

Comparison of pH Study for Humic Acid in L-Phenylalanine and Humic Acid in Dimethylformamide by Using Thin Layer Chromatography for Separation and Estimation of Heavy Metal Ions

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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, aqueous Humic acid +DMF and aqueous Humic acid+L-phenylalanine 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^{2+} , Hg^{2+} , W^{6+} and Ni^{2+} were separated from their binary mixture. Semi-quantitative determination of Ni^{2+} by measurement of spot area was attempted.

Key words: Thin layer chromatography, separation, silica Gel -G, DMF, L-phenylalanine, toxic heavy metal ion

Introduction

Heavy metals are defined as metallic elements that have a relatively high density compared to water [1]. With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure [2]. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by these metals. Also, human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications [3]. Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources [4]. Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations [1, 3, 4].

Although heavy metals are naturally occurring elements that are found throughout the earth's crust, most environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds [4-7]. Environmental contamination can also occur through metal corrosion, atmospheric deposition, soil erosion of metal ions and leaching of heavy metals, sediment re-suspension and metal evaporation from water resources to soil and ground water [8]. Natural

phenomena such as weathering and volcanic eruptions have also been reported to significantly contribute to heavy metal pollution [1, 3, 4, 7, 8]. Industrial sources include metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants [9-11].

It has been reported that metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) are essential nutrients that are required for various biochemical and physiological functions [12]. Inadequate supply of these micro-nutrients results in a variety of deficiency diseases or syndromes [12]. Heavy metals are also considered as trace elements because of their presence in trace concentrations (ppb range to less than 10ppm) in various environmental matrices [13].

Thin layer chromatography is a versatile technique for the identification and separation of organic and inorganic compounds [14-15]. Heavy metals have received considerable attention from analysts, because of their physical and environmental importance [16-17].

Principle of TLC

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate and a liquid mobile phase, which is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate. The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied. The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups. The solubility rule "Like Dissolves Like" is followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind [16].

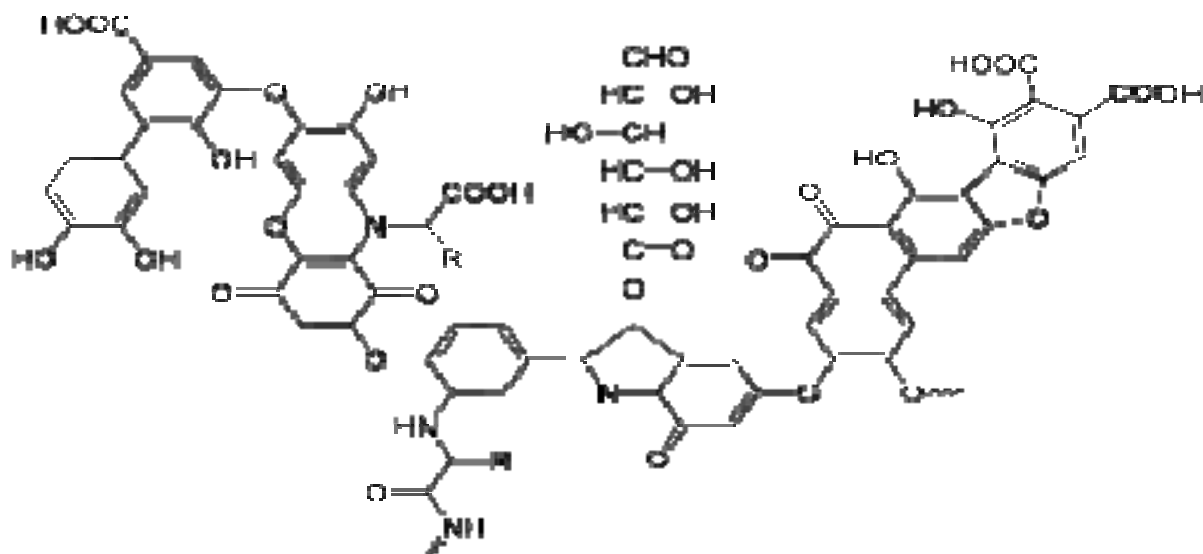
This paper compares the effect of pH, on the R_f value obtained for various metal ion by using aqueous humic acid with DMF and aqueous humic acid with L-phenylalanine as a mobile phase. The structure of humic acid, DMF (dimethylformamide) and L-phenylalanine is given below.

Materials and Methods

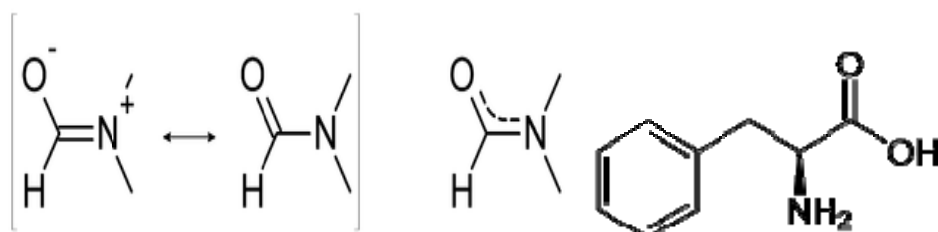
Apparatus - Glass plates of 4 x 20 cm size (coated with silica gel 'G'), 20 x 25 cm glass jars for the development of glass plates, glass sprayer for spraying reagents and EI pH meter.

Chemicals and Reagents - Humic acid (Merck, India), L-phenylalanine, DMF, 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)



Structure of aqueous humic acid



Structure of DMF idimethylformamide Structure of Lphenylalanine

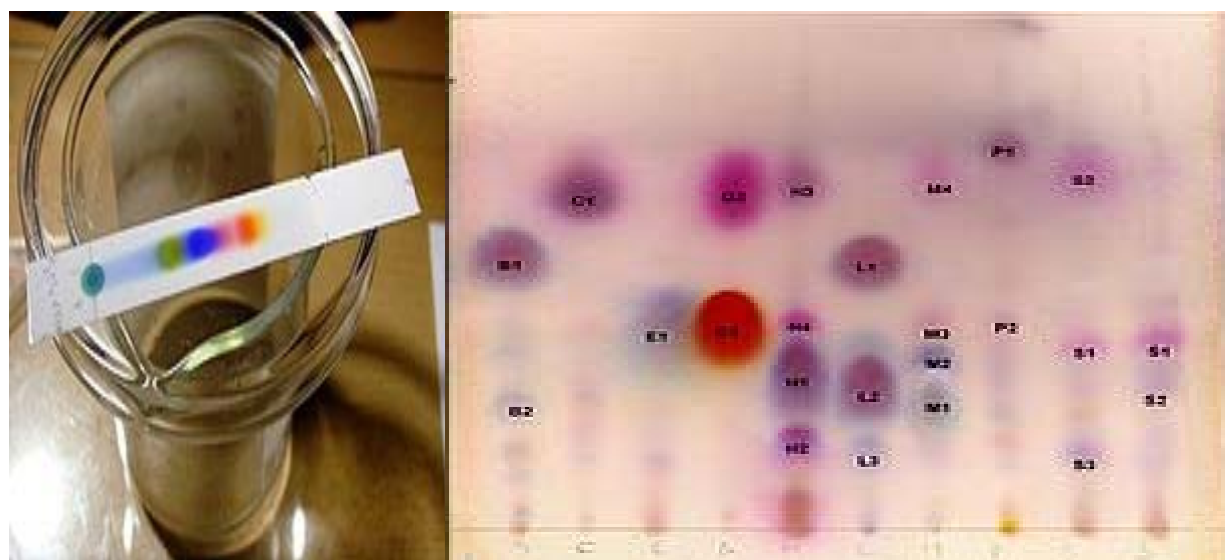


Fig.1.1 : Pictures of thin layer chromatograph

Stock Solutions:

Stock solutions of 1% of following salts were prepared in the 0.1 M hydrochloric acid.

- i. Potassium salt of Cr(VI),
- ii. Chloride of Cr(III), Ni(II), Zn(II) and Fe(III),
- iii. Sulphate of Cu(II).
- iv. Trioxide of Mo(VI).
- v. Nitrate of Co(II).

The mobile phase was prepared in double distilled water.

Detection Reagents

For the detection of various cations, the following reagents were used

- i. 0.05 % Dithiozone in carbon tetrachloride.
- ii. Saturated alcoholic AgNO₃.
- iii. Saturated alcoholic alizarin red.
- iv. 1% Alcoholic solution of DMG ie. Dimethylglyoxime.
- v. 1% Aqueous potassium ferrocyanide

Stationary phase Silica gel –G.

Mobile phase: The aqueous solution of humic acid with L-phenylalanine and humic acid with DMF as the mobile phases

Thin - Layer Chromatography (TLC)**Preparation of Plates**

Slurry was prepared by mixing silica gel 'G' in double distilled water in the ratio of 1:2 with constant stirring for about 10 minutes. It was then immediately applied to the glass plate by the dipping method [21] and dried overnight at room temperature.

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 aqueous humic acid with L-histidine 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 aqueous humic acid with L-histidine solution. Approximately 2 - 3 ml of solvent was required to run the sample per plate.

Development of TLC Plates

Plates were dried and different cations were detected by spraying various spot test reagents, which are saturated alcoholic silver nitrate, saturated alcoholic alizarin red, dithiozone in carbon tetrachloride, 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 R_f values of the individual cations

Results and Discussion

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 1 which reveal variations in the R_f values with pH of various system of humic acid. The R_f value measurements were done in the pH range of 2.0 to 7.0 at 0.05 M concentration

It was noted that in case of aqueous humic acid metal ions shows a little difference in the R_f value of pH 3.5 but as the pH was increased to 4 there was maximum difference in the R_f value of different metal ions. This was required for better separation, The clear separation was observed only at pH 4.0. So separation measurement has been carried out at pH 4 in case of aqueous humic acid with DMF as a media. And pH 3.5 in case of humic acid in L-phenylalanine as a media. Ni(II) shows little spreading in all three cases at pH 4.0.

R_f value of metal ion of two systems M1 and M2 at pH 3 and 4 pH level are given below

Table 1.1 : Effect of pH on the R_f values of metal ions of system M1 and M2

Metal ion	M1- Aqueous Humic acid in DMF (dimethylformamide)		M2- Aqueous Humic acid media in L-phenylalanine		PH=4
	PH=3	PH=4	PH=3	PH=3.5	
Cr(VI)	0.98	0.98	0.92	0.93	0.94
Cr(III)	0.74	0.76	0.28	0.14	0.30
Co(II)	0.72	0.56	0.85	0.58	
Ni(II)	0.86	0.64Spr	0.73	0.75 spr	0.74
Zn(II)	0.45	0.15 T	0.33	0.18T	0.20
Cu(II)	0.43	0.15	0.63	0.57	0.58
Fe(III)	0.44	0.12	0.28	0.29	0.28
Mo(VI)	0.44	0.15	0.37	0.24 T	0.28

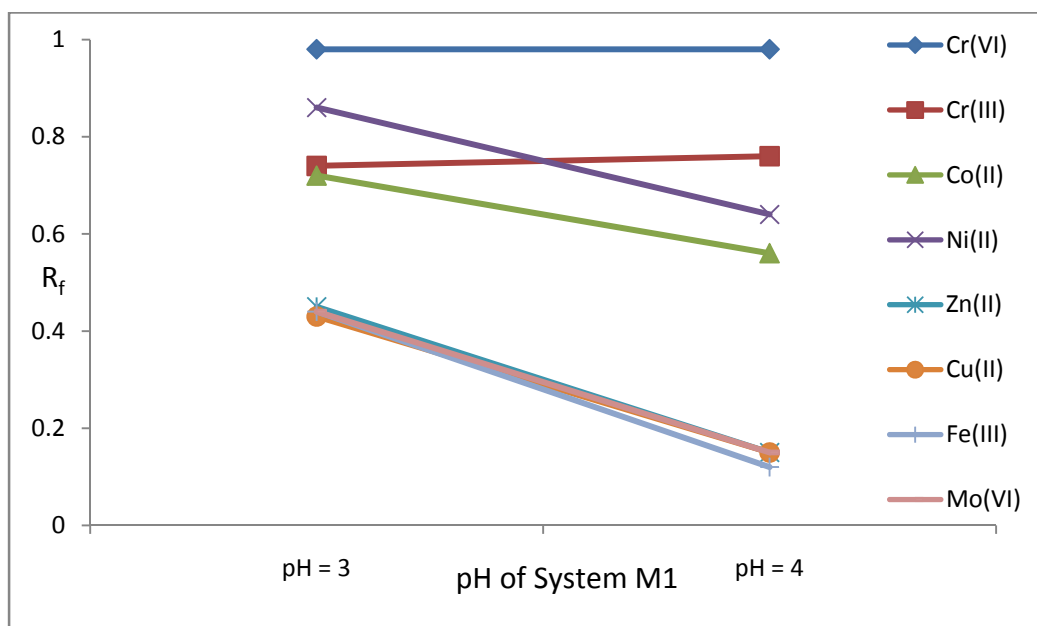


Fig. : 1.2 Effect of pH on the R_f values of metal ions of system M1

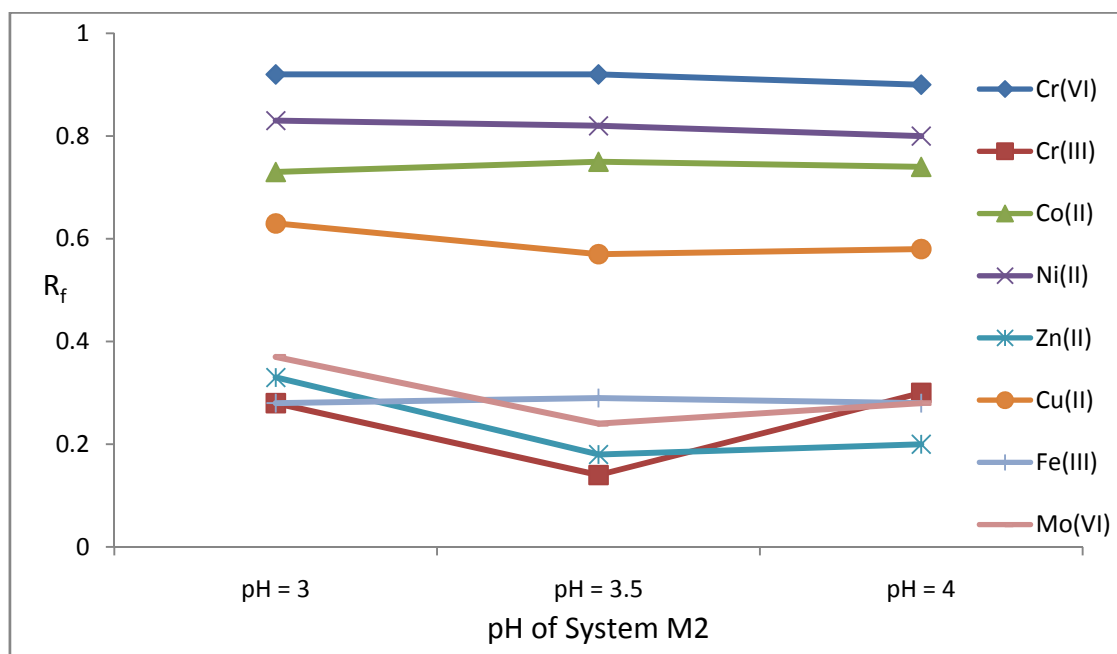


Fig. : 1.3 Effect of pH on the R_f values of metal ions of system M2

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