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The influence of addition of ion-pairing acid and organic modifier of the mobile phase on retention and migration of peptides in pressurized planar electrochromatography system with octadecyl silica-based adsorbent

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ABSTRACT

In our previous papers we have investigated the influence of the mobile phase composition on mechanism of retention, selectivity and efficiency of peptide separation in various high-performance thin-layer chromatography (HPTLC) systems with commercially available silica-based adsorbents. We have also investigated the influence of pH of the mobile phase buffer on migration and separation of peptides in pressurized planar electrochromatography (PPEC). Here we investigate the influence of concentration of ion-pairing additive, and concentration and type of organic modifier of the mobile phase on migration of peptides in PPEC system with octadecyl silica-based adsorbent, and with the same set of the solutes as before. We compare our current results with the results obtained before for similar HPTLC and PPEC systems, and discuss the influence of particular variables on retention, electrophoretic mobility of solutes and electroosmotic flow of the mobile phase. We show, that the final selectivity of peptide separation results from co-influence of all the three factors mentioned. Concentration of organic modifier of the mobile phase, as well as concentration of ion-pairing additive, affect the retention, the electrophoretic mobility, and the electroosmotic flow simultaneously. This makes independent optimization of these factors rather difficult. Anyway PPEC offers much faster separation of peptides with quite different selectivity, in comparison to HPTLC, with similar adsorbents and similar mobile phase composition. However, we also present and discuss the issue of extensive tailing of peptide zones in the PPEC in comparison to similar HPTLC systems.

1. Introduction

Pressurized planar electrochromatography (PPEC) is relatively new and constantly developed planar separation technique, which uses electric field to force the movement of the mobile phase (electroosmotic effect) and solutes (partition and electrophoretic effect) through the adsorbent layer. It is characterized by many advantages, such as: fast separation on a long distance, high performance of the system, or different selectivity in comparison to chromatographic techniques. Many examples of various modes/equipment, as well as many applications of PPEC have been presented so far [1–5]. However, there are only three papers concerning the use of this technique for peptide separation. Two of them have presented preliminary results, without detailed investigation of any separation conditions or system optimization. The first one, by Woodward et al., has been concerned with separation of peptides and oligonucleotides using a special monolithic polymer layer prepared by the authors. Anyway, as such adsorbents are not commercially available, they cannot be supposed (at least for now) to find application in common laboratory practice [6]. The second paper has been concerned with modification/improvement of PPEC equipment and preliminary results regarding system efficiency rather, than regular and detailed influence of particular variables on peptide separation and/or optimization of separation conditions [7]. The next paper has presented regular results concerning the influence of buffer pH of the mobile phase on migration distance of peptides in PPEC systems with commercial octadecyl (C18) silica-based adsorbents [8]. It proved, that influence of the mobile phase pH on selectivity of peptide separation in PPEC systems is very clear and strictly different than that in similar high-performance thin-layer

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chromatography (HPTLC) systems (including overall inversion of direction of solutes migration). These investigations have also revealed that the change of pH clearly affects the efficiency of the separation system. Therefore pH of the mobile phase is one of the main variables, which can be used for optimization of peptide separation by PPEC.

The aim of the paper is to present the influence of concentration of both ion-pairing acid and organic modifier of the mobile phase, on migration distance of peptides in PPEC systems with commercial C18 silica-based adsorbents. The significance of these variables for optimization of peptide separation has been confirmed for HPTLC systems [9–12]. They were proven to be crucial for both: efficiency of the separation system and change of selectivity of peptide separation (including overall inversion of separation system type – normal or reversed phase [9,12]. The influence of these variables on electrochromatography of peptides by PPEC may be even more complex and more important for optimization of separation conditions, as this technique combines chromatography and electrophoresis. So presence of ionic species in the mobile phase and overall polarity of this phase, may be supposed to affect not only the mechanism of retention, but also the electroosmotic flow of the mobile phase, as well as electrophoretic mobility of the solutes. Therefore, we aim to compare the influence of the variables mentioned on separation of peptides in PPEC and HPTLC, and discuss the differences. We also discuss some difficulties related to application of PPEC for peptide separation and propose possible solutions to the problems encountered.

2. Experimental

2.1. Chemicals and equipment

Ninhydrin (98%, analytical grade) was purchased from Fluka (Buchs, Switzerland). Trifluoroacetic acid (TFA, 99.5%, for biochemistry) was purchased from ACROS ORGANICS (Geel, Belgium). Acetone, methanol (analytical grade) and 1-propanol (HPLC grade) were provided by POCH (Gliwice, Poland). Glass-backed HPTLC RP–18 W plates were purchased from Merck (Darmstadt, Germany). Water used in all experiments was purified using HLP demineralizer from Hydrolab (Gdańsk, Poland). Prototype PPEC equipment, described elsewhere [7], was designed in the Department of Physical Chemistry, Medical University of Lublin (Lublin, Poland). LINOMAT 5 semi-automatic TLC sampler, TLC Scanner 4, and TLC Visualizer (with winCATS 1.4.8 software were provided by CAMAG (Muttenz, Switzerland).

2.2. Peptide standards

The synthetic peptide standards of the following sequences were used in the experiments:

1. Leu-Ile-Thr-Thr
2. Asn-Ser-Tyr-Tyr
3. Asp-Glu-Lys-Arg
4. Ser-Lys-Arg
5. Ser-Glu-Asp
6. Ser-His-His
7. Gly-Ala
8. Gly-Leu-Ile
9. Leu-Val-Val-Tyr-Pro-Trp-Thr - Spinorphin
10. Gly-Ala-Val-Ser-Thr-Ala - Necrofibrin
11. Leu-Pro-Pro-Ser-Arg - Lymphocyte activating pentapeptide
12. Tyr–Arg - Kyotorphin
13. Ala-Pro-Gly-Pro-Arg - Eneterostatin
14. c[Cys-Tyr-Phe-Gln-Asn-Cys]-Pro-Lys-Gly-NH_2 (disulfide bridge between Cys1 - Cys6)- [Lys8] Vasopressin
15. Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-NH_2 - β-neo-endorphin
16. Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH_2 - Adrenorphin
17. Asp-Arg-Val-Tyr-Ile-His-Pro-Phe - Angiotensin II
18. Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg - Bradykinin
19. Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 - Substance P
20. Thr-Lys-Pro-Arg - Tuftsin
21. Leu-Ile-Thr(p)-Thr(p) (Thr3 and Thr4 phosphorylated)
22. Leu-Ile-Tyr(p)-Tyr(p) (Tyr 3 and Tyr4 phosphorylated)

Peptides 1–13 we have synthetized in the department as described elsewhere [11]. Peptides 14–22 (purity ≥ 85%) were purchased from Lipopharm (Zblewo, Poland).

The same set of peptides was used here, as in our previous papers [8,11,12], to compare the influence of particular variables on separation of particular solutes. Peptides nos. 1–8, 21 and 22 were designed to obtain solutes of various properties (e.g. polarity, acidity/alkalinity and net charge, alkyl and/or aryl groups content). They enable to draw particular conclusions about retention/solute properties relationship. Standards 9–20 are peptides of various biological functions, found in the nature. They enable to refer overall trends of retention changes obtained for the peptides, as a whole group of compounds.

2.3. Electrochromatography of peptides

2.3.1. Preparation of chromatographic plates

Before use, the chromatographic Plates 20 × 10 cm were washed by dipping for 5 min in methanol. After that, they were dried at room temperature and activated in the oven at 105 °C for 15 min. Then they were stored in an desiccator. Additionally, for PPEC, edges of the plates were impregnated with special sealant as described elsewhere [13].

2.3.2. Application of samples

The peptides were dissolved in the mixture composed of water/methanol (1/1 v/v) to obtain 1 μg μL^{-1} solutions. 2 μL of each sample was applied 15 mm from the lower edge of the chromatographic plate as a 2 × 1 mm bands with aerosol applicator Linomat 5 at speed of 70 nL s^{-1}.

2.3.3. Development of electrochromatograms

Electrochromatograms were developed using PPEC equipment described elsewhere [7]. Before development, chromatographic plates were prewetted/conditioned for 2 min with the mobile phase. The electrical potential used was equal to 400 V (for the mobile phase containing methanol) or 600 V (for the mobile phase containing propanol), and development time was 15 min. Adsorbent layer of the chromatographic plate was pressurized by an external pressure equal to 20 bar. The temperature of the separation system was set to 25 °C. Separation systems used here were similar to those used before for HPTLC of the same set of the peptides [11,12].

2.3.4. Detection of peptides and determination of their migration distance

After development, chromatographic plates were dried at room temperature, then dipped (for about 2 s) in 2% (w/v) ninhydrin solution in acetone/methanol/glacial acetic acid (125/125/10 v/v/v) as described elsewhere [14]. After that, the plates were dried and kept in darkness at room temperature till distinct peptide zones appeared (usually, for good detection – till the next day). Then they were scanned with TLC Scanner 4 at 520 nm wavelength and