

NOVEL MOBILE PHASE FOR SEPARATION OF Cr^{6+} FROM Cr^{3+} AND ASSOCIATED HEAVY METAL CATIONS BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

A. Mohammad and Y. H. Sirwal

Analytical Laboratory, Department of Applied Chemistry, Faculty of Engineering
and Technology, Aligarh Muslim University, Aligarh-202002, India

SUMMARY

Eleven heavy metal cations have been chromatographed on silica gel high-performance thin-layer chromatography plates with pure organic, mixed organic, and mixed aqueous–organic mobile phases. Mobile phases such as methanol–dimethylamine, 8:2 (v/v), and methanol–dimethylamine–formic acid, 8:8:2 (v/v), were found most suitable for rapid separation and identification of mixtures of Cr^{6+} and Cr^{3+} and of Cr^{6+} , Ni^{2+} , and Co^{2+} , respectively. The effect of impurities such as inorganic ions, phenols, and surfactants on the separation of Cr^{6+} and Cr^{3+} was examined. Mutual separation of Cr^{6+} , Ni^{2+} , and Co^{2+} was investigated at different sample solution pH. The limit of detection for Ni^{2+} , Co^{2+} , Cu^{2+} , and Pb^{2+} on HPTLC plates was evaluated and semiquantitative determination of Cr^{6+} and Ni^{2+} by spot-area measurement was attempted. The proposed method was successfully used for identification of Cr^{6+} , Cr^{3+} , Ni^{2+} , and Co^{2+} and for mutual separation of Cr^{6+} from Cr^{3+} and of Cr^{6+} from Ni^{2+} and Co^{2+} from a variety of industrial wastewater samples.

INTRODUCTION

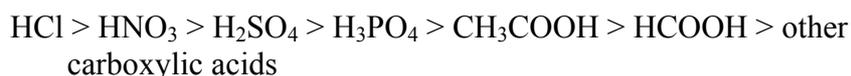
Heavy metals have recently received considerable attention from analysts, because of their physical and environmental importance [1,2]. Metals such as Pb, Cd, Hg, Ni, Cu, Zn, As, and Cr^{6+} are toxic and harmful to human health. They can form stable complexes with bio-ligands containing oxygen, nitrogen, or sulphur atoms [3] which control several redox processes in living organisms. The substantial increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in aquatic life. Industrial waste is the

major source of different kinds of metal pollution in aqueous systems. The major sources of chromium in the aquatic environment are electroplating and metal-finishing industrial effluents, sewage and wastewater treatment plant discharge, and chromates from cooling water. Chromium occurs in several oxidation states (e.g. di-, tri-, penta-, and hexa-) but only Cr^{3+} and Cr^{6+} are biologically important. Chromium in the aquatic environment tends to speciate into Cr^{3+} and Cr^{6+} , with the trivalent ion being oxidized to the hexavalent form or precipitating from solution.

The different analytical techniques available for the detection, determination, and separation of chromium include normal-phase and reversed-phase thin-layer chromatography [4–6], ion chromatography [7,8], extraction chromatography [9], ion-exchange chromatography [10,11], reversed-phase high-performance liquid chromatography [12,13], micellar electrokinetic chromatography [14], precipitation flotation [15], solid-phase extraction [16], titrimetry [17,18], capillary electrophoresis [19,20], spectrophotometry [21,22], atomic-absorption spectroscopy [23–25], graphite furnace atomic-absorption spectroscopy [26,27], atomic emission spectroscopy [28], neutron activation analysis [29,30], and hyphenated techniques such as ion-exchange chromatography–flame atomic absorption spectroscopy [31], ion-chromatography–thermal lens spectrometry [32,33], gas chromatography–neutron activation analysis [34], inductively coupled plasma mass spectroscopy [35,36], inductively coupled plasma mass spectroscopy–atomic emission spectroscopy [37,38], ion-exchange chromatography–flame atomic absorption spectroscopy [39], solid-phase extraction–flame atomic emission spectroscopy [40], liquid chromatography–inductively coupled plasma mass spectroscopy [41], high-performance liquid chromatography–inductively coupled plasma mass spectroscopy [42], and ion chromatography–inductively coupled plasma mass spectroscopy [43].

Of the different separation procedures, thin-layer chromatography (TLC) is probably the most versatile, because it can be used for the selective separation of metal cations on the micro and macro scales. The use of high-performance TLC plates has further enhanced the efficiency of this technique. An exhaustive survey of the literature published in the last thirty years [44] shows that much progress has been made in developing rapid and selective TLC methods for separation of toxic heavy metals (Cu, Ni, Co, Pb, Cd, Zn, Hg, Cr, Fe, and Al) from interfering elements, by use of a variety of acidic developers containing mineral or carboxylic acids as one of the components. Systematic examination of published data on the

use of acidic mobile phases for analysis of metal cations shows that the number of applications of the acids decreases in the order:



That HCl is used most frequently is understandable, because it forms chloro complexes with almost all heavy metal cations. Perchloric acid has been used rarely. Use of formic acid as a mobile phase component in the TLC of metal cations has received little attention [45–49] despite several favourable properties:

- (i) it does not result in oxidation of cations during analysis;
- (ii) formic acid-containing mobile phases are less affected by the properties of silica gel than those containing other acids [50];
- (iii) it enables excellent resolution of aflatoxins [50] and metal cations [45–49]; and
- (iv) it is sufficiently acidic ($K_a(\text{H}_2\text{O})$ at $25^\circ\text{C} = 1.77 \times 10^{-4}$) to prevent hydrolysis of salts.

All studies with formic acid-containing mobile phases have been performed using conventional, laboratory made TLC plates. It was therefore decided to use the analytical potential of formic acid as mobile phase and precoated HPTLC silica plates as stationary phase for analysis of heavy metal cations. As a result several analytically important separations of heavy metals were realized. Separation of the different valence states of chromium is industrially important because Cr^{3+} is converted to Cr^{6+} in alkaline peroxide media.

EXPERIMENTAL

Chemicals and Reagents

Dimethylamine was from S.D. Fine Chemicals (India), dimethylaniline, inorganic salts, amines, and phenols were from CDH (India), *o*-aminophenol was from Loba Chemie (India), formic acid was from Merck (India), and methanol and acetone were from Qualigens (India). All reagents were analytical reagent grade.

Test Solutions

Test solutions, prepared in double-distilled water, contained 1.0% aqueous solutions of the nitrates of Cd^{2+} , Pb^{2+} , Tl^+ , Bi^{3+} , Al^{3+} , and Ag^+ , the chlorides of Ni^{2+} , Co^{2+} , Hg^{2+} , and Cr^{3+} , the sulphate of VO^{2+} , and the

potassium salt of Cr^{6+} . The solutions of the nitrates of lead, silver, and bismuth, and the chloride of mercury contained small quantities of the corresponding acid to limit the extent of hydrolysis.

Detection Reagents

Yellow ammonium sulphide reagent was used for detection of Cd^{2+} , Ag^+ , Pb^{2+} , Tl^+ , Bi^{3+} , and Hg^{2+} , a 1% aqueous solution of potassium ferrocyanide for detection of VO^{2+} ; dimethylglyoxime (0.2% in ammonia) for detection of Ni^{2+} and Co^{2+} , a saturated alcoholic solution of AgNO_3 for detection of Cr^{6+} , and a 1% methanolic solution of alizarin red for detection of Cr^{3+} .

Chromatography

Chromatography was performed on silica gel 60 F₂₅₄ HPTLC plates (Merck, Darmstadt, Germany). Test solutions (5 μL) were applied approximately 2.0 cm above the lower edge of the plates by means of a micropipette. The spots were dried and the plates were developed in glass jars by the one-dimensional ascending technique. The mobile phases investigated are listed in Table I. Before development of the plates the glass jars containing the mobile phases were covered with a lid for approximately 20 min to enable presaturation of the glass jars with mobile phase vapour. The mobile phase migration distance was always 10 cm from the starting line. After development the plates were dried and the cations were visualized as coloured spots by spraying with the appropriate detection reagents. The cations were identified on the basis of their R_F values, calculated from R_L (R_F of the leading front) and R_T (R_F of trailing front) for each spot.

Separations

Test solutions (10 μL) containing the metal ions to be separated were spotted on the HPTLC plates and chromatography was performed with a variety of mobile phases. The resolved spots for these metal cations were observed on the plates after spraying with chromogenic reagents and the R_F values of the separated metal ions were determined.

Interference

To investigate the effect of interference of inorganic ions, phenols, and surfactants on the R_F values (mobility) of Cr^{6+} and Cr^{3+} a solution of the impurity (10 μL) was spotted with each metal ion, as a mixture, on the

HPTLC plate and chromatography was performed as described above. The spots were detected and the R_F values of separated metal ions were determined.

Table I

The mobile phases investigated

Symbol	Composition
M ₁	Methanol (MeOH)
M ₂	Dimethylamine (DMA)
M ₃	Formic acid (FA)
M ₄	Acetone
M ₅	Dimethylaniline (DMAL)
M ₆	<i>ortho</i> -Aminophenol (o-APH)
M ₇	Methanol–dimethylamine, 8:2
M ₈	Methanol–formic acid, 8:2
M ₉	Water–dimethylamine, 8:2
M ₁₀	Water–formic acid, 8:2
M ₁₁	Dimethylamine–methanol–formic acid, 2:8:2
M ₁₂	Dimethylamine–methanol–formic acid, 4:8:2
M ₁₃	Dimethylamine–methanol–formic acid, 8:8:2
M ₁₄	Dimethylamine–methanol–formic acid, 10:8:2
M ₁₅	Dimethylamine–acetone–formic acid, 2:8:2
M ₁₆	Dimethylamine–acetone–formic acid, 4:8:2
M ₁₇	Dimethylamine–acetone–formic acid, 8:8:2
M ₁₈	Dimethylamine–acetone–formic acid, 10:8:2
M ₁₉	Dimethylamine–water–formic acid, 2:8:2
M ₂₀	Dimethylamine–water–formic acid, 4:8:2
M ₂₁	Dimethylamine–water–formic acid, 8:8:2
M ₂₂	Dimethylamine–water–formic acid, 10:8:2
M ₂₃	Dimethylaniline–methanol–formic acid, 2:8:2
M ₂₄	Dimethylaniline–methanol–formic acid, 4:8:2
M ₂₅	Dimethylaniline–methanol–formic acid, 8:8:2
M ₂₆	Dimethylaniline–methanol–formic acid, 10:8:2
M ₂₇	<i>o</i> -Aminophenol–methanol–formic acid, 2:8:2
M ₂₈	<i>o</i> -Aminophenol–methanol–formic acid, 4:8:2
M ₂₉	<i>o</i> -Aminophenol–methanol–formic acid, 8:8:2
M ₃₀	<i>o</i> -Aminophenol–methanol–formic acid, 10:8:2

Effect of pH

The effect of pH on the mutual separation of Cr^{6+} , Ni^{2+} , and Co^{2+} was investigated by adding required amount of acid to a mixed solution of the three metals and analysing the sample by HPTLC as described above.

Limit of Detection

Detection limits for Ni^{2+} , Co^{2+} , Cu^{2+} , and Pb^{2+} were determined by spotting different amounts of solutions of the cations on the HPTLC plates, developing the plates with mobile phase M_7 (Table I), and detecting the spots as described above. The method was repeated with successive reduction of the amounts of the cations until spots could no longer be detected. The minimum amount of cation that could be detected was taken as the limit of detection.

Semi-Quantitative Determination by Spot-Area Measurement

For semi-quantitative determination by spot-area measurement standard solutions (0.5–2.0%, 10 μL) of Ni^{2+} and Cr^{6+} were spotted on the HPTLC plates and the plates were developed with mobile phases M_7 and M_{13} (Table I). After detection the spots were copied on to tracing paper from the plates and then the area of each spot was calculated.

Semi-Quantitative Determination by Visual Comparison

Standard solutions of potassium dichromate of different concentrations (0.5–2.0%, 10 μL), and an industrial wastewater sample (10 μL), were spotted on HPTLC plates. After completion of the chromatography the colour intensity and R_F values of spots from the industrial wastewater sample were matched with the coloured spots obtained from standard reference solutions of potassium dichromate. The amount of chromium present in the industrial sample was determined from the colour intensity of its spot on the plate after visual comparison with the colour intensities of the spots obtained from standard solutions.

Chromatography of Spiked Wastewater Samples

Three spiked samples of industrial wastewater were prepared and chromatographed. Wastewater sample 1 containing Cr^{6+} was spiked with an aqueous solution of Cr^{3+} (1%) in a 1:1 (v/v) ratio. The resulting spiked sample (ca. 10 μL) was analysed by HPTLC with M_7 as mobile phase and the R_F values of the resolved spots of Cr^{6+} and Cr^{3+} were determined.

Industrial wastewater samples containing Ni²⁺ (samples 2 and 3) were spiked with aqueous solutions of Cr⁶⁺ (1%) and Cr³⁺ (1%) in a 1:1:1 (v/v) ratio and 10 µL of the resulting solution was analysed by HPTLC with M₁₃ as mobile phase. The *R_F* values of resolved spots of Ni²⁺, Cr⁶⁺, and Cr³⁺ were determined.

RESULTS AND DISCUSSION

The results from this study have been summarized in Tables II–VIII and Figs 1–3. The mobilities of metal cations chromatographed with pure single-component organic mobile phases (M₁–M₆), two-component mixed organic mobile phases (M₇ and M₈) and aqueous–organic mobile phases (M₉ and M₁₀) have been summarized in Table II. It is clear from this table that, except for Cr⁶⁺, only formic acid-containing mobile phases (M₃, M₈, and M₁₀) induce migration of the metal cations; Cr⁶⁺ is more mobile in some mobile phases (M₁–M₄, M₇, and M₉) which contain no formic acid. The use of pure acetone as mobile phase (M₄) resulted in the

Table II

Mobility (as *R_F* value) of heavy metal ions on silica gel HPTLC plates developed with single-component (M₁–M₆), two-component organic (M₇, M₈) and two-component aqueous–organic (M₉, M₁₀) mobile phases

Metal ion	Mobile phase									
	M ₁	M ₂	M ₃	M ₄	M ₅	M ₆	M ₇	M ₈	M ₉	M ₁₀
VO ²⁺	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00
Cr ⁶⁺	0.34	0.86 ^a	0.72	0.00 ₂ 0.97 ^b	0.01	0.00	0.86	0.63	0.85	0.78
Ag ⁺	0.00	0.00	0.15	0.00	0.03	0.02	0.00	0.15	0.00	0.09
Hg ²⁺	0.45T ^c	0.00	0.85	0.00	0.10	0.02	0.30	0.82	0.04	0.94
Pb ²⁺	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.49	0.10	0.93
Cd ²⁺	0.08	0.00	0.78	0.00	0.05	0.05	0.02	0.72	0.00	0.94
Tl ⁺	0.10	0.00	0.82	0.00	0.00	0.00	0.05	0.76	0.00	0.78
Ni ²⁺	0.05	0.00	0.87	0.00	0.00	0.04	0.02	0.81	0.00	0.84
Co ²⁺	0.04	0.06	0.75	0.00	0.00	0.03	0.04	0.68	0.00	0.88
Bi ³⁺	0.09	0.00	0.16	0.09	0.02	0.40T ^c	0.06	0.16	0.04	0.23
Cr ³⁺	0.05	0.05	0.70	0.05	0.05	0.05	0.05	0.60	0.05	0.75

^a Detection clarity is poor

^b Double spot

^c Tailed spot ($R_L - R_T > 0.3$)

formation of double spots for Cr^{6+} . The peculiar behaviour of formic acid as tailing reducer and mobility activator results in new opportunities for separation of metal cations. With this in mind we added a third component – methanol, acetone, or water – to the mixtures of amines and formic acid and the resulting three-component mixtures (M_{11} – M_{26}) were investigated as mobile phases for TLC of metal cations. Amines were selected on the basis of our past experience [51] that they furnish highly compact and well-resolved spots of metal cations on silica layers.

The results obtained by use of mixed aqueous–organic mobile phases (M_{11} – M_{22}) containing different concentrations of DMA and fixed concentrations of formic acid and organic modifier (M_{11} – M_{18}) or water (M_{19} – M_{22}) are tabulated in Table III. On the basis of these results, the metal

Table III

Migration behaviour of heavy metal cations on precoated silica gel HPTLC plates with dimethylamine, formic acid, and/or acetone, methanol, water in different volume ratios as mobile phases

Metal ion	Mobile phase											
	M_{11}	M_{12}	M_{13}	M_{14}	M_{15}	M_{16}	M_{17}	M_{18}	M_{19}	M_{20}	M_{21}	M_{22}
VO^{2+}	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.05	0.02	0.00	0.00
Cr^{6+}	0.62	0.65	0.91	0.92	0.55	0.63	0.68	0.90	0.53	0.53	0.59	0.89
Ag^+	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.11	0.00
Hg^{2+}	0.82	0.72	0.62	0.52	0.88	0.86	0.79	0.76	0.95	0.59	0.17	0.10
Pb^{2+}	0.63	0.52	0.28	0.15	0.84	0.81	0.77	0.00	0.86	0.57	0.02	0.00
Cd^{2+}	0.89	0.66	0.42	0.29	0.84	0.80	0.78	0.76	0.93	0.84	0.26	0.09
Tl^+	0.86	0.78	0.13	0.10	0.78	0.61	0.09	0.04	0.78	0.64	0.35	0.02
Ni^{2+}	0.87	0.68	0.44	0.25	0.77	0.68	0.56	0.42	0.93	0.84	0.23	0.00
Co^{2+}	0.81	0.66	0.05	0.00	0.73	0.67	0.42	0.00	0.89	0.81	0.35	0.00
Bi^{3+}	0.15	0.08	0.00	0.00	0.14	0.13	0.05	0.00	0.15	0.11	0.06	0.03
Cr^{3+}	0.60	0.68	0.90	0.90	0.53	0.75	0.70	0.90	0.50	0.55	0.60	0.86

cations can be grouped into three categories. The metal cations VO^{2+} , Ag^+ , and Bi^{3+} were strongly retained by the stationary phase and remained near the point of application, irrespective of the concentration of dimethylamine and the nature of the organic modifier (methanol or acetone). The mobility of the metal cations Cr^{3+} and Cr^{6+} increased with increasing concentration of dimethylamine in the mobile phase, irrespective of whether the mobile phase contained methanol (M_{11} – M_{14}), acetone (M_{15} – M_{18}), or water (M_{19} – M_{22}) in combination with formic acid and

dimethylamine. The R_F of the metal cations Hg^{2+} , Pb^{2+} , Cd^{2+} , Tl^+ , Ni^{2+} , Co^{2+} , and Bi^{3+} decreased with increasing concentration of dimethylamine in the mobile phases (M_{11} – M_{22}).

Thus, these mobile phases facilitate selective separation of several metal cations by virtue of the variable mobility of the ions. For example, Cr^{3+} and Cr^{6+} can be selectively separated from all other metal cations by use of M_{13} , M_{14} , M_{18} , M_{21} , and M_{22} , because their mobility in these mobile phases is higher than that of the other metal cations. Similarly, M_{13} and M_{18} can be used to achieve the analytically important separation of Ni^{2+} , Co^{2+} , and Cr^{6+} from their mixtures. This separation could not be achieved with pure organic (M_1 – M_6), two-component mixed organic (M_7 , M_8), or mixed aqueous–organic (M_9 , M_{10}) mobile phases.

To examine the effect of the nature of the amino compounds on the mobility of metal cations, dimethylamine was replaced by dimethylaniline (M_{23} – M_{26}) and *ortho*-aminophenol (M_{27} – M_{30}) in mobile phases M_{11} – M_{14} while maintaining the volume ratio of methanol and formic acid the same. The R_F values of the metal cations were determined after use of the resulting mobile phases (M_{23} – M_{30}). The results obtained are encapsulated in Table IV. It is clear from this table that the nature of amino compound has a pronounced effect on the R_F (or mobility) of the metal cations.

Table IV

R_F values of metal cations on silica gel HPTLC plates developed with mixed three-component mobile phases consisting of methanol, formic acid, and dimethylaniline or *o*-aminophenol in different volume ratios

Metal ion	Mobile phase							
	M_{23}	M_{24}	M_{25}	M_{26}	M_{27}	M_{28}	M_{29}	M_{30}
VO^{2+}	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Cr^{6+}	0.60	0.56	0.07	0.05	0.41	0.81	0.83	0.86
Ag^+	0.15	0.15	0.12	0.05	0.15	0.12	0.04	0.02
Hg^{2+}	0.72	0.66	0.56	0.42	0.80	0.64	0.52	0.40
Pb^{2+}	0.61	0.27	0.15	0.06	0.25	0.25	0.30	0.32
Cd^{2+}	0.48	0.39	0.28	0.15	0.55	0.65	0.75	0.85
Tl^+	0.85	0.80	0.62	0.41	0.70	0.60	0.48	0.32
Ni^{2+}	0.72	0.64	0.52	0.16	0.64	0.87	0.90	0.92
Co^{2+}	0.70	0.52	0.42	0.30	0.60	0.64	0.70	0.76
Bi^{3+}	0.16	0.10	0.09	0.07	0.15	0.17	0.17	0.18
Cr^{3+}	0.60	0.55	0.10	0.05	0.40	0.60	0.80	0.82

VO^{2+} , Ag^+ , and Bi^{3+} are poorly mobile in all the mobile phases. Several changes in the R_F values of the cations resulted from substitution of dimethylamine with dimethylaniline – the R_F of Cr^{6+} dropped from 0.91 (M_{13}) and 0.92 (M_{14}) to 0.07 (M_{25}) and 0.05 (M_{26}), respectively; the R_F of Pb^{2+} decreased from 0.52 (M_{12}) to 0.27 (M_{24}); the R_F of Cd^{2+} decreased from 0.89 (M_{11}), 0.66 (M_{12}), 0.42 (M_{13}), and 0.29 (M_{14}) to 0.48 (M_{23}), 0.39 (M_{24}), 0.28 (M_{25}), and 0.15 (M_{26}), respectively; the R_F of Tl^+ increased from 0.13 (M_{13}) and 0.10 (M_{14}) to 0.62 (M_{25}) and 0.41 (M_{26}), respectively; and the R_F of Cr^{3+} decreased from 0.90 (M_{13} and M_{14}) to 0.10 (M_{25}) and 0.05 (M_{26}), respectively.

Similarly, substitution of dimethylaniline in M_{11} – M_{14} by *o*-aminophenol caused a decrease in the R_F value of Pb^{2+} from 0.63 (M_{11}) and 0.52 (M_{12}) to 0.25 (M_{27} , M_{28}) whereas the R_F of Cd^{2+} increased from 0.29 (M_{14}) to 0.85 (M_{30}). The R_F values of Ni^{2+} and Co^{2+} increased from 0.00 (M_{14}) to 0.92 and 0.76 (M_{30}), respectively. A decrease in the R_F of Cr^{3+} from 0.60 (M_{11}) to 0.40 (M_{27}) was also observed.

It is clear from these observations that amine–methanol–formic acid mobile phases have enormous analytical potential for achieving selective separations of heavy metal cations from their multi-component mixtures, because the nature of the added amine has a profound influence on the mobility of cations. Some separations of metal cations achieved experimentally using different mobile phases have been encapsulated in Table V.

Tables VI and VII summarize the effects of a variety of inorganic ions, surfactants, and phenolic impurities on the separation of mixtures of Cr^{6+} and Cr^{3+} . It is evident from Table VI that inorganic ions result in a slight change in the mobility of Cr^{6+} without influencing the mobility of Cr^{3+} . Thus, separation is always possible. Among the heavy metal ions, Hg^{2+} and Al^{3+} affected the mobility of Cr^{6+} , resulting in the formation of tailed spot. The presence of MoO_4^{2-} significantly reduced the R_F value of Cr^{6+} – from 0.85 to 0.71. Cr^{6+} also formed a tailed spot in the presence of Br^- . The results presented in Table VII clearly show that the effect of surfactants on the mobility of chromium ions is similar to that of inorganic ions. The mobility of Cr^{3+} was unaffected whereas that of Cr^{6+} changed slightly. The compact Cr^{6+} spot became elongated in the presence of surfactant, irrespective of the nature of the latter (anionic, cationic, or non-ionic). Despite this, separation of Cr^{6+} from Cr^{3+} was always possible. Phenolic impurities also affected the mobility of Cr^{6+} slightly whereas the mobility of Cr^{3+} remained unaffected. Separation of Cr^{6+} from Cr^{3+} was

Table V

Experimentally achieved separations of heavy metal cations on silica gel HPTLC plates developed with different mobile phases

Mobile phase	Separations
Formic acid	Hg ²⁺ or Ni ²⁺ (0.86)/Cd ²⁺ or Co ²⁺ (0.75)/Cr ⁶⁺ or Cr ³⁺ (0.71)/Pb ²⁺ (0.67)–VO ²⁺ (0.00)/Bi ³⁺ (0.15)
MeOH–DMA, 8:2	Cr ⁶⁺ (0.85)–Cr ³⁺ (0.05) Cr ⁶⁺ (0.85)–VO ²⁺ , Ag ⁺ , or Pb ²⁺ (0.00)/Cd ²⁺ or Ni ²⁺ (0.02)/Co ²⁺ , Tl ⁺ or Bi ³⁺ (0.05)
MeOH–formic acid, 8:2	Hg ²⁺ or Ni ²⁺ (0.81)/Cd ²⁺ (0.71)/Tl (0.75)/Cr ⁶⁺ or Cr ³⁺ (0.76)–Bi ³⁺ or Ag ⁺ (0.15)/VO ²⁺ (0.00)
Water–formic acid, 8:2	Hg ²⁺ , Pb ²⁺ , or Cd ²⁺ (0.93)/Ni ²⁺ or Co ²⁺ (0.86)/Cr ⁶⁺ or Cr ³⁺ (0.76)–Bi ³⁺ (0.21)/Ag ⁺ (0.10)/VO ²⁺ (0.00)
DMA–MeOH–formic acid, 8:8:2	Cr ⁶⁺ (0.90)–Ni ²⁺ (0.45)–Co ²⁺ (0.00) Cr ⁶⁺ or Cr ³⁺ (0.90)–Hg ²⁺ (0.60)/Cd ²⁺ –VO ²⁺ , Ag ⁺ , or Bi ³⁺ (0.00)
DMA–acetone–formic acid, 2:8:2	Hg ²⁺ (0.88)/Pb ²⁺ or Cd ²⁺ (0.85)/Tl ⁺ or Ni ²⁺ (0.78)–Cr ⁶⁺ or Cr ³⁺ (0.54)–Bi ³⁺ (0.15)/VO ²⁺ or Ag ⁺ (0.00)
DMA–acetone–formic acid, 10:8:2	Cr ⁶⁺ (0.90)–Ni ²⁺ (0.40)–Co ²⁺ (0.00) Cr ⁶⁺ or Cr ³⁺ (0.90)/Hg ²⁺ or Tl ⁺ (0.75)–VO ²⁺ , Ag ⁺ , Pb ²⁺ , or Bi ³⁺ (0.00)
DMA–water–formic acid, 10:8:2	Cr ⁶⁺ or Cr ³⁺ (0.87)–Hg ²⁺ or Cd ²⁺ (0.10)/VO ²⁺ , Ag ⁺ , Pb ²⁺ , Ni ²⁺ , or Co ²⁺ (0.00)
o-APH–MeOH–formic acid, 10:8:2	Ni ²⁺ (0.92)/Cr ⁶⁺ or Cd ²⁺ (0.85)–Hg ²⁺ (0.39)/Pb ²⁺ or Tl ⁺ (0.33)–Bi ³⁺ (0.16)/VO ²⁺ (0.05) or Ag ⁺ (0.02)

possible in the presence of all the phenols except *p*-aminophenol, which caused significant tailing of Cr⁶⁺ and hampered its separation from Cr³⁺.

The effect of pH on the mutual separation of Ni²⁺, Co²⁺, and Cr⁶⁺ is shown in Fig. 1. It is clear from this figure that the best separation of a mixture of Ni²⁺, Co²⁺, and Cr⁶⁺ was achieved in the pH range 2.5–3.5. At pH 1.5 the mobility of Ni²⁺ was reduced such that it could not be separated from Co²⁺, whereas it is well separated from Cr⁶⁺. Conversely, at pH 6.0 the *R_F* of Ni²⁺ was increased to such an extent that its separation from Cr⁶⁺ was not possible but its separation from Co²⁺ is always possible. At pH 8.0 the mobility of Ni²⁺ was further increased and the ion co-migrated with Cr⁶⁺.

The lowest detectable amounts (µg) and dilution limits of metal ions achieved on HPTLC plates developed with mobile phase M₇ were,

Table VI

Effect of inorganic impurities on the separation of Cr⁶⁺ and Cr³⁺ from their mixture on silica gel HPTLC plates developed with mobile phase M₇

Impurities	Separation (<i>R_F</i> value)	
	Cr ⁶⁺	Cr ³⁺
Ni ²⁺	0.83	0.05
Co ²⁺	0.83	0.05
Cd ²⁺	0.78	0.05
Zn ²⁺	0.79	0.05
Ag ⁺	0.84	0.05
Pb ²⁺	0.84	0.05
Tl ⁺	0.88	0.05
Bi ³⁺	0.78	0.05
Hg ²⁺	0.80T ^a	0.05
Al ³⁺	0.80T ^a	0.05
NaNO ₂	0.75	0.05
NaNO ₃	0.79	0.05
NaH ₂ PO ₄	0.79	0.05
KI	0.81	0.05
KIO ₃	0.90	0.05
KBr	0.67T ^a	0.05
K ₃ [Fe(CN) ₆]	0.81	0.05
K ₄ [Fe(CN) ₆]	0.86	0.05
NH ₄ SCN	0.81	0.05
(NH ₄) ₂ MoO ₄	0.71	0.05
Without impurity	0.85	0.05

^a Tailed spot ($R_L - R_T > 0.30$)

respectively, 0.028 and $1:3.5 \times 10^5$ for Ni²⁺, 0.11 and $1:9 \times 10^4$ for Co²⁺, 2.09 and $1:4.784 \times 10^3$ for Cu²⁺, and 0.08 and $1: 1.25 \times 10^5$ for Pb²⁺. It is clear from these data that the proposed method is a highly sensitive means of detection of the cations.

In addition to qualitative analysis, quantitative evaluation of the metal ions is often required to ascertain the levels of toxic metals in environmental samples. An approximate but simple method of quantitation is based on measurement of spot size by drawing the outline of the spot on a piece of tracing paper. Semi-quantitative determination of metal ions by measurement of spot area was therefore attempted. The linear relationship

Table VII

Effect of surfactants and phenolic impurities on the separation of Cr⁶⁺ and Cr³⁺ from their mixture on silica gel HPTLC plates developed with mobile phase M₇

Impurity	Retention (R _F value)	
	Cr ⁶⁺	Cr ³⁺
Sodium dodecyl sulphate (SDS)	0.671 ^a	0.05
<i>N</i> -Cetyl- <i>N,N,N</i> -trimethylammonium bromide (CTAB)	0.641 ^a	0.05
Polyoxyethylene dodecyl ether (Brij-35)	0.751 ^a	0.05
Polyoxyethylene (20) cetyl ether (Brij-58)	0.671 ^a	0.05
Polyoxyethylene (20) stearyl ether (Brij-78)	0.701 ^a	0.05
Polyoxyethylene (20) oleyl ether (Brij-98)	0.671 ^a	0.05
Polyoxyethylene (20) sorbitan monolaurate	0.75	0.05
Polyoxyethylene (4) sorbitan monopalmitate	0.671 ^a	0.05
Sorbitan monostearate (Span-60)	0.691 ^a	0.05
Orcinol	0.80	0.05
Resorcinol	0.76	0.05
Pyrocatechol	0.80	0.05
Phloroglucinol	0.83	0.05
Pyrogallol	0.81	0.05
<i>o</i> -Aminophenol	0.80	0.05
<i>m</i> -Aminophenol	0.86	0.05
<i>p</i> -Aminophenol	0.501 ^a	0.05
Without impurity	0.85	0.05

^a Tailed spot ($R_L - R_T > 0.30$)

obtained when the amount of the sample spotted was plotted against the area of the spot (Figs 2 and 3) followed the empirical equation $\zeta = k \times m$, where ζ is the area of the spot, m the amount of sample, and k a constant. Linearity is maintained up to 200 $\mu\text{g}/\text{spot}$ of Ni²⁺ and Cr⁶⁺. At higher concentrations a negative deviation from the linear law was observed for both metals. The standard curve constructed for semi-quantitative determination of Cr⁶⁺ (Fig. 2) was used to determine the amount of chromium present in a sample of industrial wastewater. Accuracy and precision were below $\pm 15\%$.

Semi-quantitative determination by visual comparison was used to estimate the amount of Cr⁶⁺ present in industrial wastewater. The industrial wastewater samples analysed (chrome wastewater) were found to have a chromium content in the range 5–75 $\mu\text{g L}^{-1}$.

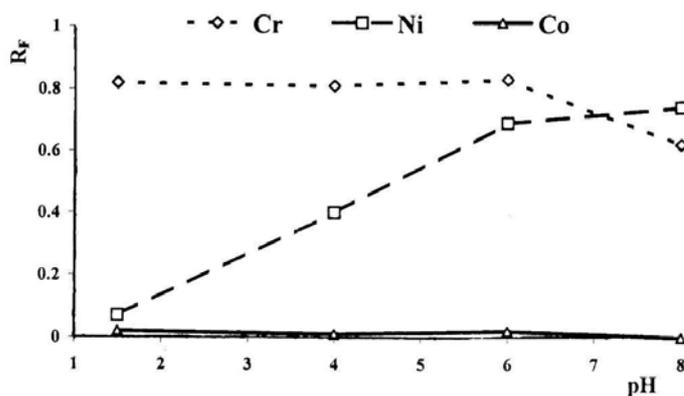


Fig. 1

Effect of pH on the separation of Cr^{6+} , Ni^{2+} , and Co^{2+} by use of in M_{13} as mobile phase on silica gel HPTLC plates

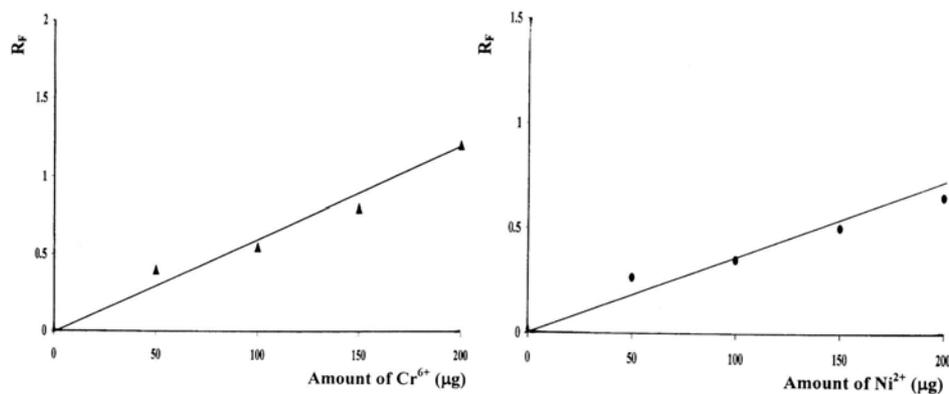


Fig. 2

Calibration plots for semiquantitative determination of Cr^{6+} and Ni^{2+} on silica gel HPTLC plates developed with mobile phase M_7

The proposed method was successfully applied for identification and separation of heavy metal ions in spiked industrial wastewater samples. The results presented in Table VIII clearly demonstrate the applicability of the method for identification of Cr^{6+} , Cr^{3+} , Ni^{2+} , and Co^{2+} in a variety of industrial wastewater samples.

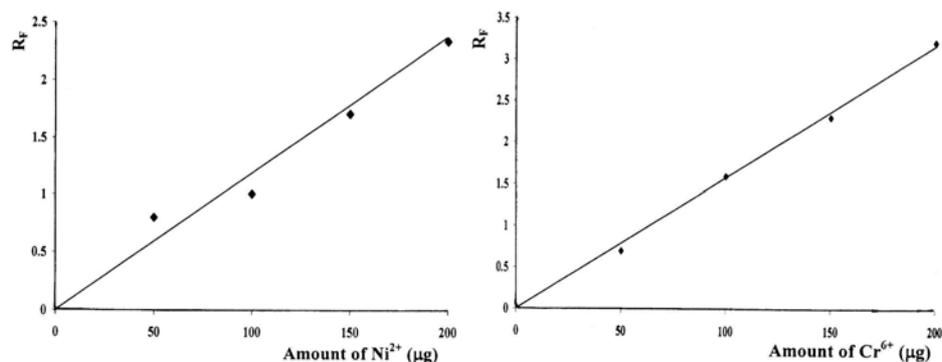


Fig. 3

Calibration plots for semiquantitative determination of Ni^{2+} and Cr^{6+} on silica gel HPTLC plates developed with mobile phase M_{13}

Table VIII

Application of proposed method (HPTLC on silica gel with mobile phases M_7 and M_{13}) for separation and identification of heavy metals from industrial wastewater

Mobile phase	Industrial waste	Separation (R_F)
M_7	Sample 1	Cr^{6+} (0.86)– Ni^{2+} (0.45)– Co^{2+} (0.00)
M_{13}	Sample 2	Cr^{6+} (0.87)– Ni^{2+} (0.41)– Co^{2+} (0.03)
M_{13}	Sample 3	Cr^{6+} (0.87)– Ni^{2+} (0.43)– Co^{2+} (0.00)

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REFERENCES

- [1] H. Siegel, Metal Ions in Biological Systems, Vols. I and II, Marcel Dekker, New York, USA, 1986
- [2] V. Venngopal and T. Luckey, Metal Toxicity of Metals and Metalloids, Plenum, New York, USA, 1987

- [3] M. Bukowska – Strzyżewska, W. Maniukewicz, G. Bazylak, and J. Masłowska, *J. Crystallogr. Spectrosc. Res.*, **21**, 157 (1991)
- [4] G. Schubert, V. Alan, J. Zirko – Babic, and S. Turina, *J. Planar Chromatogr.*, **11**, 460 (1998)
- [5] S. Przeszlakowski and M. Maliszewska, *Chem. Anal.*, **37**, 547 (1992)
- [6] S.D. Sharma and S.C. Sharma, *J. Chromatogr., A* **841**, 263 (1999)
- [7] M. Mizobuchi, Y. Horie, and K. Saito, *Nara-ken Eisei Kenkyusho Nenpo*, **26**, 56 (1991) (Published 1992); *Chem. Abstr.*, **118**, 20248q (1993)
- [8] Y. Zhou, G. Shao, and S. Mu, *Huanjing Huaxue*, **15**, 446 (1996); *Chem Abstr.*, **126**, 36660z (1997)
- [9] R.O. Crubellati and A.G. Ledesma, *Analyst*, **118**, 529 (1993)
- [10] S.A. Nabi, A. Gupta, and A. Sikarwar, *Ann. Chim.*, **89**, 419 (1999)
- [11] M. Ghannadi – Marageh, S.W. Husain, A.R. Khanchi, and S.J. Ahmady, *Sep. Sci. Technol.*, **34**, 219 (1999)
- [12] M.L. Marina, P. Andres, and J.C. Diez – Masa, *Chromatographia*, **35**, 621 (1993)
- [13] Y. Tomita, Y. Aoyama, and E. Aoyama, *Ibaraki Kogyo Koto Senmon Gakko Kenkyu Iho*, **36**, 65 (2001); *Chem. Abstr.*, **135**, 50686e (2001)
- [14] E.F. Hilder, M. Macka, and P.R. Haddad, *Analyst*, **123**, 2865 (1998)
- [15] K.S. Lee, H.S. Choi, and Y.S. Kim, *Anal. Sci. Technol.*, **3**, 419 (1990)
- [16] V. Ostruba, J. Pivnicka, and V. Kanicky, *Collect. Czech. Chem. Commun.*, **65**, 1865 (2000)
- [17] L. Zheng and S. Wang, *Yejin Fenxi*, **16**, 49 (1996); *Chem. Abstr.*, **126**, 165878a (1997)
- [18] K.K. Paliwal, D.K. Gorji, S. Kumar, N. Naulakha, A.K. Goswami, and D.N. Purohit, *Asian J. Chem.*, **13**, 299 (2001)
- [19] M.C. Mehra and C.A. Lucy, *Orient. J. Chem.*, **12**, 231 (1996)
- [20] Z. Chen, R. Naidu, and A. Subramanian, *J. Chromatogr. A*, **927**, 219 (2001)
- [21] W. Shi and T. Hu, *Huaxue Fence*, **34**, 447 (1998); *Chem. Abstr.*, **130**, 85763b (1999)
- [22] Z.G. Xu, X.H. Wen, and Q.Z. Wu, *Chin. Chem. Lett.*, **12**, 635 (2001)
- [23] Z. Shen, Y. Li, and Y. Li, *Guangdong Weiliang Yuansu Kexue*, **5**, 69 (1998); *Chem. Abstr.*, **130**, 147940t (1999)

- [24] D. Qin, Z. Lin, and J. Wang, *Huaxue Fenxi Jiliang*, **9**, 17 (2002); *Chem. Abstr.*, **135**, 24284c (2001)
- [25] A. Gaspar, C. Songor, and J. Posta, *Fresenius J. Anal. Chem.*, **363**, 480 (1999)
- [26] S.T. Sauerhoff, Z.A. Grosser, and G.R. Carrick, *At. Spectrosc.*, **17**, 225 (1996)
- [27] T.W. Lin and S.D. Huang, *Anal. Chem.*, **73**, 4319 (2001)
- [28] E.S. Zolotovitskaya, L.V. Glushkova, Z.V. Shititelman, and A.B. Blank, *Khim. Tekhnol. Vody*, **15**, 255 (1993)
- [29] T.M. Bahrainwala and Z.R. Tural, *J. Radioanal. Nucl. Chem.*, **214**, 199 (1996)
- [30] H. Rausch and T. Braun, *Fullerene Sci. Technol.*, **5**, 407 (1997)
- [31] B. Demirata, I. Tar, H. Filik, and H. Afsar, *Fresenius J. Anal. Chem.*, **356**, 375 (1996)
- [32] M. Sikovec, M. Franko, and M. Novic, *AIP Conf. Proc.*, **463**, 682 (1999)
- [33] M. Sikovec, M. Franko, M. Novic, and M. Veber, *J. Chromatogr. A*, **920**, 119 (2001)
- [34] G.S. Sattarov and A.A. Kist, *Czech. J. Phys.*, **49**, 303 (1999)
- [35] T. Etoh, M. Yamada, and M. Matsubara, *Kankyo Kagaku*, **3**, 398 (1993); *Chem. Abstr.*, **119**, 102860y (1993)
- [36] J.C. Yu, X.-J. Wu, and Z. Chen., *Anal. Chim. Acta*, **436**, 59 (2001)
- [37] J. Guan, Y. Shi, B. Gao, and Y. Liu, *Yuanzineng Kexue Jishu*, **25**, 8 (1991); *Chem. Abstr.*, **118**, 15539m (1993)
- [38] H. Chen, *Huaxue Shijie*, **41**, 488 (2000); *Chem. Abstr.*, **134**, 36428v (2001)
- [39] V. Stresko, J. Polakovicova, and A. Celkova, *Chem. Pap.*, **55**, 100 (2001)
- [40] V. Ostruba, J. Pivnicka, and V. Kanicky, *Proc. – Semin. At. Spectrochem.*, **14**, 247 (1998)
- [41] Y.-L. Chang and S.J. Jiang, *J. Anal. At. Spectrom.*, **16**, 858 (2001)
- [42] Y. Martinez – Bravo, A.F. Roig – Navaro, F.J. Lopez, and F. Hernandez, *J. Chromatogr., A* **926**, 256 (2001)
- [43] H. Guerleyuek and D. Wallschlaeger, *J. Anal. At. Spectrom.*, **16**, 926 (2001)
- [44] A. Mohammad, M. Ajmal, S. Anwar, and E. Iraqi, *J. Planar Chromatogr.*, **9**, 318 (1996)
- [45] N. Fatima and A. Mohammad, *Sep. Sci. Technol.*, **19**, 429 (1994)

- [46] M. Ajmal, A. Mohammad, and N. Fatima, *Microchem. J.*, **37**, 314 (1988)
- [47] A. Mohammad and N. Fatima, *Chromatographia*, **22**, 109 (1986)
- [48] A. Mohammad and M.A.M. Khan, *J. Planar Chromatogr.*, **8**, 134 (1995)
- [49] A. Mohammad, M. Ajmal, and S. Anwar, *Acta Chromatogr.*, **9**, 113 (1999)
- [50] I. Balzer, C. Bogdanic, and C. Pepeljnjak, *J. Assoc. Anal. Chem.*, **61**, 584 (1978)
- [51] M. Ajmal, A. Mohammad, N. Fatima, and J. Ahmad, *J. Planar Chromatogr.*, **1**, 329 (1988)