu(II), Fe(III), Zn(II) and Mo(VI) from their two, three and four component mixtures. The separations were performed on thin layer of silica gel 'G' using aqueous Humic acid as mobile phase. Thin layer of Silica Gel-G was used to study the chromatographic behavior of metal ions in surfactant mixed solvents. Effect of presence of humic acid at various concentrations, presence of strong and weak electrolytes, and effect of concentration of surfactant, effect of acidity and basicity of aqueous surfactant on mobility of metal cations were also studied. By using surfactant and with various additives mixed in mobile phase, metal ions such as Cu(II), Mo(VI) , Zn(II) and Ni2+were separated from their binary mixture.

Key words: Thin layer chromatography, separation, silica Gel –G, Glysin, Humic Acid , toxic heavy metal ion
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INTRODUCTION

Thin layer chromatography (TLC) is a very convenient and effective technique for the separation and identification of inorganic ions. It permits selective separations, simple detection and easy manipulation of the mobile phase. As a result, numerous sorbents and even greater number of mobile phases have been developed for achieving improved chromatographic performance in terms of selectivity, resolution, rapidity and reproducibility. From literature [1-3], the mobile phases used in inorganic solvents; or aqueous solution of acids, bases and salts. Although organic solvents such as benzene, chloroform, acetone, methanol, acetonitrile, carbon tetrachloride, dioxane, acetic acid, phenols, cyclohexane and hexane are quickly removed from the sorbent layer after development, most of these toxic to some extent .The use of aqueous solution as a mobile phase in TLC was pioneered by Armstrong and Terrill [4]. Using a surfactant as the mobile phase gained popularity and became more widely applied due to its operational simplicity, cost effectiveness, relative non-toxicity and enhanced separation efficiency [5-8]. The use of silica gel and an alumina layer with surfactant –mediated mobile phase systems [9-15] has been used to separate various inorganic species. Number of metal ions was systematically chromatographed on thin layer of urea formaldehyde polymer which is already used for the separation of amino acid [16].

MATERIALS AND METHODS

Apparatus
Glass plates of 4 x 20 cm size (coated with silica gel 'G'), 20 x 25 cm glass jars for the development of glass plates, glass sprayer for spraying reagents and EI pH meter.

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Chemicals and Reagents
Humic acid (Merck, India), Glysin, silica gel- G (Merck, India), hydrochloric acid and sodium hydroxide.

Metal ion studied
Cr (VI), Cr (III), Ni (II), Co (II), Cu (II), Fe(III), Zn (II) and Mo (VI)

Stock Solutions:
Stock solutions of 1% of following salts were prepared in the 0.1 M hydrochloric acid.
1. Potassium salt of Cr(VI),
2. Chloride of Cr(III), Ni(II), Zn(II) and Fe(III),
3. Sulphate of Cu(II).
4. Trioxide of Mo(VI).
5. Nitrate of Co(II).
The mobile phase was prepared in double distilled water.

Detection Reagents
For the detection of various cations, the following regents were used
1) 0.05 % Dithiozone in carbon tetrachloride.
2) Saturated alcoholic AgNO3.
3) Saturated alcoholic alizarin red.
4) 1% Alcoholic solution of DMG ie. Dimethylglyoxime.
5) 1% Aqueous potassium ferrocyanide

Stationary phase   Silica gel –G.
Mobile phase: The aqueous solution of humic acid and Glysin as the mobile phases.
Thin - layer chromatography (TLC)
1. Preparation of plates
Slurry was prepared by mixing silica gel 'G' in double distilled water in the ratio of 1:2 with constant steering for about 10 minutes. It was then immediately applied to the glass plate by the dipping method[21] and dried over night at room temperature.

2. Running of TLC plates
The test solutions were spotted on the silica gel-G plates using fine glass capillaries and they were blow-dried with hot air. The humic acid and DMSO of varying concentration was adjusted to the desired pH using sodium hydroxide and hydrochloride acid solution. The plates were developed for about 15 min in the glass jar containing 15 ml humic acid and DMSO solution. Approximately 2 -3 ml of solvent was required to run the sample per plate.

3. Development of TLC plates:-
Plates were dried and different cations were detected by spraying various spot test reagent, which are saturated alcoholic silver nitrate, saturated alcoholic alizarin red , dithiozone in carbon tetra chloride, dimethylglyoxime and potassium ferrocyanide for Cr(IV), Cr(III), and other metal ions i.e. Mo(VI), Zn(II), Ni(II), Co(II), Cu(II) and Fe(III) respectively. All experiments were carried out at room temperature. The R_f values were measured in triplicate for each set of determinations. Various experiments were carried out to study the mobile phase (0.005M - 0.1 M) ; pH (1.0 -7.0) and time (5 - 20 min) for the Rf values of the individual cations.

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RESULTS AND DISCUSSION

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Effect of pH: -
This section deals with effect of pH on R_f values of metal ions. The effect of pH on the R_f values of different metal ions was carefully studied by conducting several sets experiments. The plates were run near about to 10 cm above from the base line. The results are tabulated in table 2 which reveal variations in the R_f values with pH of Glycine with humic acid. The R_f value measurements were done in the pH range of 2.0 to 7.0 at 0.05 M concentration.

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Table 1:- Effect of pH on the R$_f$ values of metal ions

Metal Ion	pH of Humic acid +Glysin									
	2	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**7**
Cr(VI)	0.94	0.94	0.94	0.92	0.92	0.91	0.91 T	0.92 T	0.93 T	0.93 T
Cr(III)	0..72	0.73	0.70	0.75	0.55	0.24	0.23	0.23	0.23	0.20
Co(II)	0.88	0.88	0.88	0.87	0.87	0.85	0.86	0.86	0.86	0.84
Ni(II)	0.92	0.92	0.92	0.90	0.88spr	0.87spr	0.88spr	0.87spr	0.88	0.88
Zn(II)	0.90	0.90	0.86	0.51T	0.33 T	0.34 T	0.33 T	0.33 T	0.32 T	0.32 T
Cu(II)	0.94spr	0.94spr	0.92	0.80	0.84	0.82	0.81	0.81	0.80	0.80
Fe(III)	0.80spr	0.81spr	0.65	0.33	0.31	0.13	0.15	0.14	0.13	0.13
Mo(VI)	0.80	0.80	0.71	0.65T	0.26	0.25 T	0.24T	0.24T	0.23	0.23

Notation: - T- Tailing; Spr- Spreading; N.D. - Not Detected

 It can be observed from Fig. 2 and 3 (combined graph of all metal ions) that, at low pH, all the six metal ions move with the solvent. It is noted that all metal ions showed very little difference in the R$_f$ values at pH 3.5, but as we increase the pH to 4.0, maximum difference in the R$_f$ values of different metal ions could be achieved, which was required for better separation. However, the behavior of cations changed after the increase above pH 4.0 and especially at pH 5.0 and above and the metal ions Cr(VI); Cr(III); Zn (II); and Mo(VI) show tailing whereas Ni(II) shows spreading . From the observed values, pH 4.0 has been found out to be ideal for bringing about maximum separation. Hence, separation measurements have not been carried beyond pH 7.0. Therefore, pH 4.0 has been fixed for further R$_f$ measurements in Glysin with Humic acid media.

Effect of Concentration
This section deals with the separation of Cr(IV), Cr(III), Mo(VI), Zn(II), Ni(II), Co(II), Cu(II) and Fe(III).. Various experiments were carried out at different pH and at different concentration of Glycine and Humic acid for determining optimum separation concentration for the metal ions. The results dealing with the effect of concentration of mobile phase, i.e. Glycine with Humic acid on the Rf values of different metal ions such as for Cr(IV), Cr(III), Mo(VI), Zn(II), Ni(II), Co(II), Cu(II) and Fe(III) are tabulated in Table 1. The variations in the R$_f$ values with concentration in the range of 0.005 to 0.1 M were studied in pH range of 1 to7[16-17].

Table 2:- Effect of Concentration on the R$_f$ values of metal ions

Concentration M	Metal ion							
	Cr(VI)	Cr(III)	Co(II)	Ni(II)	Zn(II)	Cu(II)	Fe(III)	Mo(VI)
0.1%Humic acid + 0.1N Glysin	0.89	0.47	0.87	0.93	0.51	0.80	0.18	0.67
0.01%Humic acid + 0.01N Glysin	0.79	0.64	0.83	0.89	0.45	0.74	0.16	0.37
0.02%Humic acid + 0.02N Glysin	0.77	0.65	0.84	0.88	0.45	0.80	0.16	0.38
0.005%Humic acid + 0.005N Glysin	0.94	0.72	0.84	0.89	0.44	0.65	0.15	0.29

Effect of Time
This section deals with effect of time on R$_f$ values of metal ions. The effect of time on the Rf values of different metal ions was carefully studied by conducting several sets experiments. The plates were run near about to 10 cm above from the base line. The results are tabulated in table 2 which reveal variations in the R$_f$ values with different time slot of Glycine with humic acid. The R$_f$ value measurements were done in the pH range of 2.5 at 0.01 M concentration.

Table 3:- Effect of Time on the R$_f$ values of metal ions

Metal Ion	Time in Second			
	5 Min	10 Min	15 Min	20 Min
Cr(VI)	0.95	0.91	0.94	0.94
Cr(III)	0.67	0.86	0.87	0.93
Co(II)	0.85	0.87	0.88	0.91
Ni(II)	0.89	0.89	0.92	0.92
Zn(II)	0.76	0.87	0.90	0.93
Cu(II)	0.79	0.87	0.91	0.87
Fe(III)	0.75	0.76	0.81	0.81
Mo(VI)	0.92	0.91	0.82	0.82

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Table No 4. Binary separations

| Sr. No	Components of Binary mixture	Metal ions with their Rf Values
1	Cr(VI) Co(II)	Cr(VI) - 0.94; Co(II) - 0.88
2	Ni(II) Zn(II)	Ni(II) – 0.88; Zn(II) – 0.46
3	Ni(II) Mo(VI)	Ni(II) – 0.89; Mo(VI) – 0.38
4	Ni(II) Cr(III)	Ni(II) – 0.80; Cr(III) – 0.32
5	Co(II) Zn(II)	Co(II) - 0.86; Zn(II) – 0.28
6	Cr(VI) Cr(III)	Cr(VI) – 0.90;Cr(III) – 0.28
7	Ni(II) Co(II)	Ni(II) – 0.88; Co(II) – 0.86
8	Ni(II) Cu(II)	Ni(II) – 0.81; Cu(II) – 0.81
9	Ni(II) Zn(II)	Ni(II) – 0.87; Zn(II) – 0.32
10	Ni(II) Cr(VI)	Ni(II) – 0.86; Cr(VI) - 0.94

Table No 5. Ternary separations

Sr.No.	Components of Ternary mixture	Metal ions with their Rf Values
1	Ni(II); Co(II); Cr(III)	Ni(II) – 0.93; Co(II) – 0.88; Cr(III) – 0.32
2	Ni(II); Co(II); Zn(II)	Ni(II) – 0.91; Co(II) - 0.85; Zn(II) – 0.32
3	Ni(II); Cr(VI); Cu(II)	Ni(II) - 0.89; Cr(VI) – 0.97; Cu(II) – 0.80
4	Ni(II); Cr(VI); Co(II)	Ni(II) – 0.87; Cr(VI) – 0.97; Co(II) – 0.81
5	Ni(II); Cr(VI); Mo(VI)	Ni(II) – 0.93; Cr(VI) – 0.97; Mo(VI) – 0.33
6	Ni(II); Cr(VI); Zn(II)	Ni(II) – 0.92; Cr(VI) – 0.96; Zn(II) – 0.36

**Table No 6- Quaternary separations**

Sr. No	Components of Quaternary mixture	Metal ions with their R_f Values
1	Cr(VI); Ni(II); Co(II) Mo(VI)	Cr(VI) – 0.98; Ni(II) – 0.92 Co(II) – 0.84; Mo(VI) – 0.34
2	Cr(VI); Ni(II); Co(II) Cr(III)	Cr(VI) – 0.97; Ni(II) – 0.91 Co(II) – 0.84; Cr(III) – 0.26
3	Cr(VI); Ni(II); Co(II) Cr\\Fe(III)	Cr(VI) -0.97; Ni(II) – 0.92 Co(II) – 0.85; Fe(III) -0.33
4	Cr(III); Ni(II); Co(II) Zn(II)	Cr(III) – 0.23; Ni(II) – 0.89 Co(II) – 0.84; Zn(II) – 0.30
5	Cr(VI); Ni(II); Co(II) Zn(II)	Cr(VI) – 0.97; Ni(II) – 0.91 Co(II) – 0.86; Zn(II) – 0.32

It was observed from table no 2, at low pH, all the six metal ions move with the solvent front. It is noted that all metal ions showed very little difference in the R_f values at pH 3.0, but as we increase the pH to 3.5, maximum difference in the R_f values of different metal ions could be achieved, which was required for better separation. However, the behavior of cations changed after the increase above pH 4.0 and especially at pH 5.0 and above and the metal ions Cr (VI) shows double spotting due to formation of hetero poly acid ; Cr (III); Mo (VI); and Co(II) show tailing whereas Ni (II) and Zn (II) shows spreading. But Cu (II) shows compact spot at all pH except pH 1 and pH 2. From the observed values, pH 3.5 has been found out to be ideal for bringing out maximum separation. R_f of all mention metal ions was not possible to measure above pH 7. Hence, separation measurements have not been carried beyond pH 7.0 and pH 3.5 fixed for further R_f measurement in glycine with Humic acid as a media.
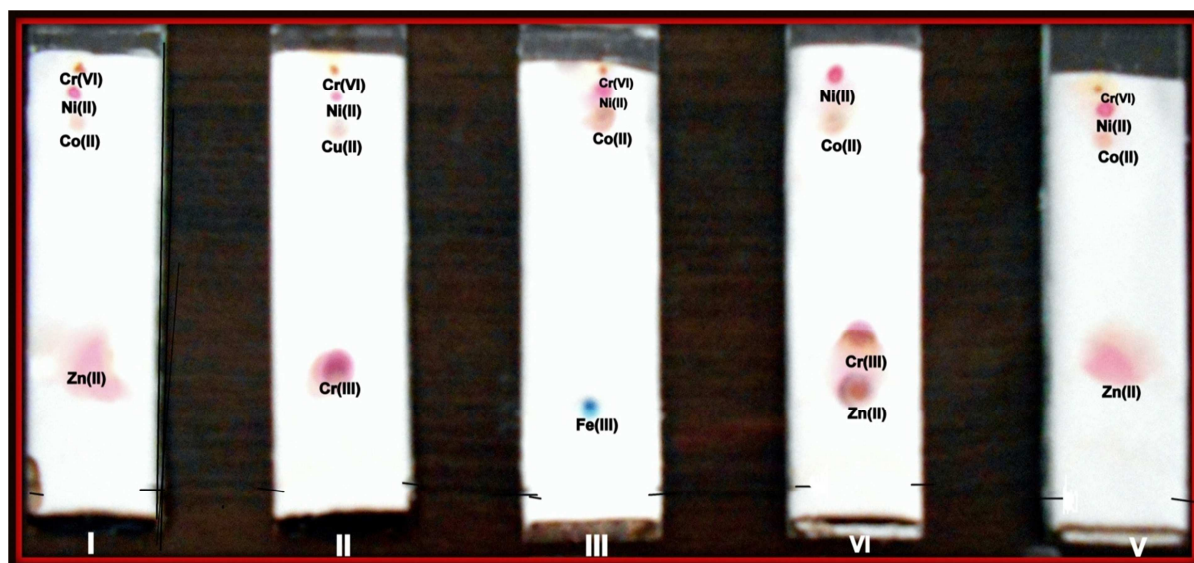
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Bionary Images
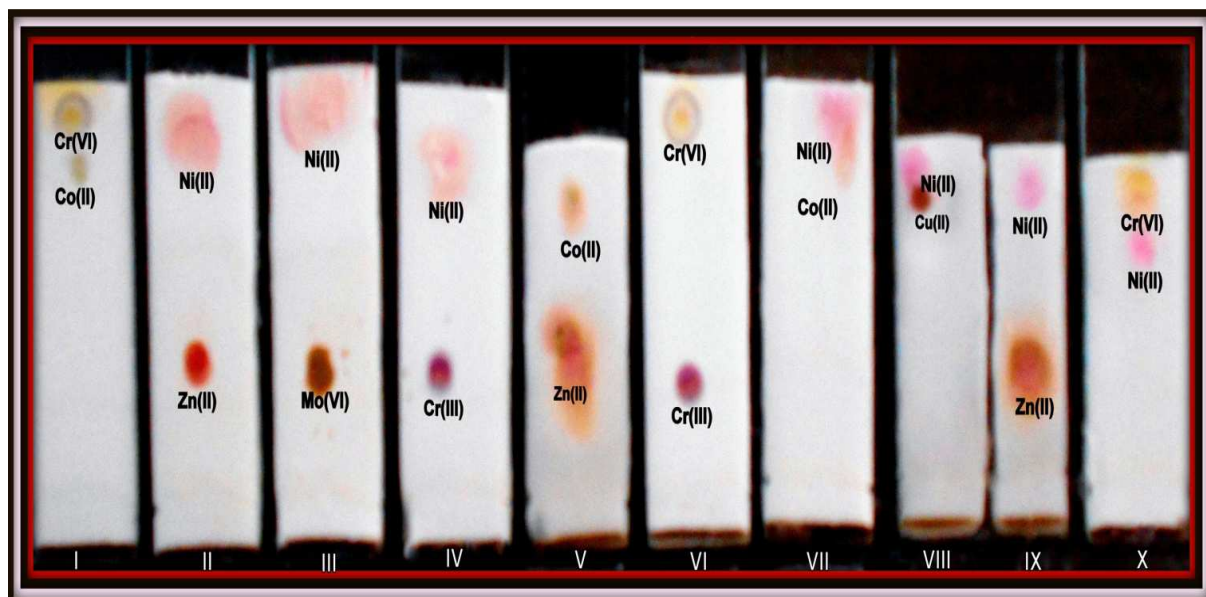
Ternary Images

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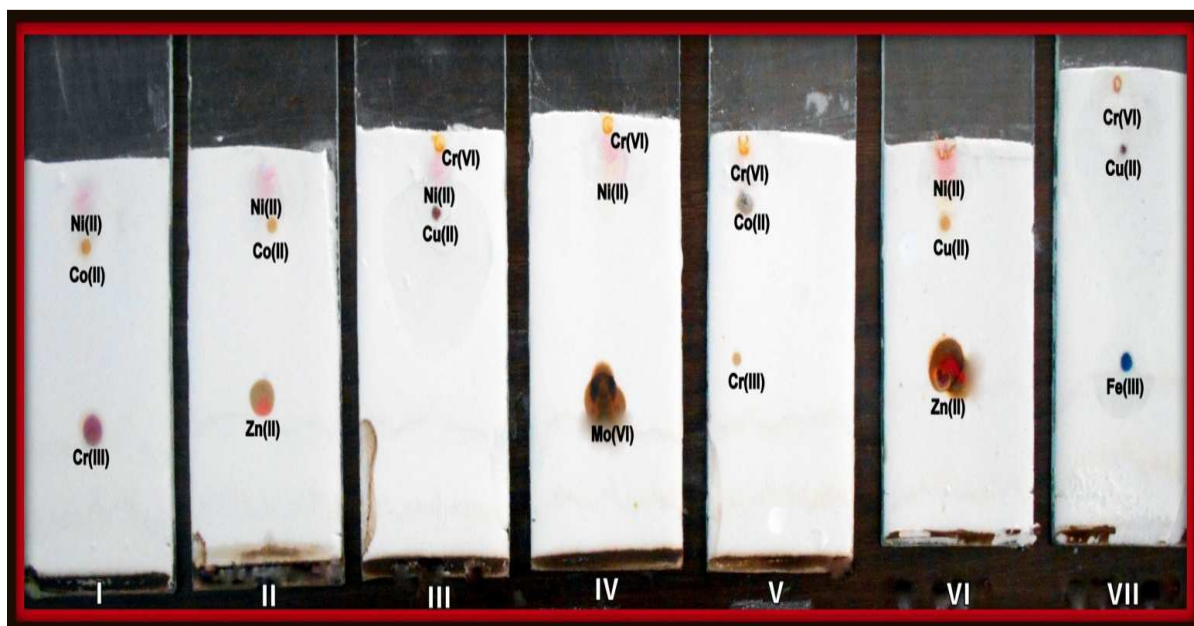
Quaternory images

It was observed that From Table No. 2 as the time increases at pH 2.5 for 0.005N glysin with Humic acid as a media R$_f$ value of all metal ions is slightly increases

CONCLUSION

Using the above mentioned conditions qualitative separation of ten binary mixtures; seven ternary mixtures; and five quaternary mixtures of metal ions have been carried out. The R$_f$ values of metal cations are given in top to bottom format, as they appear on the chromatographic plate. Experimentally achieved separations on silica gel 'G' layers developed with Glysin with Humic acid as mobile phase. The R$_f$ values of metal ions in their mixture are slightly change from their individual R$_f$ values because of mutual interactions.

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