Pressurized Planar Electrochromatography – Challenges and Perspectives

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Planar electochromatography is a separation mode in which mobile phase is driven into movement by electroosmotic effect relative to adsorbent layer of the chromatographic plate.
Conceptual view of the device for planar electrochromatography

(1) chamber for PPEC, (2) high voltage power supply, (3) chromatographic plate, (4) electrodes, (5) reservoir of the mobile phase, (6) cabinet for PPEC chamber, (7) ammeter.
Two modes of planar electrochromatography:

• PEC - planar electrochromatography in open system,

• PPEC – planar electrochromatography in closed system under pressure.
Planar electrochromatography in open system - PEC

Planar electrochromatography in closed system under pressure - PPEC
Horizontal DS chamber adapted for planar electrochromatography (PEC)

Conceptual view of the device for planar electrochromatography in a completely closed system.

Chamber for PPEC, side view of the elements marked by dotted rectangle in previous slide.

The chamber for planar electrochromatography: (a) with opened lid (2 x 10 cm chromatographic plate with separated sample mixture is placed inside the chamber) and (b) with closed lid.

Apparatus for performing PPEC.
Advantages of PPEC:

• short time,
• high performance,
• change of separation selectivity.
A separation of test solutes on a LiChrospher C18 plate,
(a) TLC, 24 min,
(b) PPEC, 9 kV; 55% ACN in acetate buffer pH 4.7; 1-min.

Separation of a nine-component mixture in 2 min. PPEC was performed at 9 kV and 63 atm using 55% aqueous acetonitrile containing 5 mM acetate buffer at a pH of 4.7 on a high-performance layer.

Electrochromatogram of pesticide mixture, 60% acetonitrile in water, HPTLC RP18W F254s plate (Merck).

**TLC**, 1 - propyphenasone, 2 – caffeine, 3- paracetamol
HPTLC RP 18W (Merck), 20% ACN in buffer (pH 4.1), time **7.5 min**

**PPEC**
HPTLC RP 18W (Merck), 25% ACN in buffer (pH 4.9), 0.5 kV, time **8 min**.

**PPEC**
HPTLC RP 18W (Merck), 20% ACN in buffer (pH=4.1) 3.5 kV, time **30 s**.
PPEC separation of (1) rhodamine, (2) malachite green, using 20 cm long plate, HPTLC RP 18W (Merck),

80% ACN i buffer (pH 3), 2.5 kV, 30 min.

Migration distance of the first zone 15 cm

6 kV, 11 min
Plate height vs. flow rate of the mobile phase for PPEC systems with RP18 F254s HPTLC plate (Merck). The various modes of sample application: (a) – application with hand operated 5 μL micro-syringe, (b) – application of the sample as a scrap of adsorbent layer, (c) – application with aerosol applicator.

Electrochromatograms of test mixture
(a) TLC RP18 F254s plate (Merck), 4.5kV, $u_{lin} = 19.0$ mm/min, $t = 2.7$min;
(b) HPTLC RP18 F254s plate (Merck), 3.5 kV, $u_{lin} = 20.6$ mm/min, $t = 2.5$min.

Separation of the enantiomers of tryptophan

(a) PPEC, (B) TLC;

Chiral plates with D-4-hydroxyproline as the chiral selector, from Macherey–Nagel (Düren, Germany);

The mobile phase was 70% acetonitrile, 10% methanol, 10% bidistilled water, 10% buffer (acetic acid + sodium acetate, buffer concentration 5 mM, pH 5.57);

the polarization potential was 1.7 kV

HPTLC
HPTLC RP18W plate (Merck), 20% acetonitrile in buffer, pH 3
1 - aspirin, 2 – caffeine, 3 – paracetamol

PPEC
HPTLC RP18W plate, 20% acetonitrile in buffer, pH 3, polarization voltage 1.6 kV,
A. Hałka, T.H. Dzido, in press.
The problems:

- equilibration of the separating system,
- Joule heat generation,
- sample application,
- chromatographic plates,
- pressure used to press plastic foil to the adsorbent layer.
Equilibration of the separating system

• chromatographic plate have to be prewetted,

• when to apply the sample: before prewetting or after prewetting the chromatographic plate?
Sample application on prewetted chromatographic plate
Prewetting time of the chromatographic plate with the mobile phase solution:

3 sec  1 min  5 min  10 min  30 min

Prewetting of the chromatographic plate
(a sample is spotted on the plate)

Longitudinal sections of the reservoir for prewetting the chromatographic plate during soaking process; (1) U-shaped partition, (2) covering glass plate, (3) silicon gasket, (4) walls of the reservoir, (5) chromatographic carrier plate, (6) adsorbent layer, (7) solution of the mobile phase, (8) margin of silicon sealant on the adsorbent layer, (9) capillary for equation of pressure;

Repeatability of migration distance, $Z_S$, of the solutes in PPEC systems, 80% acetonitrile in buffer, pH 6, HPTLC RP18 plates (MERCK), time 5 min, polarization voltage 2.5 kV.

<table>
<thead>
<tr>
<th>Solute</th>
<th>$N$</th>
<th>Prewetting the chromatographic plate followed by sample application with hand operated microsyringe</th>
<th>Prewetting the chromatographic plate followed sample application with aerosol applicator</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-(2-pyridylazo)-2-naphthol</td>
<td>7</td>
<td>8.88, 0.078</td>
<td>11.57, 0.037</td>
</tr>
<tr>
<td>4-(diethyloamino)azobenzene</td>
<td></td>
<td>20.89, 0.049</td>
<td>27.73, 0.032</td>
</tr>
<tr>
<td>1-(4-hydroxyphenylazo)-2-naphthol</td>
<td></td>
<td>34.86, 0.041</td>
<td>46.30, 0.034</td>
</tr>
</tbody>
</table>

Joule heat generation:

• cooling the electrochromatographic system,
Passages for flow of water in die block.

The upper plot is for a separation without temperature control and the lower plot is for a separation with temperature control (regular plates conditioned at 120 °C for 20 min).

Chromatographic plate
Decrease of chromatographic plate thickness should lead to reduction of Joule heat generation
Sample application mode

- microsyringe,
- strip of adsorbent layer,
- aerosol applicator.
Plate height vs. flow rate of the mobile phase for PPEC systems with TLC RP18 F254s plates (Merck). The various modes of sample application: (a) – application with hand operated 5 μL micro-syringe, (b) – application of the sample as a scrap of adsorbent layer, (c) – application with aerosol applicator.

Plate height vs. flow rate of the mobile phase for PPEC systems with RP18 F254s HPTLC plate (Merck). The various modes of sample application: (a) – application with hand operated 5 μL micro-syringe, (b) – application of the sample as a scrap of adsorbent layer, (c) – application with aerosol applicator.
