

Thin-Layer Chromatographic Specification and Separation of Cu^{1+} , Cu^{2+} , Ni^{2+} , and Co^{2+} Cations

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Abstract

The $\text{M}(\text{PyDTC})_2$ (M: Cu, Co, or Ni) and CuPyDTC complexes, prepared by reactions of ammonium pyrrolidinedithiocarbamate with metal nitrates, are examined for qualitative analysis, speciation, and mutual separation using thin-layer chromatography systems. These complexes and their mixtures are spotted to the activated and non-activated thin layers of silica gel 60GF₂₅₄ (Si-60GF₂₅₄) with a 250- μm thickness. Toluene–dichloromethane mixtures (4:1, 1:1, 1:4 v/v) are used as mobile phases for running of the complexes. All of these chromatographic systems are successfully used for speciation of Cu^{2+} and Cu^{1+} cations. The best analytical separation for the qualitative analysis of corresponding metal cations and mutual separation of components in $\text{M}(\text{PyDTC})_2$ and CuPyDTC complexes are obtained when using pure toluene–dichloromethane (1:1 v/v) on the activated layer. This study shows that it is possible to qualitatively analyze and satisfactorily separate a mixture of Cu^{1+} , Cu^{2+} , Ni^{2+} , and Co^{2+} cations on cited chromatographic systems. These results may be also said for the adaptability or validity on column chromatography.

Introduction

The species are ions with different oxidation states, isotopes of an element, and different forms of a molecule. The speciation is the qualitative and quantitative analyses of the species in a sample. In the speciation of an element, it is carried out to determine the distribution of identified chemical species of the element in a sample (1). High-performance liquid chromatography, gas chromatography, and capillary electrophoresis have been the predominant methods for elemental speciation (2–5). Extraction, spectroscopic, and electrochemical analysis methods have been used for elemental and molecular speciation (6,7).

Chromatography is one of the most important analytical techniques used to separate components of mixtures. Thin-layer chromatography (TLC) is a quick, easy, and simple separation

method extensively used for organic species but rarely used for inorganic cations. Although TLC is not common for inorganic samples, some researchers have recently revealed that its utility is also valid for speciation (8–13), qualitative and quantitative analyses, and mutual separations of inorganic cations and anions (14–29). The quantitative analysis of cited metal species in rock samples (28) and the identification, estimation, and separation of toxic metals in environmental samples (21,22,24) are performed frequently.

The unidentate or polydentate ligands that contain hard N, S or O, S donor atoms are used extensively in the separation of transition metal cations because these ligands form electrically neutral and coordinatively saturated stable complexes with cations at high formation rates. Because of these properties, the molecules of $\text{M}(\text{PyDTC})_2$ and CuPyDTC complexes cannot interact chemically with the stationary and the mobile phases in TLC systems because of their thermodynamic properties. The formation rates of complexes are very high, which also means saving time. These complexes are colored and can be easily visualized in the chromatograms (27–32).

On TLC applications, three different procedures can be used to prepare the complexes of these cations. In the first procedure, it is performed by injections of analyte and ligand solutions at the same origin onto the layer. In this procedure, two successive injections might cause punching of the layers. Therefore, this procedure has a disadvantage because of two successive injections. In the second procedure, the solutions of standard cations and sample are spotted onto the layer individually. Then, ligand solution is added to the mobile phase in the tank. Finally, chromatographic development is performed. This procedure is simpler and more rapid than the first procedure. Furthermore, it is more excellent than the first procedure because it has no disadvantage like the first procedure. However, this procedure may not be appropriate for every analyte's concentration because it is not possible to preconcentrate the analyte. The third procedure is based on using TLC following the solvent extraction and the complexation of these cations in aqueous solution. This procedure has advantages such as preconcentration and cation selectivity to prevent chromatographic tailing and scattering of retention factor (R_f) values (28).

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The chromatographic behaviors of $M(\text{DEDTC})_2$ and $M(\text{PyDTC})_2$ (M : Cu or Co) complexes on various activated and non-activated thin layers of silica gel 60GF₂₅₄ (Si-60GF₂₅₄) and flax-calcinated diatomaceous earth modified with acid (FCDE-I) using two different mobile phases are discussed in the context of variation of the stationary and mobile phase properties, retention mechanism, and the nature of the metal, ligand, and complexes (30,31).

Additionally, crystal field theory (CFT) is an appropriate tool to explain the difference between the R_f values of complexes and their chromatographic behavior. The chromatographic behaviors and parameters of $M(\text{DEDTC})_2$ (M = Cu, Co, or Ni) and $M(\text{PyDTC})_2$ (M = Cu or Co) complexes with four coordination numbers are discussed in the context of the TLC and CFT linkage (32).

On analytical applications, it is a situation that requires a solution for the speciation, qualitative, and quantitative analyses of elements in a sample because of their similar physical and chemical properties. Therefore, in this study, mixtures of $M(\text{PyDTC})_2$ and CuPyDTC complexes were run on activated and non-activated thin layers of Si-60GF₂₅₄ using three different mobile phases. The separability parameter (r) and R_f of these complexes were determined and are discussed in the context of the complexation and TLC applications, the effect of stationary phase activation, mobile phase polarity, retention mechanism, linkage between TLC and CFT, Cu(I) and Cu(II) speciation, and expected resolution on column chromatography (CC) of the mutual separation of Cu^{1+} , Cu^{2+} , Ni^{2+} , and Co^{2+} cations on TLC.

Experimental

Chemicals, reagents, and materials

Si-60GF₂₅₄, chloroform, toluene, dichloromethane, NH_4PyDTC , $\text{Cu}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2$, $\text{Co}(\text{NO}_3)_2$, and CuNO_3 were purchased from Merck (Darmstadt, Germany).

$\text{Cu}(\text{PyDTC})_2$, CuPyDTC , $\text{Ni}(\text{PyDTC})_2$, and $\text{Co}(\text{PyDTC})_2$ complexes were prepared by the reactions of NH_4PyDTC with metal nitrates. Toluene–dichloromethane mixtures (1:4, 1:1, 4:1 v/v) were used as the mobile phases. The layers of Si-60GF₂₅₄ were prepared using Loughborough-Griffin & George TLC unikit (Leicestershire, England). All the chemicals were analytical-grade.

Synthesis of $M(\text{PyDTC})_2$ and CuPyDTC complexes

0.1 mol/L solutions of metal nitrates ($M(\text{NO}_3)_2$, and CuNO_3) at pH 5.5–6.0 (adjusted by acetic acid-sodium acetate buffer) were prepared. From these solutions, a 1.0-mL aliquot was poured into a beaker, and 1.0 mL (0.5 mL for only CuNO_3) of 0.1 mol/L NH_4PyDTC solution was added to it and then shaken. Four milliliters of pure chloroform was added to the beaker and was shaken for 1 min. This mixture was transferred into a separatory funnel and shaken. The phases were allowed to separate for 5 min. The aqueous phase was separated from the chloroform phase and discarded. Subsequently, the chloroform phase containing the complex was dried by treating with anhydrous Na_2SO_4 . The dried phase was used as standard sample for TLC

applications. The same procedure was also applied to prepare each of the standard complex solution. In this procedure, the chloroform was used as the organic phase because of its complex solubility and solvent volatility advantages (28).

Preparation of thin-layer plates

Slurries of Si-60GF₂₅₄ in water (1:2 w/v) were spread onto clean glass plates (7.5 × 15 cm) with a thickness of 250 μm using a spreader kit. Non-activated plates were obtained by keeping them in a closed oven at 25°C for 12 h. After the non-activated plates were taken from the oven, they were immediately used for TLC. Plates were activated by heating them in an oven at 110°C for 2 h.

TLC applications

Two microliter aliquots from each of the complex solutions and their mixtures were spotted with micropipettes on the starting line, which was 2.0 cm from the bottom of the non-activated plates. The original spots on the layers were dried at room temperature for 3 min. A pencil line was marked 5.5 cm above the starting line of each plate. Three developing chambers with 10 × 50 × 20 cm dimensions were used for running. Sixty milliliters of the toluene–dichloromethane mixtures (4:1, 1:1, 1:4 v/v) were individually poured into the each chamber. The lids of the chambers were closed, and the chambers were allowed to stand for 15 min to ensure that saturation of the air in each chamber with solvent vapor occurred. Then, the non-activated plates containing the spotted samples were carefully immersed in the developing chambers. When the solvent fronts reached 5.5 cm above the starting line of each plate, the plates were removed and dried. The migration distance of the solvent (Z_f) and of each spot (Z_x) were measured. R_f (from $R_f = Z_x/Z_f$) was calculated (27–30). The same procedure was also applied to activated layers. R_f values of these complexes are given in Table I.

Table I. R_f Data of $M(\text{PyDTC})_2$ (M : Cu, Ni, Co) and CuPyDTC Complexes*

Mobile phase	Complex	$R_f \pm \text{SD}$	
		Non-activated layer	Activated layer
Toluene–dichloromethane (4:1 v/v)	$\text{Cu}(\text{PyDTC})_2$	0.50 ± 0.01	0.40 ± 0.01
	$\text{Ni}(\text{PyDTC})_2$	0.37 ± 0.01	0.30 ± 0.01
	CuPyDTC	0.37 ± 0.01	0.28 ± 0.01
	$\text{Co}(\text{PyDTC})_2$	0.27 ± 0.01	0.14 ± 0.01
Toluene–dichloromethane (1:1 v/v)	$\text{Cu}(\text{PyDTC})_2$	0.59 ± 0.01	0.52 ± 0.01
	$\text{Ni}(\text{PyDTC})_2$	0.55 ± 0.01	0.44 ± 0.01
	CuPyDTC	0.45 ± 0.01	0.34 ± 0.01
	$\text{Co}(\text{PyDTC})_2$	0.35 ± 0.01	0.27 ± 0.01
Toluene–dichloromethane (1:4 v/v)	$\text{Cu}(\text{PyDTC})_2$	0.63 ± 0.01	–
	$\text{Ni}(\text{PyDTC})_2$	0.61 ± 0.01	–
	CuPyDTC	0.50 ± 0.01	–
	$\text{Co}(\text{PyDTC})_2$	0.49 ± 0.01	–

* Number of repeated runs = 9. %RSD = 2.3.

Result and Discussion

Samples from different natural and synthetic sources contain various compounds as major and minor components. Many of these compounds may have very similar physical and chemical properties. In such cases, sample components generate mutual interference spectra in qualitative, quantitative, and structural analyses. Therefore, the challenger Cu^{1+} , Cu^{2+} , Ni^{2+} , and Co^{2+} in the qualitative and quantitative analysis require satisfactory separation. In this study, the qualitative analysis, the speciation, and mutual separability of Cu^{1+} , Cu^{2+} , Co^{2+} , and Ni^{2+} cations and their complexes have been examined using TLC following the solvent extraction and the complexation of these cations with PyDTC ligand.

The complexation and TLC applications

In this study, the third procedure (28) was selected because of advantages cited. On complexation reactions, PyDTC ligand was selected as the limiting reagent in complexation reactions to prevent the extraction into the chloroform phase of ligand. As for the chromatographic applications, although there were the spots of standard complexes on the chromatograms, the spot of the ligand was not on the chromatograms. These qualitative indicators show that the standards were successfully prepared as pure.

The effect of stationary phase activation

As seen from Table I, the R_f values of the $\text{M}(\text{PyDTC})_2$ and $\text{Cu}(\text{PyDTC})$ complexes increase when the activated Si-60GF_{254} layer is replaced by the non-activated Si-60GF_{254} layer when using the same mobile phase. The surface of the non-activated layer was covered by water molecules. The effectiveness of the Si-OH groups on a non-activated layer was lower than on an activated layer because the Si-OH groups were masked by water molecules. Thus, the activity of a non-activated layer was also lower. In this context, it is concluded that the increase of the R_f values stemmed from the weakening of the interactions responsible for the retention of complex components because of the decrease in the activity of the layer. When this result is compared with previous results (30) in the context of stationary phase activation, it can be suggested that they were same.

The effect of mobile phase polarity

As seen in Table I, the R_f values of the $\text{M}(\text{PyDTC})_2$ and $\text{Cu}(\text{PyDTC})$ complexes decreased when the toluene-dichloromethane mixture (1:4 v/v) was replaced by the toluene-dichloromethane mixture (4:1 v/v) when using the same stationary phase. In this context, the decrease in the R_f values stems from decreasing the mobile phase polarity. This can be explained in the following way: the polarity of dichloromethane is higher than that of toluene because of the dielectric constant. Consequently, when the percentage of dichloromethane decreases, the polarity of the solvent system, the interaction of the complex molecules with the mobile phase, and R_f also decrease.

The effect of retention mechanism

In a chromatographic application, the retention mechanism depends on the liquid pre-adsorbed on the layer's surface, the

nature of the mobile phase, and the properties of the sample components (30). In this context, the adsorbed water on the surface of non-activated Si-60GF_{254} layers was not miscible with the toluene-dichloromethane mixtures (4:1, 1:1, 1:4 v/v), and the separation of complex molecules was carried out via Nerst distribution equilibriums in liquid-liquid chromatography (LLC). In contrast, the surface of the activated Si-60GF_{254} layer was not covered by water, and adsorption equilibriums were established instead of distribution equilibriums between the stationary and mobile phases in solid-liquid chromatography (SLC). Therefore, the retention mechanisms of the $\text{M}(\text{PyDTC})_2$ and $\text{Cu}(\text{PyDTC})$ complexes are different on activated and non-activated layers.

As seen in Table I, the R_f values of $\text{M}(\text{PyDTC})_2$ and $\text{Cu}(\text{PyDTC})$ complexes changed when the activated layer was replaced by the non-activated layer and when using toluene-dichloromethane mixture (4:1 v/v) as the mobile phase. The change in the R_f values is because of differences in the retention mechanism. This chromatographic behavior pattern is also valid when using toluene-dichloromethane mixtures (1:1, 1:4 v/v) as the mobile phase. This result is compared with previous results in literature (30) in the context of retention mechanism, and it can be suggested that they were same.

Linkage between TLC and crystal field theory

As seen in Table I, the R_f values of $\text{M}(\text{PyDTC})_2$ complexes shows significant difference when the ligands, coordination numbers, geometries of molecules, and the mobile and stationary phases are the same. This results from the difference of crystal field stabilization energies, crystal field splitting energies in this context of the effect of electronic configurations of central metal atoms.

As seen in Table I, the R_f value of $\text{Cu}(\text{PyDTC})_2$ complex decreased when toluene-dichloromethane (1:4 v/v) was replaced by the toluene-dichloromethane (4:1 v/v) as the mobile phase, when stationary phase, coordination number, geometry of molecule, and metal atom of complex were kept the same. This results from the variation of crystal field stabilization energies, crystal field splitting energies in this context of the difference in polarity of toluene-dichloromethane (1:4 v/v) and toluene-dichloromethane (4:1 v/v) as CFT. The result was also valid for other $\text{M}(\text{PyDTC})_2$ complexes in all the other chromatographic systems. When this result is compared with previous results in literature (32) in the context of variation of the mobile phase properties, it can be suggested that they were same.

As seen in Table I, the R_f value of $\text{Cu}(\text{PyDTC})_2$ complex decreased when the non-activated layer was replaced by the activated layer as the stationary phase and when mobile phase, coordination number, geometry, and metal atom of complex were the same. This results from the variation of crystal field stabilization energies, crystal field splitting energies in this context of the difference in the activity of non-activated layer and activated layer as CFT. The result was also valid for other $\text{M}(\text{PyDTC})_2$ complexes in all the other chromatographic systems. When this result is compared with previous results in literature (32) in the context of stationary phase activation, it can be suggested that they were same.

Cu(I) and Cu(II) speciation

Cu^{2+} and Cu^{1+} cations are elemental species of copper atom

only because of their different charges or electronic configurations when $\text{Cu}(\text{PyDTC})_2$ and CuPyDTC complexes prepared from these cations are molecular species. Although the nucleus charges, radii, and charges density of Cu^{2+} and Cu^{1+} ions in the aqueous solutions are very similar, their d^9 and d^{10} electronic configuration leads to different physical and chemical properties of complex molecules. As seen in Table I, the R_f values of $\text{Cu}(\text{PyDTC})_2$ and CuPyDTC complexes show significant difference when the ligands and the mobile and stationary phases are the same. This results from the variation of crystal field stabilization energies, crystal field splitting energies in this context of the difference in coordination numbers, geometries of molecules, and charges of metal atoms of complexes as CFT. According to the data in Table I, all of the obtained results cited are also valid for the chromatographic behaviors of $\text{Cu}(\text{PyDTC})_2$ and CuPyDTC complexes in the context of effect of the stationary phase activation and mobile phases polarity, retention mechanism, and electronic configurations to their R_f values.

Separability on TLC and expected resolution on CC

On the TLC application, the resolution is relative to separability parameter (r) in the context of differences between R_f values of complex couple. In addition, the separations by TLC are a precursor for the applications on CC. The retention factors and separability of components in the sample using TLC is related to the elution time and separability on CC. In this context, the r parameter is quantitatively the measurement of expected resolution on CC in this context of the separability of components on TLC. It is calculated using the following (33):

$$r = \frac{a}{b + 0.1a} \quad \text{Eq. 1}$$

where a is R_f value of the fast moving substance A, and b is R_f value of the slow moving substance B on the chromatograms, respectively. The separability parameter (r) is a dimensionless number, which indicates whether separation on CC is carried out or not. When mobile and stationary phases are the same for CC and TLC systems, it is said that the separation of two components may be possible when $r > 1$ (33). In this study, r values of $\text{M}(\text{PyDTC})_2$ and CuPyDTC complex couples obtained on TLC applications are given in Table II.

As seen in Table II, r parameters of $\text{Cu}(\text{PyDTC})_2$ – CuPyDTC , $\text{Cu}(\text{PyDTC})_2$ – $\text{Co}(\text{PyDTC})_2$, and $\text{Ni}(\text{PyDTC})_2$ – $\text{Co}(\text{PyDTC})_2$ complex couples were bigger than 1.000 on all chromatographic systems. Therefore, it can be said that these complex couples were successfully separated on all TLC systems because of $r > 1.000$. In addition, the best analytical separations of $\text{Cu}(\text{PyDTC})_2$ – $\text{Co}(\text{PyDTC})_2$ and $\text{Ni}(\text{PyDTC})_2$ – $\text{Co}(\text{PyDTC})_2$ complex couple were obtained when using toluene–dichloromethane (4:1 v/v) as mobile phase on activated layer. The best analytical separation of $\text{Cu}(\text{PyDTC})_2$ – CuPyDTC complex couple was obtained when using toluene–dichloromethane (1:1 v/v) as mobile phase on the activated layer.

As seen in Table II, r parameters of $\text{Cu}(\text{PyDTC})_2$ – $\text{Ni}(\text{PyDTC})_2$, $\text{Ni}(\text{PyDTC})_2$ – CuPyDTC , and CuPyDTC – $\text{Co}(\text{PyDTC})_2$ were smaller than 1.00 on some chromatographic systems. On these chromatographic systems, the ratios of toluene–dichloromethane mixtures as the mobile phase were (1:1 and 1:4 v/v) for $\text{Cu}(\text{PyDTC})_2$ – $\text{Ni}(\text{PyDTC})_2$, (1:1 v/v) for $\text{Ni}(\text{PyDTC})_2$ – CuPyDTC , and (1:4 v/v) for CuPyDTC – $\text{Co}(\text{PyDTC})_2$ complexes when the stationary phase was non-activated phase. In addition, r value for the $\text{Cu}(\text{PyDTC})_2$ – $\text{Ni}(\text{PyDTC})_2$ complex couple was smaller than 1.0 when using toluene–dichloromethane (4:1 v/v) as mobile phase on the activated layer. As a result, although these chromatographic systems were not successfully separated complexes couples cited, other chromatographic systems successfully separated complex couples. The mutual separation for all of complexes was obtained when using toluene–dichloromethane (1:1 v/v) as mobile phases on the activated layer. As a result, the best analytical separation is carried out on this chromatographic system. It can be said that this result is also valid for CC in the context of r parameters of complex couples.

Conclusions

This study was carried out on mixtures of $\text{M}(\text{PyDTC})_2$ and CuPyDTC complexes in order to understand the effects of stationary phase activation, mobile phase polarity, and retention mechanism, as well as the nature of the metal, the ligand, and the complexes on the chromatographic parameters (e.g., R_f and r). This also reveals the linkage between the TLC and the CFT and determines the separability of cation mixtures using TLC following the complexation of the cations with PyDTC ligand. In light of this study, the following conclusions can be made:

This study shows that it is possible to qualitatively analyze and satisfactorily separate a mixture Cu^{1+} , Cu^{2+} , Ni^{2+} , and Co^{2+} cations using TLC following the complexing of the cations with PyDTC ligand. The best analytical separation for $\text{M}(\text{PyDTC})_2$ and CuPyDTC complex mixtures is obtained when using toluene–dichloromethane mixture (1:1 v/v) on an activated layer.

The R_f values of the $\text{M}(\text{PyDTC})_2$ and CuPyDTC complexes increase when the acti-

Table II. Expected Resolution Data on CC of $\text{M}(\text{PyDTC})_2$ and CuPyDTC Complex Couples

Complex	Non-activated layer			Activated layer		
	r^*	r^\dagger	r^\ddagger	r^*	r^\dagger	r^\ddagger
$\text{Cu}(\text{PyDTC})_2$ – $\text{Co}(\text{PyDTC})_2$	1.563	1.443	1.139	2.222	1.615	–
$\text{Cu}(\text{PyDTC})_2$ – $\text{Ni}(\text{PyDTC})_2$	1.190	0.969	0.936	1.177	1.057	–
$\text{Cu}(\text{PyDTC})_2$ – CuPyDTC	1.191	1.159	1.119	1.250	1.327	–
$\text{Ni}(\text{PyDTC})_2$ – $\text{Co}(\text{PyDTC})_2$	1.205	1.358	1.107	1.765	1.401	–
$\text{Ni}(\text{PyDTC})_2$ – CuPyDTC	0.909	1.089	1.087	0.968	1.146	–
CuPyDTC – $\text{Co}(\text{PyDTC})_2$	1.205	1.139	0.926	1.667	1.118	–

* Toluene–dichloromethane (4:1 v/v) mixtures. Number of repeated runs = 9.

† Toluene–dichloromethane (1:1 v/v) mixtures. Number of repeated runs = 9.

‡ Toluene–dichloromethane (1:4 v/v) mixtures. Number of repeated runs = 9.

vated Si-60GF₂₅₄ layer is replaced by the non-activated Si-60GF₂₅₄ layer when using the same mobile phase. The R_f values increase because the interactions responsible for the retention of the complex molecules weaken due to the decrease in activity of the layer.

The R_f values of the M(PyDTC)₂ and CuPyDTC complexes decreased when the toluene–dichloromethane mixture (1:4 v/v) was replaced by the toluene–dichloromethane mixture (4:1 v/v) while using the same stationary phase. In this context, the decrease in the R_f values stems from decreasing the mobile phase polarity because of the decrease on the percentage of dichloromethane.

The separation of M(PyDTC)₂ and CuPyDTC molecules on the non-activated layer was carried out via Nerst distribution equilibria in LLC. However, because there is no water covering the activated layer, adsorption equilibria are established instead of distribution equilibria between stationary and mobile phases in SLC.

The R_f values of Cu(PyDTC)₂ and CuPyDTC complexes show significant difference when the ligands and the mobile and stationary phases are the same because of the variation of crystal field stabilization energies, crystal field splitting energies in this context of the difference in coordination numbers, geometries of molecules, and charges of metal atoms of complexes as CFT.

The mutual separation for all of complexes was obtained when using toluene–dichloromethane (1:1 v/v) as mobile phases on the activated layer. It can be said that this result is also valid for CC in context of r parameters of complex couples.

References

- D.M. Templeton, F. Ariese, R. Cornelis, L.G. Danielson, H. Muntau, H.P.V. Leeuwen, and R.O. Ski. Guidelines for terms related to chemical speciation and fractionation of elements: definitions, structural aspects and methodological approaches (IUPAC recommendations 2000). *J. Pure Applied Chem.* **72**: 1453–1470 (2000).
- R. Rubio, A. Padro, and G. Rauret. LC-HG-QCAAS versus LC-HG-ICP/OES in arsenic speciation. *Fresenius J. Anal. Chem.* **331**: 351 (1995).
- J. Szpunar, S. McShyehy, K. Polec, V. Vacchina, S. Mounicou, I. Rodriguez, and R. Lobinski. Determination of total antimony and antimony(V) by inductively coupled plasma mass spectrometry after selective separation of antimony(III) by solvent extraction with N-benzoyl-N-phenylhydroxylamine. *Spectrochim. Acta B* **55B**: 779 (2000).
- B. Sun, M. Macka, and R.R. Haddad. Speciation of tin, lead, mercury, arsenic and selenium compounds by capillary electrophoresis. *Int. J. Environ. Anal. Chem.* **81**: 161 (2001).
- K. Pyrzynska. Analysis of selenium species by capillary electrophoresis. *Talanta* **55**: 657 (2001).
- W. Lund. Speciation analysis—why and how? *Fresenius J. Anal. Chem.* **337**: 557–564 (1990).
- A. Ringbom. *Complexation Methods in Analytical Chemistry*, Interscience Publishers, New York/London (1963).
- A. Mohammad and Y. Sirwal. Novel mobile phase for separation of Cr⁶⁺ from Cr³⁺ and associated heavy metal cations by high-performance thin-layer chromatography. *Acta Chromatogr.* **13**: 117–134 (2003).
- A. Mohammad and N. Fatima. TLC separation of microgram quantities of iron (II) from milligram quantities of iron (III). *J. Liquid Chromatogr.* **10**(7): 1349–1358 (1987).
- A. Mohammad and V. Agrawal. Thin-layer chromatography of anions separation of coexisting hexacyanoferrate (II), hexacyanoferrate (III) and thiocyanate with a new mobile phase. *J. Planar Chromatogr. Modern TLC* **14**(5): 371–377 (2001).
- A. Mohammad and V. Agrawal. Micelles activated planar chromatographic separation of coexisting iodide, iodate and periodate ions. *Quim. Anal.* **20**(4): 251–254 (2002).
- A. Mohammad, J. Chahar, E. Iraqi, and V. Agrawal. A new chromatographic-iodometric method for the separation and determination of iodide and its oxyanions. *J. Planar Chromatogr. Modern TLC* **13**(1): 12–15 (2000).
- A. Mohammad and S. Tiwari. Chromatography of anionic pollutants on silica-gel layers selective microgram separation of NO₂⁻ and IO₃⁻. *Microchem. J.* **44**(1): 39–48 (1991).
- S. Ergül. Modification of diatomaceous earth and thin layer chromatographic applications. Ph.D Thesis, University of Balikesir, Balikesir, Turkey, 2003.
- A. Mohammad and H. Shahab. Use of micellar anionic surfactant solutions with added carbohydrates as mobile phases in thin-layer chromatography of heavy metal cations. Separation of mixtures of aluminium (III), manganese (II), and chromium (VI). *Acta Chromatogr.* **15**: 192–205 (2005).
- A. Mohammad, V. Agrawal, and S. Hena. Adsorption studies of metal cations on a silica static flat bed using anionic micellar mobile-phase systems containing carboxylic acids: separation of co-existing iron (III), copper (II) and nickel (II) cations. *Adsorp. Sci. Technol.* **22**: 89–105 (2004).
- A. Mohammad and N. Jabeen. Soil thin-layer chromatography of heavy metal cations with surfactant-modified mobile phases: mutual separation of zinc (II), cadmium (II), and mercury (II). *J. Planar Chromatogr. Modern TLC* **16**: 137–143 (2003).
- A. Mohammad and V. Agrawal. Use of cationic micellar mobile phases in normal-phase TLC for enhanced selectivity in the separation of transition metal ions: simultaneous separation of mixtures of zinc, nickel, mercury, and cadmium or manganese cations. *Acta Chromatogr.* **12**: 177–188 (2002).
- A. Mohammad, E. Iraqi, and I.A. Khan. Use of nonionic poly(ethylene glycol) p-isooctyl-phenyl ether (triton x-100) surfactant mobile phases in the thin-layer chromatography of heavy-metal cations. *J. Chromatogr. Sci.* **40**: 162–169 (2002).
- A. Mohammad and V. Agrawal. Applicability of surfactant-modified mobile phases in thin-layer chromatography of transition metal cations: identification and separation of zinc (II), cadmium (II), and mercury (II) in their mixtures. *J. Planar Chromatogr. Modern TLC* **13**: 210–216 (2000).
- A. Mohammad. Thin-layer chromatographic methods for the identification, estimation, and separation of toxic metals in environmental samples. *J. Planar Chromatogr. Modern TLC* **10**: 48–54 (1997).
- A. Mohammad and M. Khan. Thin-layer chromatographic-separation of zinc from Cd (II), Hg (II) and Ni (II) in environmental-samples using impregnated silica layers. *J. Chromatogr. Sci.* **33**: 531–535 (1995).
- M. Ajmal, A. Mohammad, and N. Fatima. Sodium molybdate impregnated silica-gel layers as stationary phase for chromatographic-separation of metal-ions. *J. Ind. Chem. Soc.* **66**: 425–426 (1989).
- M. Ajmal, A. Mohammad, N. Fatima, and A.H. Khan. Determination of micro-quantities of mercury (II) with preliminary thin-layer chromatographic-separation from mercury (I), lead (II), nickel (II), and copper (II) on acid-treated silica-gel layers - recovery of mercury (II) from river waters and industrial wastewater. *Microchem. J.* **39**: 361–371 (1989).
- M. Ajmal, A. Mohammad, and N. Fatima. Separation of microgram quantities of cadmium (II) from milligram quantities of zinc (II) and of copper (II) from nickel (II) and cobalt (II) with aqueous sodium formate halogen anion systems. *Microchem. J.* **37**: 314–321 (1988).
- A. Mohammad and N. Fatima. Cation thin-layer chromatography in mixed organic-solvents containing s-butylamine - separation of Zn (II) from Cd (II) and Cu (II). *Chromatogr.* **23**: 653–656 (1987).
- S. Ergül. Qualitative analysis of Cu²⁺, Co²⁺ and Ni²⁺ cations using thin layer chromatography. *J. Chromatogr. Sci.* **42**: 121–124 (2004).
- Ş. Şavascı and M. Akçay. The determination of some heavy metal cations by TLC/Photodensitometry. *Tr. J. Chem. TUBITAK* **20**: 146–152 (1996).
- R. Gürkan and Ş. Şavascı. Investigation of separation and identification possibilities of some metal-DEDTC complexes by sequential TLC-IR systems. *J. Chromatogr. Sci.* **43**: 324–330 (2005).
- S. Ergül. Investigation of thin-layer chromatography properties of some transition metal complexes based on ditiocarbamates. *J. Chromatogr. Sci.* **44**: 543–547 (2006).
- S. Ergül and Ş. Şavascı. Acid modified diatomaceous earth - A sorbent material for thin layer chromatography. *J. Chromatogr. Sci.* **46**: 308–315 (2008).
- S. Ergül. Linkage between separation of Cu²⁺, Co²⁺, Ni²⁺ on TLC and crystal field theory. *J. Chromatogr. Sci.* **46**: 907–911 (2008).
- G.R. Duncan. The transfer of chromatoplate resolutions to large scale separations on adsorption columns A new apparatus for producing thin layers. *J. Chromatogr. A* **8**: 37 (1962).

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