Influence of Inorganic Mobile Phase Additives on the Retention and Separation Efficiency of Selected Amino Acids in Thin-Layer Chromatography on Cellulose Layers

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Abstract

Selected amino acid standards are investigated on cellulose layers using organic–aqueous eluent systems modified with neutral and chaotropic salts: chlorides, iodides, nitrates, thiocyanates, perchlorates, and hexafluorophosphates at low concentrations ranging from 10 up to 80mM in whole mobile phase. The effect of salts used as the mobile phase modifier is analyzed by the comparison of densitograms, peak symmetry coefficient, and theoretical plate number. The efficiency of chromatographic systems modified with inorganic salts additives depends primarily on the kind of salt and organic solvent in the mobile phase. The best efficiency is obtained through the addition of ammonium thiocyanate to the mobile phase containing acetonitrile as an organic modifier.

Introduction

In salting-out thin-layer chromatography (SOTLC), amino acids and peptides are adsorbed onto neutral solid support in the presence of high concentrations of alkaline phosphates or other salts promoting adsorption, known as kosmotropic, antichaotropic, or water-structuring according to the Hofmeister series (1,2). Adsorption is, therefore, effected directly with salt presented in the mobile phase, usually at near neutral pH. Changes in retention parameters could be achieved by alterations in the concentration of an organic modifier or added salt causing the changes in surface tension of the surrounding solvent. Retention arises from the existence of all interactions, such as: hydrophobic, charge-charge attraction, and dipole–dipole, which are enhanced in the presence of high concentrations of antichaotropic salts (3–5).

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In 1948, Tiselius (6) published some research considering separations of amino acids and peptides using SOTLC. Since that time, the physicochemical basis of the retention mechanism has been investigated in details by other authors (7–10).

Currently, this analytical method has been found to be particularly useful for the purification of proteins (11,12). However, SOTLC may also be applied in resolution of transition metal complexes, for instance *cis*-trans isomeric Co (III) complexes containing diamine rings. This method appeared to have been useful not only for separation of complexes but it also enabled discrimination according to the chelate ring size (13,14).

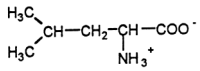
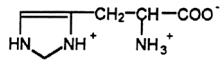
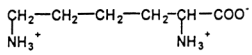
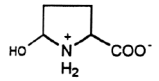
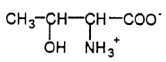
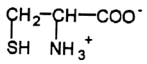
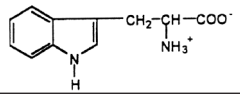
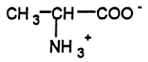
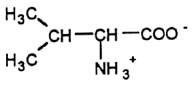
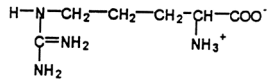
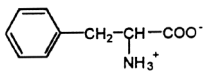
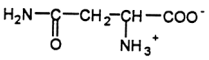
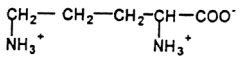
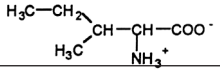
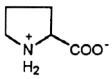
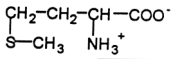
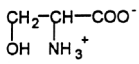
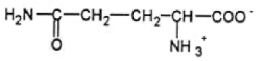
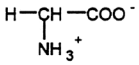
The previous paper concerned the application of the salting-out effect in the analysis of sulphonamides (15). Chromatographic parameters achieved using SOTLC on silica gel were suitable in quantitative structure-activity relationship (QSAR) studies.

The effect of inorganic salts as mobile phase additives at low concentrations (smaller than 0.1M) was investigated according to their influence on the hydrophobicity parameters (R_{MW}, S, f_0) of sulfosuccinic acid esters (16), 1,2,4-triazole derivatives with potential antitubercular activity (17), aniline derivatives (18) determined by reversed-phase thin-layer chromatography. The improvement of correlations between chromatographically determined lipophilicity was observed either for the neutral anionic surfactants or chaotropic ones.

Inorganic salts as the mobile phase additives were also applied in thin-layer chromatography of dansylated amino acids in a reversed-phase system (19), nucleobases and nucleosides on cellulose layers (20).

The aim of this work is application of quite opposite to SOTLC conditions in the analysis of amino acid patterns on cellulose layers. Chromatographic operations are made in solvent modified with neutral and chaotropic salts: chlorides, iodides, nitrates, thiocyanates, perchlorates, and hexafluorophosphates, the electrolyte strength of which does not differ much from common biological environments (at milimolar concentration). The usefulness of the

Table I. Investigated Compounds

No.	Amino acid	Chemical Formula	No.	Amino acid	Chemical Formula
1	Leucine		11	Histidine	
2	Lysine		12	Hydroxyproline	
3	Threonine		13	Cysteine	
4	Tryptophan		14	Alanine	
5	Valine		15	Arginine	
6	Phenylalanine		16	Asparagine	
7	Ornithine		17	Isoleucine	
8	Proline		18	Methionine	
9	Serine		19	Glutamine	
10	Glycine				

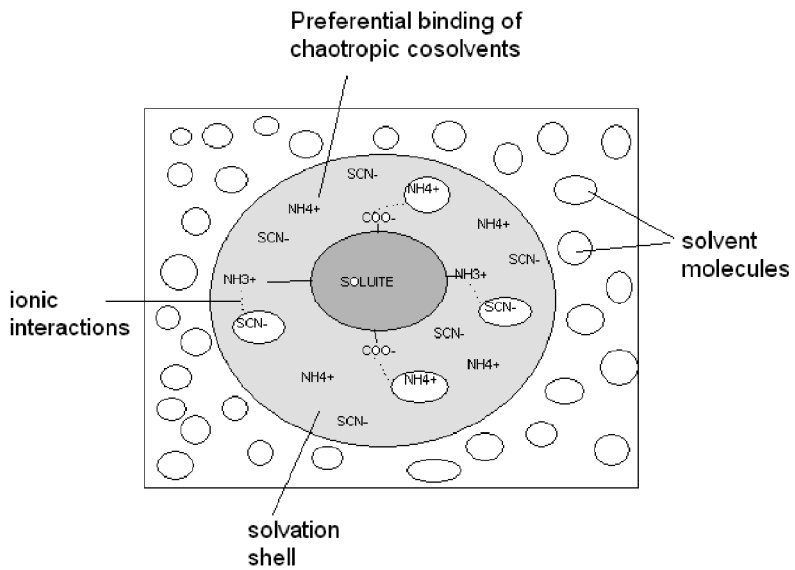


Figure 1. Scheme of preferential binding of chaotropic cosolvent molecules by solvation shell of solute with polar groups.

proposed modification is estimated according to their influence on separation selectivity, peak symmetry, and the theoretical plate number parameters. The chromatographic system offering the best separation selectivity and efficiency was used for resolution of the amino-acids mixture.

Experimental

Chromatography was performed on 10 cm × 20 cm high-performance thin-layer chromatographic plates precoated with 0.1-mm layers of cellulose (Merck, Darmstadt, Germany). The investigated compounds were dissolved in 2-propanol–water (1:1 v/v) at concentration of 3 mg/mL and samples (3 µL) of the solutions were spotted on the plates as 2 mm bands by means of a Linomat 5 automatic TLC sampler (Camag, Muttenz, Switzerland) controlled by ATS3 software. The plates were developed in a 20 cm × 20 cm horizontal Teflon DS

chambers (Chromdes, Lublin, Poland) and after drying, visualized by spraying the layers with ninhydrin solution. Chromatograms were developed at 20°C to a distance of 9 cm.

Amino acids standards contained in Table I were supplied by Merck. All solvents used were of analytical reagent grade from Merck: methanol, acetonitrile, and P.O.Ch. 2-propanol was from Gliwice, Poland. Evaluation of the developed TLC plates was performed densitometrically by the use of a TLC-HPTLC scanner (J&M, Aalen, Germany) equipped with a diode-array detector. The scanner was operated at the optimized absorbance wavelength of 565–580 nm. The wavelength for individual compound was selected after in situ recording of the spectra from λ = 200 to 700 nm.

Results and Discussion

Retention model

It has already been proved that low concentrations of chaotropic cosolvents cause increase the solubility of nonpolar solutes in aqueous media. This phenomenon is connected with randomizing the structure of liquid water. Changing the structure of water in the direction of greater disorder is associated with large positive entropies of the hydrated forms of these anions. Considering details of the described effects as being a consequence of the presence of chaotropic salts in solution, it should be stressed that an essential role is preferentially played by the binding of chaotropic cosolvents into the shell regions of the solute, which will be enhanced owing to electrostatic attraction forces of ionic additives with polar functional groups of opposite charges in solute molecules (21,22). A schematic representation of the described processes is presented on Figure 1.

In aqueous solution, which is transformed into a disordered state in the presence of chaotropic salts, solute exclusion rapidly decreases. Therefore, in chromatographic systems, these additives promote elution in entropically driven adsorption–desorption processes.

We assumed that the presented processes could be exploited in chromatographic systems causing improvement of efficiency and separation selectivity.

Table II(A). R_f, As, and N/m Values for Investigated Compounds Obtained on Cellulose in Different Eluent Systems Modified with NaCl.

	Acetonitrile–water (7:3)								
	0mM NaCl			40mM NaCl			80mM NaCl		
Investigated compounds	R_f	As	N/m	R_f	As	N/m	R_f	As	N/m
Leucine	0.50	1.0	8244	0.53	1.3	3022	0.48	0.8	3622
Lysine	0.05T	1.1	5211	0.16	0.7	3467	0.17	0.8	1014
Threonine	0.24	1.1	8456	0.19	0.9	6578	0.23	0.9	1068
Tryptophan	0.42T	0.9	4167	0.39T	0.7	1244	0.35	0.8	1156
Valine	0.39	1.2	5133	0.35	1.1	5756	0.32	0.9	7189
Phenylalanine	0.53	1.0	4456	0.48	1.0	3233	0.44	1.0	1100
Ornithine	0.06T	1.3	2556	0.12	0.9	4722	0.15	0.7	3622
Proline	0.39	1.1	1911	0.28	1.0	1711	0.30	–	–
Serine	0.25	1.0	6167	0.15	1.0	6167	0.18	0.9	2100
Glycine	0.27	1.1	13133	0.15	1.0	2100	0.18	0.9	4622
Histidine	0.06T	1.1	6667	0.12T	0.9	4989	0.16	1.0	7078
Hydroxyproline	0.25	0.8	4678	0.22	0.9	3022	0.22	0.9	278
Cysteine	0.23	1.0	2656	0.28	0.9	3122	0.25	0.9	3144
Alanine	0.26	1.1	9522	0.23	0.9	5311	0.24	0.8	4056
Arginine	0.03T	1.0	6578	0.22	0.8	5178	0.16	0.8	4522
Asparagine	0.33T	–	–	0.08	0.8	200	0.12	–	–
Isoleucine	0.48	1.1	8867	0.42	1.3	7267	0.41	–	–
Methionine	0.47	1.1	4622	0.36	1.1	6667	0.37	1.1	1178
Glutamine	0.36	–	–	0.14	0.9	244	0.20	0.9	1067

* (–) Criterion for asymmetry or theoretical plates not satisfied. T = tailing spot.

Table II(B). R_f, As, and N/m Values for Investigated Compounds Obtained on Cellulose in Different Eluent Systems Modified with NaCl.

	Tetrahydrofuran–water (6:4)								
	0mM NaCl			20mM NaCl			50mM NaCl		
Investigated compounds	R_f	As	N/m	R_f	As	N/m	R_f	As	N/m
Leucine	0.76	0.9	3022	0.77	0.7	586	0.65	0.9	8144
Lysine	0.39T	0.9	922	0.47T	0.9	2222	0.41	1.1	4967
Threonine	0.60	0.9	1289	0.53	1.1	2111	0.39	1.0	4678
Tryptophan	0.63	0.8	389	0.69	0.9	1756	0.60	0.9	9111
Valine	0.65	1.0	833	0.60	0.9	3944	0.53	1.3	5178
Phenylalanine	0.70	1.2	622	0.72	0.9	3944	0.62	1.0	4622
Ornithine	0.29T	0.6	3511	0.33T	0.8	3700	0.32	0.8	3400
Proline	0.51	1.1	278	0.49	0.9	689	0.42	0.9	5178
Serine	0.43	1.1	1067	0.43	1.0	1867	0.35	0.8	2222
Glycine	0.40	1.0	1644	0.37	0.9	1367	0.32	–	–
Histidine	0.28T	0.6	689	0.35T	0.8	4622	0.27	0.8	8044
Hydroxyproline	0.45	1.0	600	0.47	1.1	2733	0.39	1.0	3467
Cysteine	0.61T	0.8	500	0.63T	1.1	189	0.49	1.0	1356
Alanine	0.48	0.9	2222	0.49	0.9	2733	0.41	0.8	3233
Arginine	0.22T	0.6	756	0.37	0.8	1389	0.31	0.8	5856
Asparagine	0.39	0.8	267	0.32T	–	–	0.25	–	–
Isoleucine	0.67	1.0	4367	0.69	–	–	0.60	0.8	3467
Methionine	0.65T	0.9	1189	0.67	–	–	0.58	–	–
Glutamine	0.53	–	–	0.52	–	–	0.41	–	–

* (–) Criterion for asymmetry or theoretical plates not satisfied. T = tailing spot.

Similarly to salting-out chromatography based on precipitation caused by high concentration of kosmotropic salts sometimes closed to the saturation point, this chromatographic technique could be named salting-in TLC (SITLC) according to the chromatographic conditions (i.e., use of mobile phase modified with low concentration of chaotropic salts).

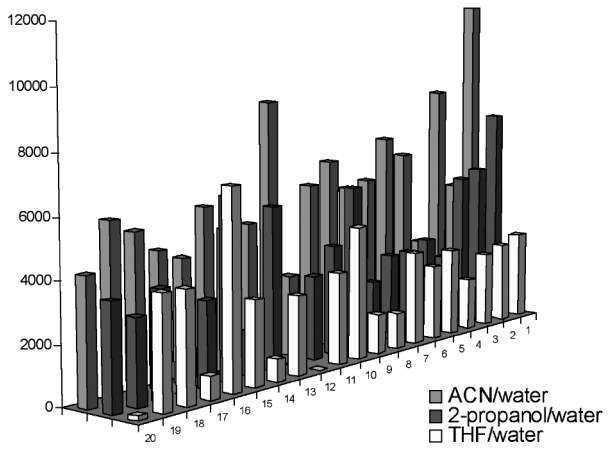


Figure 2. Influence of the kind of organic modifier in the mobile phase on the values of N/m parameters obtained for investigated amino acids on cellulose by the use of eluent modified with 20mM NH_4SCN.

Effect of organic solvent in the mobile phase

All experiments were conducted on cellulose layers by the use of an organic aqueous mobile phase. In accordance with the liquid–liquid partition mechanism, partitioning occurs between the mobile phase and mixed stationary liquid formed by preferential solvation of sorbent. It was studied that an increase in polarity of the mobile phase causes a decrease in retention. Such behavior is characteristic of normal phase chromatography. In this system, tailing peaks were obtained, especially for basic amino acids. It means that the retention is strongly affected by their charge slowing down the rate of mass transfer. Improvement of chromatographic systems could be achieved by an increase of ionic strength of the mobile phase owing to the addition of inorganic salts.

In weakly acidic and weakly basic solutions, amino acids occur as dipolar ions (zwitterions). Mobile phase enriched with inorganic ions generate charge–charge interactions. Electrostatic attraction between solute ions and ions comes from dissociation of the added salt, which competes with the solvation processes. Taking into account electrostatic forces in organic–aqueous mobile phases, the dominant role as for their strength will be played by the value of solvent dielectric constant. This relationship is expressed by the following equation (23):

$$F = q_1 q_2 / 3\pi d^2 \varepsilon$$

where q_1 and q_2 are the charges of ions, d is the distance between them; ε is the dielectric constant of solvent system.

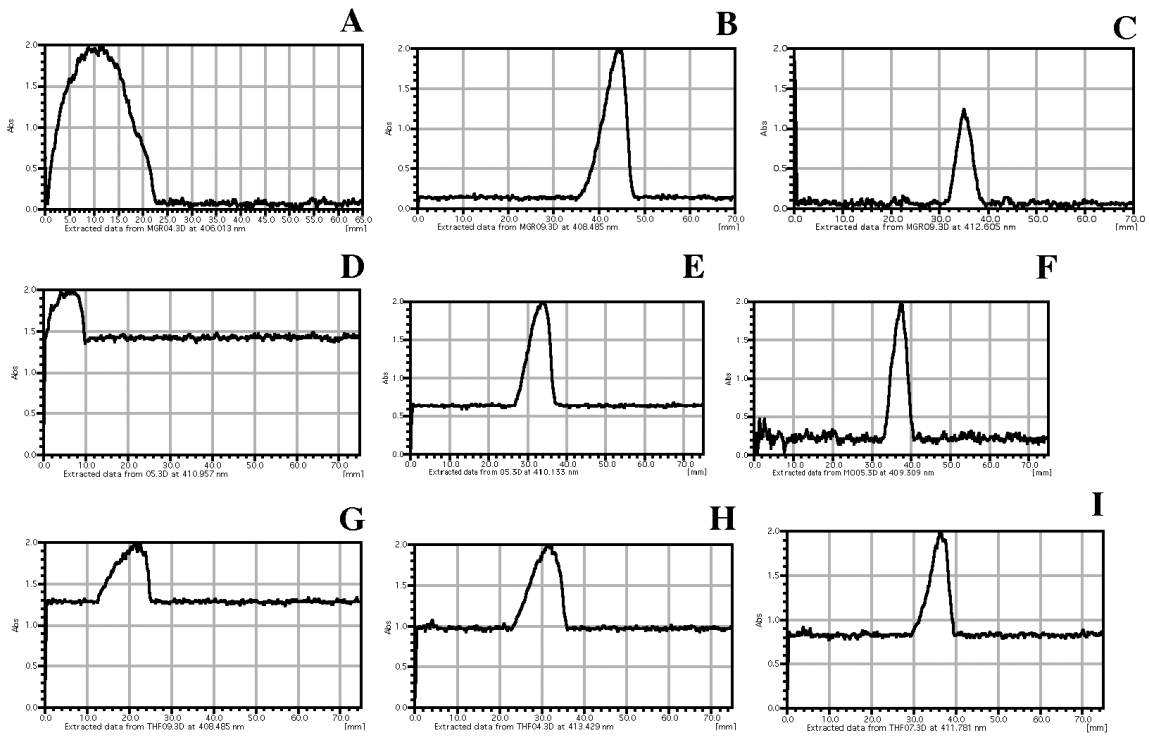


Figure 3. Influence of the kind of organic modifier on salt concentration in the mobile phase. Analyzed compound: histidine. For A, B, and C: ACN–H_2O (6:4). 0mM NH_4SCN (A) 30mM NH_4SCN (B) 80mM NH_4SCN (C) As = 1.0; N/m = 3456. For D, E, and F 2-propanol–H_2O (6:4). 0mM NH_4SCN (D), 20 mM NH_4SCN (E), 80 mM NH_4SCN As = 1.0; N/m = 13500 (F). For G, H, and I THF–H_2O (6:4). 0mM NH_4SCN (G), 10mM NH_4SCN (H), and 20 mM NH_4SCN As = 0.73; N/m = 3144 (I).

Organic solvents added to the mobile phase in a visible way change the dielectric constant of water, which is 78.08, because they differ in value of their dielectric constant: acetonitrile-35, 95, 2-propanole-20, 44, tetrahydrofuran-7, 5. The strongest electrostatic interactions will take place in mobile phases containing tetrahydrofuran as an organic modifier. This assumption is confirmed by the experimental data concerning the efficiency of the examined mobile phase presented in Tables II and III and additionally in Figure 2.

The obtained results indicate that a higher value of dielectric constant and smaller participation in solvation is more advantageous for achieving better efficiency of chromatographic systems enriched with chaotropic additives. That is why the most satisfactory efficiency was obtained for acetonitrile as an organic modifier.

It appears that the type of organic solvent influences the concentration of salt, giving improvement of peaks characteristics. Tetrahydrofuran requires the smallest concentration of the added salt—approximately 20mM, whereas 2-propanol requires 80mM of additives to achieve comparative peak parameters [i.e., symmetry and theoretical plates number (Figure 3)].

Table III(A). R_f, As, N/m Values for Investigated Compounds Obtained on Cellulose with Different Mobile Phases Modified with Ammonium Thiocyanate

	Acetonitrile–water (6:4)								
	0mM NH_4SCN			30mM NH_4SCN			80mM NH_4SCN		
No.	R_f	As	N/m	R_f	As	N/m			
1	0.66	0.9	5856	0.72	1. 0	11500	0.67	1.1	2067
2	0.07	0.9	1600	0.52	0.8	4844	0.53	0.8	6878
3	0.41	1.0	4622	0.48	0.9	8444	0.47	0.9	1041
4	0.53	0.8	3844	0.59	0.8	3011	0.52	–	–
5	0.53	1.0	4500	0.63	0.9	6333	0.53	0.8	3556
6	0.65	1.0	4867	0.70	1.1	7000	0.64	1.1	6156
7	0.09	1.0	1289	0.50	0.8	5622	0.50	–	–
8	0.50	0.9	1089	0.59	1.0	5467	0.53	0.9	3544
9	0.33	0.9	4989	0.44	0.9	6511	0.40	1.0	1300
10	0.33	0.9	9078	0.44	0.7	5811	0.40	1.3	1533
11	0.12	1.3	1000	0.49	0.7	2778	0.47	1.0	3456
12	0.39	1.0	5233	0.49	1.0	4867	0.43	0.9	8044
13	0.42	0.8	3744	0.50	0.8	4844	0.48	0.9	1622
14	0.39	1.0	4867	0.50	0.8	5700	0.48	0.8	7444
15	0.05	1.0	4122	0.50	0.8	4156	0.50	0.9	7844
16	0.65	0.8	2667	0.29	0.9	4556	0.22	–	–
17	0.61	1.0	4989	0.69	1.0	5311	0.65	1.0	9878
18	0.56	1.0	5111	0.64	1.0	5811	0.61	1.0	6878
19	0.60	0.8	1911	0.39	0.9	4222	0.36	–	–

* (–) Criterion for asymmetry or theoretical plates not satisfied. T = tailing spot.

Table III(B). R_f, As, N/m Values for Investigated Compounds Obtained on Cellulose with Different Mobile Phases Modified with Ammonium Thiocyanate

	Tetrahydrofuran–water (6:4)								
	0mM NH_4SCN			10mM NH_4SCN			20mM NH_4SCN		
No.	R_f	As	N/m	R_f	As	N/m			
1	0.76	0.9	3022	0.80	1.2	6367	0.75	0.9	3022
2	0.39	0.9	922	0.53	0.9	1956	0.56	0.9	2778
3	0.60	0.9	1289	0.58	0.9	2556	0.52	1.1	2600
4	0.63	0.8	389	0.67	0.9	1444	0.71	0.9	1800
5	0.65	1.0	833	0.63	0.9	2222	0.65	1.0	3022
6	0.70	1.2	622	0.70	1.0	3467	0.74	0.9	2600
7	0.29	0.6	3511	0.42	0.7	2867	0.48	0.8	3233
8	0.50	1.1	278	0.50	1.0	1289	0.51	1.2	1244
9	0.43	1.1	1067	0.44	1.0	3467	0.37	0.9	1367
10	0.40	1.0	1644	0.40	0.8	4144	0.40	0.7	4556
11	0.28	0.6	689	0.38	0.6	1278	0.44	0.7	3144
12	0.49	1.0	600	0.47	1.2	1344	0.47	1.1	2700
13	0.61	0.8	500	0.64	1.0	800	0.64	1.0	756
14	0.48	0.9	2222	0.49	0.9	1178	0.50	0.9	2900
15	0.22	0.6	756	0.41	0.8	600	0.50	0.9	6700
16	0.39	0.8	267	0.30	0.9	1667	0.30	1.2	789
17	0.67	1.0	4367	0.67	0.9	1367	0.71	1.0	3744
18	0.65	0.9	1189	0.65	0.8	1978	0.68	0.9	3778
19	0.53	–	–	0.50	1.3	1422	0.50	1.1	156

* (–) Criterion for asymmetry or theoretical plates not satisfied. T = tailing spot.

Effect of salt added to the mobile phase

Salts containing chaotropic anions are weakly hydrated and they exhibit a small change in viscosity with concentration, having a negative Jones-Dole B coefficient. Taking into account potential chromatographic application, this property is very advantageous, as it makes penetration of sorbent and equilibration of sorption–desorption processes much easier. Among the analyzed salts used as a mobile phase modifier, the best result according to the values of asymmetry factor and the theoretical plate number was obtained for thiocyanate and nitrate ions. Considering their physicochemical properties, it should be stressed that these ions possess the highest polarizability, reflecting their significant contribution in dispersion forces. A common property of these ions is also a non-spherical structure in comparison to spherical ions, such as halides and perchlorates.

Table III(C). R_f, As, N/m Values for Investigated Compounds Obtained on Cellulose with Different Mobile Phases Modified with Ammonium Thiocyanate

	2-propanol–water (6:4)								
	0mM NH_4SCN			20mM NH_4SCN			80mM NH_4SCN		
No.	R_f	As	N/m	R_f	As	N/m			
1	0.73	0.8	6667	0.73	1.0	7456	0.75	0.8	24644
2	0.03	0.9	14178	0.48	0.9	5556	0.57	0.9	7844
3	0.43	1.0	6167	0.49	0.9	5311	0.50	0.9	10144
4	0.41	1.0	1533	0.43	0.9	2522	0.43	–	–
5	0.57	0.9	1533	0.60	1.1	3311	0.61	0.9	8144
6	0.69	0.9	2600	0.62	0.9	3022	0.62	0.9	7456
7	0.06	0.8	2222	0.41	0.8	3022	0.45	0.9	9622
8	0.48	0.9	200	0.51	1.0	2222	0.52	1.1	10411
9	0.36	0.9	989	0.41	1.0	5678	0.42	0.8	13133
10	0.36	0.9	1344	0.40	1.1	3778	0.39	0.8	17800
11	0.06	–	–	0.36	0.8	2900	0.43	1.0	13500
12	0.43	0.9	833	0.44	0.8	5556	0.44	0.9	10956
13	0.42	1.0	300	0.21	0.8	1544	0.22	–	–
14	0.46	1.1	3022	0.48	1.1	6167	0.49	0.8	10411
15	0.02	–	–	0.41	0.8	2900	0.45	0.9	12078
16	0.60	–	–	0.24	–	–	0.21	–	–
17	0.65	0.9	900	0.67	1.0	3622	0.68	1.1	8289
18	0.58	1.1	2467	0.59	0.8	2878	0.61	1.1	9622
19	0.67	0.9	1700	0.34	0.7	3556	0.34	–	–

* (–) Criterion for asymmetry or theoretical plates not satisfied. T = tailing spot.

In searching for the system with the best efficiency, different salts were added in various concentrations to mobile phases containing different organic modifiers. The influence of different salt concentrations on the peak symmetry and system efficiency can be estimated by comparison of values of N/m and A_s given in Table IV and in Figure 4. As can be seen with the increasing of the salt concentration, improvement of efficiency and peak symmetry occurs. Generally, the advantageous effects of additives were observed only up to 80mM, after which lowering of the system efficiency appeared.

The best improvement of peak characteristic was observed for the following amino acids: lysine, ornithine, proline, serine, histidine, arginine, and methionine.

The retention of amino acids decreases with increasing salt concentration in the mobile phase. This effect is connected with a salting-in effect, especially visible for chaotropic salts (thiocyanates, nitrates) at low salt concentrations. Figure 5 shows the dependency of R_f values on

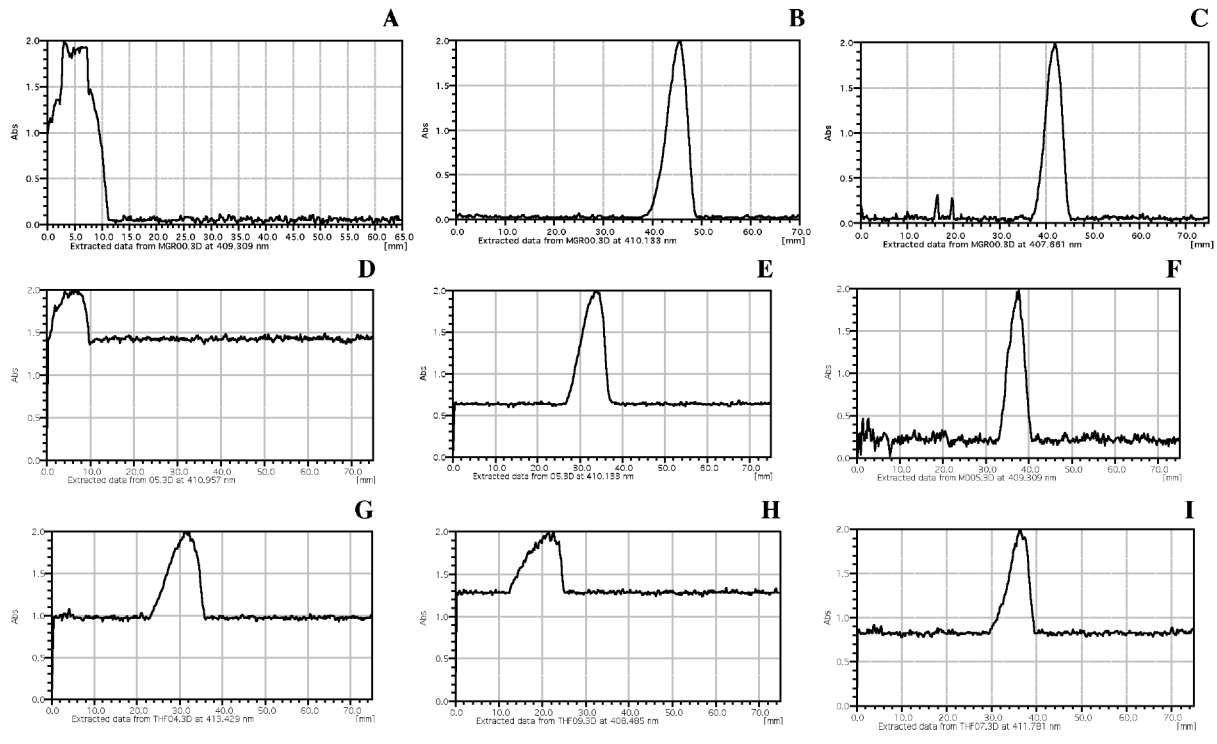


Figure 4A. Densitograms of chosen amino acid standards obtained on cellulose by the use of different eluent systems modified salt additives. Lysine: ACN–H_2O (6:4) (A); ACN–H_2O (6:4)–30mM NH_4SCN (B); ACN–H_2O (6:4)–40mM NH_4SCN (C); Ornithine: 2-propanol–H_2O (6:4) (D); 2-propanol–H_2O (6:4)–20 mM NH_4SCN (E); 2-propanol–H_2O (6:4)–80mM NH_4SCN (F); Histidine: THF–H_2O (6:4) (G); THF–H_2O (6:4)–10mM NH_4SCN (H) THF–H_2O (6:4)–20mM NH_4SCN (I).

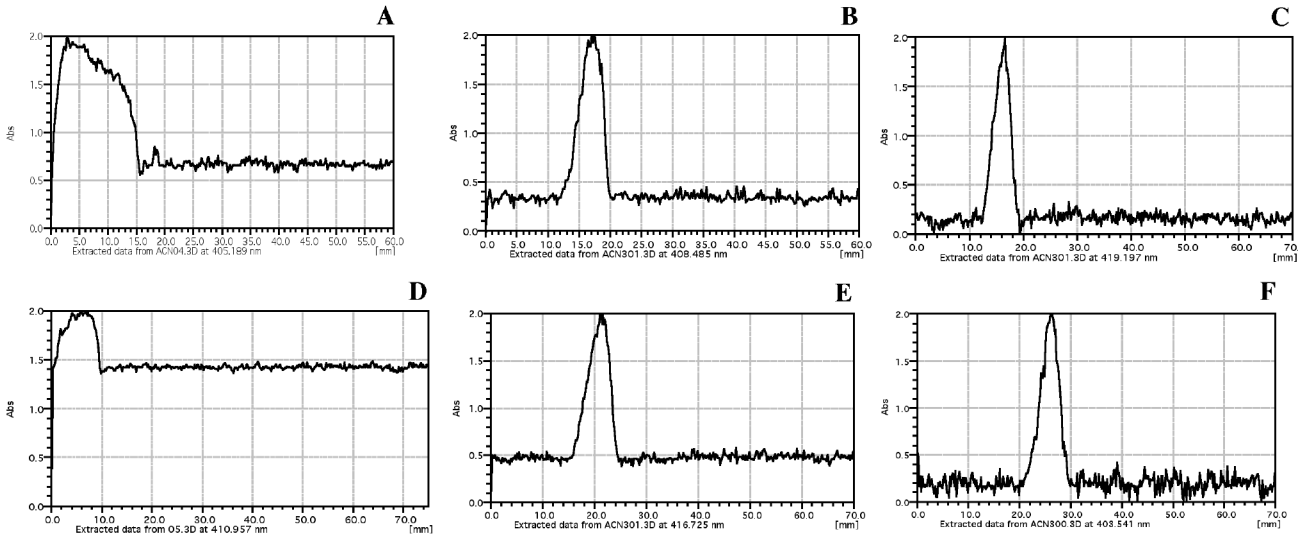


Figure 4B. Densitograms of chosen amino acid standards obtained on cellulose by the use of different eluent systems modified salt additives. Ornithine: ACN–H_2O (7:3) (A); ACN–H_2O (7:3)–40mM NH_4NO_3 (B) ACN–H_2O (7:3)–80mM NH_4NO_3 (C); and Ornithine: 2-propanol–H_2O (6:4) (D); 2-propanol–H_2O (6:4)–20mM NH_4NO_3; (E) 2-propanol–H_2O (6:4)–80mM NH_4NO_3 (F).

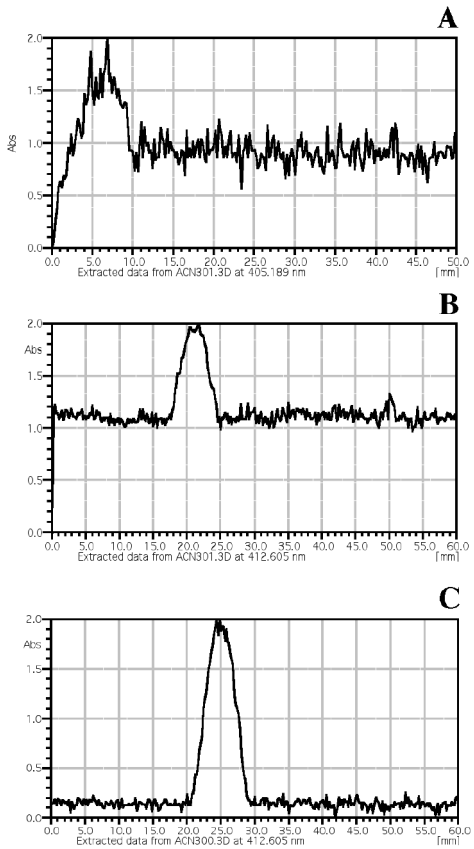


Figure 4C. Densitograms of chosen amino acid standards obtained on cellulose by the use of different eluent systems modified salt additives. Ornithine: 2-propanol–H_2O (5:5) (A); 2-propanol–H_2O (5:5)–50mM $NaClO_4$; 2-propanol–H_2O (5:5)–80 mM $NaClO_4$ (C) As = 1.0; N/m = 5111.

the concentration of salts ammonium thiocyanate and sodium chloride added to the mobile phase for chosen amino acids: histidine, lysine, and arginine.

Resolution of amino acid mixture by the use of salting-in TLC effect

The system containing ammonium thiocyanate in organic–aqueous mobile phase is the most efficient for the separation of amino acid mixtures. Single development enables resolution eight of them whereas the incremental multiple development (IMD) technique can improve separation efficiency. The results obtained for the acetonitrile–water mobile phase are illustrated in the densitograms shown in Figure 6A and 6B. As has been observed, amino acids with nonpolar long chains like valine, methionine, phenylalanine, tryptophan, leucine, and isoleucine exert strong affinity to the mobile phase, which is connected either with higher solubility in organic solvent or the presence of chaotropic cosolvent in the mobile phase. Polar substituents or shorter nonpolar chains in the molecules of such amino acids as praline, arginine, alanine, histidine, and lysine involve in turn higher solubility in polar stationary phase and stronger retention.

Conclusion

In spite of the fact that many laboratory techniques have been exercised for the determination of amino acids, sensitive analytical methods for the resolution of amino acid mixtures are still required, considering their excellent diagnostic role.

The benifits of applying salting in TLC are: (*i*) fast, owing to organic-aqueous mobile phase with polar sorbent; (*ii*) efficient, thanks to chaotropic cosolvents additives; (*iii*) and sufficiently selective, by changing the mode of development (IMD). Adding chaotropic (thiocyanates and nitrates) and neutral (cholides and iodides) cosolvents leads to the improvement of efficiency of the chromatographic system, causing an increase of theoretical plate number. The peaks which are wide and asymmetric become narrow, with satisfied symmetry factor between 0.8–1.2.

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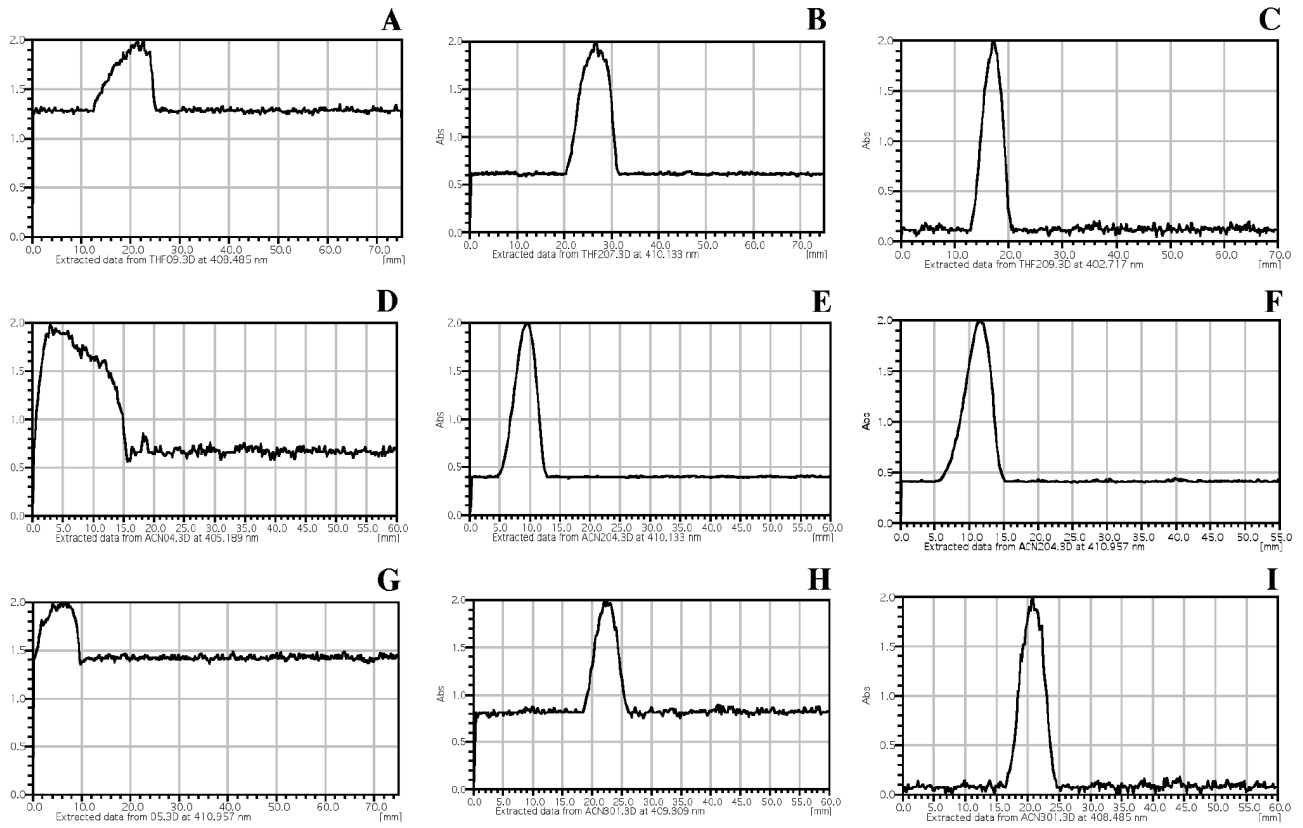

Figure 4D. Densitograms of chosen amino acid standards obtained on cellulose by the use of different eluent systems modified salt additives. Histidine: THF–H_2O (6:4) (A); THF–H_2O (6:4)–20mM NaCl (B); THF–H_2O (6:4)–50mM NaCl (C); and Ornithine: ACN–H_2O (7:3) (D) ACN–H_2O (7:3)–40mM NaCl (E) ACN–H_2O (7:3)–80mM NaCl (F); and Ornithine: 2-propanol–H_2O (6:4) (G) 2-propanol–H_2O (6:4)–40mM NaCl (H); 2-propanol–H_2O (6:4)–80mM NaCl.

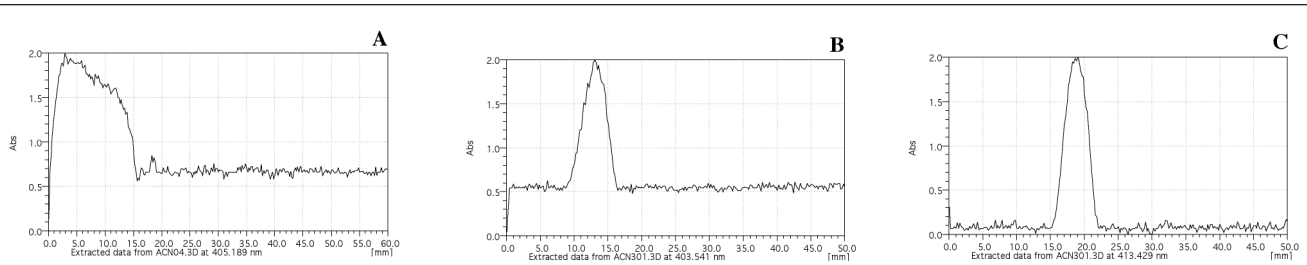

Figure 4E. Densitograms of chosen amino acid standards obtained on cellulose by the use of different eluent systems modified salt additives. Ornithine: ACN–H_2O (7:3) (A); ACN–H_2O (7:3)–30mM KI (B) ACN–H_2O (7:3)–60mM KI (C) As = 0.86; N/m = 5756.

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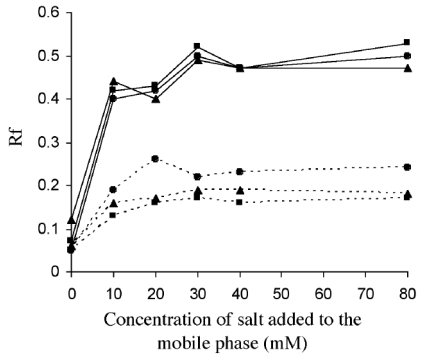

Figure 5. Graphical comparison of Rf values obtained for lysine (■), arginine (•) and histidine (▲) in chromatographic systems: cellulose, acetonitrile–water (6:4) modified with NH_4SCN (-) and NaCl (…).

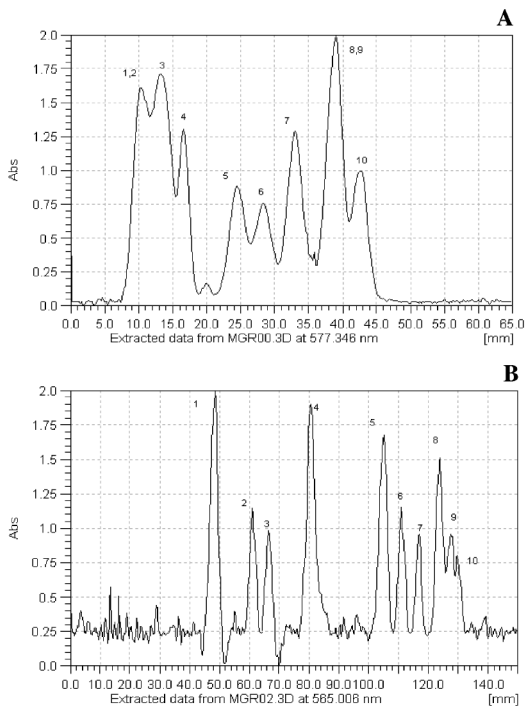

Figure 6. Densitograms of cellulose plates with the separated mixture of selected amino acid standards: 1, lysine; 2, histidine; 3, alanine; 4, arginine; 5, proline; 6, valine; 7, methionine; 8, phenylalanine; 9, leucine; and 10, isoleucine. Single development with ACN–H_2O (8:2) containing 40mM of NH_4SCN (A). Incremental multiple development (IMD) with eight developments by the use of ACN–H_2O (8:2), ninth development using ACN–H_2O (8:2) containing 40mM of NH_4SCN, development increment 20 mm, total development distance 180 mm (B).

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