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Thin Layer Chromatographic Separation of Cr(VI), Cr(III), Ni(II), Co(II), Cu(II), Fe(III), Zn(II) and Mo(VI) Toxic 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. Silica gel- G is acting as an good adsorbent so various separations were performed on thin layer of silica gel G using aqueous humic acid and L – histidine as a mobile phase. The effect of concentration and pH of mobile phase on the Rf values of individual metal ions were studied and the optimum conditions for separation of metal ions from their mixture were determined. By using surfactant and with various additives mixed in mobile phase, metal ions such as Cu(II), Cr(VI), Cr(III), Co(II), and Ni(II) were separated from their binary mixture.
Keywords: Thin layer chromatography, separation, silica Gel –G, aqueous humic Acid, L-histidine, toxic heavy metal ion.

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INTRODUCTION

Metal ions occur naturally in the earth's crust and their contents in the environment can vary between different regions resulting in spatial variations of background concentrations. The distribution of metals in the environment is governed by the properties of the metal and influences of environmental factors[1] . Of the 92 naturally occurring elements, approximately 30 metal ions and metalloids are potentially toxic to humans, Be, B, Li, Al, Ti, V, Cr, Mn, Co, Ni, Cu, As, Se, Sr, Mo, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, W, Pt, Au, Hg, Pb, and Bi. Heavy metals is the generic term for metallic elements having an atomic weight higher than 40.04 (the atomic mass of Ca) [2].

Heavy metals enter plant, animal and human tissues via air inhalation, diet and manual handling. Motor vehicle emissions are a major source of airborne contaminants including arsenic, cadmium, cobalt, nickel, lead, antimony, vanadium, zinc, platinum, palladium and rhodium.[3] Water sources (ground water, lakes, streams and rivers) can be polluted by heavy metal ions leaching from industrial and consumer waste; acid rain can exacerbate this process by releasing heavy metal ions trapped in soils[4]. Plants are exposed to heavy metals through the uptake of water; animals eat these plants; ingestion of plant and animal based foods are the largest sources of heavy metal ions in humans[5]. Absorption through skin contact, for example from contact with soil, is another potential source of heavy metal ions contamination[6]. Toxic heavy metal ions can bioaccumulate in organisms as they are hard to metabolize (process and eliminate)[7]. Chrominum(III) compounds and chromium metal are not considered a health hazard, while the toxicity and carcinogenic properties of chromium(VI) have been known since at least the late 19th century[8]. In 1890, Newman described the elevated cancer risk of workers in a chromate dye company[9]. Chromate induced dermatitis was reported in aircraft workers during World War II[10]. In 1963 an outbreak of dermatitis ranging from erythema to exudative eczema, occurred amongst 60 automobile factory workers in England. The workers had been wet-sanding chromate-based primer paint that had been applied to car bodies[11].

Some elements regarded as heavy metals are essential in small quantities for human health. These elements include vanadium, manganese, iron, cobalt, copper, zinc, selenium, strontium and molybdenum[12]. A deficiency of these essential metals may increase susceptibility to heavy metal poisoning [13].

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 humic acid with L-histidine as a mobile phase. Humic acid is a principal component of humic substances which are the major organic constituents of soil (humus), peat, coal, many upland streams, dystrophic lakes, and ocean water [14]. It is produced by biodegradation of dead organic matter. It is not a single acid rather, it is a complex mixture of many different acids containing carboxyl and phenolate groups so that the mixture behaves functionally as a dibasic acid. The structure of humic acid and L-histidine re as follows:
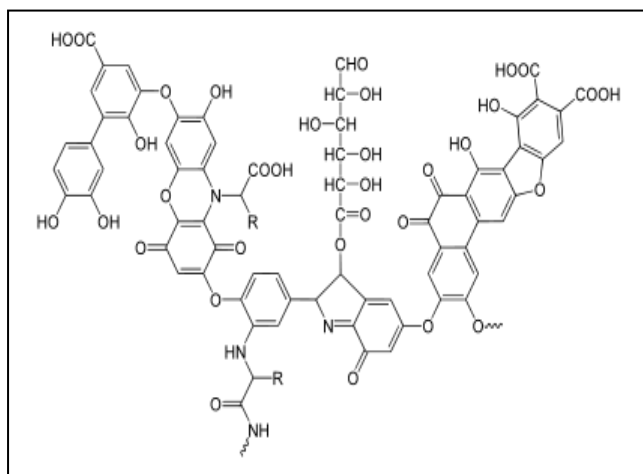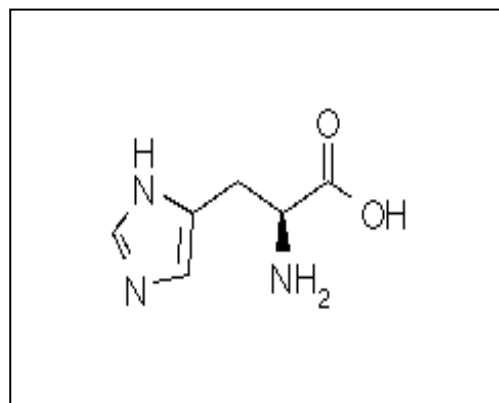
Structure of Humic Acid   **Structure of L-Histidine**

Histidine (abbreviated as His or H) is a proteinogenic, α-amino acid with an imidazole functional group. Initially thought essential only for infants, longer-term studies shown it's essential for adults also[15]. It is one of the 23 proteinogenic amino acids. Its codons are CAU and CAC. Histidine was first isolated by German physician Albrecht Kossel in 1896 The imidazole ring of histidine is aromatic at all pH values[16]. It contains six pi electrons, four from two double bonds and two from a nitrogen lone pair. It can form pi stacking interactions[17] but is complicated by the positive charge[18]. It does not absorb at 280 nm in either state, but does in the lower UV range more than some amino acids[19-20].

MATERIAL 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

The chemical such as humic acid (Merck, India), (Merk India), silica gel- G (Merck, India), hydrochloric acid and sodium hydroxide , L-histidine were used. All other solvents were of the analytical grade.

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 0.1M 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 AgNO$_3$.
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 with L-histidine as the mobile phases

Soil sample preparation for heavy metal analysis

The soil samples were oven dried at 105°C for 24 h, followed by grinding and sieving using 0.18 mm sieve. 0.5 g of dry soil sample was poured into a graduated test tube and mixed with 2 ml of aquaregia 1:3 (1 conc. HCl: 3 conc. HNO$_3$). The mixture was digested on a hot plate at 95°C for 1 h and allowed to cool to room temperature. The sample was then diluted to 10 ml using distilled water and left to settle overnight. The

supernatant was filtered prior to analysis using atomic absorption spectroscopy(AAS) as specified in Adams (1991) [21].

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 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.

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 hydrochloride acid solution. The plates were developed for about 15 min in the glass jar containing 15 ml aqueous humic acid 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 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 R_f values of the individual cations

RESULTS AND DISCUSSION

Effect of concentration: This section deals with the separation of Cr (VI), Cr (III), Ni (II), Co(II), Cu(II), Fe(III), Zn(II) and Mo(VI). Various experiments were carried out at different pH and at different concentration of aqueous humic acid for determining optimum separation concentration for the metal ions. The results dealing with the effect of concentration of mobile phase, i.e. aqueous humic acid with L-histidine on the Rf values of different metal ions such as Cr(VI), Cr(III), Ni(II), Co(II), Cu(II), Fe(III), Zn(II) and Mo(VI) are tabulated in Table 1 and graphically represented in Fig. 1. The variations in the R_f values with concentration in the range of 0.005 to 0.1 % were studied in pH range of 1 to7. It is seen from the Fig.1.0 at low concentration in the range of 0.01 to 0.02 of humic acid and L-histidine Cr(III), Fe(III), Mo(VI), Zn(II), Co(II) and Ni(II) shows tailing spot . But as concentration of aqueous humic acid with L- hitidine was decrease to 0.005M better separation was achieved with clear and distinct spots .

Effect of pH : This section deals with the separation Cr (VI), Cr (III), Ni (II), Co(II), Cu(II), Fe(III), Zn(II) and Mo(VI). Various experiments were carried out at different run time, different pH and at different concentration of aqueous humic acid with L-histidine for determining optimum conditions for separation of the metal ions. The variation in the R_f values of metal ions with run time are shown in Table 2 and graphically represented in Fig. 2.

It was noted that metal ions shows a maximum difference in the R_f value of pH 2.0 to pH 2.5 and the metal ions such as but as Cr(III), Ni(II), Zn(II), Co(II)   shows tailed spot and Cu(II) and Fe(III) shows spread nature. The pH was increased to 3.5 to 4 shows little difference in their R_f value and for pH 4 maximum metal ions give compact and clear spot. This was required for better separation. However the behavior of metal ions changes after pH 5.0

Separation studies in synthetic sample

The present work of separation in aqueous humic acid with L-histidine media was applied to the quantitative separation and detection of metal ions in the synthetic sample and industrial Fe- Ti granular soil sample from Amg super alloy Pvt. Ltd Hingna industrial area Nagpur. The AAS studies of metal ions were done before and after running chromatogram for the comparative study which is listed in Table No.3 and 4 for synthetic samples and industrial soil sample respectively.

Table 1: Effect of concentration on the R_f value of metal ions
Mobile phase : 0.005% Aqueous Humic acid +0.005M L-Histidine
Adsorbent : Silica gel –G , pH=2.5

Metal Ion	Time in Second for 0.005% Humic acid +0.005M L-Histidine			
	5 Min	10 Min	15 Min	20 Min
Cr(VI)	0.75	0.85	0.86	0.90
Cr(III)	0.42	0.23	0.23	0.21
Co(II)	0.78	0.75	0.74 T	0.70 T
Ni(II)	0.88	0.85	0.84 T	0.79 T
Zn(II)	0.39	0.31	0.26	0.22
Cu(II)	0.42	0.41	0.39	0.41
Fe(III)	0.33	0.34	0.33	0.32
Mo(VI)	0.34	0.33	0.32	0.30

Notation : T – Tailing , Spr – Spreading , DS – Double spot
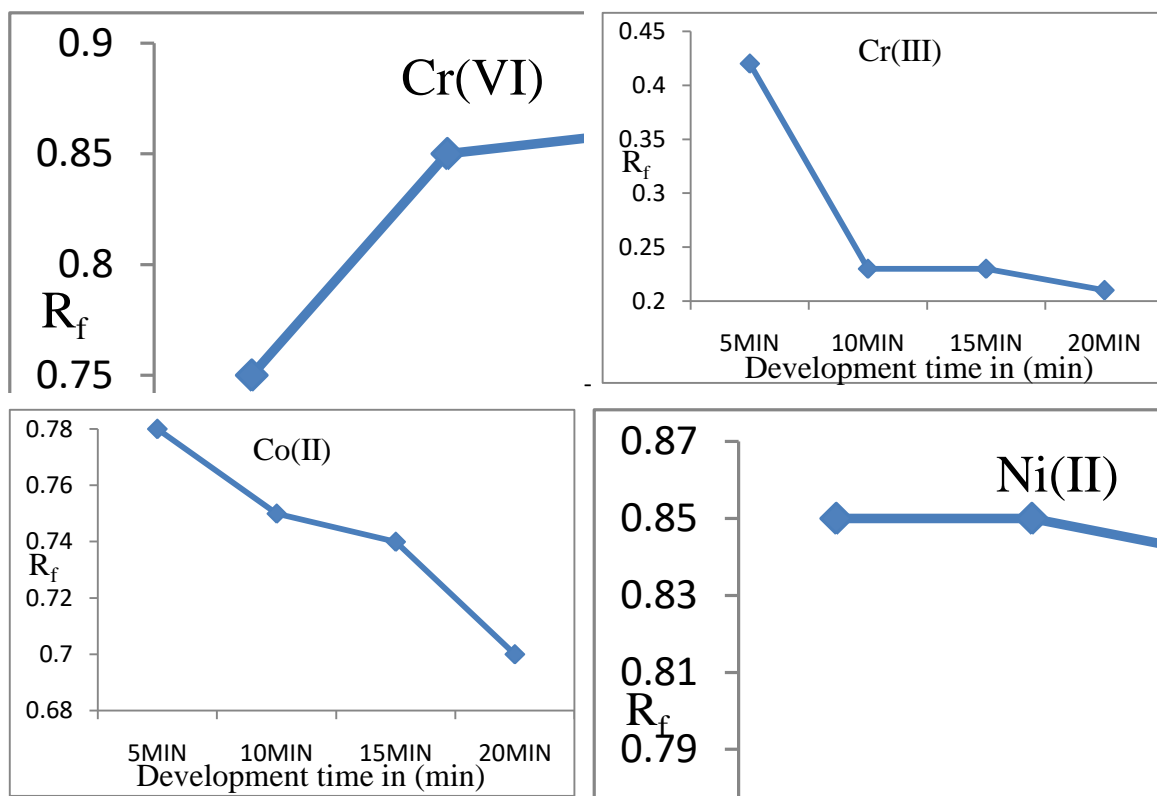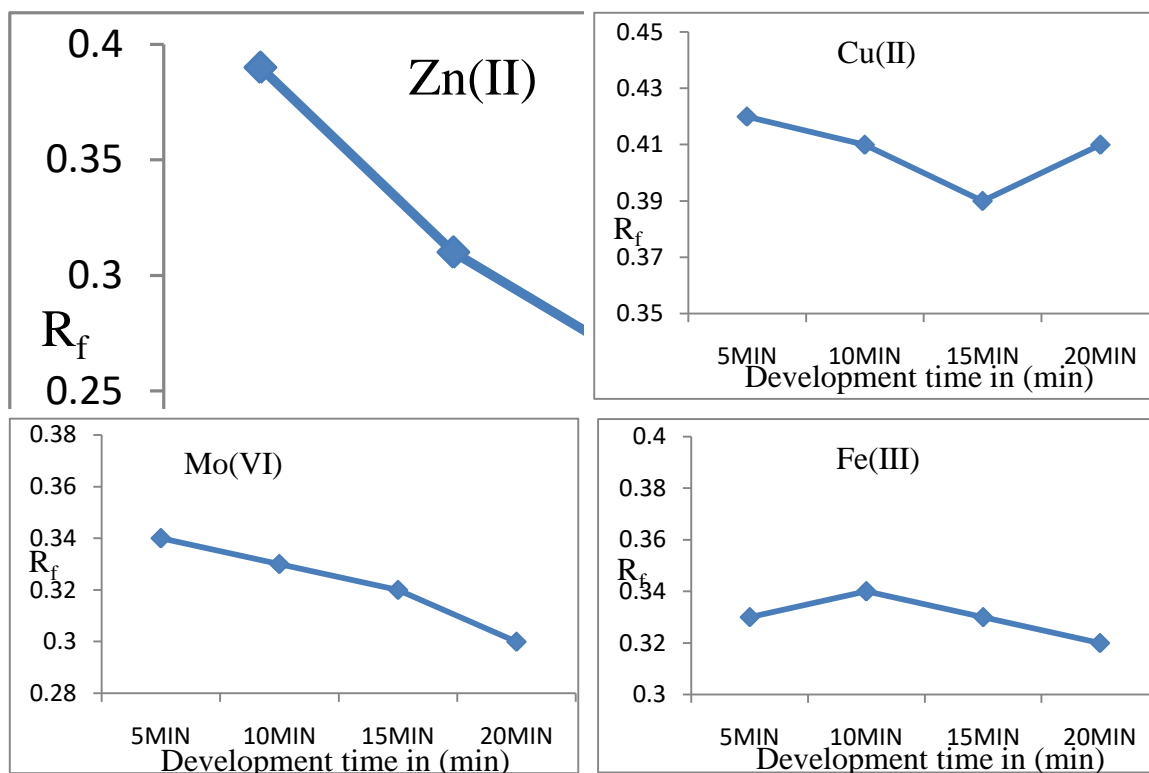
Fig 1: Effect of concentration on R_f value on metal ion

Table 2: Effect of pH on the R_f values of metal ion

Metal Ion	pH of Humic acid + L- Histidine									
	2	2.5	3	3.5	4	4.5	5	5.5	6	7
Cr(VI)	0.92	0.94	0.93	0.93	0.94	0.93	0.92 T	0.92 T	0.93 T	0.93 T
Cr(III)	0.57	0.24 T	0.21	0.20	0.14	0.14	0.15	0.15	0.15	0.14
Ni(II)	0.86	0.85 T	0.85	0.84	0.81	0.80	079	0.78	0.78	0.77
Co(II)	0.84	0.82 T	0.78	0.75	0.74spr	0.73spr	0.72spr	0.73spr	0.72	0.72
Zn(II)	0.61	0.42 T	0.24	0.16T	0.17 T	0.17 T	0.18 T	0.17 T	0.18T	0.19 T
Cu(II)	0.79spr	0.49spr	0.47	0.41	0.45	0.44	0.43	0.41	0.41	0.42
Fe(III)	0.74spr	0.42spr	0.39	0.32	0.20	0.17	0.15	0.16	0.17	0.15
Mo(VI)	0.64	0.58	0.35	0.28T	0.28	0.25 T	0.23T	0.22T	0.21	0.21

Notation : T – Tailing , Spr – Spreading , DS – Double spot
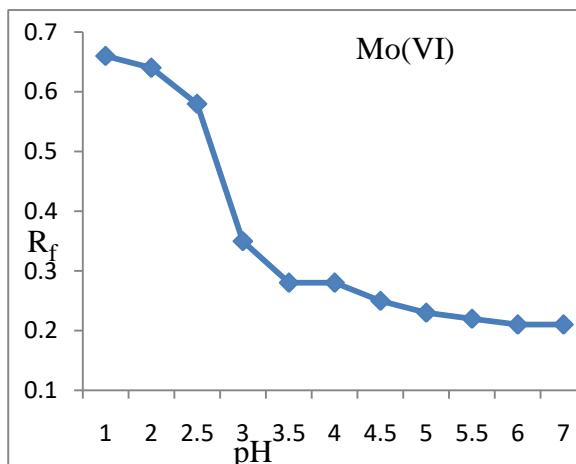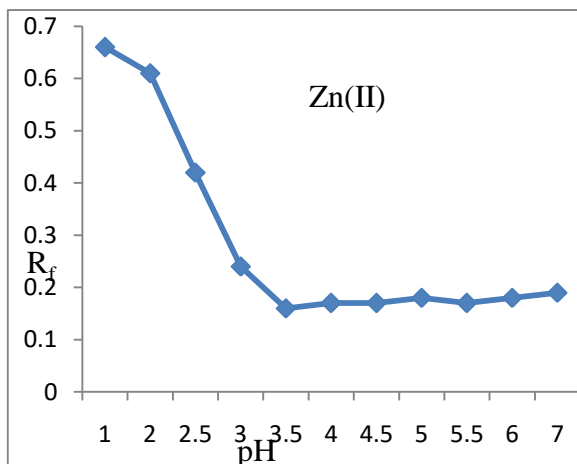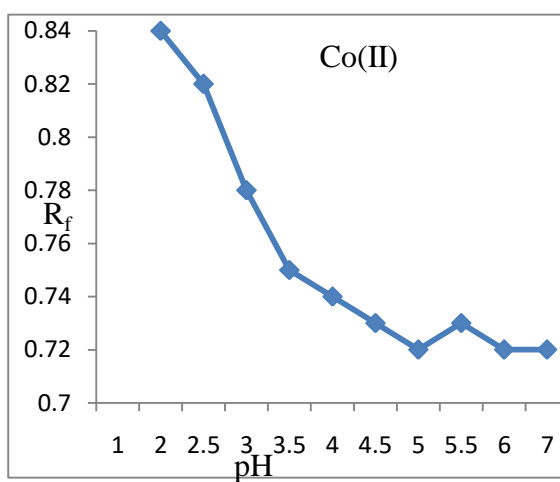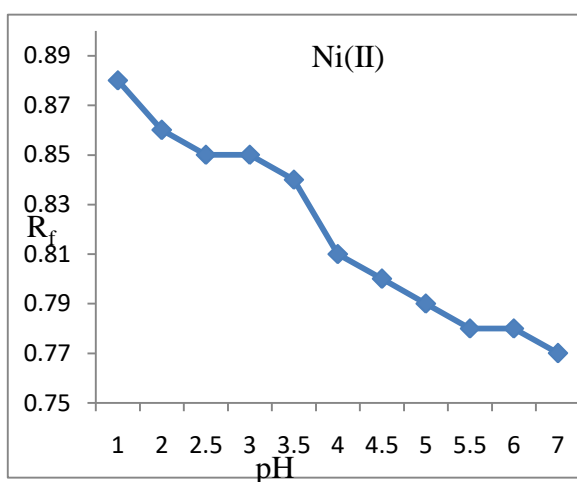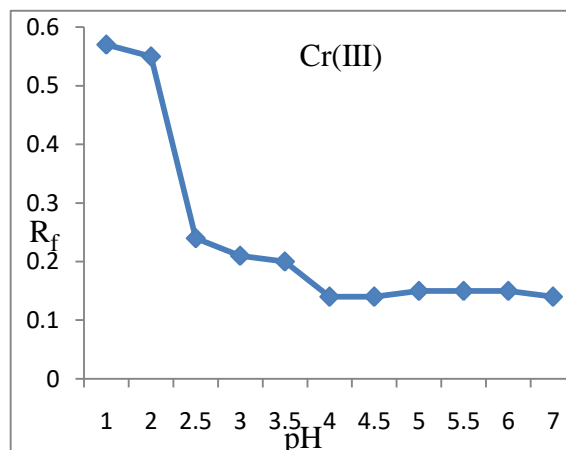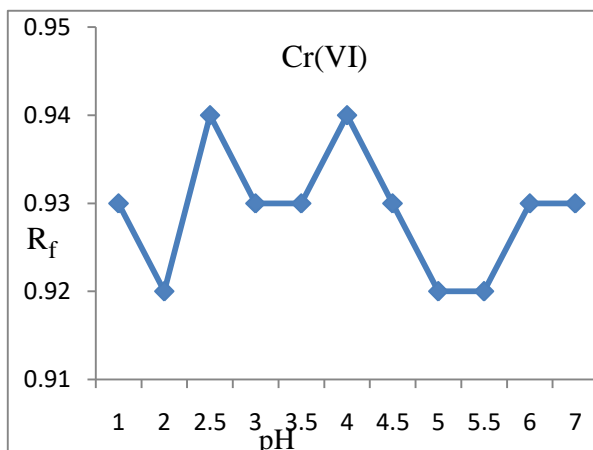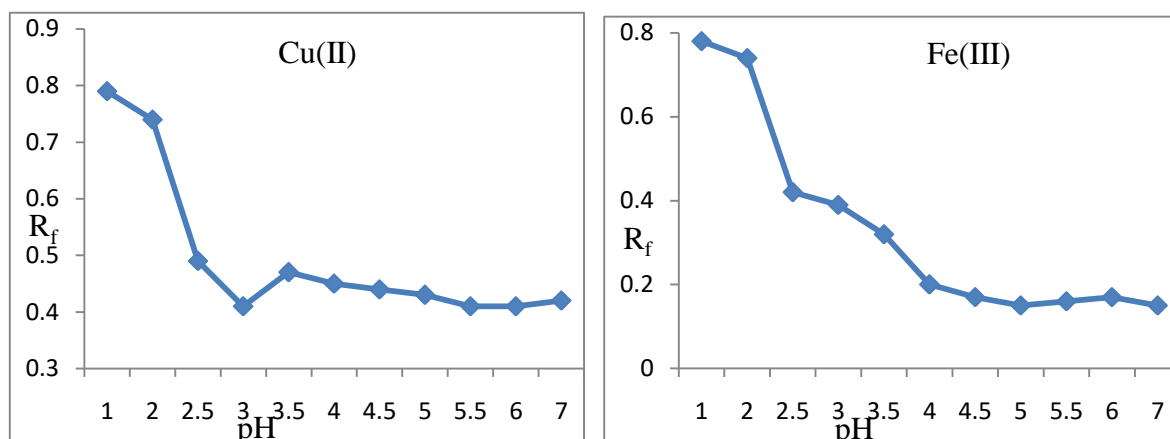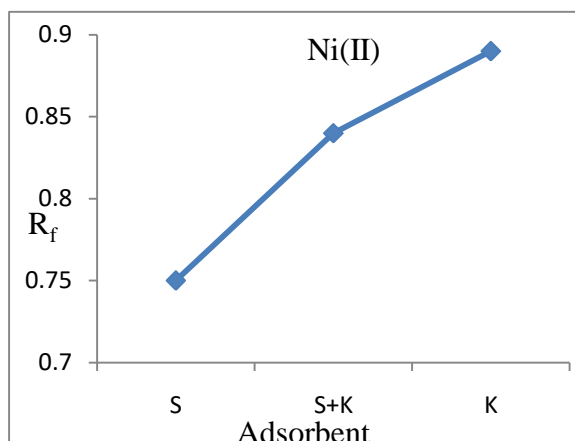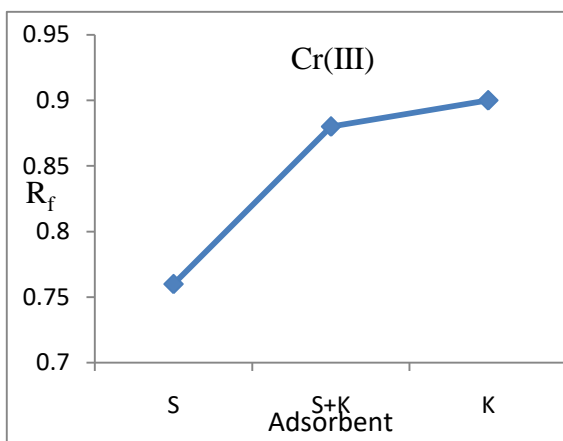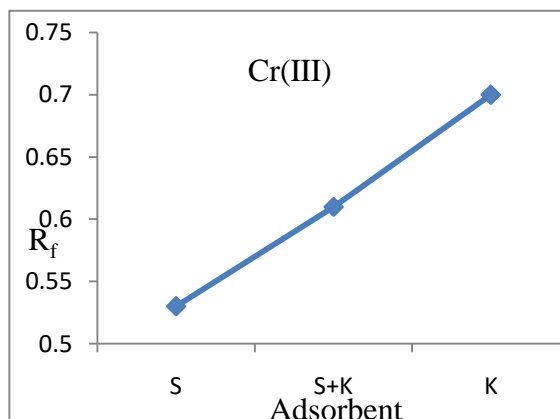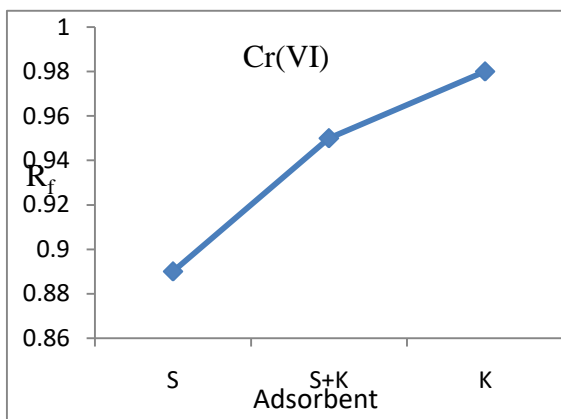
Fig 2: Effect of pH on the R_f values of metal ion

Effect of Adsorbent : - This section deals with the separation of Cr (VI), Cr (III), Ni (II), Co(II), Cu(II), Fe(III), Zn(II) and Mo(VI) metal ions, Various experiments were carried by using different adsorbent such as silica gel –G, keiselghur and mixture of silica gel –G and keiselguhr . with aqueous humic acid with L-Hitidine as a mobile phase for determining optimum separation concentration for the metal ions. The results   obtained   are summarized   in   Table 3 .   From   Table   it is revealed   that   Silica gel –G is best adsorbent for the separation of binary , ternary and quaternary mixtures of Cr(VI) , Cr(III) , Ni(II) , Cu(II) , Mo(VI) , Zn(II) , Co(II) and Fe(III)   metal ions as the spot obtained with silica gel G were quite compact as compared with keiselguhr and mixture of silica gel –G and keiselguhr .

Table 3: Effect of Adsorbent on the R_f value of metal ions

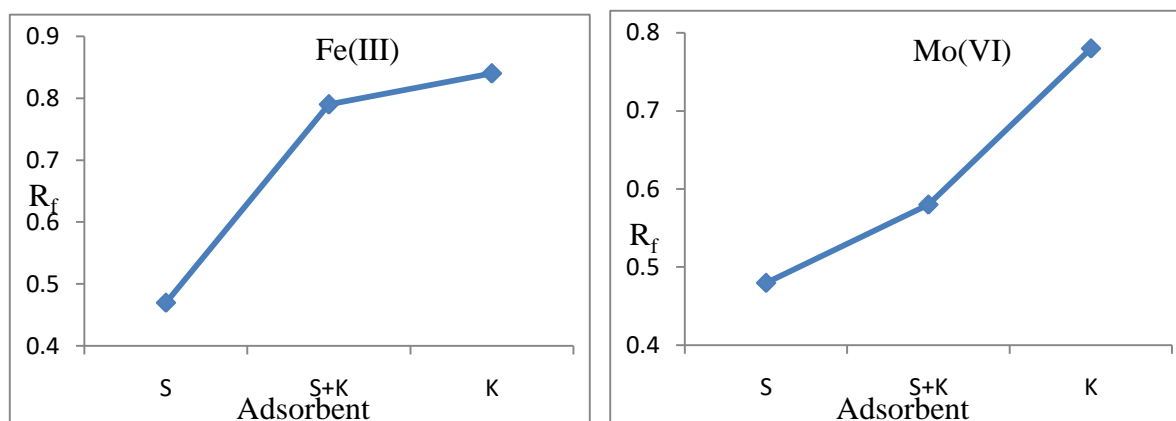| Metal ions	Silica gel -G	Silica gel-G +Keiselghur	Keiselghur
Cr(VI)	0.89	0.95	0.98 Spr
Cr(III)	0.53	0.61	0.70 Spr
Co(II)	0.76	0.88	0.90
Ni(II)	0.75 Spr	0.84 Spr	0.89 Spr
Zn(II)	0.70	0.75	0.82
Cu(II)	0.50 T	0.81 T	0.87 T
Fe(III)	0.47 T	0.79 T	0.84 T
Mo(VI)	0.48 T	0.58 T	0.78 T
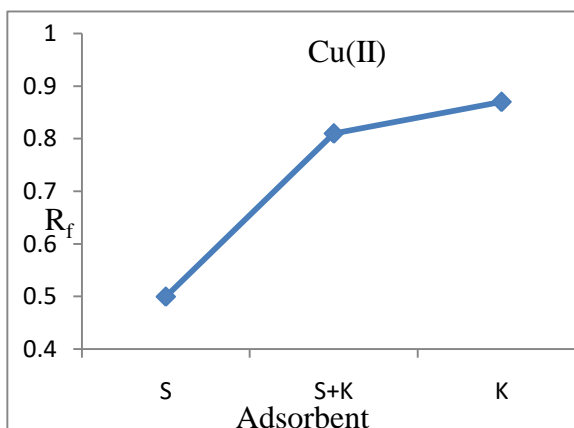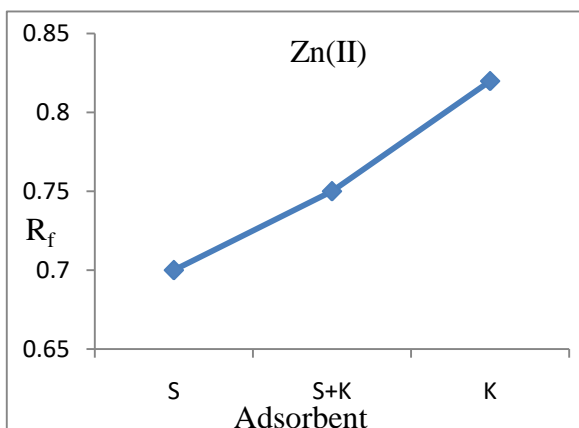
Fig 3: Effect of Adsorbent on the R_f value of metal ion experimentally achieved separations of various mixture using optimum separating conditions

Table 4: Binary separation

Sr. No	Components of Binary mixture	Metal ions with their R_f Values
1	Cr(VI)Co(II)	Cr(VI) - 0.95; Co(II) - 0.76
2	Ni(II); Zn(II)	Ni(II) – 0.85; Zn(II) – 0.20
3	Ni(II); Mo(VI)	Ni(II) – 0.84; Mo(VI) – 0.26
4	Cr(VI); Cr(III);	Cr(VI)– 0.95; Cr(III) – 0.17
5	Co(II); Zn(II)	Co(II) - 0.77; Zn(II) – 0.20
6	Ni(II); Cr(III)	Ni(II)– 0.84; Cr(III) – 0.18
7	Ni(II); Co(II);	Ni(II) – 0.78; Co(II) – 0.71
8	Ni(II); Cu(II)	Ni(II) – 0.79; Cu(II) – 0.55
9	Cr(VI); Zn(II)	Cr(VI) -- 0.96; Zn(II) – 0.18
10	Cr(VI); Ni(II)	Cr(VI) – 096; Ni(II) -- 0.80
11	Co(II); Fe(III)	Co(II) - 0.75; Fe(III) – 0.13

Table 5: Ternary separations

Sr.No.	Components of Ternary mixture	Metal ions with their R_f Values
1	Ni(II); Co(II); Cr(III)	Ni(II) – 0.79; Co(II) – 0.72; Cr(III) – 0.19
2	Ni(II); Co(II); Zn(II)	Ni(II) – 0.79; Co(II) - 0.74; Zn(II) – 0.19
3	Ni(II); Cr(VI); Cu(II)	Ni(II) - 0.80; Cr(VI) – 0.92; Cu(II) – 0.40
4	Ni(II); Cr(VI); Mo(VI)	Ni(II) – 0.80; Cr(VI) – 0.93; Mo(VI) 0.28
5	Ni(II); Cr(VI); Co(II)	Ni(II) – 0.80 ; Cr(VI) – 0.93; Co(II) –0.72
6	Ni(II); Cr(VI); Zn(II)	Ni(II) – 0.81 ; Cr(VI) – 0.94; Zn(II) –0.20
7	Fe(III); Co(II); Cu(II)	Fe(III) - 0.17 ; Co(II) – 0.76; Cu(II) –0.44

Table 6: Quaternary separation

Sr. No	Components of Quaternary mixture	Metal ions with their R_f Values
1	Cr(VI); Ni(II); Co(II); Zn(II)	Cr(VI) − 0.93; Ni(II) − 0.61; Co(II) − 0.51; Zn(II) − 0.14
2	Cr(VI); Ni(II); Co(II); Cr(III)	Cr(VI) − 0.93; Ni(II) − 0.63; Co(II) − 0.55; Cr(III) − 0.16
3	Cr(VI); Ni(II); Zn(II); Mo(VI)	Cr(VI) -0.94; Ni(II) − 0.60; Zn(II) − 0.13; Mo(VI) -0.17
4	Cr(III); Ni(II); Co(II); Zn(II)	Cr(III) − 0.16; Ni(II) − 0.65; Co(II) − 0.53; Zn(II) − 0.12
5	Cr(VI); Ni(II); Co(II); Fe(III)	Cr(VI) − 0.93; Fe(III) − 0.10; Co(II) − 0.50; Zn(II) − 0.17
6	Cr(VI); Ni(II); Cu(II); Zn(II)	Cr(VI) − 0.93; Ni(II) − 0.63; Cu(II) − 0.35; Zn(II) − 0.16

Table 7: Metal Ions analysis by AAS

| Name of Sample	Metal ion analysis	Metal ion present in mixture (ppm) before chromatogram	Metal ion Analysis by AAS (ppm) after chromatogram
Amg super alloy of Fe-Ti granular industrial soil sample	Cr(VI) Cr (III) Ni(II) Co(II) Zn(II) Mo(VI) Fe(III)	Cr(VI) - 0.202 ppm Cr (III) − 0.08 ppm Ni(II) 0.154 ppm Cu(II) − 0.071 ppm Co(II) − 0.124 ppm Mo(VI) − 0.236 ppm Fe(III) − 26.34 ppm	Cr(VI) - 0.199 ppm Cr (III) − 0.06 ppm Ni(II) 0.152 ppm Cu(II) − 0.068 ppm Co(II) − 0.122 ppm Mo(VI) − 0.233 ppm Fe(III) − 26.17 ppm

Plate 1.1: Binary separation
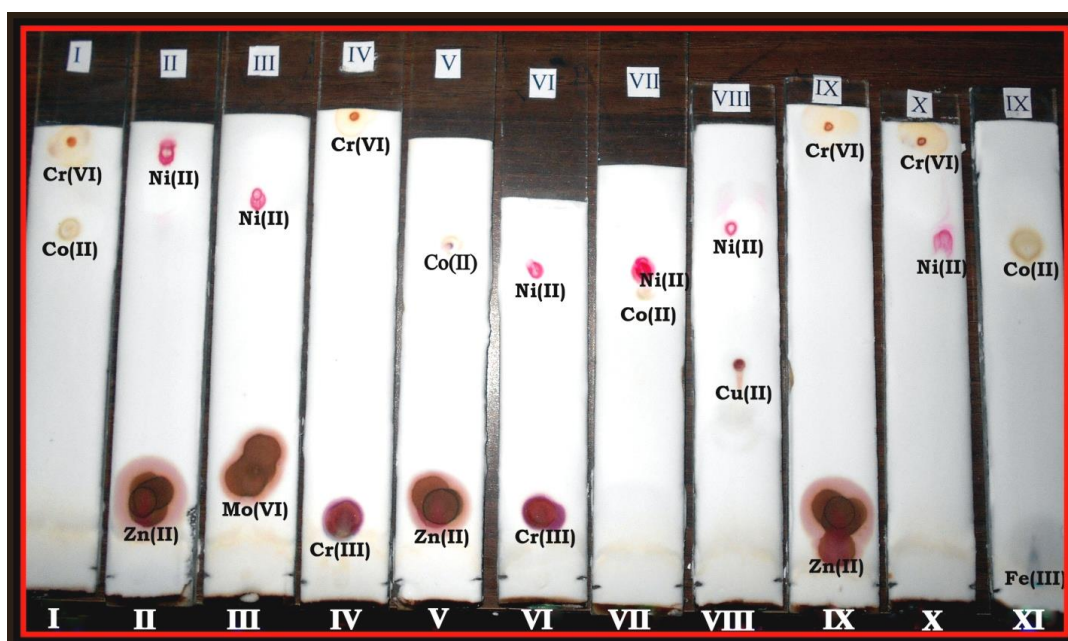
Plate 1.2: Ternary separation

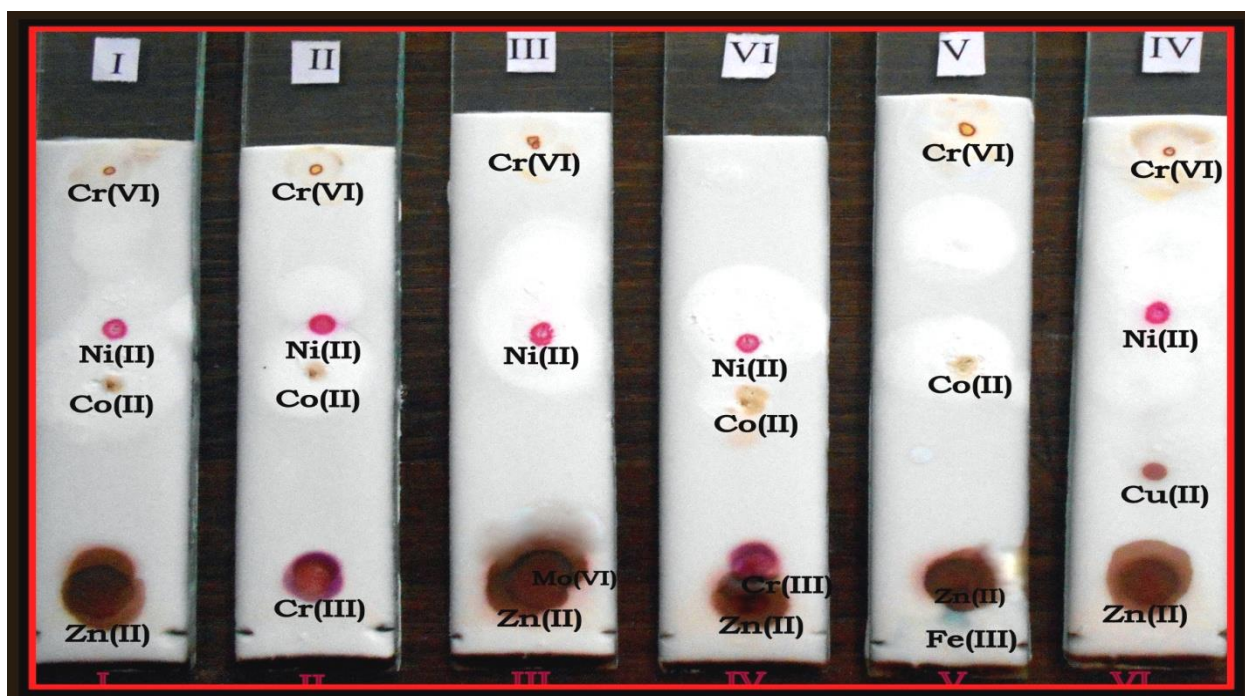**1.3 Quaternary separation**

Fig 4 Pictures of Binary, Ternary and Quaternary Separations using optimum separating conditions

CONCLUSION

Using the above mentioned optimum separating conditions, that is 0.005% aqueous humic acid with 0.005ML histidine at pH 4.0, qualitative separation of eleven binary mixtures; six ternary mixtures; and five quaternary mixtures of metal ions have been carried out. The R_f values of various binary, ternary and quaternary separations have been listed in Table 4, 5 and 6 respectively. The R_f values of metal cations are given in top to bottom format, as they appear on the chromatographic plate. Photograph of achieved binary separations were given in Fig. 1, for ternary separations in Fig. 2 and quaternary separations in Fig.3. R_f values of experimentally achieved separations on silica gel 'G' layers developed with aqueous humic acid with L-histidine as mobile phase.

ACKNOWLEDGEMENT

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