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Comparative study of different Amino Acid as mobile phase for thin layer Chromatographic separation of toxic heavy metal ions

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Abstract

Thin layer chromatographic method has been developed for the separation of metal ions such as Cr^{6+} , Cr^{3+} , As^{3+} , Cd^{2+} , Tl^{3+} and Hg^{2+} from their two, three and four component mixtures. The separations were performed on thin layer of silica gel ‘G’ using aqueous L - Valine as mobile phase. The effect of concentration and pH of mobile phase on the R_f values of individual metal ions were studied and the optimum conditions for separation of metal ions from their mixture were determined.

Keywords: Thin layer chromatography, Separation, Silica gel-G, L - Valine, Toxic Heavy Metal Ions

1. Introduction

Heavy metals have received considerable attention from analysts, because of their physical and environmental importance [1, 2]. Metal such as Cr, As, Hg, Tl, Cd, Ni, Tl, Cu, Fe, Zn, Pb are toxic and harmful to human health. These metal ions can form complexes with bioligands containing Oxygen, Nitrogen and Sulphur atoms [3], which causes many problems by their redox processes in living organisms.

In the past few decades, there is substantial increase in the use of heavy metals, due to the industrialization; this resulted in an increased flux of metallic substances. Industrial waste is the major source of different kinds of metal pollution in aquatic systems. The major sources of pollution of Chromium in the aquatic environment are electroplating stainless steel industries, metal finishing industrial effluents, sewage and waste-water treatment plants discharge and chromates from cooling water. Chromium occurs in several oxidation states such as di, tri, penta and hexavalent, but only Cr^{3+} and Cr^{6+} are biologically important. Chromium in the aquatic tends to speciate into Cr^{3+} & Cr^{6+} , with the trivalent ion being oxidized to the hexavalent form or precipitating from solution.

There are different analytical techniques of separation and detection of chromium including graphite furnace atomic-absorption spectroscopy [4, 5], neutron activation analysis [6, 7], atomic emission spectroscopy [8], normal phase and reverse phase thin layer chromatography [9, 11], ion chromatography [12, 13], precipitation floatation [14], titrimetry [15, 16], and hyphenated techniques such as ion-exchange chromatography - flame atomic absorption spectroscopy [17], solid - phase extraction - flame atomic emission spectroscopy [18].

Out of these different separation procedures, thin layer chromatography is probably the most versatile, because it can be used for the selective separation of metal cations on the micro and as well as macro scales. Exhaustive survey off the literature published in the last thirty years [19] shows that much progress has been made in developing rapid and selective thin layer chromatographic method for separation of toxic heavy metals from interfering elements, by use of a variety of acidic developer containing mineral or carboxylic acid as one of the component.

Using of amino acid as mobile phase is not common for chromatographic separations using conventional laboratory made TLC plates. Therefore it was decided to use the analytical potential of amino acid that is aqueous L - Valine and L- iso Leucine as mobile phase and silica gel-G as stationary phase for analysis of heavy metal ions. As a result several analytically important separations of heavy metals were realized. Separation of the chromium is industrially important, because Cr^{+3} are converted to Cr^{+6} in alkaline peroxide medium.

This paper deals with the rapid separation of heavy metal ions present in three, as well as four component mixtures on non - impregnated silica gel 'G' coated plates, using aqueous solution of L - Valine and L- iso Leucine as a mobile phase and date has been compared for better mobile phase amongst two mobile phase.

2. 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 & EI pH meter.

Chemicals and Reagents

L - Valine (E. Merck; India), L- iso Leucine (E. Merck; India); Silica gel- G (E. Merck; India); Hydrochloric acid and Sodium hydroxide.

Stock Solutions:

The stock solutions of 0.05 M of following salts were prepared in the 0.1 M Hydrochloric acid.

1. Potassium salt of Cr^{6+}
2. Chloride of Cd^{2+} and Hg^{2+}
3. Sulphate of Cr^{3+}
4. Trioxide of As^{3+} and Tl^{3+} .

The mobile phase was prepared in double distilled water.

Detection Reagents

For the detection of various cations, the following reagents [20] were used

1. 0.05 % Dithiozone in Carbon tetra chloride.
2. Saturated Alcoholic AgNO_3 .
3. Saturated Alcoholic Alizarin red.

Procedure:

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 L - Valine and L- iso Leucine [22, 23] of varying concentration was adjusted to the desired pH using sodium hydroxide and hydrochloric acid solution. The plates were developed for about 15 min in the glass jar containing 15 ml L - Valine solution and L- iso Leucine separately. Approximately 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 and dithiozone in carbon tetra chloride, for Cr^{6+} , Cr^{3+} , and other metal ions i.e. As^{3+} , Cd^{2+} , Tl^{3+} , & Hg^{2+} 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 to 7.0) and time (5 to 20 min) for the R_f values of the individual cations.

3. Results and Discussion

Effect of Concentration: - This section deals with the separation of Cr^{6+} , Cr^{3+} , As^{3+} , Cd^{2+} , Tl^{3+} and Hg^{2+} . Various experiments were carried out at different concentration of L -Valine and L- iso Leucine separately for determining optimum condition for separation of the metal ions.

The results dealing with the effect of concentration of mobile phase, i.e. on the R_f values of different metal ions such as Cr^{6+} , Cr^{3+} , As^{3+} , Cd^{2+} , Tl^{3+} and Hg^{2+} 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 to 7.

Table 1: Effect of Concentration on the R_f values of metal ions

Mobile phase	Conc. (M)	Metal Ions					
		Cr^{6+}	Cr^{3+}	As^{3+}	Cd^{2+}	Tl^{3+}	Hg^{2+}
L -Valine	0.005	0.94 (L.T.)	0.32	0.92	0.68 (L.T.)	0.46 (L.T.)	0.77
	0.01	0.95 (S.T.)	0.15	0.90	0.46 (S.T.)	0.25	0.70 (S.T.)
	0.05	0.93	0.26	0.85	0.57	0.43	0.77
	0.1	0.95 (spr)	0.46	0.97	0.63 (spr)	0.50	0.91 (spr)
L- iso Leucine	0.005	0.94	0.33	0.62	0.47	0.40	0.88
	0.01	0.95 (spr)	0.20	0.65	0.46 (spr)	0.39	0.70 (spr)
	0.05	0.93	0.26 (L.T.)	0.67	0.57	0.43 (L.T.)	0.77 (spr)
	0.1	0.95	0.31 (L.T.)	0.67	0.63	0.50 (L.T.)	0.91 (spr)

Note: L.T. – long tailing, S.T. – short tailing, spr- spreading

It was observed that, at 0.005 M. and 0.01 M concentration of L-Valine, Cr^{6+} ; Cd^{2+} ; and Tl^{3+} shows tailing and short tailing. At 0.05 M concentration of L-Valine, clear and distinct spots were seen. At 0.1 M, L-Valine spots were not

compact and shows little spreading, especially Cr^{6+} ; Cd^{2+} ; Hg^{2+} .

In case of L -Iso Leucine, at concentration 0.1 M, Cr^{6+} was not detected except pH 2, Cr^{3+} and Tl^{3+} shows long tailing

while Hg²⁺ shows spreading. At 0.05 M concentration of L-Iso Leucine, almost same results were observed. At 0.01 M, L-Iso Leucine spots were not compact and shows little spreading, especially Cr⁶⁺; Cd²⁺; Hg²⁺. However 0.05 M concentration of L-Valine and 0.005 M concentration of L-Iso Leucine was selected as the optimum concentration for further studies with.

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 L-Valine and L-iso Leucine. The R_f value measurements were done in the pH range of 1.0 to 7.0 at 0.05 M L-Valine and 0.01 M L-iso Leucine concentration.

Table 2: Effect of pH on the R_f Values of metal ions at conc. 0.05M L-Valine

Mobile phase	Metal ions	pH of Mobile Phase									
		1	1.5	2	2.5	3	3.5	4	5	6	7
L-Valine	Cr ⁶⁺	0.95	0.95	0.95	0.95	0.95	0.96	0.93	0.92 D.S	0.94 D.S	0.94 D.S
	Cr ³⁺	0.70	0.73	0.76	0.54	0.27	0.20	0.15	0.06 T	0.07 T	0.08 T
	As ³⁺	0.83	0.84	0.85	0.84	0.86	0.89	0.88	0.84	0.87	0.79
	Cd ²⁺	0.90	0.87	0.83	0.69	0.57	0.46	0.37 T	0.35 T	0.33 T	0.37 T
	Tl ²⁺	0.80	0.82	0.85	0.61	0.43	0.32	0.22 T	0.18 T	0.22 T	0.16 T
	Hg ²⁺	0.95	0.94	0.93	0.91	0.79	0.71	0.72	0.67spr	0.69 spr	0.66 spr
L-iso Leucine	Cr ⁶⁺	0.95	0.92	0.94	0.89	0.89	0.92	0.89D.S	0.9 D.S	0.92 D.S	0.92 D.S
	Cr ³⁺	0.54	0.44	0.32	0.17	0.14	0.16	0.16 T	0.14 T	0.16 T	0.14 T
	As ³⁺	0.72	0.72	0.7	0.73	0.66	0.64	0.68	0.67	0.64	0.67
	Cd ²⁺	0.68	0.64	0.43	0.28	0.26	0.28	0.29 T	0.18 T	0.19 T	0.17 T
	Tl ²⁺	0.58	0.52	0.37	0.24	0.24	0.22 T	0.22 T	0.24 T	0.25 T	0.20 T
	Hg ²⁺	0.85	0.83	0.88	0.87	0.88	0.91	0.98 Spr	0.88 Spr	0.95 Spr	0.9 Spr

Note: L.T. – long tailing, S.T. – short tailing, spr- spreading, D.S. – Double spotting.

It can be observed that at low pH, all the six metal ions move with the solvent front. It was noted that all metal ions showed very little difference in the R_f values at different pH. In case of L-Valine, the pH 3, maximum difference in the R_f values of different metal ions could be achieved, which was required for better separation. In case of L-iso Leucine, Maximum difference in the R_f values of different metal ions were seen at pH 2.

However, the behavior of cations changed after the increase in pH 4.0 and above, especially at pH 5.0, Cr⁶⁺ shows double spotting due to formation of hetero poly acid; Cr³⁺; Cd²⁺; & Tl³⁺ show tailing whereas Hg²⁺ shows spreading. But As³⁺ shows compact spot at all pH except pH 1 & pH 2.

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. pH 3 fixed for aqueous media of L-Valine and pH 2 for L-iso Leucine further R_f measurement.

Effect of runtime: -The variation in the R_f values of metal ions with run time was studied in this section. The R_f values have been studied at pH 3 having 0.05 M concentration of L-Valine and, at pH 2 and 0.01M for L-iso Leucine.

From obtained data it revealed that, when the migration runtime was kept at 10 minutes, the metal ion shows remarkable difference in their R_f values which is required for the separation of binary, ternary and quaternary mixtures of metal ions. The R_f of metal ions were also studied at 15 and 20 min as run time, but separation of metals has been found out to be good at only 10 min. Further increase in the run time did not affect the separation of binary and ternary separation but for quaternary separations, run time, 15 minutes was consider.

4. Conclusion

Using the above mentioned optimum separating conditions of 0.05 M L-Valine at pH 3, and 10 minutes as run time, qualitative separation of eight binary mixtures; seven ternary mixtures; and five quaternary mixtures of metal ions have been carried out.

In 0.01M. l- iso leucine, pH 2 and run time was 10 and 15 minutes, ten binary mixtures; ten ternary mixtures; and four quaternary mixtures were successfully separated.

The R_f values of various binary, ternary and quaternary separations have been listed in Table 3, 4, and 5 respectively.

From table no. 3, 4 and 5, it is concluded that, for binary and ternary separation l-iso leucine is better mobile phase, but quaternary l-valine is good mobile phase.

Table 3: Binary separations

Sr. No	Metal ions with their R _f Values	
	l-Valine	l-iso leucine
1.	Cr ⁶⁺ - 0.97; Cr ³⁺ - 0.38.	Cr ⁶⁺ - 0.96; Cr ³⁺ - 0.39.
2.	Cr ⁶⁺ - 0.97; As ³⁺ - 0.80.	Cr ⁶⁺ - 0.95; As ³⁺ - 0.71.
3.	Cr ⁶⁺ - 0.97; Cd ²⁺ - 0.44.	Cr ⁶⁺ - 0.96; Cd ²⁺ - 0.52.
4.	Cr ⁶⁺ - 0.98; Hg ²⁺ - 0.18.	Cr ⁶⁺ - 0.94; Hg ²⁺ - 0.41.
5.	Hg ²⁺ - 0.71; Cr ³⁺ - 0.35	As ³⁺ - 0.76; Cr ³⁺ - 0.45.
6.	As ³⁺ - 0.85; Cd ²⁺ - 0.44.	Hg ²⁺ - 0.89; Cr ³⁺ - 0.42.
7.	Hg ²⁺ - 0.74; Cd ²⁺ - 0.53.	As ³⁺ - 0.79; Cd ²⁺ - 0.51.
8.	Cr ⁶⁺ - 0.97; Hg ²⁺ - 0.76	Hg ²⁺ - 0.94; As ³⁺ - 0.75.
9		Hg ²⁺ - 0.93; Cd ²⁺ - 0.51.
10		Cd ²⁺ -0.42; Cr ³⁺ - 0.54.

Table 4: Ternary separations

Sr. No	Metal ions with their R _f Values	
	I-Valine	I-iso leucine
1.	Cr ⁶⁺ - 0.96; As ³⁺ - 0.29; Cr ³⁺ - 0.37.	Cr ⁶⁺ - 0.95; As ³⁺ - 0.77; Cr ³⁺ - 0.37.
2.	Cr ⁶⁺ - 0.98; Cd ²⁺ - 0.59; Cr ³⁺ - 0.64.	Cr ⁶⁺ - 0.96; Cd ²⁺ - 0.54; Cr ³⁺ - 0.38.
3.	Hg ²⁺ - 0.85; Cd ²⁺ - 0.34; Hg ²⁺ - 0.23.	Hg ²⁺ - 0.94; As ³⁺ -0.77; Hg ²⁺ - 0.43.
4.	Cr ⁶⁺ - 0.97; Cd ²⁺ - 0.39; Hg ²⁺ - 0.25.	Hg ²⁺ - 0.92; Cd ²⁺ - 0.56; Hg ²⁺ - 0.40.
5.	As ³⁺ - 0.83; Hg ²⁺ - 0.62; Cd ²⁺ - 0.36.	Cr ⁶⁺ - 0.95; Cd ²⁺ - 0.56; Hg ²⁺ - 0.35.
6.	Cr ⁶⁺ - 0.98; As ³⁺ - 0.80; Cd ²⁺ - 0.50.	Cr ⁶⁺ - 0.97; As ³⁺ - 0.78; Hg ²⁺ - 0.45.
7.	As ³⁺ - 0.81; Cd ²⁺ - 0.50; Cr ³⁺ - 0.31.	Hg ²⁺ - 0.95; As ³⁺ - 0.78; Cr ³⁺ - 0.44.
8.		Hg ²⁺ - 0.96; As ³⁺ - 0.76; Cd ²⁺ - 0.49.
9.		Cr ⁶⁺ - 0.97; As ³⁺ - 0.77; Cd ²⁺ - 0.54.
10.		As ³⁺ - 0.78; Cd ²⁺ - 0.51; Cr ³⁺ - 0.38.

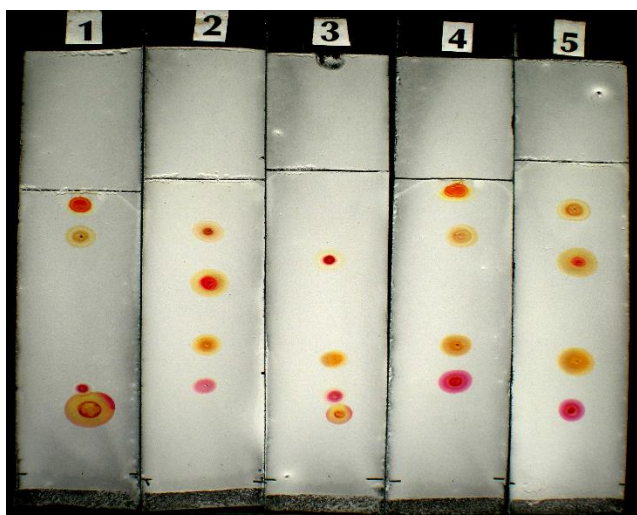
Table 5: Quaternary separations

Sr. No.	Metal ions with their R _f Values	
	I-Valine	I-iso leucine
1.	Cr ⁶⁺ - 0.97; As ³⁺ - 0.23; Cr ³⁺ - 0.22; Hg ²⁺ - 0.30.	Cr ⁶⁺ - 0.96; As ³⁺ - 0.75; Cr ³⁺ - 0.40; Hg ²⁺ - 0.48.
2.	Hg ²⁺ - 0.67; As ³⁺ -0.83; Cd ²⁺ - 0.46; Hg ²⁺ - 0.32.	Hg ²⁺ - 0.94; As ³⁺ - 0.79; Cd ²⁺ - 0.79; Hg ²⁺ - 0.38.
3.	Hg ²⁺ - 0.73; Cd ²⁺ - 0.40; Cr ³⁺ - 0.22; Tl ³⁺ - 0.26.	Cr ⁶⁺ - 0.95; As ³⁺ - 0.79; Cd ²⁺ - 0.53; Cr ³⁺ - 0.37.
4.	Cr ⁶⁺ - 0.96; As ³⁺ - 0.81; Cd ²⁺ - 0.45; Cr ³⁺ - 0.33.	Hg ²⁺ - 0.91; As ³⁺ - 0.78; Cd ²⁺ - 0.37; Cr ³⁺ - 0.37;
5.	Hg ²⁺ - 0.70 As ³⁺ - 0.86; Cd ²⁺ - 0.49; Cr ³⁺ - 0.23;	

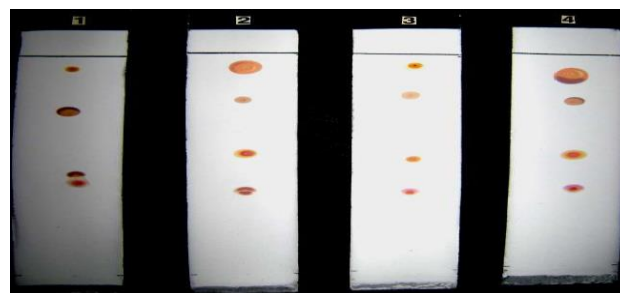
There is slight difference in the R_f values of metal ions during the separations from their mixtures, due to the mutual interaction of metal ions. The R_f values of metal cations are given in top to bottom format, as they appear on the chromatographic plate.

Photograph of achieved quaternary separations were given in picture no 1, using I-Valine as mobile phase in picture no. 2, using I-iso leucine.

Picture No. 1 for the achieved separations using above mention optimum separating conditions.



Picture 1: For Quaternary Separations using I-valine as mobile phase



Picture 2: For Quaternary Separations using I-iso leucine as mobile phase

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