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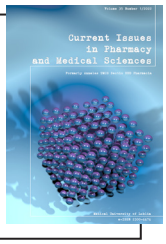

Thin-layer chromatography of DNS amino acids derivatives in systems with silica gel and silanized silica gel plates

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ABSTRACT

Investigations into separation selectivity of High-Performance Thin-layer chromatography (HPTLC) of dansyl (DNS) derivatives of amino acids in silica gel (Silica gel 60 F_{254s} plates) and silanized silica gel (RP-18 W plates) systems are presented. The results have been obtained for mobile phases containing different concentrations of acetonitrile (ACN) in formic acid (FA) water solution (final concentration of FA in the mobile phase was equal to 265 mmol/dm^3). The data obtained show differences in separation selectivity of the solutes between employment of HPTLC silica gel and RP-18 W systems.

INTRODUCTION

Isolation and separation of amino acids is a current problem, especially in biomedical analysis. Amino acids determination is used for the diagnosis of various diseases, such as cancer, neurological disorders, metabolic diseases, as well as disorders in the functioning of the liver or kidneys [1-6]. These studies can also be used to monitor the progress of patients treatment by determining the effect of metabolites of various drugs on the human body.

Electrophoresis, high-performance liquid chromatography (HPLC), ion exchange chromatography (IEC) and thin-layer chromatography (TLC) are commonly used for amino acid separation. However, it is still challenging to obtain a good resolution of all 20 biogenic amino acids, especially with planar techniques.

Silica gel and cellulose adsorbent layers are most often employed in TLC [7-12]. For separation of amino acids in the normal phase system, two or three components mobile phase are usually used. The most popular mobile phase components are ethanol, butanol, water, acetic and formic acid. Sleckman and Sherma comprehensively compared the separation of amino acids on silica gel, cellulose and ion exchange plates [11]. Vasta *et al*. [12] also presented comprehensive research on the separation of amino acids. Reversed-phase TLC is, as well, extensively used for the separation of amino acids, e.g. reports by Sleckman and Sherma [13] and Baranowska and Kozłowska [14]. Polak *et al*., in turn, reported on the application of pressurized planar electrochromatography (PPEC) to separate the amino acids enantiomers, and diastereoisomers [15-19]. In previous papers, our group contributed an exploration into two-dimensional separation of some amino acids by HPTLC and PPEC on HPTLC RP 18W plates [20] and the comparison of separation selectivity of 20 biogenic amino acids in TLC and PPEC in systems with silica gel and water mobile phase [21].

In literature, there is many examples of one and two dimensional DNS amino acids TLC separations. In such work, silica gel and polyamide adsorbent layers have been most commonly used as stationary phases [22-27]. Indeed, Crowshaw *et al*. [25], Bhushan and Reddy [23] published extensive research on the separation of DNS amino acid derivatives in the normal phase system (silica gel). Still, the TLC/HPTLC reverse phase system is not popular/widely used for the separation of DNS derivatives [28], and it is specially difficult to find any publications concerning DNS aminoacids separation on RP-18W plates. However, there are more examples of the separation of DNS amino acids enantiomers using these plates [19,29-31].

Unfortunately, the majority of the cited articles were published many years ago. Nowadays, for TLC/HPTLC, new adsorbent layers are available, such as monolithic thin-layer sorbents based on silica gel (MERCK) or sorbents of greater/better purity e.g. MS-Grade Plates (MERCK) dedicated to coupling TLC plates with mass spectrometry (TLC-MS). Therefore, it is reasonable to conduct further research on the separation of amino acids and their derivatives.

EXPERIMENTAL

Materials used

Acetonitrile (analytical grade), acetone (analytical grade), diethyl ether (analytical grade), formic acid 98-100% (analytical grade), sodium bicarbonate (analytical grade),

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hydrochloric acid (35-38% analytical grade), sodium sulfate (analytical grade) were supplied by POCh (Gliwice, Poland); dansyl chloride (analytical grade) from Sigma–Aldrich (St Louis, USA). The deionized water was produced in the department using a 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-18 W 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) and citrulline (Cit), all from Sigma-Aldrich (St Louis, USA).

DNS derivatives of amino acids

DNS derivatives of amino acids were obtained according to the LeFevre procedure [32,33]. 2 mg of each amino acid was dissolved in 6.7 ml of 0.2 M sodium bicarbonate. The obtained solution was subsequently mixed with 6.7 mL of 5.5 mM dansyl chloride, the solution pH was then modified to be within the optimal range for the dansylation reaction (8.5-10.5), and was shaken for 1 min, then allowed to settle for 90 min in the dark, at room temperature. After this time, hydrochloric acid was added until the pH equal to 4.0. Following this, the obtained samples were three times extracted by diethyl ether. The obtained solutions were combined and filtered through anhydrous sodium sulfate crystals, and the solution was allowed to evaporate the solvent. The obtained DNS amino acids were then dissolved in 2 ml of acetone.

TLC

All TLC experiments were performed with a DS-II-20x10 chamber from Chromdes (Lublin, Poland). Before use, the plates were washed by dipping in methanol for 1 minute and dried in the air for 1 min and in the oven at a temperature range of 105-110°C for 15 min. Solutions of the twenty-one DNS amino acids were applied on both opposite sides of the plate using Automatic TLC Sampler (CAMAG, Switzerland) at the distance of 8 mm from the edge. The chamber atmosphere and chromatographic plate therein were conditioned for 15 minutes before chromatogram development. All TLC experiments were performed in triplicate.

Detection and documentation

Chromatograms were taken with TLC Visualizer (CAMAG, Switzerland). The retardation factor values were determined using VideoScan TLC/HPTLC Evaluation Software (CAMAG, Switzerland).

RESULTS AND DISCUSSION

The retardation factor values of DNS amino acids, dependent on the concentration of acetonitrile in the water mobile phase in systems with HPTLC Silica gel 60 F_{254} and HPTLC RP-18 W plates, are presented in Table 1 and 2, respectively.

Table 1. DNS amino acids R_f values in a HPTLC system with Silica gel 60 F_{254s} plates (Merck) and acetonitrile in the concentration range from 0% to 50% in water formic acid solution (final concentration of FA in the mobile phase equal to 265 mmol/dm^3)

% acetonitrile	0%	10%	20%	30%	50%
DNS-Tyr	0.58	0.74	0.75	0.77	0.76
DNS-Gly	0.42	0.60	0.71	0.67	0.75
DNS-Ala	0.42	0.60	0.72	0.67	0.75
DNS-Asn	0.45	0.70	0.83	0.75	0.76
DNS-Arg	0.08	0.71	0.76	0.75	0.77
DNS-Lys	0.08	0.12	0.68	0.75	0.78
DNS-Glu	0.55	0.68	0.71	0.67	0.77
DNS-Val	0.32	0.61	0.69	0.67	0.79
DNS-Phe	0.25	0.56	0.71	0.75	0.79
DNS-His	0.05	0.08	0.66	0.74	0.78
DNS-Ile	0.22	0.60	0.69	0.75	0.78
DNS-Met	0.29	0.61	0.70	0.68	0.76
DNS-Leu	0.27	0.59	0.67	0.74	0.75
DNS-Asp	0.50	0.65	0.72	0.65	0.75
DNS-Pro	0.21	0.52	0.65	0.63	0.73
DNS-Ser	0.47	0.63	0.73	0.66	0.71
DNS-Thr	0.37	0.59	0.69	0.66	0.70
DNS-Trp	0.31	0.60	0.69	0.79	0.70
DNS-Gln	0.20	0.72	0.76	0.79	0.76
DNS-Cys	0.11	0.58	0.74	0.76	0.70
DNS-Cit	0.13	0.67	0.84	0.76	0.72

Table 2. DNS amino acids R_f values in a HPTLC system with RP-18 W plates (Merck) and acetonitrile in the concentration range from 10% to 85% in water formic acid solution (final concentration of FA in the mobile phase equal to 265 mmol/dm^3)

% acetonitrile	10%	25%	40%	55%	70%	85%
DNS-Tyr	0.08	0.07	0.32	0.41	0.64	0.70
DNS-Gly	0.10	0.20	0.38	0.45	0.61	0.68
DNS-Ala	0.08	0.27	0.33	0.40	0.61	0.68
DNS-Asn	0.13	0.22	0.48	0.53	0.63	0.68
DNS-Arg	0.11	0.35	0.29	0.37	0.59	0.69
DNS-Lys	0.01	0.20	0.08	0.19	0.52	0.68
DNS-Glu	0.14	0.01	0.44	0.52	0.64	0.67
DNS-Val	0.05	0.33	0.22	0.34	0.59	0.69
DNS-Phe	0.03	0.12	0.16	0.32	0.57	0.69
DNS-His	0.11	0.07	0.37	0.40	0.33	0.14
DNS-Ile	0.03	0.28	0.18	0.32	0.57	0.70
DNS-Met	0.03	0.14	0.25	0.38	0.60	0.70
DNS-Leu	0.03	0.07	0.17	0.30	0.59	0.70
DNS-Asp	0.14	0.36	0.45	0.52	0.64	0.67
DNS-Pro	0.03	0.11	0.23	0.32	0.57	0.67
DNS-Ser	0.15	0.38	0.47	0.52	0.63	0.66
DNS-Thr	0.03	0.10	0.21	0.31	0.55	0.66
DNS-Trp	0.02	0.06	0.21	0.30	0.59	0.69
DNS-Gln	0.10	0.18	0.28	0.36	0.60	0.67
DNS-Cys	0.00	0.03	0.19	0.32	0.60	0.68
DNS-Cit	0.12	0.19	0.28	0.33	0.58	0.72

As indicated by the data obtained, the solutes demonstrate the decrease of retention with the increase of acetonitrile concentration in the mobile phase. In the normal phase system, when the stationary phase is more polar than the mobile phase, the retention of solutes based on adsorption in silica gel system should increase with concentration increase of the less polar component of the eluent. As can be seen in our data, the effect of concentration increase of the less polar solvent does not obey this rule. This suggests partition or a mixed mechanism with regard to solute retention. At low pH, about 2, of the mobile phase, the dissociation of the silanol groups of the stationary phase and carboxyl groups of the solutes is significantly suppressed. It is unlikely that under these conditions solute molecules can compete with water molecules for sites on the silica gel surface, so the notion of retardation factor increase of the solutes with concentration increase of the organic modifier with lower polarity to water is reasonable.

For the RP-18 W plates, the solutes demonstrate a retention decrease with the increase of acetonitrile concentration – this is a characteristic feature of reverse-phase systems. In comparison to silica gel systems, the RP-18 W type is characterized by more compact shape of solutes bands on the developed chromatograms.

Based on the data obtained, it can be stated that the largest diversity of solute retention, *i.e*. the largest group separation selectivity, was noticed for silica gel plates with formic acid water solution as the mobile phase. ACN addition to silica gel system leads to considerable decrease of solute retention and makes the system inappropriate for separating DNS amino acid derivatives. In contrast, for RP-18W plates, the largest group separation selectivity of the solutes was observed in the system with 40% acetonitrile in a water solution of formic acid. The correlation of the retardation factor values of the solutes obtained in the systems mentioned is presented Figure 1. The following regression equation describes it: y = 0.8508x + 0.0569. We observed that the group separation selectivity demonstrated by both systems is quite different (R=0.597), hence this means that such systems could be applied to two-dimensional separations.

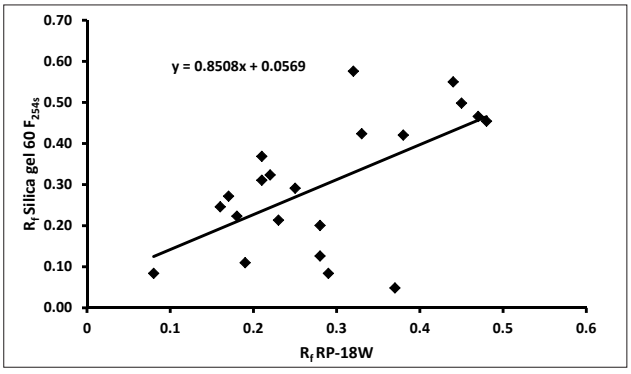

Figure 1. Comparison of retention of amino acids in a HPTLC system with Silica gel 60 F_{254s} plate and the mobile phase containing water formic acid solution (with final concentration of FA in the mobile phass equal to 265 mmol/dm^3) *vs.* HPTLC system with RP-18W plate and the mobile phase composed of 40% acetonitrile in water formic acid solution (final concentration of FA in the mobile phass equal to 265 mmol/dm^3)

CONCLUSION

The data obtained show differences in the separation selectivity between HPTLC systems with Silica gel 60 F_{254s} and RP-18 W as the stationary phase. In HPTLC Silica gel 60 F_{254s}, partition and/or mixed mechanism of solute retention was observed. The range of the solute retention change in dependence on the organic modifier (ACN) concentration was narrow. Indeed, large differences in retardation factors were observed only for a water formic acid solution as the mobile phase. Our work indicated that even a small addition of the organic modifier (10% ACN in the mobile phase) induced the majority of amino acid derivatives to migrate near the front of the mobile phase. Contrary to the silica gel systems, RP-18W systems demonstrated a wider range of the R_f coefficient dependence on organic modifier concentration in the mobile phase. The group separation selectivity between these two HPTLC systems (especially silica gel with FA water solution and RP-18 W with 40% ACN in FA water solution) is quite different, hence, such systems could be applied to two-dimensional separations.

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