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Review

Pressurized planar electrochromatography

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ABSTRACT

Theoretical backgrounds, development, examples of separations, constructional details and principle of action of devices of pressurized planar electrochromatography (PPEC) are presented. Development of the mode is described in respect of operating variables (composition of the mobile phase, pressure exerted on adsorbent layer, mobile phase flow velocity, temperature of separating system, etc.) influencing separation efficiency (kinetic performance, repeatability, separation time). Advantages of PPEC such as high kinetic performance, short separation time and different separation selectivities, especially relative to conventional thin-layer chromatography, are described. Examples of two-dimensional separations are demonstrated to show high separation potential of the mode when combined with conventional thin-layer chromatography (TLC). The PPEC mode is in infancy stage of development, so its challenges are presented as well.

Contents

1. Introduction

Development of planar chromatography under potential (in electric field), when electroosmotic effect drives mobile phase against adsorbent layer of a chromatographic plate, can be divided into two stages. The first one is characterized by application of electric field to a chromatographic plate when gas phase is involved in separation process. Then the separation process is performed in open system. The mode has been named as planar electrochromatography (PEC) [1]. The second stage is characterized by application of electric field to the prewetted chromatographic plate under conditions of closed system, when chromatographic plate is covered with plastic foil, or plastic block, under pressure. Then the adsorbent layer is pressurized between carrier plate (usually glass) and plastic foil. So the separating system comprises two phases, stationary and mobile, what is contrary to conventional thin-layer chromatography (TLC) and planar electrochromatography in open system mentioned above. This mode has been named as pressurized planar electrochromatography (PPEC). Nurok and coworkers as the first introduced PPEC to scientific literature in 2004 [2]. In the mode electroosmotic effect drives mobile phase relative to adsorbent layer on chromatographic plate. Conceptual pictures of the devices with chromatographic plate in vertical position and horizontal position are presented in Fig. 1a and b,

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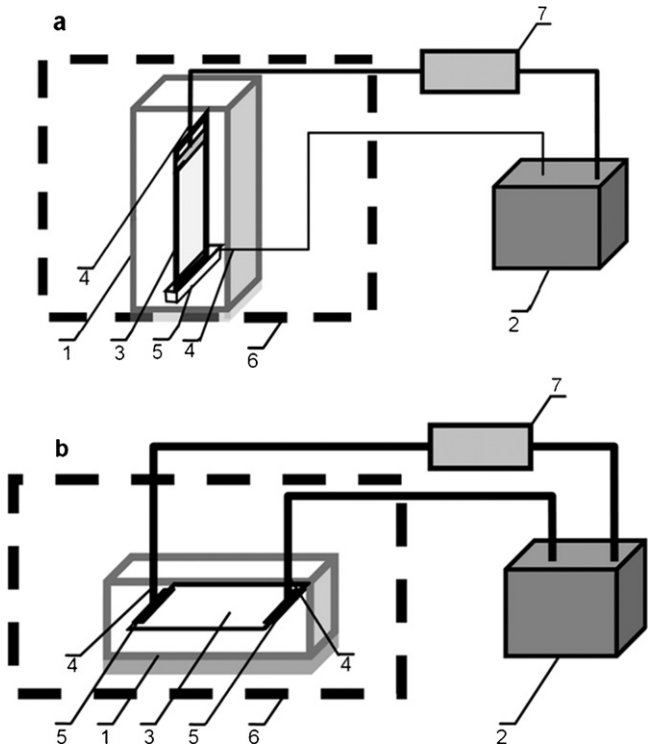

Fig. 1. Conceptual view of the device for PPEC with the chromatographic plate (a) vertically and (b) horizontally positioned in a chamber for PPEC: chamber for PPEC (1), high voltage power supply (2), chromatographic plate (3), electrodes (4), reservoir of the mobile phase (5), cabinet for PPEC chamber (6), and ammeter (7).

respectively. Nurok and coworkers [2,3] have designed the former device and Tate and Dorsey [4,5] and our group [6–9] have worked out the latter. Both device types are described in details in the paper.

Thirty years before publication of the first paper on PPEC [2] the stage of development of planar electrochromatography started with the paper by Pretorius et al. [10]. The authors used the name high-speed thin-layer chromatography for the new mode. In the paper, application of electroosmotic effect to drive mobile phase on prewetted adsorbent layer of chromatographic plate for separation of some steroids has been described. The chromatographic plate was inserted into a conventional rectangular chamber for TLC that was equipped with two electrodes; the one was dipped in solution on the bottom part of the chamber and the second was pressed against upper part of the adsorbent layer of the chromatographic plate. When polarization voltage was switched on the separation process of mentioned solutes started. The time of separation under polarization voltage applied was 15-fold shorter than by conventional thin-layer chromatography using the same stationary and mobile phases. Twenty years later Prosek et al. presented the next paper on application of electroosmotic effect to planar chromatography separations [1]. In the paper the name "planar electrochromatography" was introduced for the first time. However, the authors applied initially dry adsorbent layer to perform chromatogram development in horizontal developing chamber simultaneously from both sides of the plate under polarization voltage in the range 0–25 kV. The obtained results were interesting in respect of different times of chromatogram development from anode and cathode sides. Chromatogram development from anode side was considerably shorter than that from cathode side. In addition, the authors obtained different separation selectivities of solutes when development was performed under conditions of polarization voltage in comparison to conventional development of thin-layer chromatogram.

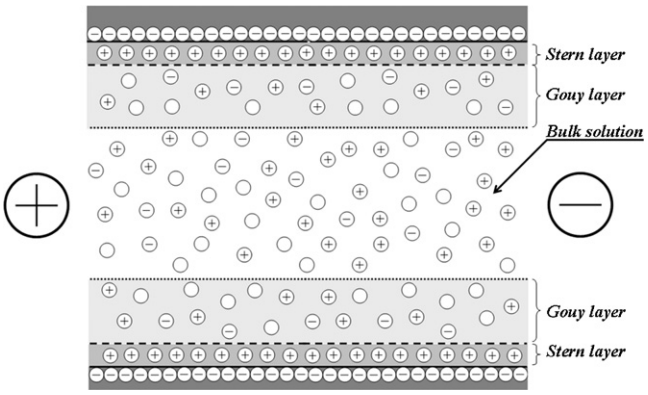

Fig. 2. Scheme of electrical double layer in silica capillary.

The next important paper by Poole and Wilson [11] considerably influenced on further development of planar electrochromatography. In the paper the authors encouraged, based on theoretical considerations, to involve the chromatographic community in planar chromatography separations using electroosmotic flow of the mobile phase.

Since then there has been considerable increase of number of papers on planar electrochromatography [12–22], including excellent review by Nurok [23]. The investigations performed on PEC revealed some disadvantages of the mode. The main disadvantages were concerned with generation of Joule heat and flux of the mobile phase to the surface of the adsorbent layer during separation process. The former effect was responsible for evaporation of solvent from the chromatographic plate and the latter for dispersion of solute zones. Both effects were responsible for low repeatability of migration distance of solutes and kinetic performance of PEC systems. In few papers some reduction of these disadvantages was described [16,20]. However, the results obtained were not still fully satisfactory. The mentioned disadvantages have been overcome when Nurok and coworkers introduced the new mode, PPEC, [2] as it is mentioned above.

2. Theoretical

Electroosmotic effect is defined as migration of liquid solution towards solid state in the applied electric field. It is easy to explain this effect in silica capillary, which is filled up with water solution. In such case the boundary between silica surface and water solution comprises electrical double layer. Solid surface becomes negatively charged due to dissociation of silanol groups when pH of water solution is higher than 1.5:

$$\equiv SiOH \leftrightarrow \equiv SiO^- + H^+ \quad (1)$$

Otherwise, solution near the solid surface is positively charged. According to Stern model the electrical double layer consists of two parts of solution layer. The first one, which is by the border of the solid state, possesses dimension approximately equal to diameter of single ion and the second part comprises diffusion layer, which is extended some distance into bulk solution. Model of this double layer is demonstrated in Fig. 2. Potential is dramatically changed in the first part of the electrical double layer but at the diffusion layer it decreases monotonously due to no uniform distribution of cations and anions, Fig. 3. Only uniform cation and anion distribution takes place in bulk solution. If potential drop is applied to the terminals of the capillary presented in Fig. 2 then created electrical field exerts force to the ions, which are in diffuse layer (excess of cations is presented in the diffuse layer as demonstrated in Fig. 2). It causes the diffuse layer moves in the direction of cathode and together with it the bulk solution in the capillary is moved, too. Profile of

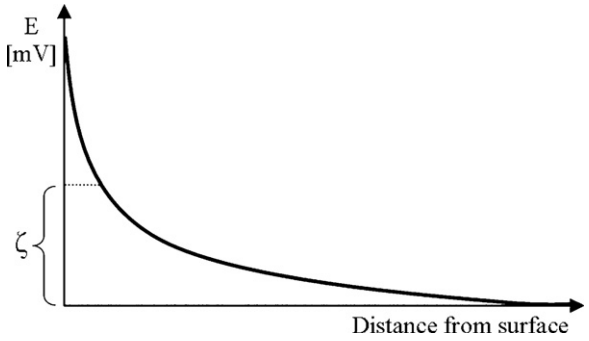

Fig. 3. Potential drop in stationary–mobile phase interface.

such liquid flow is characteristic because it shows almost the same value of stream flow across the capillary section, Fig. 4a. Otherwise, laminar flow profile in capillaries, in which hydrodynamic forces drive liquid, shows parabolic shape, Fig. 4b. It means that liquid near the capillary wall flows with lower velocity than in its middle part. Such flow profile demonstrated by electrochromatography systems is very advantageous for band broadening. It has been well documented in scientific literature that much higher plate number of capillary electrochromatography systems was obtained in comparison to conventional HPLC or capillary liquid chromatography [24].

Electroosmotic flow is mainly dependent on potential zeta, ζ, which reflects potential drop in diffusion part of electrical double layer, Figs. 2 and 3. Its value directly influences on linear electroosmotic flow velocity, υ_{eo}, of the mobile phase which is expressed according to Smoluchowski equation:

$$\upsilon_{eo} = \frac{\varepsilon\zeta E}{\eta} \tag{2}$$

We can see that linear electroosmotic flow velocity is additionally dependent on dielectric constant of the mobile phase, ε, applied potential gradient, E, and the solvent viscosity, η. This flow velocity is not dependent on capillary length, its diameter, and particle diameter of the stationary phase. It means that no pump is necessary to drive mobile phase but only potential gradient applied to the both opposite terminals of the separating column or the chromatographic plate. So it is responsible for simplification of the PPEC device relative to that with mobile phase driven by pressure.

On the other hand, in conventional thin layer chromatography flow velocity of the mobile phase, υ_{tlc}, is not constant, its value diminishes with development distance according to the equation:

$$\upsilon_{tlc} = 0.5\kappa(Z_f)^{-1} \tag{3}$$

where Z_f, and κ, are the migration distance of solvent front, and the velocity constant, respectively. This feature stands for the main disadvantage of TLC because, in principle, flow velocity of the mobile phase cannot be optimized. For this reason the TLC technique may not be fully recognized as the high-performance separation mode.

There is no parameter described pressure drop in Eq. (2) otherwise to the HPLC and overpressured layer chromatography (OPLC) systems in which mobile phase velocity, υ_c, is expressed by the equation:

$$\upsilon_c = \frac{d_p^2\,\Delta P}{\theta\eta L} \tag{4}$$

where θ is the flow resistance parameter, ΔP is the inlet pressure, d_p is the particle size, η is the mobile phase viscosity, and L is the column length.

It should be mentioned that Eq. (2) was derived under assumption that electrical double layer represents electrical condenser with parallel screens. This restricts application of the equation to the cases in which diameter of the capillary is substantially greater in comparison to the thickness of the electrical double layer. In open capillary electrochromatography systems the capillary diameter is approximately equal to 50 μm and the thickness of the electrical double layer is lower than 10 nm then this requirement is fulfilled. On the other hand, stationary phase particles form channels of various diameters in packed bed that can lead to overlap of electrical double layers especially for stationary phase of poor particle size distribution and in its regions of higher packing densities what was discussed by Wan [25,26]. Consequence of this effect can be reflected in disturbance of flat profile of the electroosmotic flow, reduction of the mobile phase flow and decrease of efficiency of the separating system. According to Knox et al. [27,28] particle size diameter is not critical parameter, which can restrict application of very fine particles such as these applied in HPLC to create electroosmotic flow with its flat profile. However, the results obtained by Nurok et al. [18], in their investigations of electroosmotic flow in planar electrochromatography systems, were interpreted in terms of Wang's approach with success.

In typical electrochromatography systems the electroosmotic flow velocity is characterized by higher value in comparison to electrophoretic flow velocity. Equation describing electrophoretic flow velocity, υ_{ep}, of charged species is very similar to that presenting electroosmotic flow velocity:

$$\upsilon_{ep} = \frac{\varepsilon\zeta E a}{\eta} \tag{5}$$

where a is the parameter representing shape and size of the charged species. Meanings of remaining parameters are the same as in Eq. (2).

For an easier comparison of experimental data obtained with different field strengths, E, the ionic mobility (electrophoretic mobility), μ_{ep}, has been introduced as:

$$\mu_{ep} = \frac{\upsilon_{ep}}{E} \tag{6}$$

Analogously electroosmotic mobility, μ_{eo}, can be defined as:

$$\mu_{eo} = \frac{\upsilon_{eo}}{E} \tag{7}$$

Usually the electroosmotic mobility in silica capillaries is larger than the electrophoretic mobility. This implies that all neutral, cation and anion solutes usually migrate towards the negative electrode with following velocities:

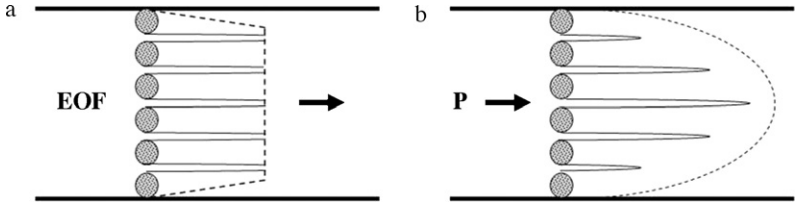

Fig. 4. Flow profiles. (a) Electroosmotic flow and (b) laminar (hydrodynamic) flow.

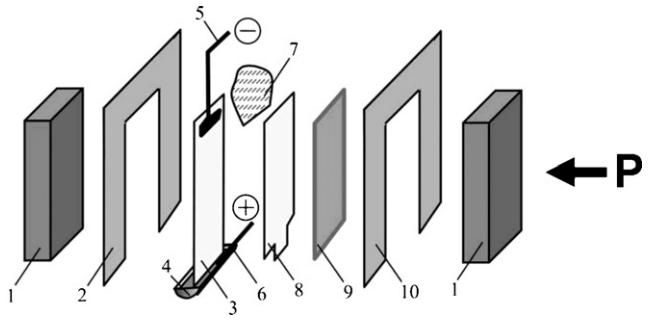

Fig. 5. Exploded view of the elements of PPEC chamber by Nurok et al.: (1) metal die block, (2) frame (first part) for the chromatographic plate, (3) chromatographic plate, (4) mobile phase, (5) cathode, (6) anode, (7) paper wick, (8) Teflon foil, (9) ceramic sheet, and (10) frame (second part) for the chromatographic plate [2].

for positive ions: $\upsilon_+ = (\mu_{eo} + \mu_{ep+})\, E$
for negative ions: $\upsilon_- = (\mu_{eo} - \mu_{ep-})\, E$
for neutral solutes: $\upsilon = \mu_{eo}\, E$

It means that positive ions migrate with the largest velocity; negative ions with the lowest and neutral solutes migrate with the velocity of the mobile phase. It must be pointed out that the behavior discussed above takes place if molecular interactions of the solute with capillary wall or stationary phase are neglected. It is especially the case when open tubular capillaries or microchip devices are considered. However, if packed bed stationary phase (e.g. packed column or TLC plate) is a part of electrochromatography system then participation of molecular interactions to migration velocity of solutes throughout the separating column or plate can lead to a change of migration order of mixture components to be separated. So it is clear that such system offers new separation selectivity in comparison to the systems in which mobile phase is driven by hydrodynamic or capillary forces.

3. Development of equipment to PPEC

Three research groups designed equipment to PPEC [2–9]. The first one was by Nurok et al. as it is mentioned above [2]. An adsorbent layer of the chromatographic plate is sealed with special plastic foil and aluminum nitride ceramic sheet between two die blocks. Exploded view of the chamber for PPEC is presented in Fig. 5. The lower edge of the plate and platinum wire electrode (anode) are dipped in solution in the mobile phase reservoir. Upper part of the adsorbent layer is equipped with platinum electrode (cathode) and blotting paper, which accumulates solution of the mobile phase during separation process. In the next paper Nurok and coworkers described modification of their equipment, of which die blocks had been equipped with channels for circulating water. The complete device for PPEC is demonstrated in Fig. 6 [3]. The last modification is very advantageous with respect to temperature control of separating system—Joule heat generated during separation process leads to temperature change of this system. The mentioned equipment can maintain temperature of the separating PPEC system within ±1 °C. However, it seems that the authors will further improve the construction of the device for PPEC in respect of making it more familiar to operators. Nurok and coworkers have confirmed this assumption because they have founded the new analytical instrumentation design and manufacturing company, InChromatics (www.inchromatics.com). Primary focus of this company is the development and commercialization of PPEC.

The first of our paper on device for PPEC has been concerned with adaptation of horizontal DS (Dzido and Soczewiński) developing chamber for TLC [6]. However, some part of the chromatographic plate extended beyond the cover of the adsorbent layer. It could lead to some evaporation of the mobile phase from the plate during separation process. In the next paper [7] we presented a device for PPEC with chromatographic plate completely closed. This device was further modified what has been described in the paper [8]. This modification was mainly concerned with location and shape of electrode cells in the chamber for PPEC. Cross section of the chamber and conceptual scheme of complete device for PPEC are presented in Fig. 7. Our device enables monitoring of flow velocity of the mobile phase. This feature is very advantageous for investigations of relationships retention–mobile phase composition and kinetic performance–mobile phase flow velocity, what is described in the further part of the paper.

Tate and Dorsey also described equipment for PPEC [4,5]. Their chamber for PPEC was constructed to perform investigations mainly concerned with equilibration of the separating system. The adsorbent layer of the chromatographic plate was covered with special plastic block (Kel-F) equipped with reader electrode grid and pressed with special hydraulic press, Fig. 8 [5]. Thirty-two platinum wire electrodes were incorporated in this block to monitor potential drop between these during flushing the adsorbent layer with the mobile phase driven by electroosmotic effect.

The PPEC devices presented above suffer one disadvantage concerned with manual transportation of the chromatographic plate from the prewetting device, or from spotting device of prewetted plate, to the PPEC chamber. Some solvent components of the mobile phase can evaporate from the chromatographic plate during this operation what can lead somewhat to disturbance of equilibration of separation system and, as a consequence of it, to lowering

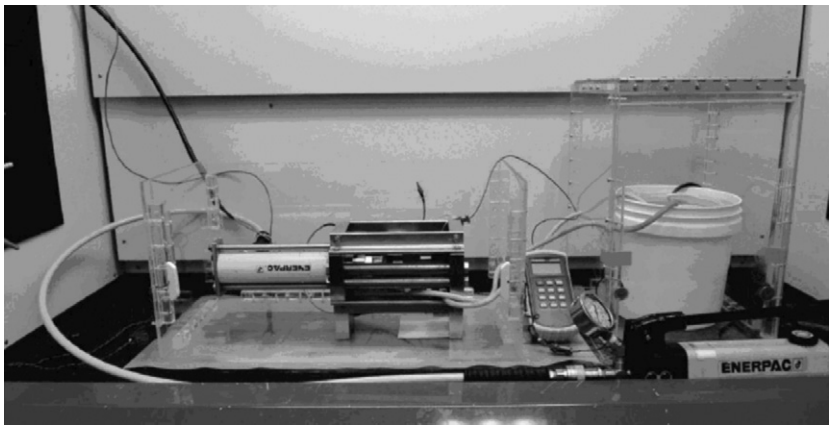

Fig. 6. Apparatus for PPEC by Nurok et al. [2].

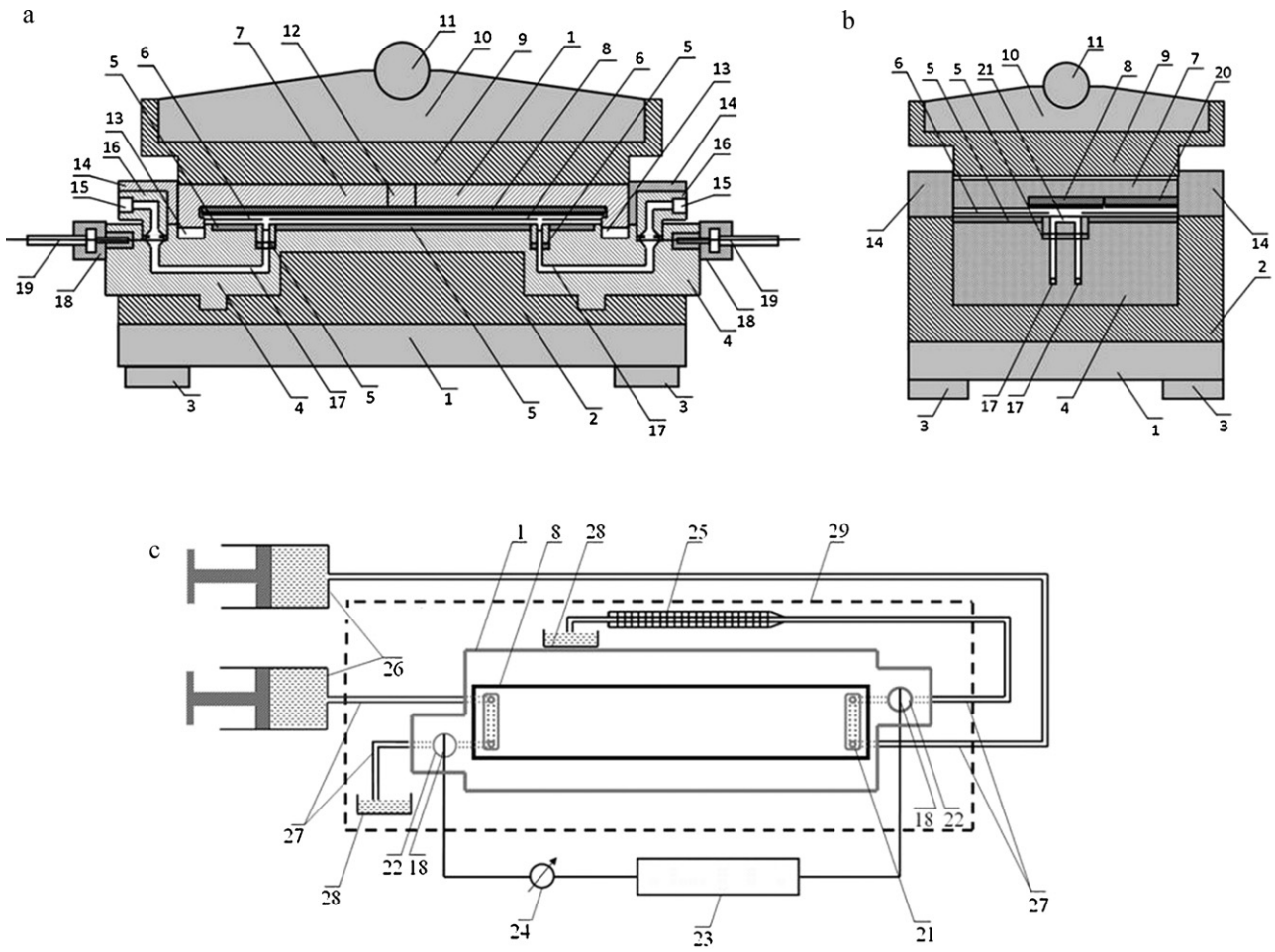

Fig. 7. Schematic view of the device for PPEC: (a) longitudinal section of the chamber for PPEC, (b) cross section of the chamber for PPEC, and conceptual view of the complete device for PPEC. (1) Steel base plate, (2) Tarnoform body of the chamber, (3) wheel legs, (4) Teflon block, (5) silicone gasket, (6) Teflon foil, (7) the adaptor of the chromatographic plate, (8) chromatographic plate, (9) polyacetal body of the lid, (10) steel body of the lid, (11) steel ball, (12) the hole for thermocouple, (13) the recess for the adaptor of the chromatographic plate, (14) electrode cell plug, (15) mobile phase in/out, (16) channel unit, (17) channel for the mobile phase solution, (18) electrode housing, (19) electrode, (20) supplement glass plate, (21) trough, (22) electrode cell, (23) high-voltage power supply DC, (24) ammeter, (25) micro-pipette, (26) reservoir, (27) Teflon tube, (28) waste, and (29) the dashed rectangle represents a Plexiglas cabinet, which stands as a safety interlock
Adapted from [8].

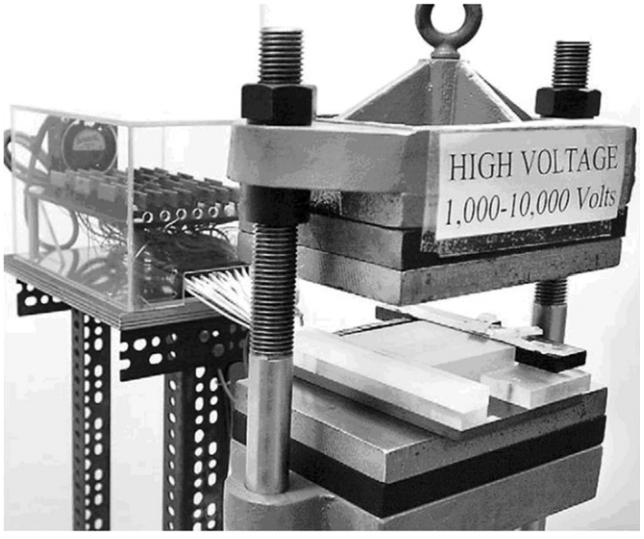

Fig. 8. Picture of the PPEC device by Tate and Dorsey [5].

of repeatability of migration distances of solute zones. In our last paper we have proposed a new PPEC chamber in which prewetting of adsorbent layer of the chromatographic plate and PPEC separation process are performed in the individual device [9]. It means that sample spots previously applied on the chromatographic plate are on the adsorbent layer during prewetting process in the PPEC device. These spots are prevented from dispersion by the partition (4), which tightly covers the spots during prewetting, Fig. 9 [9]. In this figure the complete device for PPEC is presented during the stage of electrochomatogram development. However, this device is featured by one weak point concerned with manual removal of prewetting solution from the electrode compartments (9) and from the compartment formed between partitions (4) before covering the adsorbent layer with the cover (7). We have worked out the next design of PPEC device in which mentioned inconvenience has been eliminated. We are now preparing a patent application on this matter.

In spite of many constructional modifications contemporary PPEC prototypes still need a lot of manual operations, which make the PPEC equipments are not fully familiar for operators. If these operations will be eliminated or considerably reduced then future application of PPEC to laboratory practice seems to be more prosperous.

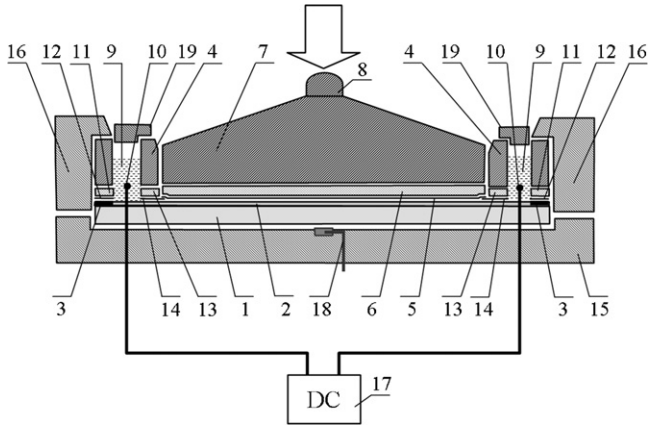

Fig. 9. Schematic view of the device for PPEC with details of the chamber, which stands as the prewetting and electrochromatogram development unit; (1) chromatographic plate, (2) adsorbent layer, (3) margin on the adsorbent layer, (4) partition, (5) Teflon foil, (6) silicone rubber, (7) cover, (8) external hydraulic press, (9) electrode compartments, (10) electrodes, (11) silicone rubber, (12) Teflon foil, (13) silicone rubber, (14) Teflon foil, (15) Teflon base of the chamber, (16) Teflon body of the chamber, (17) high-voltage power supply, (18) temperature sensor, and (19) electrode compartment covers.
Adapted from [9].

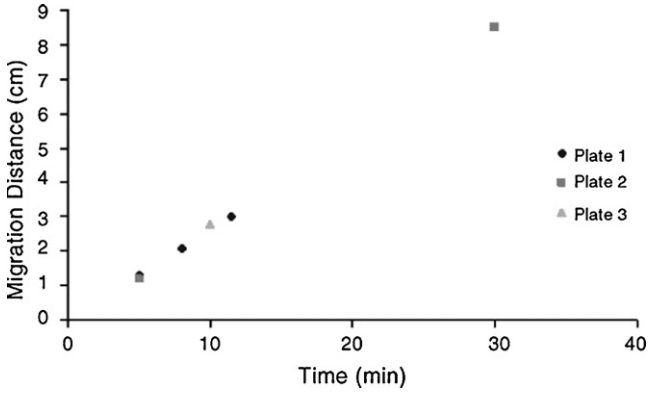

Fig. 11. Migration of rhodamine B marker on three TLC plates [5].

4. Repeatability of migration distance in PPEC

Weakness of planar electrochromatography in open system was due to low repeatability of migration distance and significant dispersion of solute zones. The former effect was pertained to evaporation of the mobile phase solution caused by Joule heat and the latter to flux of the mobile phase to the surface of the adsorbent layer. Since introduction of PPEC these disadvantages have been considerably eliminated. Covering the adsorbent layer with plastic foil under pressure prevented mobile phase evaporation and its flux to the surface of adsorbent layer. Higher repeatability of migration distance was obtained when time of adsorbent layer prewetting was expanded [6]. In Fig. 10a PPEC chromatograms are presented when chromatographic plate was prewetted for 3 s. The values of migration distance of the components of test dye mixture are spread in relatively broad range. Expanding of the prewetting time to 1 min, and longer, leads to considerable improvement of repeatability of migration distance of the solutes, Fig. 10b–e. Nurok et al. confirmed these results in the paper [3]. They compared the values of relative standard deviation of migration distance, when the plates had been soaked in the mobile phase for 20 min before PPEC, to these previously [2] obtained, when the plates had been briefly dipped in the mobile phase before PPEC, Table 1. Repeatability of migration distance was considerably better in the former case than in the latter. Tate and Dorsey also reported high repeatability of migration distance when adsorbent layer of the chromatographic plate was pre-equilibrated with running buffer for 30 min, prior to applying sample (rhodamine B) solution with a 10 μL syringe on the chromatographic plate, Fig. 11 [5].

Performing of experiments on prewetted chromatographic plates is very advantageous with regard to equilibration of the separation system of PPEC. In this way any electrochromatogram development can be performed under equilibrated conditions, similarly as it is in column liquid chromatography and capillary electrochromatography (CEC). This is contrary to conventional TLC and HPTLC (high performance thin layer chromatography). In these modes separation process is performed under non-equilibrated conditions—mobile phase migrates throughout the adsorbent layer initially dry. However, the procedure of chromatographic plate prewetting for PPEC experiments is characterized by a weak link concerned with transferring of the chromatographic plate from prewetting device to PPEC chamber what is also mentioned above.

5. Kinetic performance of PPEC

Theoretical plate height of the separating system obtained with PPEC mode was reported to be close to $2d_p$ where d_p is average particle diameter of an adsorbent layer of the high performance chromatographic plate (HPTLC plate) [3,29,30]. Nurok and coworkers reported plate height equal to 10.6 μm [3] for migration distance 69.3 mm of 2-nitroaniline on HPTLC plate what corresponds to 94 000 plates/m. The volume of sample solution was reported to be 10 nL (application with hand operated 0.5 μL syringe) what was advantageous in respect of narrow width of

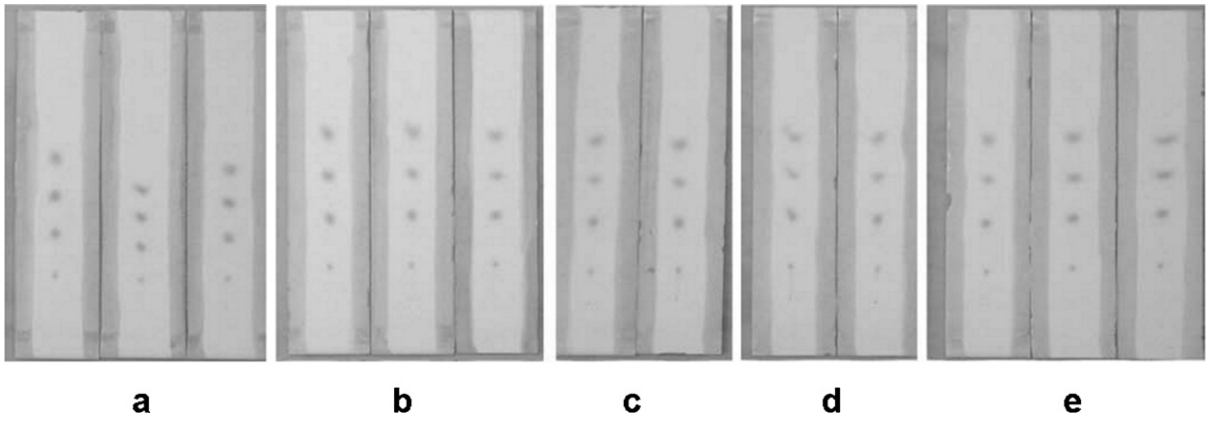

Fig. 10. PPEC chromatograms of test dye mixture. Adsorbent layer prewetting time: (a) 3 s, (b) 1 min, (c) 5 min, (d) 10 min, and (e) 30 min [6].

Table 1
Values of relative standard deviation, %, of migration distances of the solutes when TLC plates were soaked for 20 min or briefly dipped in the mobile phase before PPEC [3].

Solute	RP-18 F254s plates				LiChrospher RP-18 WF254s plates	
	Briefly dipped	20 min soaking, first set replicates	20 min soaking, second set replicates	20 min soaking, third set replicates	Briefly dipped	20 min soaking, first set replicates
17α-Acetoxyprogesterone	9.1	4.2	4.1	3.1	3.3	2.7
2′-Acetonaphthone	7.5	3.0	3.6	2.6	4.6	2.1
Benzanilide	6.1	1.7	3.1	2.0	3.7	1.6
o-Nitroaniline	4.0	1.7	2.8	1.9	2.7	1.6

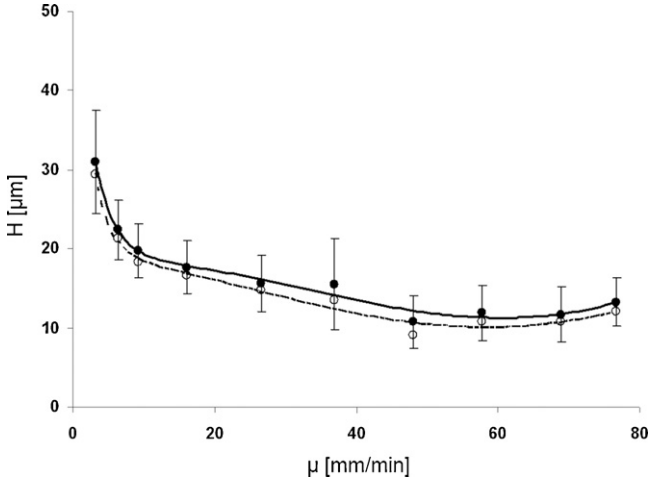

Fig. 12. Plate height vs. flow velocity of the mobile phase for PPEC system. (●) HPTLC RP-18W plate from Merck and (○) after subtracting variance concerned with starting spot width. The test solute prednisolone succinate. Mobile phase 80% acetonitrile in buffer (1.96 mM citric acid, 4.08 mM disodium hydrogen phosphate, pH = 5.0) [30].

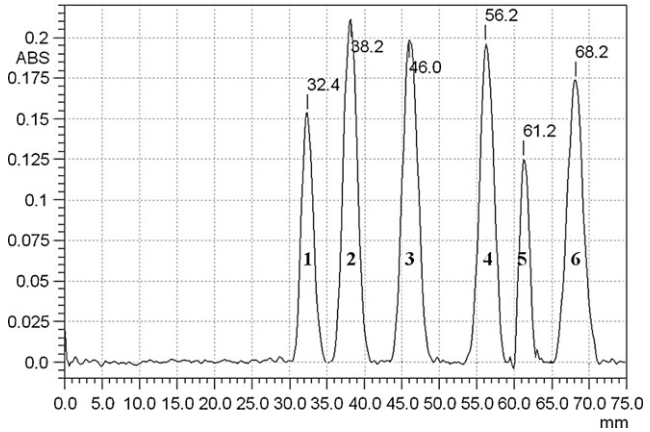

Fig. 14. Electrochromatogram (PPEC) of the test mixture; RP-18W HPTLC plate from Merck, applied polarization voltage 2.5 kV, separation time 5 min, the mobile phase 80% acetonitrile in buffer (1.96 mM citric acid, 4.08 mM disodium hydrogen phosphate, pH = 5.0), the order of mixture components according to the increase of migration distance: (1) testosterone isobutyrate, (2) testosterone acetate, (3) methandienone, (4) hydrocortisone acetate, (5) 16-dehydropregnenolone acetate, and (6) prednisolone succinate [30].

starting zone. The authors obtained even few results characterized by higher performance between 100 000 and 112 000 plates/m. However, it was not possible to routinely obtain such performance with apparatus applied in the experiments. Our group obtained similar kinetic performance of PPEC system, however, using commercially available sample applicator and considerably larger volume of solution applied on the plate [29,30]. In Figs. 12 and 13 the relationship plate height vs. mobile phase flow velocity and plate height vs. migration distance of the mobile phase front are plotted for PPEC and conventional HPTLC systems, respectively. The minimum value of plate height of HPTLC system is equal to 27 μm. These data indicate that performance increase of PPEC system in comparison to HPTLC is between twofold and threefold. In addition, based on the data presented in Fig. 12 it can be concluded that at flow velocity of the mobile phase equal to 80 mm/min plate height of the PPEC system increases about 30% relative to its minimum value, which was registered at flow velocity 50 mm/min. It means that using HPTLC RP-18W plates it is still possible to obtain relatively good kinetic performance of the system in very short time. In Figs. 14 and 15 examples of chromatograms of six component steroid mixture with PPEC and conventional HPTLC modes are

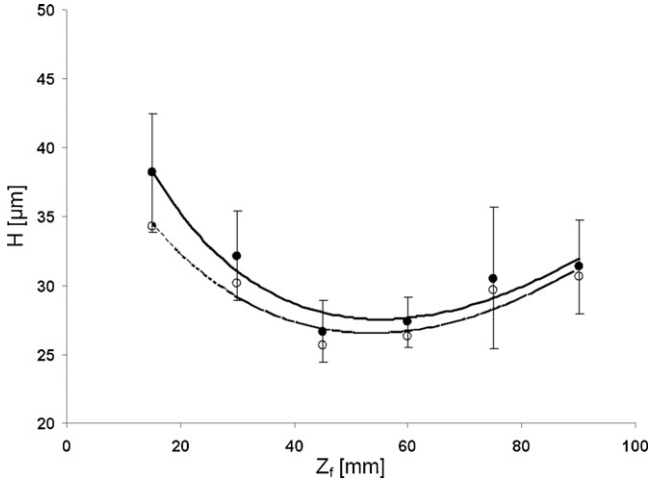

Fig. 13. Plate height vs. migration distance of solvent front for HPTLC system. The TLC chamber (DS-II-5x10 from Chromdes) with (●) chromatographic plate HPTLC RP-18W from Merck and (○) after subtracting variance concerned with starting spot width. The test solute prednisolone succinate. Mobile phase 80% acetonitrile in buffer (1.96 mM citric acid, 4.08 mM disodium hydrogen phosphate, pH = 5.0) [30].

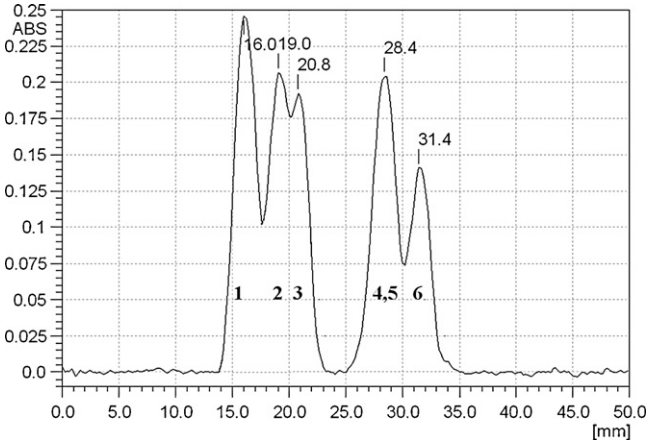

Fig. 15. Separation of the test mixture by conventional high-performance planar chromatography with HPTLC RP-18W plate (Merck), separation time 6.5 min, the mobile phase and the test solute mixture as in Fig. 13 [30].

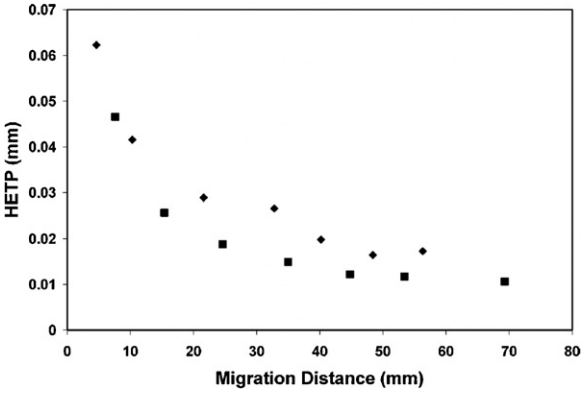

Fig. 16. Height equivalent of a theoretical plate (HETP) for 2-nitroaniline vs. migration distance. The high-performance plates conditioned at 160 °C for 20 min. PPEC was under conditions voltage 6 kV, pressure 63 atm, mobile phase 55% acetonitrile in buffer (pH 4.7, 5 mM acetate buffer) for between 1.0 and 7.5 min, depending on distance migrated. The upper plot (♦) is for the regular plates and the lower plot (■) is for the high-performance plates [3].

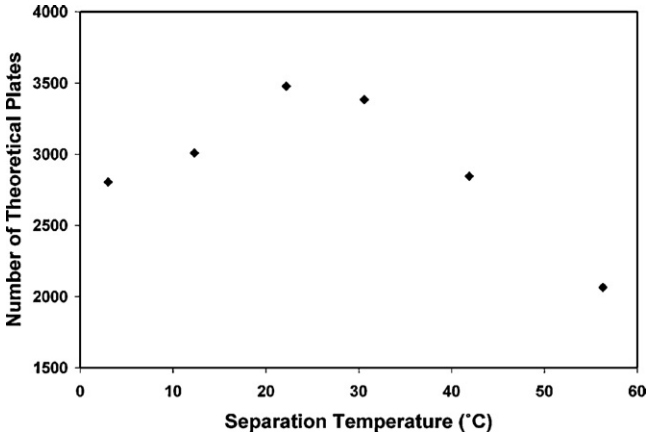

Fig. 17. Number of theoretical plates for 2-nitroaniline vs. separation temperature on HPTLC plates conditioned at 160 °C for 20 min. PPEC conditions: voltage −6 kV, pressure 63 atm, and mobile phase 55% acetonitrile in buffer (pH 4.7, 5 mM acetate buffer). Separation time was adjusted from 8 min at 3.0 °C to 3.0 min at 56 °C such that migration distance at each temperature was close to 49 mm [3].

presented, respectively, using the same mobile phase—stationary phase system in both modes. Resolution of mixture components with PPEC mode is completely contrary to that with conventional HPTLC mode. It should be mentioned that solvent front migration in HPTLC mode was equal to 50 mm, time of chromatogram development 6.5 min and the least retained solute was characterized by retardation factor 0.63. The presented PPEC chromatogram was obtained for 5.0 min applying polarization voltage 2.5 kV at flow velocity of the mobile phase 24.6 mm/min. It means that virtual front migration of the mobile phase in the PPEC system was approximately equal to 120 mm, more than double of that in HPTLC one. This is reflected by the values of migration distance of the solute bands in PPEC system in comparison to HPTLC one. The values of the former system are much higher than these of the latter. It means that considerably better resolution in PPEC mode relative to conventional HPTLC was obtained by both higher performance and longer migration distance of the solute zones in the former system. It should be underlined that migration distance of solvent front in conventional reversed phase HPTLC mode rarely exceeds 5 cm due to significant diminution of solvent front velocity and decrease of kinetic performance, compare Eq. (3). In PPEC systems this effect does not take place. Mobile phase migrates with constant velocity, which is dependent on electric field strength but not on chromatographic plate length, compare Eq. (2). This feature is the next important advantage of PPEC relative to TLC/HPTLC.

Tate and Dorsey also reported high kinetic performance of PPEC system [5]. They obtained more than 10 000 plates for HPTLC-RPS 10 cm long plates (Analtech) in system with methanol–buffer mobile phase for rhodamine B (8.5 cm migration distance) as the test solute.

In TLC and PPEC all solute bands stay on the chromatographic plate after separation process. Some solute bands migrate very short distance then contribution of starting sample width to general kinetic performance is especially important. Nurok and coworkers investigated this aspect and obtained the data of plate height vs. migration distance of the test solute (2-nitroaniline), Fig. 16 [3]. As it is demonstrated in this figure the plate height of PPEC system diminishes with increase of migration distance reaching plateau for migration distance longer than 40 mm. Similar results were obtained by our group when investigated influence of sample application mode on efficiency of PPEC system [29]. In this paper we also presented that sample application with commercially available aerosol applicator leads to the highest efficiency of PPEC system in comparison to that when sample was applied with hand operated 5 μL syringe and/or using a scrap of the adsorbent layer on which sample solution was deposited.

Nurok and coworkers investigated temperature influence on efficiency of PPEC separating system [3]. It was possible to perform these investigations when they introduced the channels for circulating water in the die blocks of the PPEC device. They found that maximum kinetic performance was obtained at 26 °C in the separation system. Diminution of system efficiency below 26 °C was attributed to predominance of mass transfer effect over molecular diffusion and above mentioned temperature reversed contribution of these effects was attributed, Fig. 17 [3].

In PPEC systems the adsorbent layer is pressurized during separation process as it is mentioned above. It is reasonable that too high pressure can lead to damage of structure of the adsorbent layer what can be reflected in efficiency lowering. Tate and Dorsey reported that optimum pressure exerted on the adsorbent layer should be in the range 1000–2000 psi [5]. Nurok and coworkers performed some experiments showing decrease of migration distance of test solute vs. pressure exerted on the adsorbent layer, Fig. 18 [2,3]. The effect was explained by diminution of diameter of inter-particle capillaries of the adsorbent layer. However, HPTLC plates demonstrate considerably lower decrease of flow velocity of the mobile phase with increase of pressure in comparison to regular plates. They also found that separation quality of the test mixture is considerably diminished under pressure 118 atm in comparison to two lower pressures (11.8 and 19.7 atm), Fig. 19 [2].

The discussion above indicates that many operation variables influence on kinetic performance of PPEC system. Especially quality of the adsorbent layer seems to be important in this regard. It is evident that PPEC technique is still in infancy stage of development and more investigations on influence of operating variables and stationary phase properties on kinetic performance of PPEC system should be performed.

6. Separation time by PPEC

As it was discussed above flow velocity of the mobile phase in PPEC system is not dependent on particle diameter of the stationary phase and is constant during experiment process contrary to that of conventional TLC/HPTLC, compare Eqs. (2) and (3). Value of flow velocity can be easily altered by applying various electric field (polarization voltage) to prewetted adsorbent layer of the chromatographic plate. This relation is demonstrated in Fig. 20 in which volume flow velocity vs. voltage applied to the chromatographic

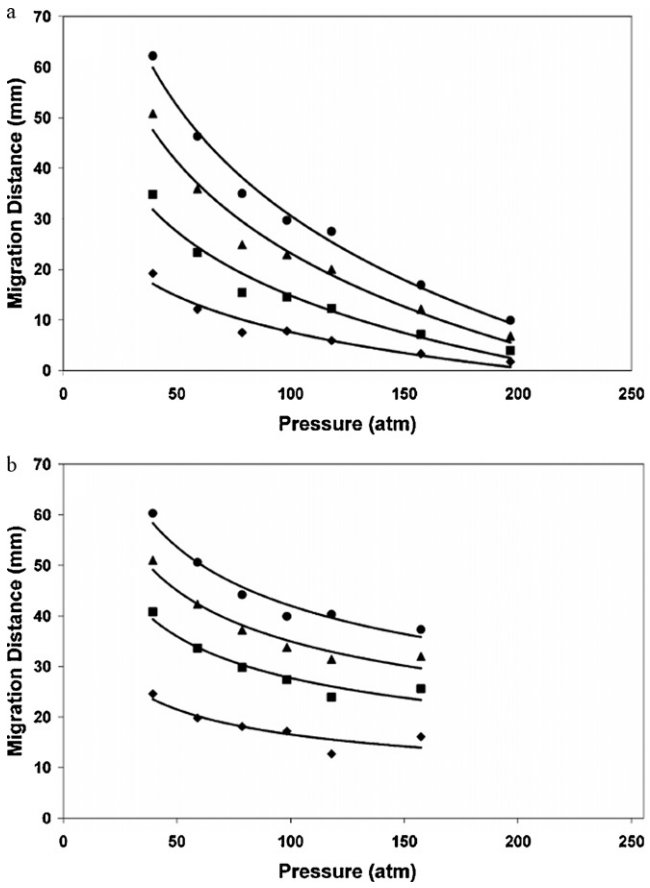

Fig. 18. (a) Migration distance vs. pressure on regular plates conditioned at 160 °C for 20 min. PPEC was for 7 min and (b) migration distance vs. pressure on HPTLC plates conditioned at 160 °C for 20 min. PPEC was 4.5 min. The analyte mixture 17α-acetoxyprogesterone (♦), 2′-acetonaphthone (■), benzanilide (▲), and 2-nitroaniline (●) and operating conditions [3].

plate is plotted [7]. The figure demonstrates linear relationship in the range 0–3.5 kV. However, the plot shows an increase of slope above 3.5 kV. It can be explained by viscosity decrease of the mobile phase solution at higher voltage attributed to stronger Joule heat generation.

Nurok's and our groups have reported few examples of very fast separations by PPEC mode [2,7]. The first group presented 1 min

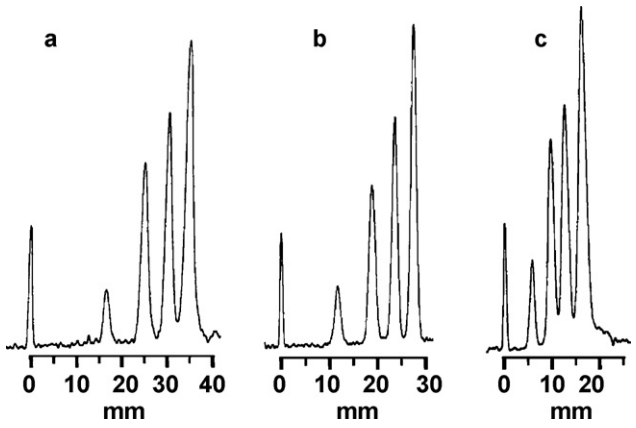

Fig. 19. A 1 min separation on a LiChrospher C_{18} plate at 9 kV and a pressure of (a) 11.8 atm, (b) 19.7 atm, and (c) 118 atm. The mobile phase 55% acetonitrile in buffer (pH 4.7; 25 mM acetate buffer). Solute mixture: 4-cholesten-3-one, 17α-acetoxyprogesterone, 2′-acetonaphthone, and benzanilide [2].

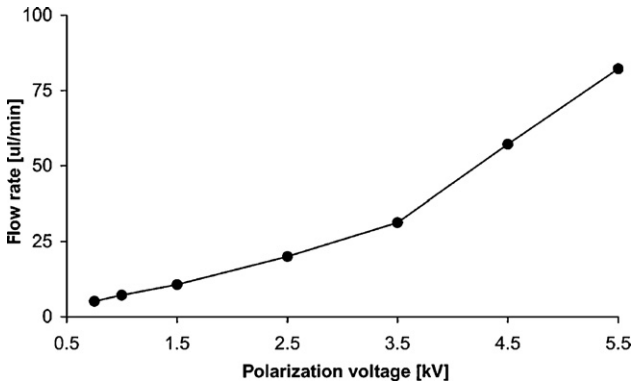

Fig. 20. Plot of mobile phase flow rate vs. polarization voltage. TLC RP-18 F_{254} plate (Merck), mobile phase: 80% acetonitrile in buffer (pH 6.0; 3.74 mM citric acid, 12.52 mM disodium hydrogen phosphate) [7].

PPEC separation of five-component test mixture whereas similar separation using conventional TLC mode was performed for 24 min.

Very fast separations by PPEC are very promising for future application of this technique in laboratory practice. However, at present such separations cannot be performed routinely due to few reasons. There is no commercially available equipment for PPEC that could effectively dissipate Joule heat generated at higher values of polarization voltage. Commercially available chromatographic plates for TLC and/or HPTLC are used in contemporary PPEC experiments. Thickness of their adsorbent layer is in the range 0.2–0.25 mm. It seems that diminution in thickness of adsorbent layer of chromatographic plates is essential for considerably lower generation of Joule heat what could enable PPEC separations to be routinely very fast.

7. Separation selectivity and two-dimensional separation with PPEC

Partitioning of the solutes between stationary and mobile phases and electrophoretic effect are involved in separation mechanism of PPEC. The electrophoretic effect is especially responsible for alteration of separation selectivity in PPEC relative to liquid chromatography systems. In Fig. 21a and b the chromatograms of acetylsalicylic acid, caffeine and acetaminophenon are demonstrated which were obtained by TLC and PPEC modes, respectively, using the same mobile phase–stationary phase systems [31]. As it can be seen separation selectivity in both systems is quite different. It should be mentioned that in PPEC system one solute, acetylsalicylic acid, migrates in opposite direction relative to two others, caffeine and acetaminophen. This feature of PPEC mode is advantageous in comparison to capillary electrochromatography. All solute bands migrating in both directions can be registered on the chromatographic plate but on the other side; solute bands migrating only to one electrode can be registered in a single run by contemporary equipment of capillary electrochromatography mode. Dramatically different separation selectivities of electrochromatography and liquid chromatography systems was applied to demonstrate strength of two-dimensional separation (2D) with PPEC involved in such procedure [8]. In Figs. 22 and 23 two-dimensional separations of test dye mixture and some amino acids, respectively, with HPTLC and PPEC (2D HPTLC/PPEC) are presented [8,32]. The presented preliminary 2D separations indicate that such mode is very promising for future analytical applications. It should be stressed that in these experiments the 5 cm × 20 cm HPTLC RP-18W plates were used and electrochromatogram developments were performed along the longer edge (20 cm) of the chromatographic plate. It is well know that conventional chro-

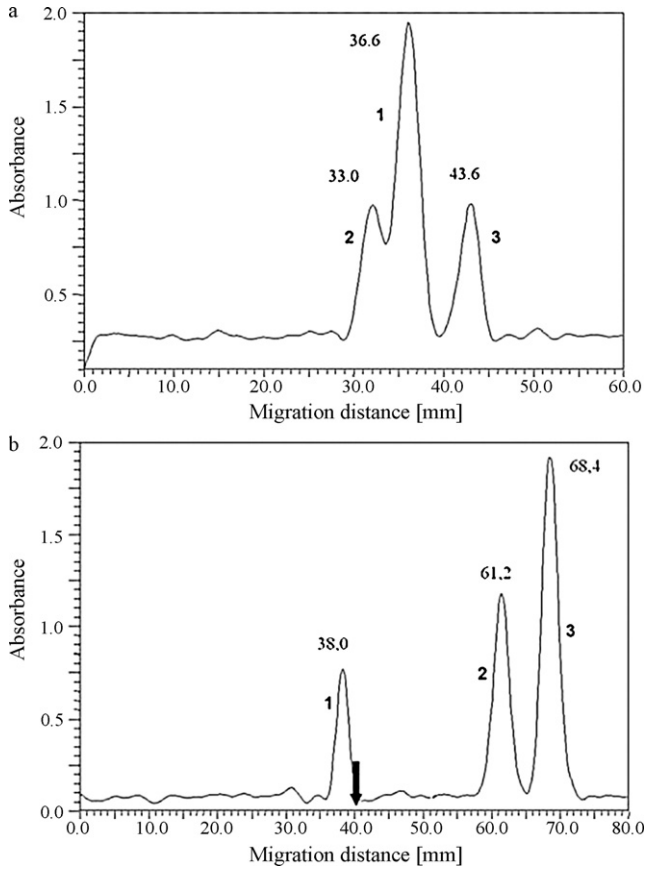

Fig. 21. TLC chromatogram (a) and PPEC chromatogram (b) of acetylsalicylic acid (1), caffeine (2) and acetaminophen (3) on RPTLC plate. Mobile phase 60% acetonitrile in buffer (pH 3.8). PPEC conditions: polarization potential 2.0 kV (the black arrow indicates the point of application) [31].

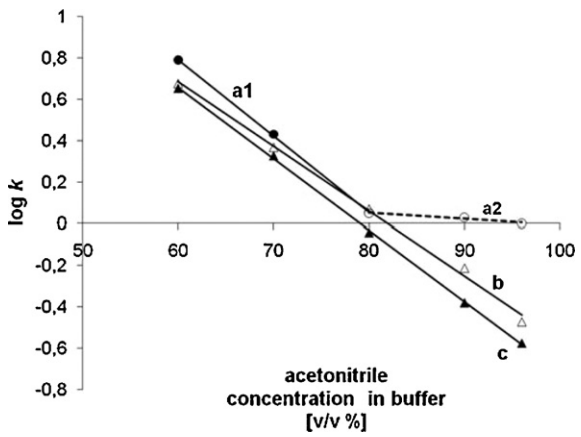

Fig. 24. Dependence of log *k* of 1-(4-hydroxyphenylazo)-2-naphthol on the concentration [% (v/v)] of acetonitrile in buffer (pH 4.8), (a1 and a2) TLC, (b) PPEC, and (c) HPLC [33].

matogram developments on such long plates are not realized in laboratory practice. It is the next advantage of PPEC relative to TLC and/or HPTLC.

8. Miscellaneous applications

8.1. Retention–mobile phase composition relationships

PPEC process can be performed under equilibrated conditions contrary to conventional thin-layer chromatography as is mentioned above. This feature of PPEC enables to determine retention vs. mobile phase composition relationships. There is one example in the literature that is concerned with this aspect of PPEC application [33]. In Fig. 24 the plots of log *k* (*k* is retention factor) vs. acetonitrile concentration in buffer mobile phase are presented for HPLC, PPEC and HPTLC systems. The data clearly indicate that relationships obtained by HPLC and PPEC techniques are very similar contrary to that by HPTLC one.

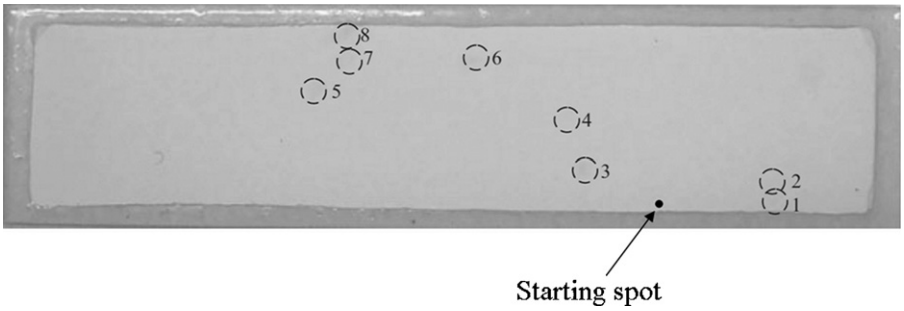

Fig. 22. Digital picture of the chromatographic plate after 2D HPTLC/PPEC separation of the mixture of rhodamine 6G (1), PAR (2), patent blue (3), Green S (4), azarubine (5), brilliant blue (6), allura red (7) and brilliant black (8). The first dimension—TLC mobile phase 45% methanol in buffer (pH 3.0), the second dimension—PPEC mobile phase 75% acetonitrile in buffer (pH 3.0), polarization voltage 2.5 kV [8].

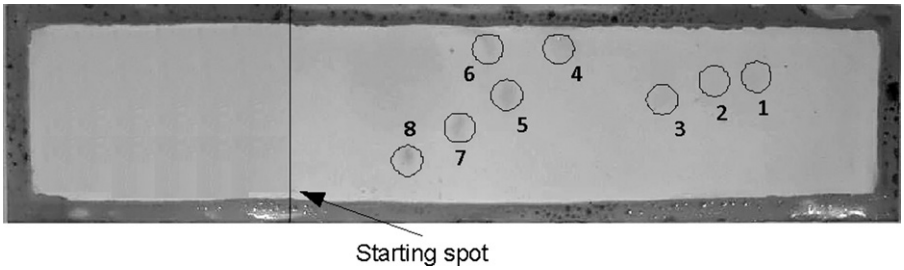

Fig. 23. Digital picture of the chromatographic plate after 2D HPTLC/PPEC separation of the amino acid mixture: (1) Lys, (2) His, (3) Arg, (4) Thr, (5) Leu, (6) Glu, (7) Phe, (8) Try; TLC/PPEC mobile phase 10% acetonitrile in buffer (pH 3.2), polarization voltage 2.5 kV [32].

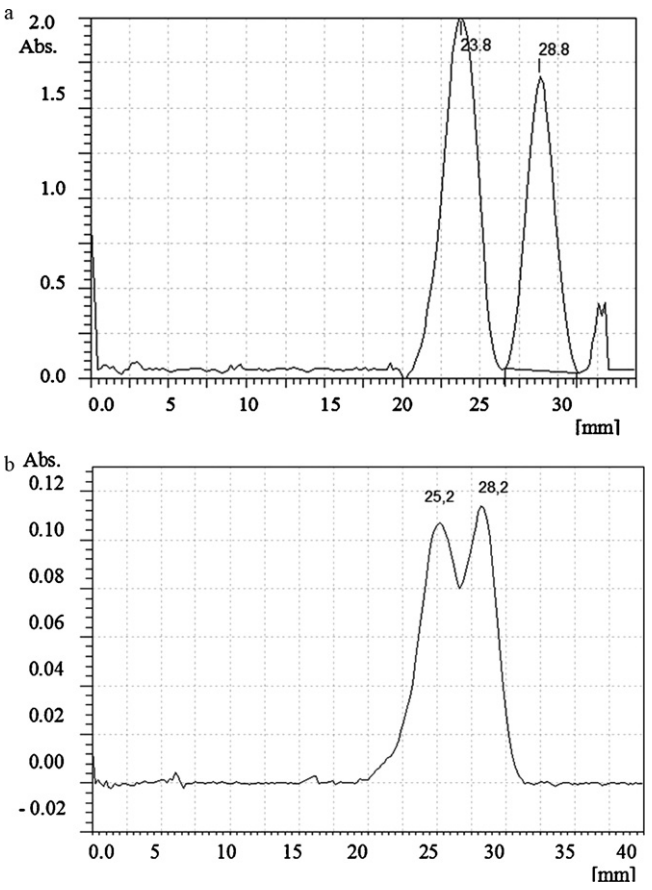

Fig. 25. (a) PPEC separation of the enantiomers of tryptophan. The mobile phase was 70% acetonitrile, 10% methanol and 20% buffer (acetic acid + sodium acetate, buffer concentration 5 mM, pH 5.57), the chromatographic chiral plate, with D-4-hydroxyproline as the chiral selector, was from Macherey–Nagel (Düren, Germany), the polarization voltage was 1.7 kV. (b) TLC separation of the enantiomers of tryptophan. The mobile and stationary phases were as for (a) [34].

8.2. Enantiomer separations

We have published two papers on separation of amino acid enantiomers with PPEC mode [9,34]. The results were compared with these of TLC mode. In Fig. 25a and b PPEC and TLC separations of the enantiomers of tryptophan are presented, respectively [34]. The chromatographic plate and the mobile phase solution were the same in both separation systems, PPEC and TLC. It is clearly demonstrated in this figure that separation efficiency obtained with PPEC mode is substantially better than with TLC. Based on the discussion performed in the papers it can be concluded that PPEC mode is promising for enantiomer separations. Variables such as buffer pH, buffer concentration and concentration of organic modifier in the mobile phase can be easily used for enantioseparation by PPEC mode. In addition separation selectivity of amino acid enantiomers investigated is more strongly influenced by the variables mentioned in PPEC mode in comparison to TLC one [9].

8.3. Combination of MS and PPEC

The late publications on combination of thin-layer chromatography and mass spectrometry have been substantially risen for the latest ten years. This is the result of introduction of few ion sources and interface devices, which easily transfer solute zones from any chromatographic plate to mass spectrometer [35–39]. However, there are only two publications on combination of mass spectrometry and planar electrochromatography. The first paper was by Patton et al. [38]. The authors described two-dimensional separation of phosphopeptides with planar electrochromatography and thin-layer chromatography followed by analysis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The second paper was by Nurok et al. who described preliminary application of DESI source to transfer solute zones from the chromatographic plate to mass spectrometer after PPEC separation [39].

8.4. New stationary phases for PPEC

Commercially available chromatographic plates for TLC/HPTLC have been practically applied in the experiments of PPEC so far. These plates are not fully appropriate for PPEC mainly due to their too high thickness and relatively low ability to generate electroosmotic flow. Production of adsorbent layers dedicated to PPEC is the one of the most important challenges for further development of PPEC. Nurok and coworkers lately published the first paper on application of monolithic polymer layer to PPEC that was used for separation of six peptides for 1 min [40]. The authors stated that the results are preliminary, however, very promising for future acquiring fast separations of compounds of biological origin with PPEC mode.

9. Conclusions

Based on the features of PPEC described above such as high performance, short time of separation and different separation selectivities in comparison to liquid chromatography it can be stated that this mode is very promising for future application in laboratory practice. However, the mode requires further development in respect of construction of the device, including sample application and prewetting of adsorbent layer of the chromatographic plate, which makes the mode familiar for the operator. In addition new chromatographic plates devoted to PPEC should be worked out which facilitate optimal generation of electroosmotic flow of the mobile phase and minimize involvement of Joule heat in separation process. Optimistic symptoms of further interest in planar separation techniques, including PPEC, seems to be concerned with their combination with mass spectrometry what is reflected in increase of number of the contemporary papers published in prestigious journals.

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