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ORIGINAL RESEARCH PAPER

Pressurized planar electrochromatography of DNS amino acids derivatives in silica gel and silanized silica gel systems with formic acid addition to the water mobile phase

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Abstract
Pressurized planar electrochromatography (PPEC) of dansyl (DNS) derivatives of amino acids in normal- and reversed-phase systems is presented. The results have been obtained for mobile phases with different acetonitrile (ACN) concentrations (0–85%). The data obtained show differences in separation selectivity between high-performance thin-layer chromatography (HPTLC) and PPEC systems. These differences originate from the electrophoretic effect which is involved in the PPEC system, contrary to the HPTLC one.

Keywords Pressurized planer electrochromatography · PPEC · Amino acids · Dansyl amino acids

1 Introduction

Amino acids are a significant group of biochemical compounds. They have many industrial applications, especially in the pharmacy or nutrition industry. Moreover, the determination of the amino acids is used for the diagnosis of various diseases, such as cancer, neurological disorders, metabolic diseases, disorders in the functioning of the liver or kidneys [1–6]. Chromatographic techniques are widely used in the study and analysis of the samples related to these aspects. High-performance liquid chromatography (HPLC) and capillary electrophoresis techniques are characterized by high efficiency and short separation time [7–12]. Thin-layer chromatography (TLC/HPTLC) of amino acids [13–20] and their dansyl (DNS) derivatives [21–27] has been widely described in the scientific literature. Pressurized planar electrochromatography (PPEC) is also included in the current research on amino acid separation. This technique is attractive to that application due to its short separation time and high performance [28–38]. The different separation selectivity in comparison with liquid chromatography and electrophoresis is also an essential advantage of PPEC [35, 36, 39, 40].

Polak et al. [41–45] reported the use of PPEC to separate amino acids enantiomers and diastereoisomers. In previous papers by our group, we reported two-dimensional separation of some amino acids by HPTLC and PPEC on HPTLC RP-18W plates [39] and the comparison of separation selectivity of 20 biogenic amino acids in TLC and PPEC in systems with silica gel and water mobile phase [40].

Gwarda et al. [46–50] presented extensive research on peptides chromatography in HPTLC and PPEC systems with silica and silica-based C18 stationary phases. They reported interesting data on the solutes retention and selectivity depending on ion-pairing reagent in the mobile phase [47, 49]. According to these papers, the addition of trifluoroacetic acid (TFA) to the mobile phase led to a considerable reduction of peptide tailing zones. It was revealed that TFA addition was more effective for RP-HPTLC peptide separation than formic acid (FA) [46]. However, the TFA addition resulted in high electric current and high Joule heat generation during the PPEC process [48]. Based on these data, it was interesting to apply analogous systems to separation of DNS amino acids derivatives. However, in order to avoid the mentioned problems with high current and Joule heat generation in PPEC systems, the FA addition to the mobile phase was applied in the research presented.

This paper stands for a continuation of the research mentioned above and shows differences in the separation

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selectivity of DNS amino acids between HPTLC and PPEC systems.

2 Experimental

2.1 Materials used

All reagents used were of analytical grade. Acetonitrile, acetone, diethyl ether, formic acid 98–100%, sodium bicarbonate, hydrochloric acid (35–38%), sodium sulfate were supplied by POCh (Gliwice, Poland), dansyl chloride was from Sigma–Aldrich (St Louis, MO, USA). The deionized water was produced in the department using demineralizer HLP 5 (Hydrolab, Straszyn, Poland). The solutions of the mobile phase were prepared by mixing acetonitrile with formic acid and deionized water. All experiments were performed with HPTLC silica gel 60 F_{254s} and HPTLC RP-18W plates, both from Merck (Darmstadt, Germany). The amino acids investigated were tyrosine (Tyr), glycine (Gly), alanine (Ala), asparagine (Asn), arginine (Arg), lysine (Lys), glutamic acid (Glu), valine (Val), phenylalanine (Phe), histidine (His), isoleucine (Ile), methionine (Met), leucine (Leu), aspartic acid (Asp), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), glutamine (Gln), cysteine (Cys), citrulline (Cit), all from Sigma-Aldrich.

2.2 Mobile phase preparation

The mobile phase solutions were prepared by adding formic acid to deionized water or to acetonitrile and deionized water mixtures (the final concentration of FA in the mobile phase was equal to 265 mmol/dm^3).

2.3 DNS derivatives of amino acids

DNS derivatives of amino acids were obtained according to the LeFevre procedure [51, 52]. A quantity of 2 mg of each amino acid was dissolved in 6.7 mL of 0.2 M sodium bicarbonate. The obtained solution was mixed with 6.7 mL of 5.5 mM dansyl chloride; the solution pH was within the optimal range for the dansylation reaction (8.5–10.5), and shaken for 1 min, then allowed to stay for 90 min in the dark at room temperature. After this time, hydrochloric acid was added until the pH was equal to 4.0. Then, the obtained samples were extracted three times by diethyl ether. The obtained solutions were combined and filtered through anhydrous sodium sulfate crystals, and then the solution was allowed to evaporate the solvent. The obtained DNS amino acids were dissolved in 2 mL of acetone.

2.4 Pressurized planar electrochromatography

2.4.1 Plate preparation

The margins of 5 mm width of impregnating agent comprised both Sarsil W and Sarsil H50 (Zakłady Chemiczne "Silikony Polskie," Nowa Sarzyna, Poland) were produced on the whole periphery of adsorbent layer of the plates [37]. Before experiments, the HPTLC silica gel 60 F_{254s} and HPTLC RP-18W plates were washed by dipping in methanol for 1 min and dried in the air and in the oven at temperature 105–110 °C for 15 min. Solutions of the amino acids were applied on the plate using Automatic TLC Sampler (CAMAG, Muttenz, Switzerland). The distance of the marginal starting spots to both left and right chromatographic plate side edges was equal to 15 mm, and the distance of start line from lower chromatographic plate edge was 16 mm.

2.4.2 Pressurized planar electrochromatography procedure

All PPEC experiments were performed with a PPEC chamber designed for 10 cm × 20 cm plates [53] according to the same procedure as presented in the previous work [40]. All PPEC experiments were performed in triplicate.

2.5 Detection and documentation

Chromatograms were taken with TLC Visualizer (CAMAG). The retardation factor values were determined with a VideoScan TLC/HPTLC evaluation software (CAMAG).

3 Results and discussion

The data of migration distance of the solutes depending on the acetonitrile concentration, in the range 0–40% (HPTLC silica gel 60 F_{254s} plates) and 10–85% (HPTLC RP-18W plates) in aqueous formic acid solution, are presented in Tables 1 and 2, respectively.

For silica gel plates (Table 1), a significant increase in the migration distance of DNS amino acids was observed with increasing concentration of acetonitrile in the mobile phase in the range of 0–25% (an increase between 9.3 mm for DNS-Cit and 43.8 mm for DNS-Cys). This is in line with the previous report [54]. For 25% ACN in the mobile phase compared to 20%, a reduction of the difference in the migration distance of DNS amino acids was observed (migration distance differences for 20% ACN ≤39.4 mm; for 25% ≤27 mm). Regarding the higher concentrations of 30% and 40% ACN, the migration distance of DNS amino

Table 1 The DNS amino acid migration distances (mm) in PPEC systems with HPTLC silica gel 60 F_{254s} plates (Merck) and acetonitrile in the concentration range from 0 to 40% in water–formic acid solution (the final concentration of formic acid in the mobile phase was equal to 265 mmol/dm^3), polarization voltage 0.500 kV, separation time 15 min

% Acetonitrile	0%	10%	20%	25%	30%	40%
DNS-Tyr	33.4	39.6	53.2	56.4	51.3	46.8
DNS-Gly	35.5	44.3	53.8	58.9	50.6	47.4
DNS-Ala	32.8	42.3	52.6	58.9	51.3	47.6
DNS-Asn	25.8	32.4	40.0	47.8	51.8	47.9
DNS-Arg	37.5	49.65	64.5	62.4	52.1	48.7
DNS-Lys	11.7	23.6	33.2	45.6	51.5	49.0
DNS-Glu	35.7	42.0	47.9	54.6	51.9	49.5
DNS-Val	26.1	39.2	46.4	56.1	52.1	51.0
DNS-Phe	22.9	35.8	44.5	53.4	52.1	51.4
DNS-His	7.1	18.7	25.7	35.4	52.5	51.8
DNS-Ile	23.7	37.4	47.9	54.9	51.7	52.2
DNS-Met	24.4	38.0	47.1	55.3	52.1	52.1
DNS-Leu	22.5	37.1	47.2	52.8	53.2	52.4
DNS-Asp	35.5	42.7	50.1	57.3	54.4	52.6
DNS-Pro	19.2	36.6	46.6	55.3	54.6	51.9
DNS-Ser	35.0	46.2	52.4	59.3	56.0	51.1
DNS-Thr	19.2	33.9	43.3	50.6	56.0	50.6
DNS-Trp	23.0	30.3	46.7	55.4	56.5	49.7
DNS-Gln	37.8	57.9	65.4	56.1	55.6	48.7
DNS-Cys	11.0	28.1	40.1	54.8	53.3	45.9
DNS-Cit	44.3	58.81	62.2	53.6	53.0	44.4

Table 2 The DNS amino acid migration distances (mm) in PPEC systems with HPTLC RP-18W plates (Merck) and acetonitrile in the concentration range from 10 to 85% in water–formic acid solution (the final concentration of formic acid in the mobile phase was equal to 265 mmol/dm^3), polarization voltage 0.500 kV, separation time 15 min

% Acetonitrile	10%	25%	40%	55%	70%	85%
DNS-Tyr	7.24	15.02	27.76	31.11	18.77	35.40
DNS-Gly	9.12	17.97	24.94	25.48	14.35	33.52
DNS-Ala	8.32	15.29	23.06	27.36	14.75	34.06
DNS-Asn	6.44	9.79	17.57	28.16	14.62	34.06
DNS-Arg	5.63	7.64	32.05	27.36	16.36	35.94
DNS-Lys	1.07	3.62	8.45	10.59	13.54	34.73
DNS-Glu	8.85	18.64	25.48	24.94	14.22	33.12
DNS-Val	5.10	7.78	15.69	20.65	14.22	34.46
DNS-Phe	2.55	6.17	11.40	18.51	14.22	33.52
DNS-His	0.81	3.22	8.32	27.76	13.68	32.19
DNS-Ile	3.09	5.63	12.74	20.25	13.68	34.33
DNS-Met	4.56	7.24	15.15	20.12	13.68	32.99
DNS-Leu	2.95	5.90	10.59	19.58	13.68	34.87
DNS-Asp	9.12	17.30	25.48	27.09	14.35	29.50
DNS-Pro	4.02	7.78	15.83	22.80	13.54	34.33
DNS-Ser	9.93	20.65	30.58	26.15	13.68	31.38
DNS-Thr	2.68	5.63	9.79	25.75	14.88	31.38
DNS-Trp	2.41	6.97	15.69	23.20	15.02	35.67
DNS-Gln	10.73	20.88	36.07	25.61	16.62	35.94
DNS-Cys	1.07	7.78	10.86	16.90	10.59	29.36
DNS-Cit	4.29	19.84	30.84	26.55	18.64	34.19

acids was of low diversity (migration distance differences for 30% ≤5.9 mm; for 40% ≤8.2 mm). The exemplary electrochromatogram of DNS amino acids is presented in Fig. 1.

In Fig. 2, the correlation of retardation factor values of the DNS amino acids in HPTLC silica gel system with formic acid–water solution (265 mmol/dm^3) [54] against their

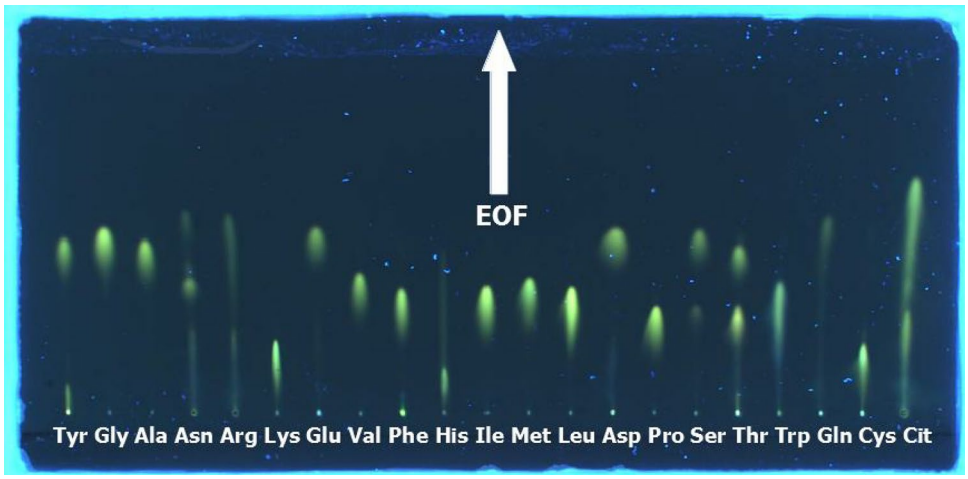

Fig. 1 Electrochromatogram of DNS amino acids, mobile phase: water solution of formic acid, the final concentration of formic acid in the mobile phase was equal to 265 mmol/dm^3, HPTLC silica gel 60 F_{254}s plate from Merck, polarization voltage 0.500 kV, separation time 15 min (EOF – electroosmotic flow)

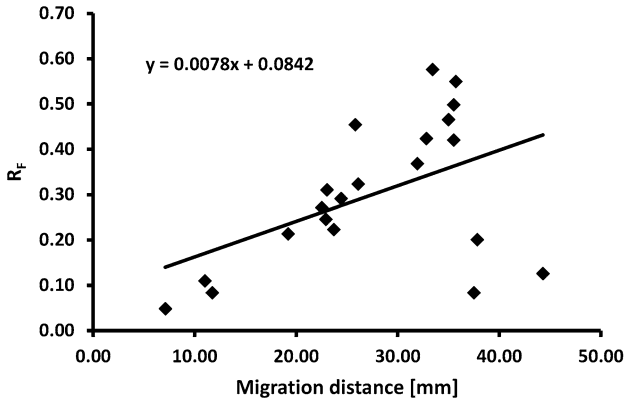

Fig. 2 Comparison of the R_F values of the DNS amino acids in HPTLC system with the migration distances in PPEC one. The silica gel 60 F_{254s} plates and formic acid–water solution (265 mmol/dm^3) were used in both techniques

migration distances values in PPEC system comprising the same adsorbent layer and mobile phase is presented. It is shown that the separation selectivities produced by the two systems are different ($R = 0.4796$). It means that the electrophoresis effect involved in the PPEC process significantly altered the separation of the solutes in comparison with HPTLC. It should be noted that despite the low pH of the mobile phase (formic acid in the mobile phase), the data obtained suggest that the acid groups of DNS amino acids were at least partially dissociated. So DNS amino acids' electrophoretic mobility affected the change in separation selectivity in PPEC in comparison with that in HPTLC.

For RP-18W plates (Table 2), an increase in the migration distance of DNS amino acids was observed with increasing concentration of acetonitrile in the mobile phase in the range of 10−55% (an increase between 9.52 mm for DNS-Lys and 26.95 mm for DNS-His). It is characteristic of the reversed-phase system. However, for 70% ACN in the mobile phase compared to 55%, except DNS-Lys, the solute migration distances decreased (a decrease between 5.9 mm for DNS-Leu and 14.80 mm for DNS-His), and in addition, they migrated distances of low diversity (migration distance differences for 55% ACN ≤ 20.52 mm; for 70% ≤ 8.18 mm). For 85% ACN in the mobile phase compared to 70% ACN, solute migration increased (an increase between 15.15 mm for DNS-Asp and 20.79 mm for DNS-Pro), and all DNS amino acids showed minor diversity in the migration distances (migration distance differences ≤6.58 mm) similarly as for 70% ACN. The discussed effect can be concerned with change of the stationary phase structure, which is dependent on modifier concentration in the mobile phase. For lower ACN concentration in the mobile phase, C18 ligands of the adsorbent surface are expelled from the mobile phase and for higher ACN concentration are embedded in it. Then, in the latter case, the adsorbent capacity of the stationary phase increases in comparison with the former. Similar effects have been previously described in publications [55, 56]. The exemplary electrochromatogram of DNS amino acids is presented in Fig. 3.

Correlation of the data obtained for HPTLC RP-18W system [54] with those for PPEC RP-18W, one revealed substantial changes of separation selectivity of the solutes. It is exemplified in Fig. 4 where the retardation factor values of the solutes investigated in HPTLC system are plotted against migration distances values in the PPEC one (the mobile phase comprised 40% ACN in FA water solution, $y = 0.0084x + 0.1118$; $R = 0.656$). These data evidence that the electrophoretic effect significantly changes the separation selectivity in the PPEC system in comparison with that in the HPTLC one.

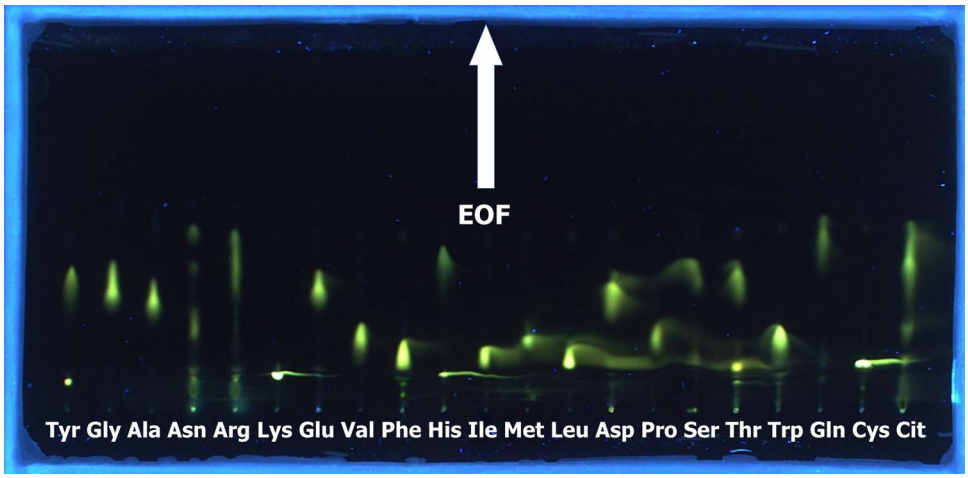

Fig. 3 Electrochromatogram of DNS amino acids, mobile phase: 40% ACN in water–formic acid solution (the final formic acid concentration in the mobile phase was equal to 265 mmol/dm^3), HPTLC RP-18W plates from Merck, polarization voltage 0.500 kV, separation time 15 min (EOF – electroosmotic flow)

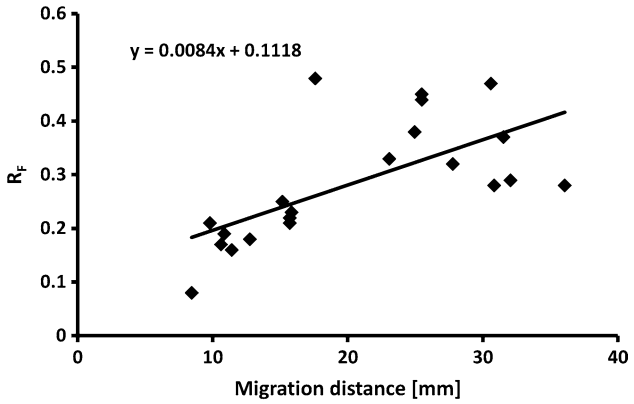

Fig. 4 Comparison of the R_F values of the DNS amino acids in HPTLC with the migration distances in PPEC. The RP-18W plates and the mobile phase comprised 40% ACN in water–formic acid solution (the final formic acid concentration in the mobile phase was equal to 265 mmol/dm^3)

4 Conclusion

The data obtained show differences in the separation selectivity between HPTLC and PPEC systems with silica gel 60 F_{254s} and RP-18W plates as the stationary phase. In PPEC, the electrophoretic effect is responsible for considerable separation selectivity differences relative to the HPTLC. It is an important issue because the data obtained lead to presume that the combination of HPTLC and PPEC in a two-dimensional separation process should substantially enhance DNS amino acid separation.

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