

Separation Mechanisms of Co(III) Complexes with EDTA-Type Ligands during Salting-Out TLC on Impregnated and Non-Impregnated Silica Gel

V. M. Živković-Radovanović¹, G. N. Vučković^{1*}, M. D. Antonijević-Nikolić² and M.I. Djuran³

¹Faculty of Chemistry, University of Belgrade, P.O.Box 118, 11158, Belgrade, Serbia, ²Higher Technological School of Professional Studies, 15000 Šabac, Serbia, and ³Faculty of Science, University of Kragujevac, P.O. Box 60, 34000 Kragujevac, Serbia

*Author to whom correspondence should be addressed. Email: gordanav@chem.bg.ac.rs

Received 14 October 2011; revised 30 December 2011

A separation mechanism of eight Co(III) complexes with ethylenediaminetetraacetate-type ligands belonging to two series during salting-out thin-layer chromatography on silica gel is investigated. The sorbent is impregnated with five poly(ethylene glycol)s with different molecular masses, and ammonium sulphate solutions are used as mobile phase. Additionally, on non-impregnated sorbent, mobile phases containing one of eight salts with ions of different lyotropic properties are used: kosmotropic Mg^{2+} accompanied with SO_4^{2-} (kosmotrope), Cl^- (border), NO_3^- (chaotrope), ClO_4^- (chaotrope), Cl^- of Li^+ (kosmotrope), Na^+ (border), K^+ (chaotrope) and NH_4^+ (chaotrope). Salting-out and salting-in effects were observed depending on salt nature. The combined retention mechanism of specific H-bonding and nonspecific hydrophobic interactions is proposed.

Introduction

Ethylenediaminetetraacetate (EDTA), a potentially hexadentate chelate ligand, and its derivatives are used in analytical chemistry for complexometric titrations due to the formation of thermodynamically and kinetically stable complexes with numerous metal ions (1). The non-toxicity of the complexes with this type of ligands, even with toxic metals, and their solubility in water enable applications in medicine for the detoxification of mammal organisms (2). Recently, there have been reports on the cytotoxic activity of such ligands and some of their metal complexes (3–7).

A successive increase of side carboxylate/diamine chains in EDTA-ligand by $-CH_2-$ group gives a series of the complexes with enhanced hydrophobic surface (Figure 1). In a previous investigation (8), two series of Co(III) complexes with such ligands were separated by thin-layer chromatography (TLC) on silica gel with $(NH_4)_2SO_4$ solutions giving enhanced retention with the increase of salt concentration. Because the complexes have longer side/back chains, they showed stronger retention (i.e., there is an order consistent with the reversed-phase mechanism). This was explained by the mechanism of non-specific hydrophobic interactions. On the same sorbent, these complexes were separated with solvent systems consisting of C_2H_5OH and H_2O in different mol% ratios, giving the same order (9). Nevertheless, the R_M values ($\log(1 - R_F)/R_F$) decreased with an increase of the mol% of H_2O in the C_2H_5OH solution, suggesting a normal-phase mechanism. The order of the complexes was explained by the positive inductive effect of the $-CH_2-$ group bonding to COO^- , which enhances specific H-bonding through silanol groups of the sorbent.

The results of the solutes' chromatographic separations on different layers with various solvents enables the prediction of their interactions with the sorbent/mobile phase. The mixed aminocarboxylato Co(III) complexes were studied using $(NH_4)_2SO_4$ solutions on silica gel impregnated with poly(ethylene glycol)s (PEGs) of different average molecular masses (10). With an increase in the molecular mass of the PEGs, hydrophobic properties of silica gel are enhanced, thus intensifying retention, even of the complexes with small hydrophobic moiety, which otherwise are not salted-out on non-modified silica gel. The same compounds were studied with different aqueous salt solutions as mobile phases on non-impregnated silica gel (11). It was found that numerous highly soluble salts, apart from their lyotropic properties, showed salting-out effects.

The aim of this paper is salting-out (SO) chromatography of Co(III) complexes with ligands of EDTA-type on silica gel impregnated with PEGs using $(NH_4)_2SO_4$ solutions as mobile phases. Additionally, the influence of different aqueous salt solutions on TLC behavior on non-impregnated silica gel is studied. The selection of salts was based on their solubility in water and various lyotropic properties of their constituents' ions (12, 13). Thus, Group 1 contains border anion (Cl^-) and cations changing lyotropic properties from kosmotrope (Li^+), over border (Na^+) to chaotropes (K^+ ; NH_4^+), and Group 2 contains very kosmotropic cations (M) and anions of kosmotrope (SO_4^{2-}), border (Cl^-) or chaotropic anions (NO_3^-/ClO_4^-). The separation mechanism is considered from all data.

Experimental

Chromatographed complexes (Table I) were synthesized and purified by the described procedures (14) and those cited in Vučković *et al.* (8). In short, $CoCl_2 \cdot 6H_2O$ and corresponding EDTA ligand were mixed in a 1:1 molar ratio in water and the pH value was adjusted to 8.0 by KOH solution. The mixture was stirred at 80°C for 3–5 h while 3% H_2O_2 solution was gradually added to oxidize Co(II) to Co(III). The ion-exchange chromatography (Dowex 1-X8, 200–400 mesh in the Cl^- form) was used for purification of Co(III) complexes and separation of the corresponding geometrical isomers. They were crystallized from water–ethanol or water–acetone solvent upon cooling, separated by suction and dried. The purity of the complexes was checked by elemental microanalyses. Their aqueous solutions ($\sim 2 \mu L$, $c = 5 \text{ mg/mL}$) were applied on the corresponding thin-layer using micro-capillaries.

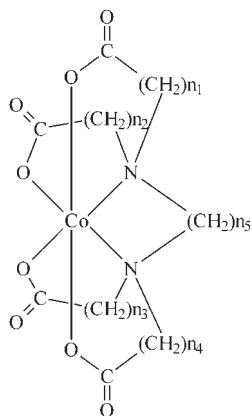


Figure 1. Structure of the complexes with EDTA-type ligands (n_1 – $n_4 = 1$ or 2 and $n_5 = 2$ or 3).

Silica gel H (type 60) for TLC (Merck; Darmstadt, Germany, Art 7736) was modified with PEGs of different average molecular masses (Carbowax 400, 1000 and 4000; Wilkens Instrument and Research, Walnut Creek, CA; 1540, Perkin-Elmer, Waltham, MA; and 5000/6000, Fluka, Buchs, Switzerland) by an immersion technique described in the literature (10). An aqueous suspension of sorbent (sorbent: water = 1: 2, w/w) was applied to glass plates (26 × 76 mm) using a manual fixed hopper spreader of Miller-Kirchners type. The layer thickness was 0.25 mm. The plates were dried at room temperature and kept in a closed dry space. $(\text{NH}_4)_2\text{SO}_4$, previously dried (at 105°C) was used for preparation of a stock solution ($c = 9.2 \text{ mol\%} = 4.0 \text{ mol/L}$). Other solutions were prepared by its dilution with deionized water in volumetric flasks.

Polygram SIL G/UV254 sheets for TLC (Macherey-Nagel, Düren, Germany) were cut into 40 × 66 mm pieces and used for the study of different salts' influence. Solid salts were of p.a. grade (Merck). Aqueous solution series of NaCl, KCl and NH_4Cl were prepared from dried salts. Due to their great hygroscopic properties, saturated stock solutions from dried LiCl, anhydrous $\text{Mg}(\text{ClO}_4)_2$ and Mg^{2+} crystallohydrates were prepared at 20°C [25 mol% for LiCl; 7.4 mol% for $\text{Mg}(\text{ClO}_4)_2$]; 9.3, 6.2 and 7.8 mol% were calculated for anhydrous salts: MgCl_2 , MgSO_4 and $\text{Mg}(\text{NO}_3)_2$, respectively, which were diluted with deionized water to desired concentrations.

The ascending technique in chambers (25 × 46 mm X 90 mm) was applied. For 15 min before development, approximately 3 mL of the solvent was kept closed in the chamber for its saturation. Experiments were performed simultaneously with all complexes of the same series. The temperature was $20 \pm 2^\circ\text{C}$. The mobile phase migration distance was 6 cm. Detection was visual, because the complexes were of intensive blue-violet color. Chromatographic developments were repeated at least three times, and calculated average R_F values were used.

For a preliminary test of normal-phase separations, organic solvents of p.a. grade were used: methanol, ethanol (Hemos, Belgrade, Serbia), acetone (Zorka, Šabac, Serbia), and their mixtures with water in (v/v) ratios of 1:3; 1:1 and 3:1, respectively.

For drawing and calculation Chem Window 3 (Figure 1) and Origin 6.1 (Figures 3–5 and data in Tables) were used. R_M

values for silica gel with 6.4 mol% (–0.162, 0.160, 0.389, 0.805, 1.103, 0.385, 0.680 and 0.921 for complexes 1–8, respectively) were calculated from fitted linear dependence of experimentally obtained R_M values taken from the study of Vučković *et al.* (8) on mol% of ammonium sulphate.

Results and Discussion

Studied complexes are listed in Table I. In series 1–5 containing five-membered 1,2-ethylenediamine back rings, side rings are increased with successive addition of $-\text{CH}_2-$ groups (up to four). Complexes 6–8 have six-membered 1,3-propanediamine back rings and one $-\text{CH}_2-$ group more than 1–3. It has been suggested (9) that during their separation by TLC on silica gel with an increased mobile phase polarity, from the system $\text{C}_2\text{H}_5\text{OH}-\text{H}_2\text{O}$ to aqueous $(\text{NH}_4)_2\text{SO}_4$ solutions, the change of the separation mechanism varied from specific H-bonding of the sorbent's $-\text{OH}$ group to COO^- oxygen of the complex to nonspecific hydrophobic interactions in SO chromatography, giving in both cases the reversed-phase order. By preliminary chromatography of the mentioned complexes, it was established that except on silica-gel, they are not salted-out with $(\text{NH}_4)_2\text{SO}_4$ on the other sorbents, because they have more pronounced hydrophobic properties (cellulose and polyacrylonitrile) (15). Thus, high R_F values of the complexes without mutual separation within one series are obtained. However, a certain retention of the complexes on silica gel in reversed-phase order appeared, even with pure H_2O . With other polar organic solvents [CH_3OH , $\text{C}_2\text{H}_5\text{OH}$ or $(\text{CH}_3)_2\text{CO}$] in which the complexes were sparingly soluble, they rested at the start position. The complex solubility decrease with decreasing of solvent polarity was in the following order: $\text{CH}_3\text{OH} > \text{C}_2\text{H}_5\text{OH} > (\text{CH}_3)_2\text{CO}$, and within the series, each of these solvents parallels the number of extended side/back rings. Additionally, separation by normal-phase chromatography on silica gel is possible only with classical reversed-phase solvent systems composed of water and CH_3OH , $\text{C}_2\text{H}_5\text{OH}$ or $(\text{CH}_3)_2\text{CO}$.

Our results of chromatographic separations of studied complexes with $(\text{NH}_4)_2\text{SO}_4$ solutions on silica gel impregnated with PEGs of different molecular mass, as bR_F ($R_F \times 100$) values, are given in Table II. The order of the complexes was analogous that obtained on non-modified sorbent (8). However, on impregnated sorbents, the R_F values were higher. Additionally, increase of molecular mass of the PEG caused an increase of the R_F value of the complex, showing a less pronounced tailing effect, giving with PEG 5500 an ideal round shape (Figure 2). These data suggest a combined separation mechanism involving specific and nonspecific interactions. The presence of PEG (especially to a greater polymerization degree) shields polar $-\text{OH}$ groups, producing weaker specific interaction. Moreover, it seems that the specific interaction of this type of compound during the SO process on non-modified silica gel is a more important factor of retention, giving selective separation. Decrease of specific interaction due to impregnation with PEG produces worse separation of the series 1–5 containing a smaller back ring. Dependence of R_M values of studied compounds obtained on silica gel impregnated with PEG 5500 on corresponding R_M values for non-modified silica

Table IInvestigated Co(III) Complexes as K⁺ Salts and Structural Formulas of their EDTA-Type Ligands

Number	Complex anion	Formula of the ligand	Ligand name (abbreviation)
1	[Co(edta)] ⁻		Ethylenediamine- <i>N,N,N',N'</i> -tetraacetate ion (edta)
2	<i>trans</i> (O ₅)-[Co(ed3ap)] ⁻		Ethylenediamine- <i>N,N,N'</i> -triacetate- <i>N'</i> -3-propionate ion (ed3ap)
3	<i>trans</i> (O ₅)-[Co(eddadp)] ⁻		Ethylenediamine- <i>N,N'</i> -diacetate- <i>N,N'</i> -di-3-propionate ion (eddadp)
4	<i>trans</i> (O ₅ ,O ₆)-[Co(eda3p)] ⁻		Ethylenediamine- <i>N</i> -acetate- <i>N,N',N'</i> -tri-3-propionate ion (eda3p)
5	[Co(edtp)] ⁻		Ethylenediamine- <i>N,N,N',N'</i> -tetra-3-propionate ion (edtp)
6	[Co(1,3-pdta)] ⁻		1,3-propanediamine- <i>N,N,N',N'</i> -tetraacetate ion (1,3-pdta)
7	<i>trans</i> (O ₅)-[Co(1,3-pd3ap)] ⁻		1,3-propanediamine- <i>N,N,N'</i> -triacetate- <i>N</i> -3-propionate ion (1,3-pd3ap)
8	<i>trans</i> (O ₅ ,O ₆)-[Co(1,3-pddadp)] ⁻		1,3-propanediamine- <i>N,N'</i> -diacetate- <i>N,N'</i> -di-3-propionate ion (1,3-pddadp)

gel (for 6.4 mol% (NH₄)₂SO₄) is presented in Figure 3. The slopes of the obtained lines (dx/dy) represent relation of separation factors, α , between adjacent complexes of one series on sorbents calculated with Equation (1), where ΔR_M is the difference between R_M values for neighbor complexes of one series on modified (SG-PEG 5500) and non-modified silica gel (SG).

$$\frac{dx}{dy} = \frac{\Delta R_M(\text{SG} - \text{PEG 5500})}{\Delta R_M(\text{SG})} = \frac{\alpha(\text{SG} - \text{PEG 5500})}{\alpha(\text{SG})} \quad (1)$$

For further investigation of the same compounds on silica gel, aqueous solutions of the salts (listed previously) with ions of

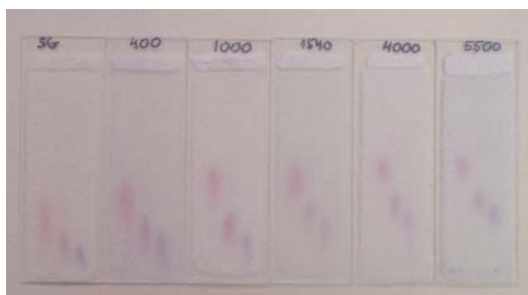


Figure 2. Photograph of the representative chromatograms obtained for series of investigated complexes 6–8 (Table I) (from left to right on each plate) with 9.2 mol% (NH₄)₂SO₄ on silica gel (SG) and impregnated silica gel with PEGs of different average molecular masses as marked on plates.

Table IIhR_F (R_F × 100) Values Obtained for the Investigated Complexes using (NH₄)₂SO₄ Solutions on Silica Gel Impregnated with Different PEGs

Average molecular mass of PEGs	Complex number*							
	1	2	3	4	5	6	7	8
400								
(NH ₄) ₂ SO ₄ mol%								
1.9	81	67	55	38	26	65	45	30
4.0	69	58	47	29	18	62	39	21
6.4	65	52	33	21	13	52	33	19
9.2	58	38	26	12	7	46	27	14
1000								
(NH ₄) ₂ SO ₄ mol%								
1.9	83	73	61	40	28	69	48	34
4.0	78	65	50	31	19	64	43	27
6.4	68	57	38	22	13	53	37	21
9.2	63	47	29	16	9	47	28	16
1540								
(NH ₄) ₂ SO ₄ mol%								
1.9	86	78	64	47	35	74	60	40
4.0	80	67	58	38	22	69	50	35
6.4	72	60	49	30	15	57	39	25
9.2	65	50	33	21	12	49	31	16
4000								
(NH ₄) ₂ SO ₄ mol%								
1.9	89	84	78	66	48	79	64	50
4.0	83	77	64	49	39	73	55	42
6.4	75	67	56	31	23	63	44	28
9.2	67	55	39	26	17	55	37	19
5500								
(NH ₄) ₂ SO ₄ mol%								
1.9	92	87	83	72	61	80	72	63
4.0	84	80	71	63	50	73	63	53
6.4	76	69	59	47	36	63	52	35
9.2	67	57	42	31	23	58	45	29

*Numbers of the complexes are the same as in Table I.

different lyotropic properties are applied. It is known (12, 13) that lyotropic property reflects the relation of an ion with molecules of water. Kosmotropes are usually small, with great ionic density, thus binding a great number of H₂O molecules in their hydration sphere, ordering the H₂O structure, and causing a salting-out effect. Alternatively, chaotropes are bigger and poorly charged. They have tendency toward adsorption on hydrophobic surfaces, causing an increase in substances' solubility (salting-in effect). During the chromatographic process with aqueous salt solutions, chaotropic ions interact with solutes, but also to some extent with the sorbent (16, 17). In Table III, hR_F values of the studied complexes thus obtained are given. For calculated R_M values, the linear dependence rule (dependence of R_M values on mol% of the salt) (15) was checked and the slopes of the lines are given in Table IV. These were assumed to be salting-out efficiency, because they represent the change of R_M value caused by a unit increase of mol% of the applied salt in the mobile phase. The effects of different salts on chromatographic behavior are shown in Figures 4 and 5. Three different cases during the increase of the salt concentration are established: (i) the retention is always enhanced by the application of MgSO₄ and earlier (8) by (NH₄)₂SO₄ (positive slopes) due to the salting-out effect; (ii) the salting-in effect always occurs with NO₃⁻ and ClO₄⁻ of Mg²⁺ solutions (negative slopes); and (iii) with Mg²⁺, Na⁺, K⁺ and NH₄⁺ Cl⁻ solutions, the salting-in effect is observed for the members with a small hydrophobic part within the complex series, whereas for the others, retention is enhanced.

The results are in accordance with previous observations (18) that anion has the most considerable role in the salting-out process. Most anions are usually more complicated and bigger than cations, so their influence on the arrangement of water molecules is greater (16–18). Additionally, an increase of chaotropic properties of anions in the order SO₄²⁻ < Cl⁻ < NO₃⁻ < ClO₄⁻ causes weaker interactions between the sorbent and the investigated complexes in the chromatographic system. It is assumed that their retention is the consequence of both specific and nonspecific interactions, acting in the same direction. Influence of individual anion could be explained by the following model: (i) extremely kosmotropic anion, SO₄²⁻ in the mobile phase efficiently brings about dehydration of both, solute and sorbent causing enhanced retention; (ii) chaotropic

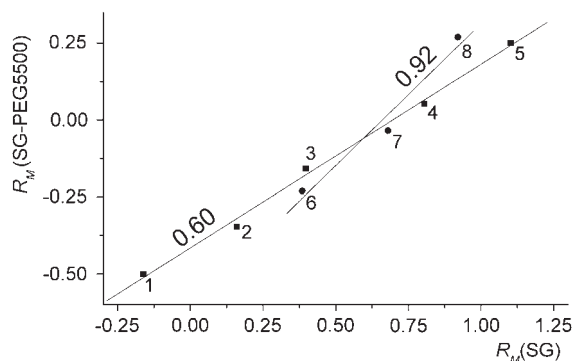


Figure 3. Dependence of R_M values of the studied complexes obtained on silica gel impregnated with PEG 5500 on the corresponding R_M values for non-modified silica gel. Numbers above lines represent their slopes and the near points complexes numerated as in Table I.

anions, NO₃⁻ < ClO₄⁻, are adsorbed on non-polar parts of solute and sorbent causing their hydration and decreasing of hydrophobic interaction. At the same time, as these ions are less hydrated, in their concentrated solutions there is possibility of H-bonding through their oxygens to –OH groups of silica gel. Thus, specific interaction of EDTA complexes with the sorbent is prevented; (iii) border anion, Cl⁻, is moderately hydrated producing moderate dehydration of solute and sorbent. Moreover, it could be adsorbed on their hydrophobic parts proportionally to contact surface. However, it could not form H-bonds with silica gels –OH groups. Thus, in the case of the complexes with bigger EDTA-type of ligands contact with the sorbent is sufficiently realized and their retention increase with increase of salt concentration (specific and nonspecific interactions). The salting-in effect occurs because of enhanced hydration by adsorption of Cl⁻ on non-polar solute parts of the complexes with smaller ligands. Therefore, it prevails over specific interactions with the sorbent. Some differences in the

Table III

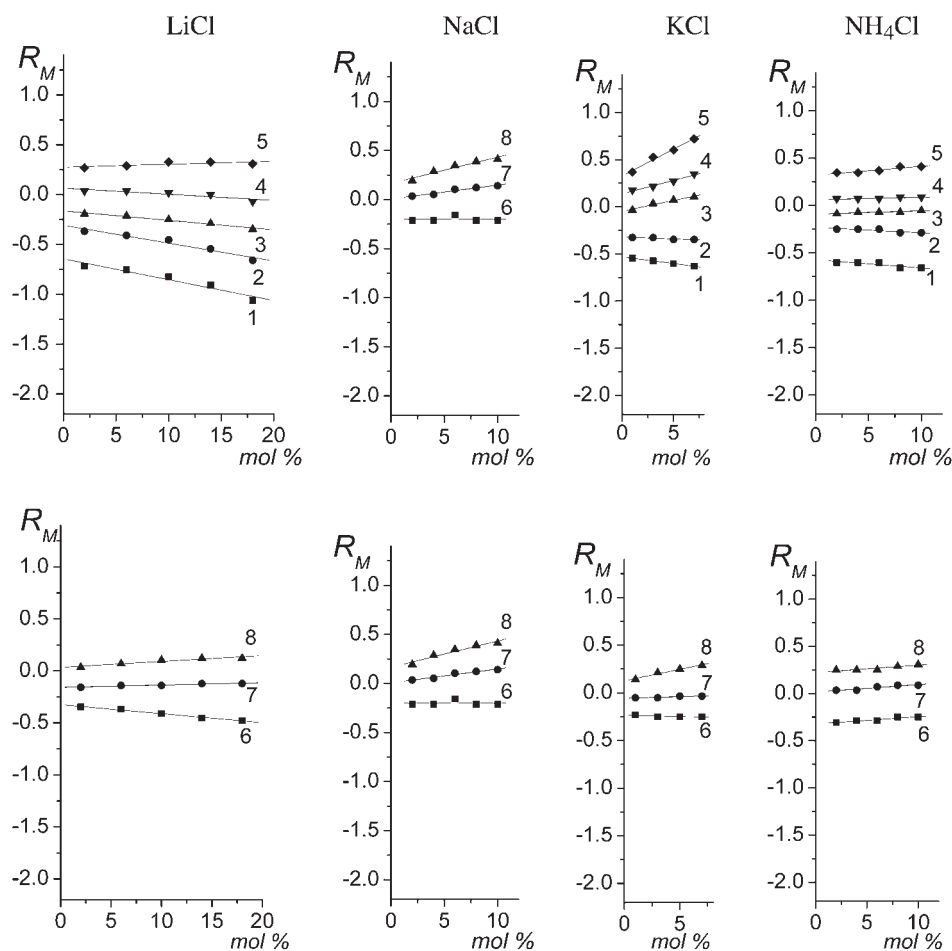
hR_F Values of the Studied Complexes on Silica Gel Obtained with H₂O and Various Salt Solutions

	Complex number*							
	1	2	3	4	5	6	7	8
Deionized water	82	74	60	41	31	72	52	42
LiCl mol%								
2.0	84	70	61	48	35	69	59	48
6.0	85	72	62	48	34	70	58	46
10.0	87	74	64	49	32	72	58	44
14.0	89	78	66	50	32	74	57	43
18.0	92	82	69	54	33	75	57	43
NaCl mol%								
2.0	78	66	55	36	26	62	48	39
4.0	78	67	54	34	24	62	47	34
6.0	79	67	52	33	20	59	44	31
8.0	79	68	51	30	19	62	43	29
10.0	80	68	50	29	17	62	42	28
KCl mol%								
1.0	78	68	52	40	30	63	53	42
3.0	79	68	48	38	23	64	53	38
5.0	80	69	46	35	20	64	52	36
7.0	81	69	44	31	16	64	52	34
NH ₄ Cl mol%								
2.0	80	64	55	46	31	67	48	36
4.0	80	64	54	46	31	66	48	36
6.0	80	64	54	46	30	66	46	36
8.0	82	66	54	45	28	64	45	34
MgSO ₄ mol%								
1.0	74	60	50	35	26	62	42	29
2.0	72	58	44	25	17	54	36	26
3.0	71	53	43	25	18	50	30	24
4.0	63	49	38	21	12	43	27	19
5.0	63	46	35	20	12	42	24	18
MgCl ₂ mol%								
1.0	82	72	59	46	32	68	46	39
3.0	85	74	61	46	34	70	47	37
5.0	87	76	64	45	30	73	47	44
7.0	90	79	70	46	32	76	51	42
9.0	92	83	74	48	28	76	51	40
Mg(NO ₃) ₂ mol%								
1.5	82	70	62	42	26	70	48	40
3.0	86	75	66	48	29	76	55	45
4.5	88	81	73	54	34	76	55	50
6.0	91	83	74	56	38	78	57	52
7.8	92	84	78	59	41	79	64	55
Mg(ClO ₄) ₂ mol%								
1.0	94	86	74	65	44	84	70	60
3.0	98	96	92	80	67	92	84	79
5.0	99	98	94	86	76	96	90	86
7.0	100	99	98	92	83	98	94	90

*Numbers of the complexes are the same as in Table I.

Table IVSalting-Out Efficiency (m) Calculated for the Investigated Salts*

Number [†]	Alkaline and NH_4^+ chloride				Mg^{2+} salts			
	LiCl	NaCl	KCl	NH_4Cl	MgSO_4	MgCl_2	$\text{Mg}(\text{NO}_3)_2$	$\text{Mg}(\text{ClO}_4)_2$
1.	-0.021	-0.007	-0.014	-0.009	0.062	-0.050	-0.065	-0.200
2.	-0.018	-0.005	0.004	-0.006	0.065	-0.034	-0.058	-0.197
3.	-0.010	0.011	0.023	0.003	0.065	-0.038	-0.053	-0.192
4.	-0.006	0.018	0.028	0.003	0.075	-0.004	-0.047	-0.128
5.	0.003	0.030	0.057	0.015	0.100	0.010	-0.049	-0.129
6.	-0.009	0	0.002	0.009	0.090	-0.024	-0.029	-0.161
7.	0.002	0.014	0.003	0.010	0.090	-0.012	-0.039	-0.136
8.	0.006	0.027	0.024	0.006	0.072	-0.006	-0.041	-0.127

*Note: calculated as the slopes of the line: $R_M = R_M^0 + m$ [mol% of the salt].[†]Numbers of the complexes are the same as in Table I.**Figure 4.** Dependence of R_M values on mol% obtained for the investigated complexes on silica gel with NH_4^+ and some alkaline chlorides. Numbers near lines correspond to the complexes numerated as in Table I.

behavior of the same series originate from the nature of the cation of the applied Cl^- salt, which are obvious from the obtained slopes (salting-out efficiencies), listed in Table IV. It seems that Cl^- is more adsorbed in the presence of highly kosmotropic cations (Li^+ and Mg^{2+}), so most of the complexes between the members of the series appeared in the presence of increased Cl^- concentration, especially with K^+ and Na^+ , bringing enhanced separation selectivity (ΔR_M is higher than in the other cases).

All investigated salts are listed in Table V in order of their decreasing salting-out/increasing salting-in effect on investigated complexes during TLC on silica gel. The lyotropic properties of their cation/anion are also shown in the table. In addition to the former conclusion that the anion has the predominant influence on retention [following the order kosmotrope (k) > border (b) > chaotrope (c)], for the production of certain effects, the combination of cation/anion is also important. Namely, stronger effects are achieved with ions of the same

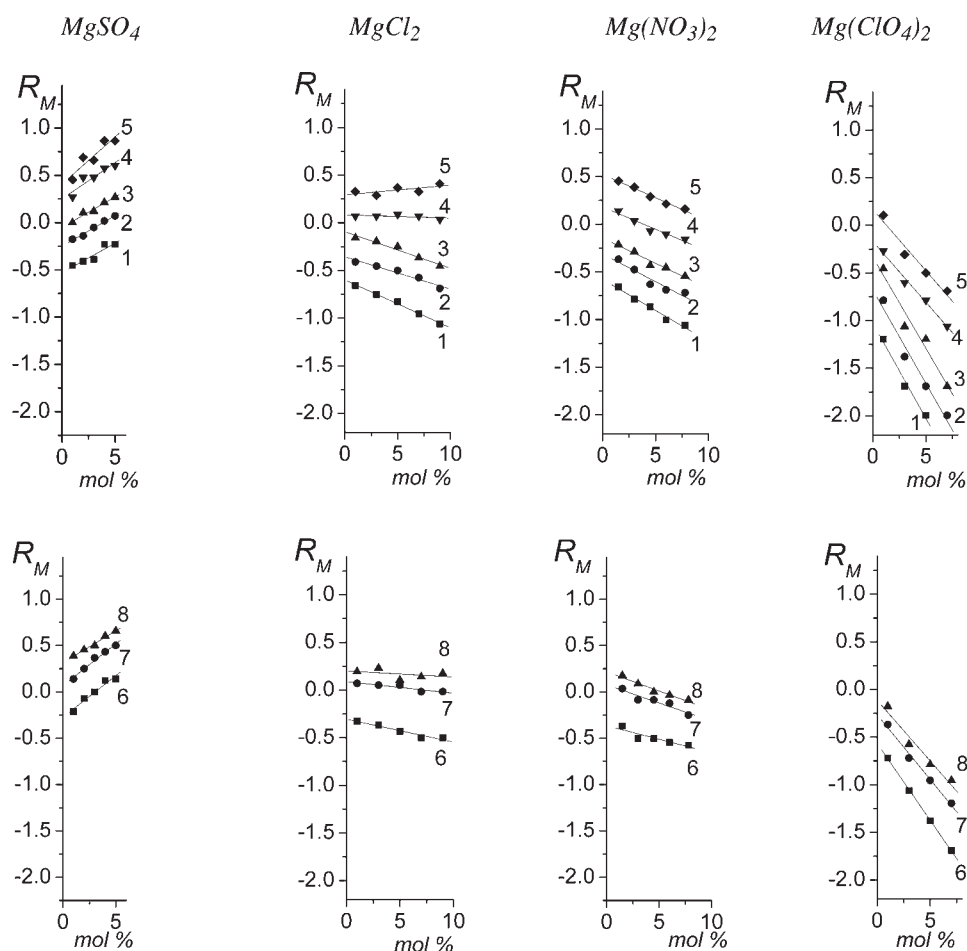


Figure 5. Dependence of R_M values on mol% obtained for the investigated complexes on silica gel with some Mg^{2+} salts. Numbers near lines correspond to the complexes as in Table I.

Table V

Influence of the Investigated Salts on Behavior of Studied Complexes Regarding Lyotropic Properties of their Constituents

Salt	MgSO ₄	NaCl	KCl	NH ₄ Cl	LiCl	MgCl ₂	Mg(NO ₃) ₂	Mg(ClO ₄) ₂
Effect	salting-out	salting-out; salting-in	salting-out; salting-in	salting-out; salting-in	salting-out; salting-in	salting-out; salting-in	salting-in	salting-in
Lyotropic property of cation	k*	b*	c*	c	k	k	k	k
Lyotropic property of anion	k	b	b	b	b	b	c	c
Combined cation-anion	kk	bb	cb	cb	kb	kb	kc	kc

*k, kosmotrope; c, chaotrope; b, border ion.

lyotropic properties in the following order of combinations: $kk > bb > cb > kb > kc$.

Conclusion

By SOTLC of Co(III) complexes with EDTA-type ligands on silica-gel in most cases, the opposite results were obtained than those found in earlier studied aminocarboxylato Co(III) complexes under the same conditions. The increase of the polymerization degree of PEGs for silica gel impregnation caused a decreased retention of these complexes when $(NH_4)_2SO_4$ solutions were used as mobile phases. However,

when salt solutions with various lyotropic properties were applied for SOTLC on non-impregnated silica gel, the influence of the anion nature was predominant. The salts with chaotropic anions (NO_3^-/ClO_4^-) produce a decrease in the complexes' retention with increase of the salt concentration in the mobile phase, i.e., a pronounced salting-in effect. With Cl^- , the behavior of the complexes depended mostly on their hydrophobic properties (salting-out or salting-in effect) and also on the cation nature. The salting-out effect was always achieved with SO_4^{2-} solutions. Based on all data for the studied complexes, the combined separation mechanism (including specific and nonspecific interactions) was assumed. This paper showed that for the discussion of separation mechanisms in SOTLC, all

factors (sorbent, solute and content of mobile phase) should be considered.

Acknowledgment

The authors are grateful to the Ministry of Education and Science (Project 172014) for the financial support.

References

1. Jeffery, G.H., Bassett, J., Mendham, J., Denney, R.C.; *Vogel's textbook of quantitative chemical analysis*. 5th edition, Longman Scientific & Technical, John Wiley & Sons, Inc, New York, NY, (1989), pp. 55–60.
2. Roat-Malone, R.M.; *Bioinorganic chemistry, A short course*. 1st edition, Wiley Interscience Edition, Hoboken, NJ, (2002), p. 267.
3. Rami, M., Winum, J.Y., Innocenti, A., Montero, J.L., Scozzafava, A., Supuran, C.T.; Carbonic anhydrase inhibitors: Copper(II) complexes of polyamino-polycarboxylamido aromatic/heterocyclic sulfonamides are very potent inhibitors of the tumor-associated isoforms IX and XII; *Bioorganic and Medicinal Chemistry Letters*, (2008); 18: 836–841.
4. Radić, G.P., Glodjović, V.V., Kaludjerović, G.N., Heinemann, F.W., Palladium(II) complexes with R2edda-derived ligands. Part V. Reaction of O,O'-diethyl-(S,S)-ethylenediamine-N,N'-di-2-(3-methyl)butanoate with K₂[PdCl₄]; *Transition Metal Chemistry*, (2011); 36: 331–336.
5. Lazić, J.M., Vučićević, Lj., Grguric-Šipka, S., Janjetović, K., Kaludjerović, G.N., Misirkić, M., *et al.*; Synthesis and in vitro anticancer activity of octahedral platinum(IV) complexes with cyclohexyl-functionalized ethylenediamine-N,N'-diacetate-type ligands; *ChemMedChem*, (2010); 5: 881–889.
6. Krajčinović, B.B., Kaludjerović, G.N., Steinborn, D., Schmidt, H., Wagner, C., *et al.*; Synthesis and in vitro antitumoral activity of novel O,O'-di-2-alkyl-(S,S)-ethylenediamine-N,N'-di-2-propanoate ligands and corresponding platinum(II/IV) complexes; *Journal of Inorganic Biochemistry*, (2008); 102: 892–900.
7. Rastogi, K., Chou, T.C., Ting, C.Y., Chen, K.T., Hwang, J., Su, T.L.; Synthesis and biological activity of AHMA-EDTA conjugates; *Medicinal Chemistry Research*, (2002); 11: 278–292.
8. Vučković, G., Miljević, D., Janjić, T.J., Djuran, M.I., Čelap, M.B.; Salting-out thin layer chromatography of transition metal complexes: A comparative study of the effect of increased number of CH₂ groups in chelate rings; *Chromatographia*, (1995); 40: 445–447.
9. Vučković, G., Miljević, D., Janjić, T.J., Djuran, M.I., Čelap, M.B.; Effect of solvent system polarity on RM values of cobalt(III) complexes obtained on silica gel thin layers; *Journal of the Serbian Chemical Society*, (1996); 61: 615–618.
10. Živković-Radovanović, V., Vučković, G.; Poly(ethylene glycol) as impregnator for silica gel in salting-out thin-layer chromatography of some Co(III) complexes; *Chromatographia*, (2005); 62: 91–97.
11. Živković-Radovanović, V., Vučković, G.; Use of different salt solutions in salting-out TLC of Co(III) complexes on silica gel; *Chromatographia*, (2008); 67: 259–267.
12. Marcus, Y.; A simple empirical model describing the thermodynamics of hydration of ions of widely varying charges, sizes, and shapes, review; *Biophysical Chemistry*, (1994); 51: 111–127.
13. Zhao, H.; Are ionic liquids kosmotropic or chaotropic? An evaluation of available thermodynamic parameters for quantifying the ion kosmotropicity of ionic liquids; *Journal of Chemical Technology and Biotechnology*, (2006); 81: 877–891.
14. Radanović, D.J., Miletić, V.D., Ama, T., Kawaguchi, H.; Synthesis and characterization of hexadentate cobalt(III) complexes with new EDTA-type ligands. 5. Structure and absolute configuration of the trans(O5)(-)-546-isomer of lithium (ethylenediamine-N,N,N'-triacetato-N'-3-propionato) cobaltate(III) monohydrate, (-)-546-trans(O5)-Li[Co(ed3ap)].H₂O. Strain analysis of [Co(EDTA-type)]-chelates in relation to their octahedral distortion; *Bulletin of the Chemical Society of Japan*, (1998); 71: 1605–1614.
15. Janjić, T.J., Živković-Radovanović, V.M., Čelap, M.B.; Planar salting-out chromatography, review; *Journal of the Serbian Chemical Society*, (1997); 62: 1–17.
16. Washabaugh, M.W., Collins, K.D.; The systematic characterization by aqueous column chromatography of solutes which affect protein stability; *Journal of Biological Chemistry*, (1986); 261: 12477–12485.
17. Collins, K.D.; Sticky ions in biological systems. *Proceedings of the National Academy of Science*, (1995); 92: 5553–5557.
18. Görgényi, M., Dewulf, J., Van Langenhove, H., Héberger, K.; Aqueous salting-out effect of inorganic cations and anions on non-electrolytes; *Chemosphere*, (2006); 65: 2949–2956.