The last achievements and prospects of pressurized planar electrochromatography

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O U T L I N E

Principle of action

Construction of devices for PPEC

Performance

Two dimensional separations

Quantitative analysis

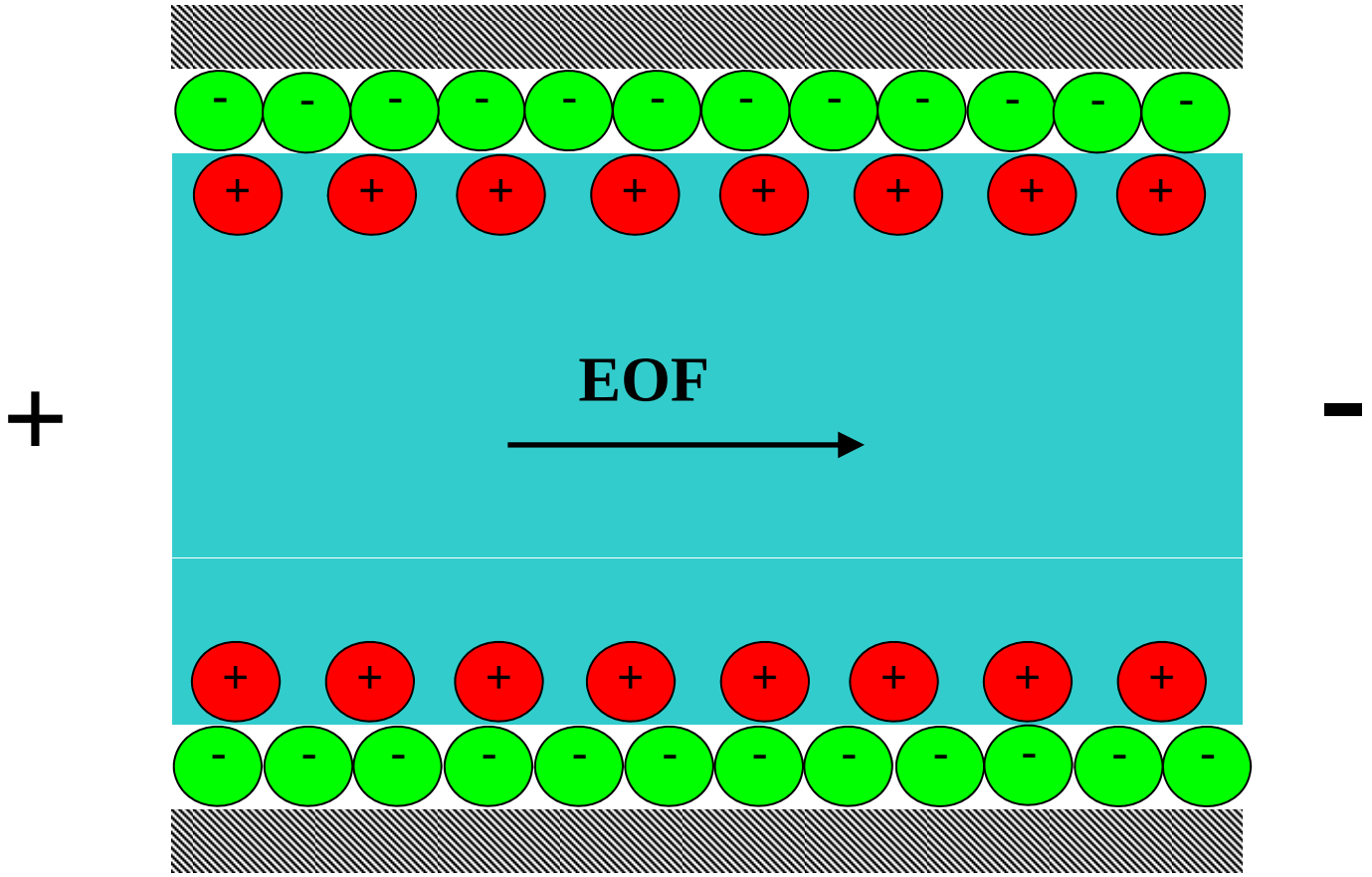
Conclusions

Principle of action

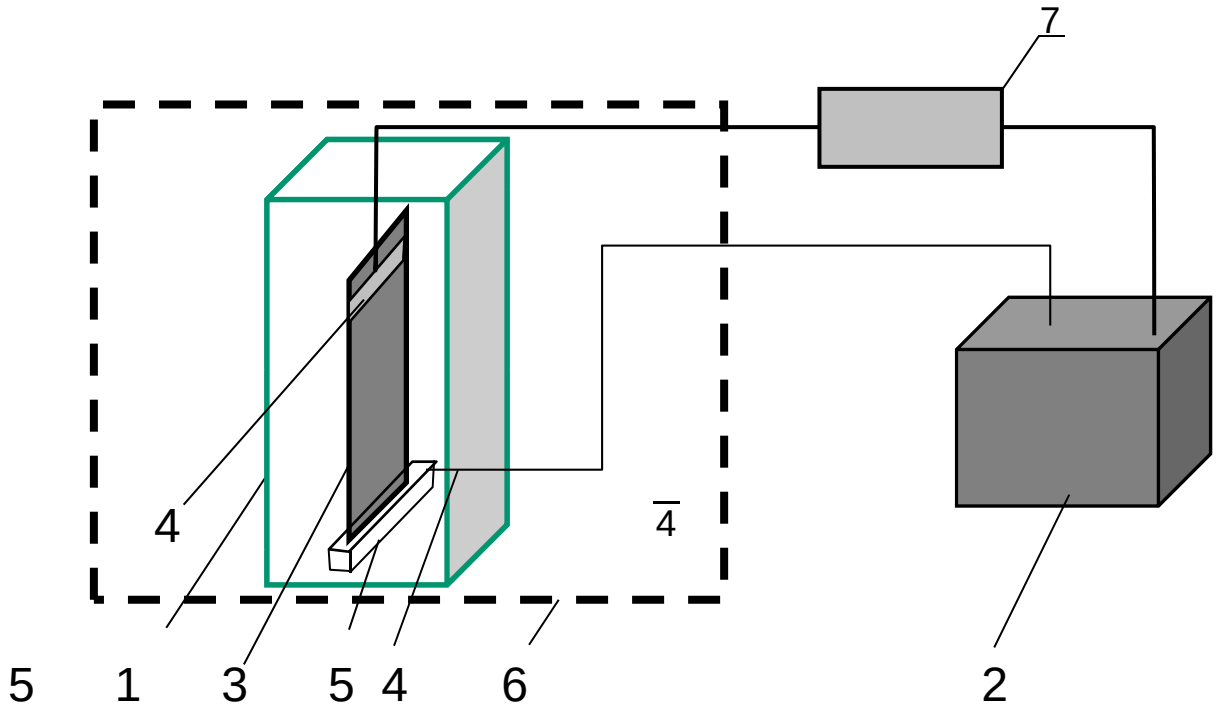
Pressurized planar electochromatography, PPEC, is a separation mode in which mobile phase is driven into movement by electroosmotic effect relative to an adsorbent layer of the chromatographic plate.

Electrical double layer is responsible for generation of electroosmotic flow

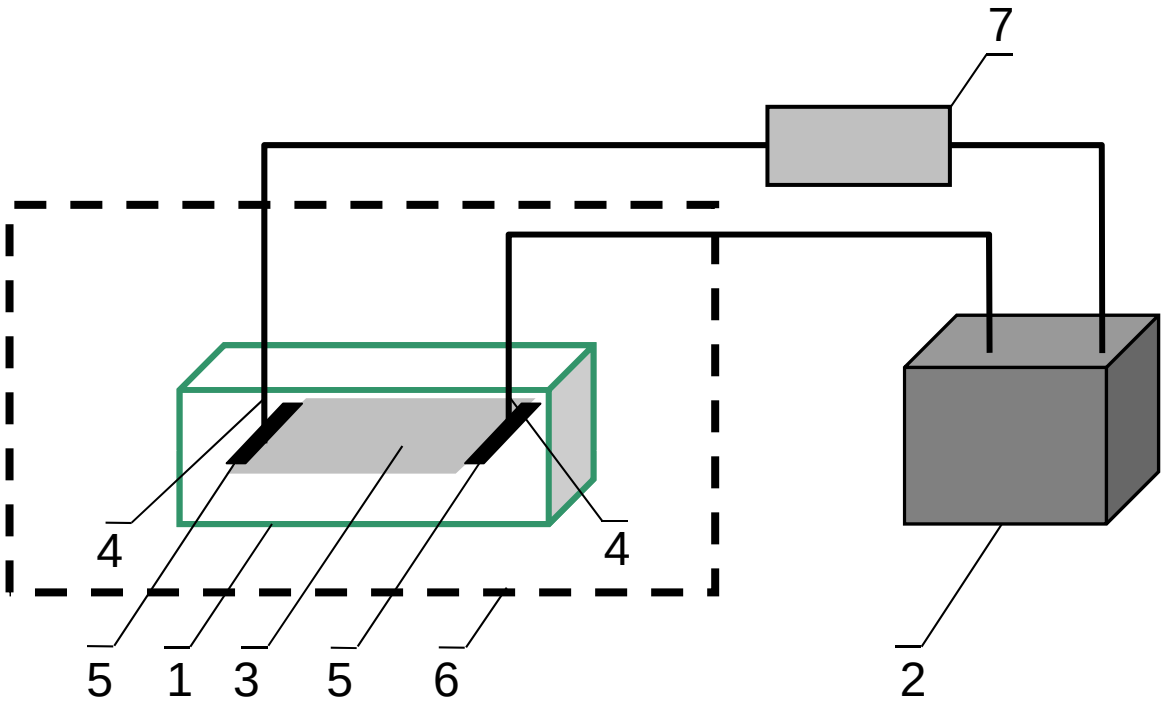
$$\equiv SiOH \leftrightarrow \equiv SiO^- + H^+$$



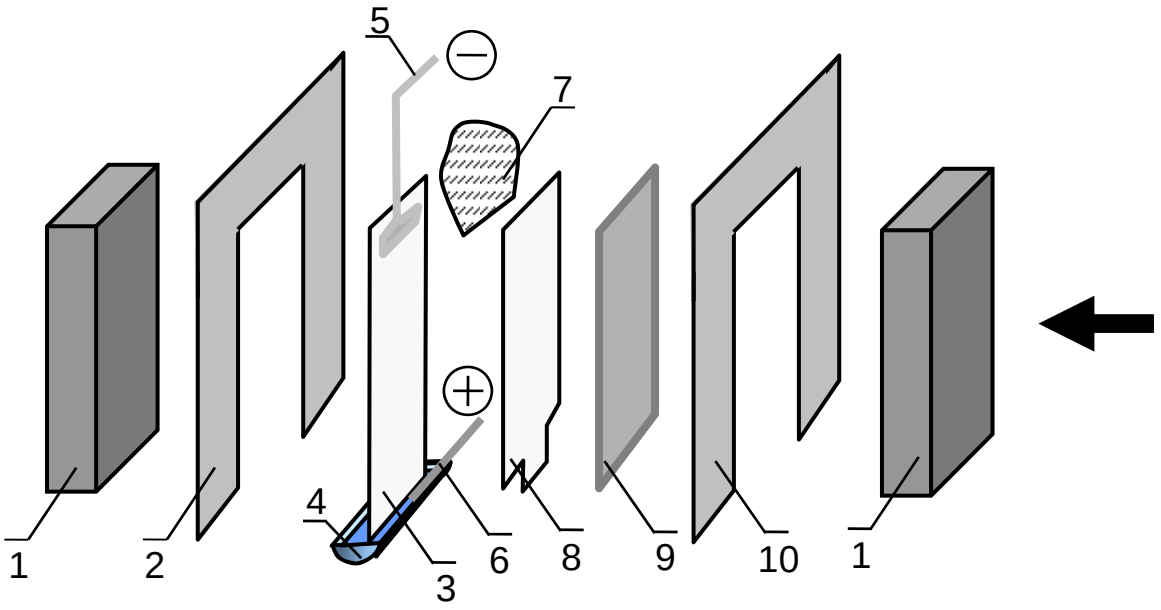
Construction of devices for PPEC



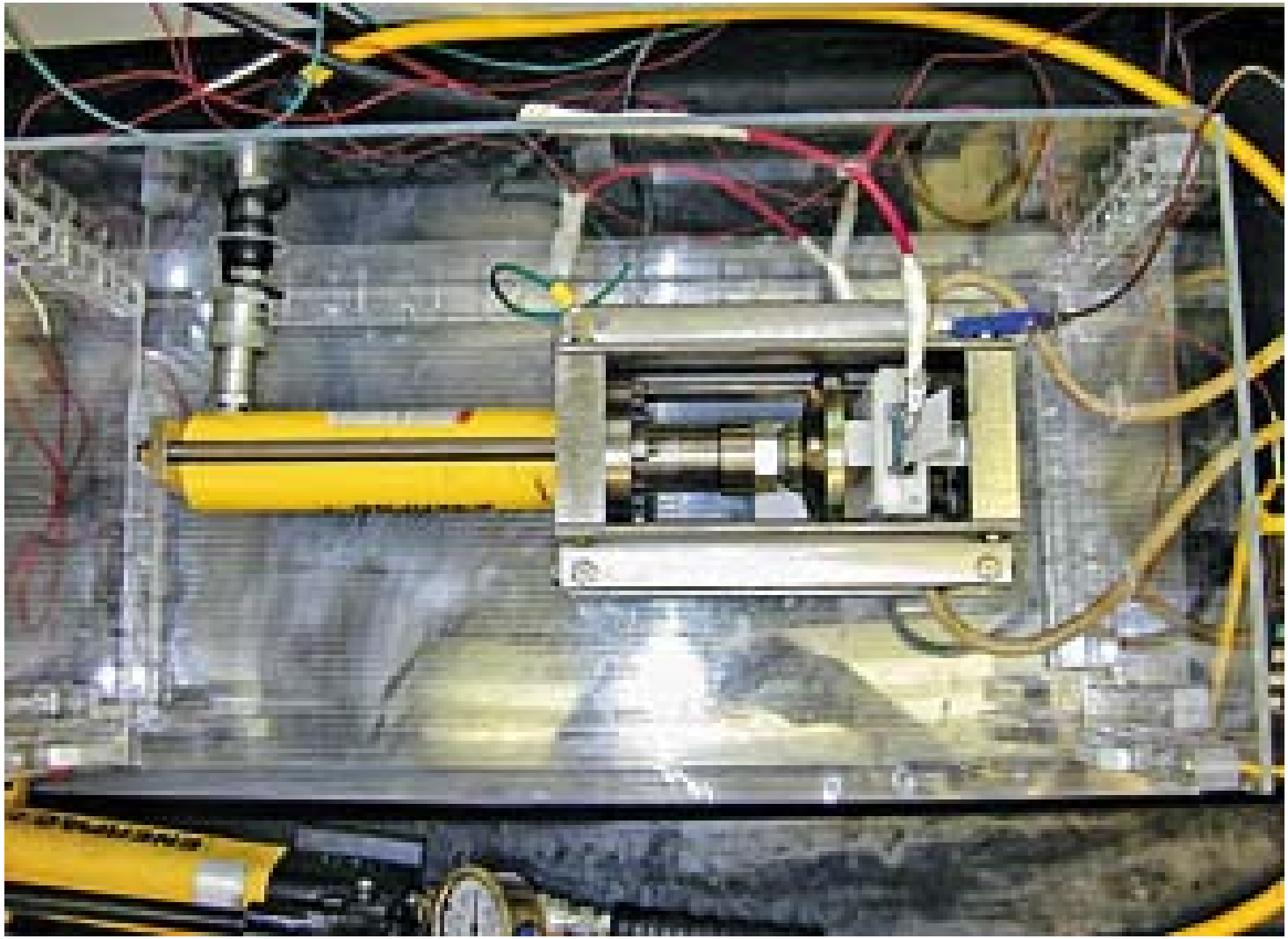
Conceptual view of a device for planar electrochromatography with chromatographic plate in vertical position; (1) chamber for PPEC, (2) high voltage power supply, (3) chromatographic plate, (4) electrodes, (5) reservoir of the mobile phase, (6) cabinet for PPEC chamber, (7) ammeter.



Conceptual view of a device for planar electrochromatography with a chromatographic plate in horizontal position; (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.

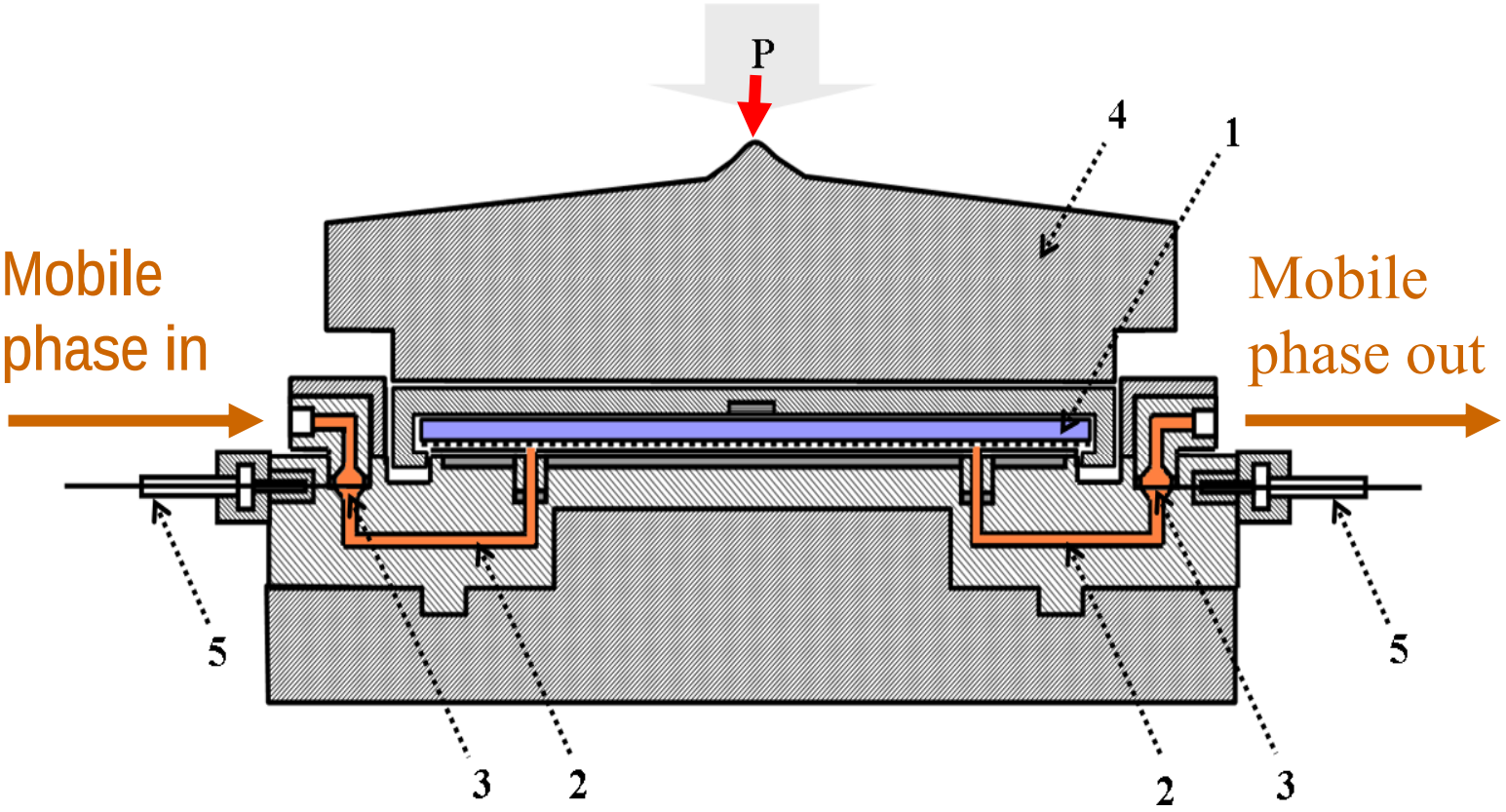


Exploded view of the elements of PPEC chamber proposed by Nurok et al.; (1) metal die block, (2) frame (first part) for the chromatographic plate, (3) chromatographic plate, (4) mobile phase, (5) cathode, (6) anode, (7) paper wick, (8) Teflon foil, (9) ceramic sheet, (10) frame (second part) for the chromatographic plate. Nurok D, Koers JM, Novotny AL, Carmichael MA, Kosiba JJ, Santini RE, Hawkins GL, Replogle RW, Anal. Chem. 76 (2004)1690–1695.



Apparatus for performing PPEC with chromatographic plate in vertical position.

D. Nurok, et al.: Anal. Chem., 78 (2006) 2823 – 2831
and Chemical & Engineering News, May 18, 2009 Issue.



Schematic view of the horizontal chamber for PPEC;
(1) chromatographic plate, (2) feed and outlet pipes, (3) electrodes, (4) cover, (5) electrode output, (P) hydraulic press.

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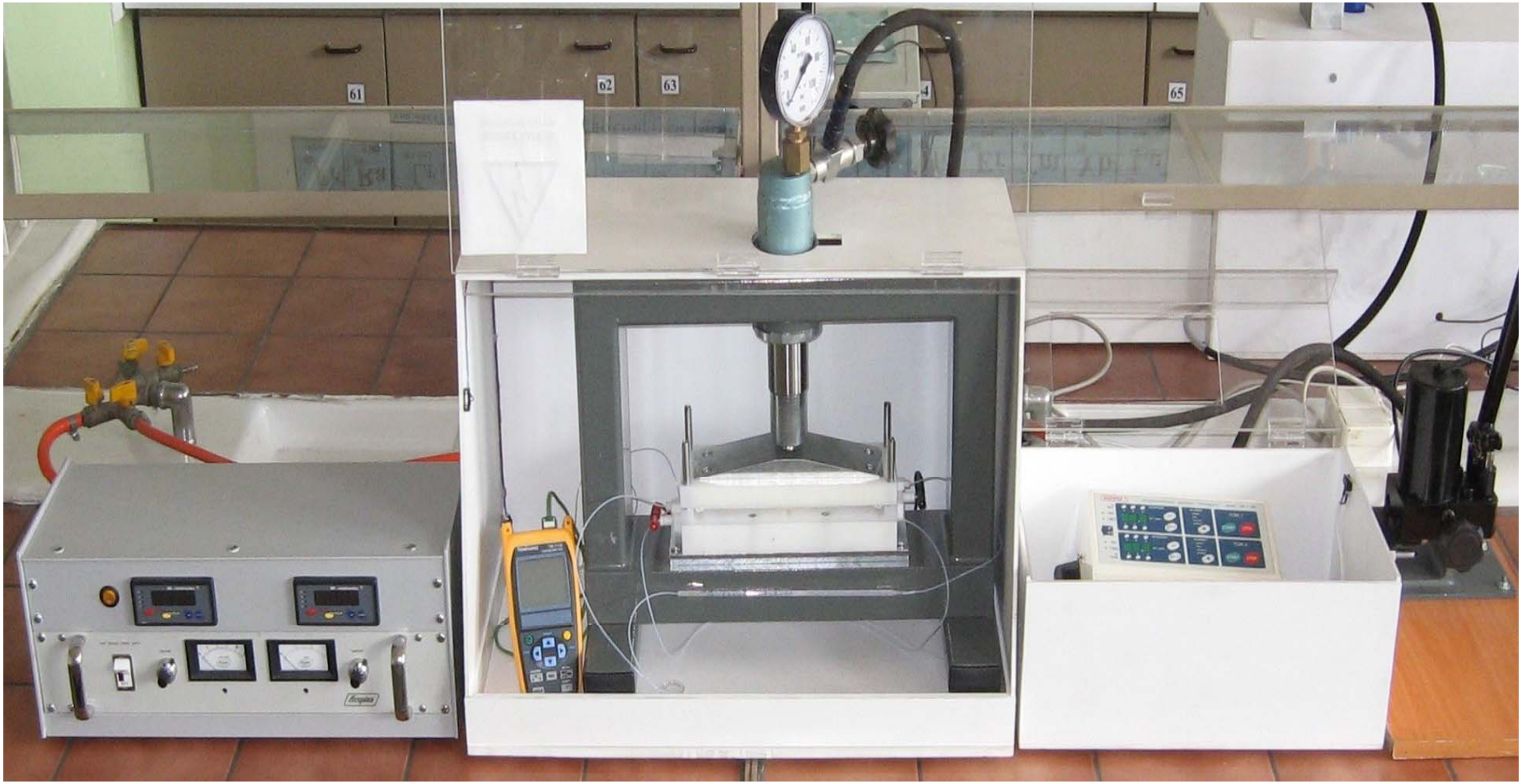
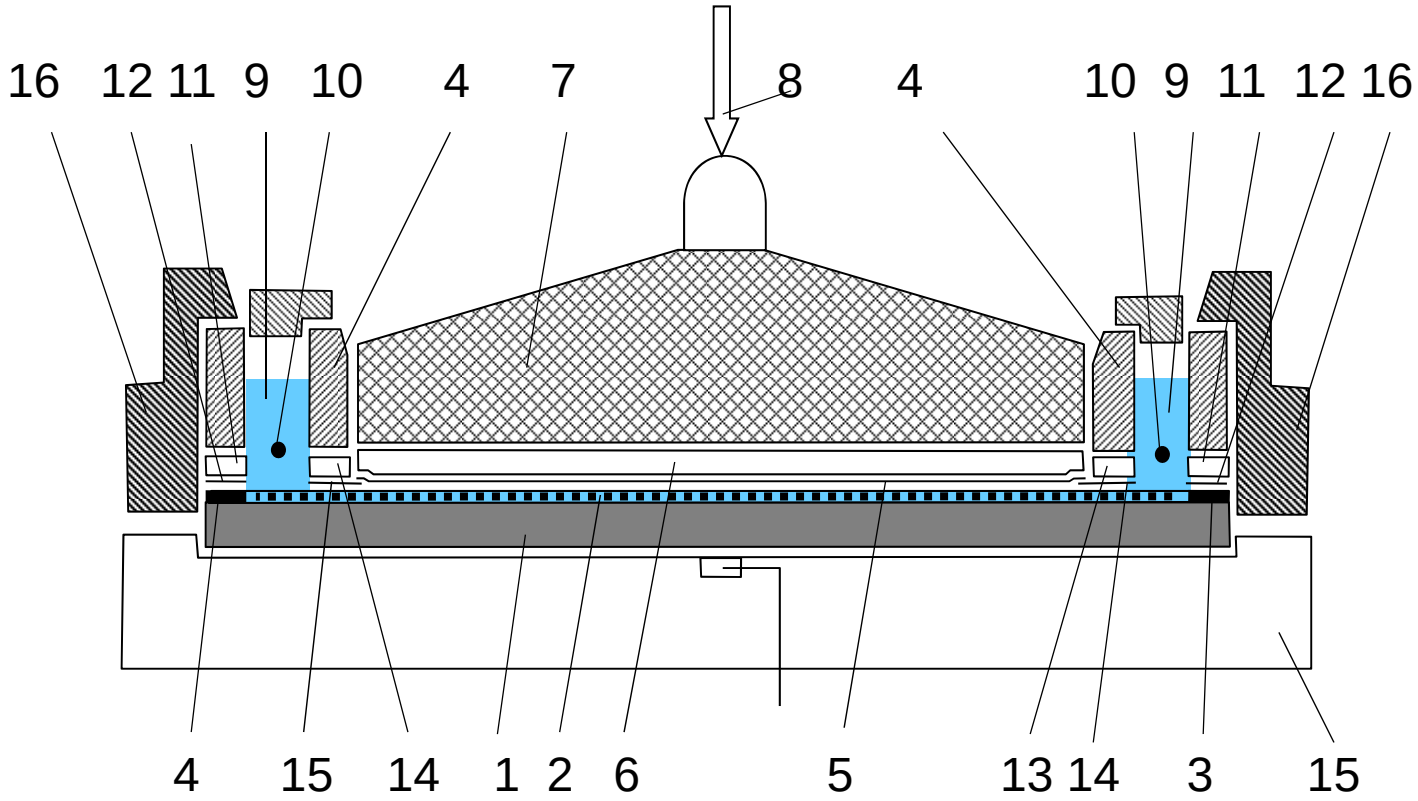
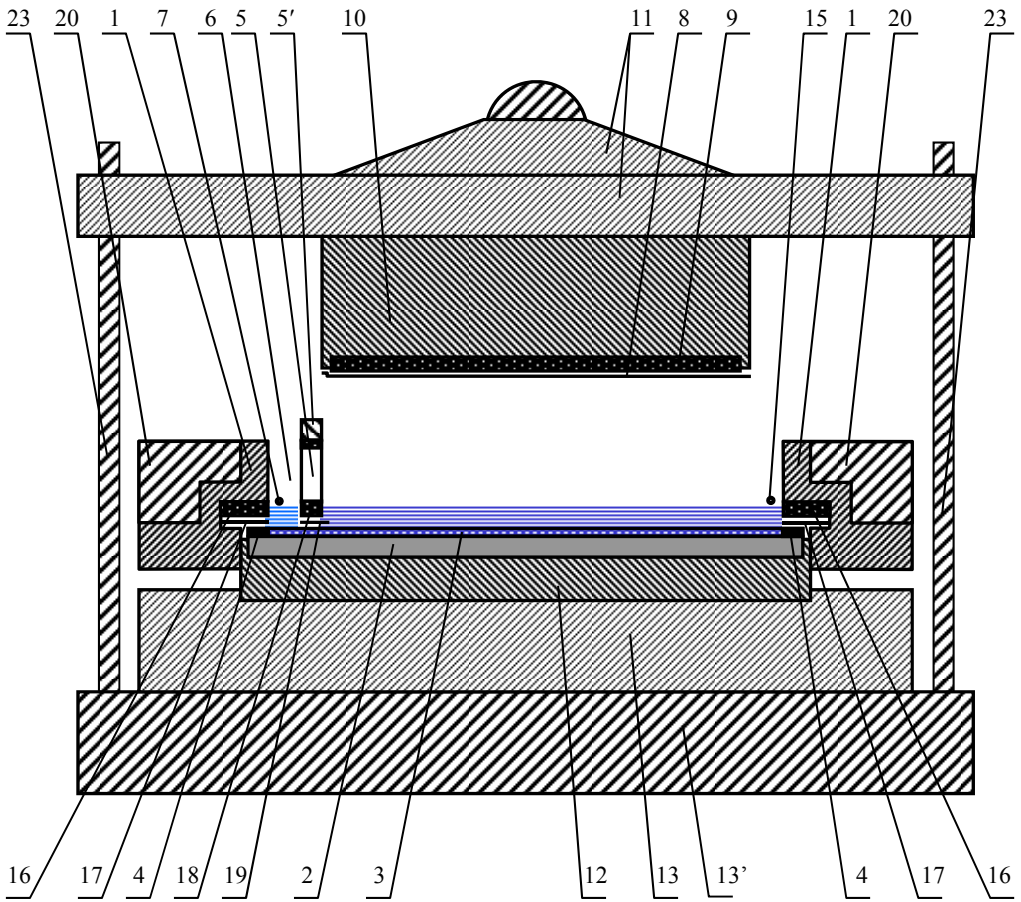


Complete device for PPEC with chromatographic plate in horizontal position.



Schematic view of the chamber for PPEC;
(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 (patent pending).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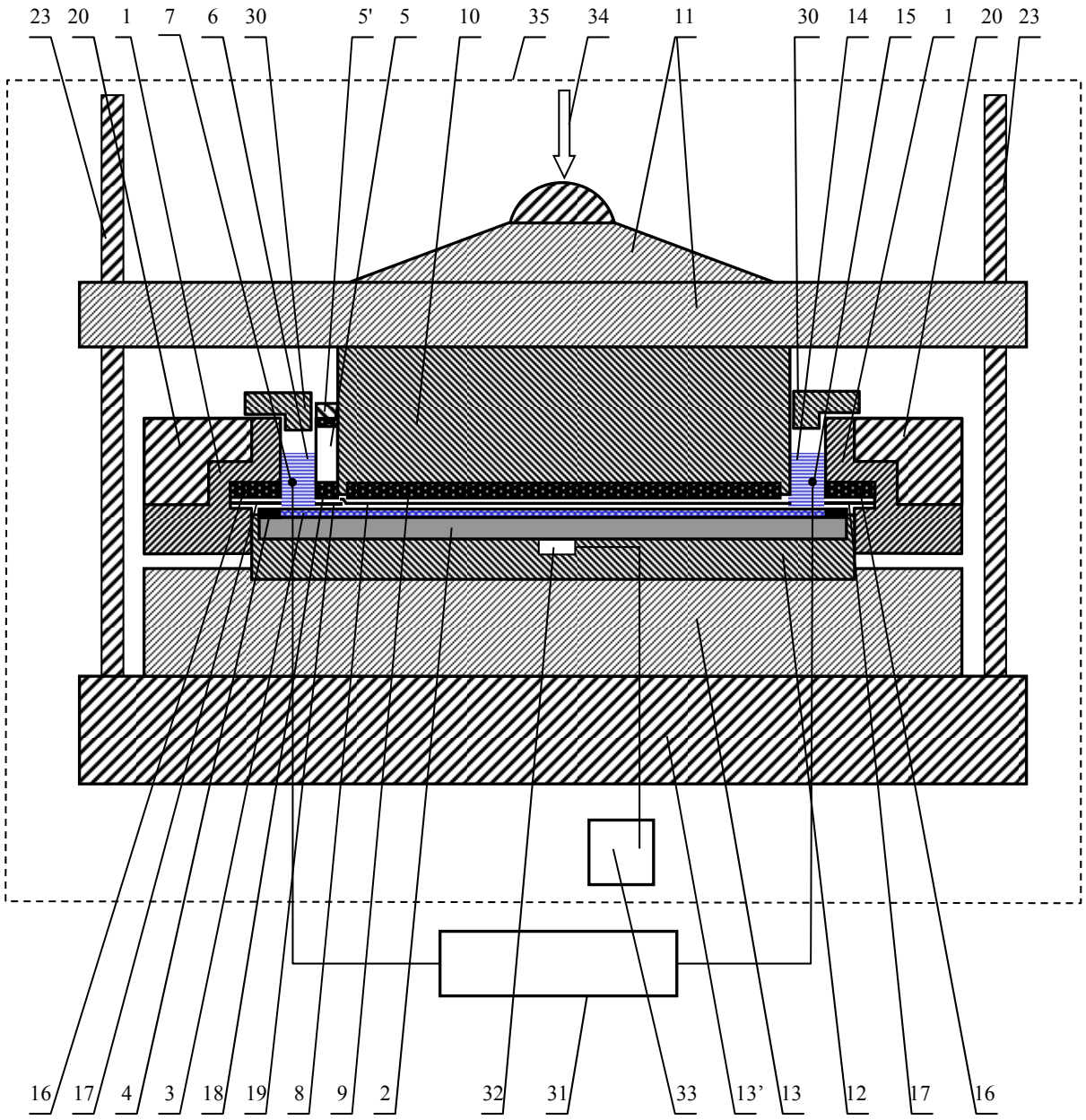
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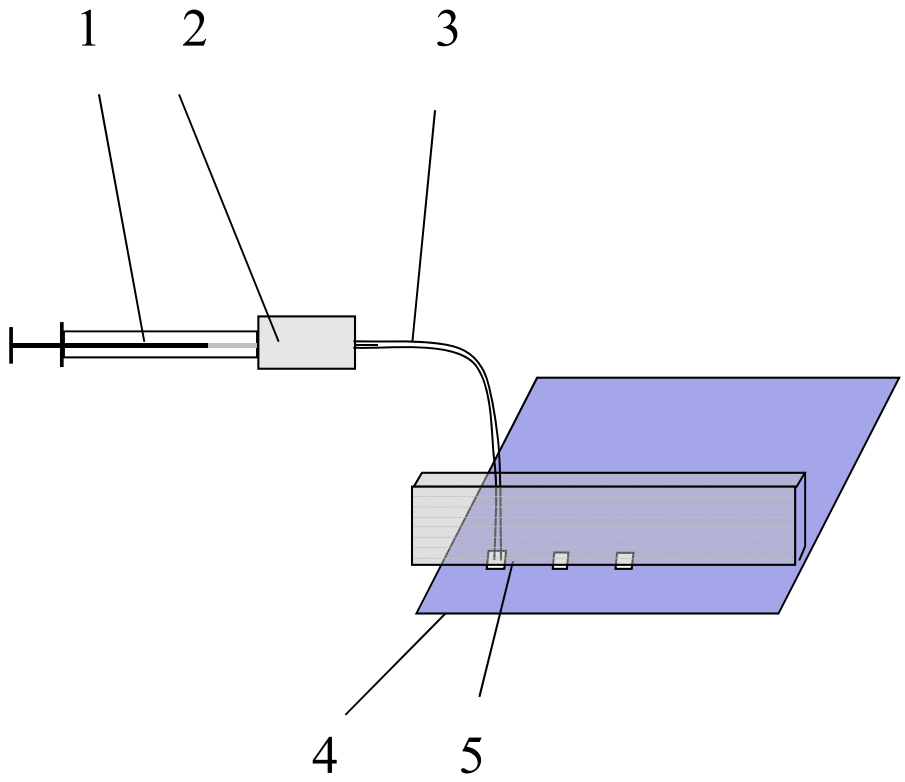
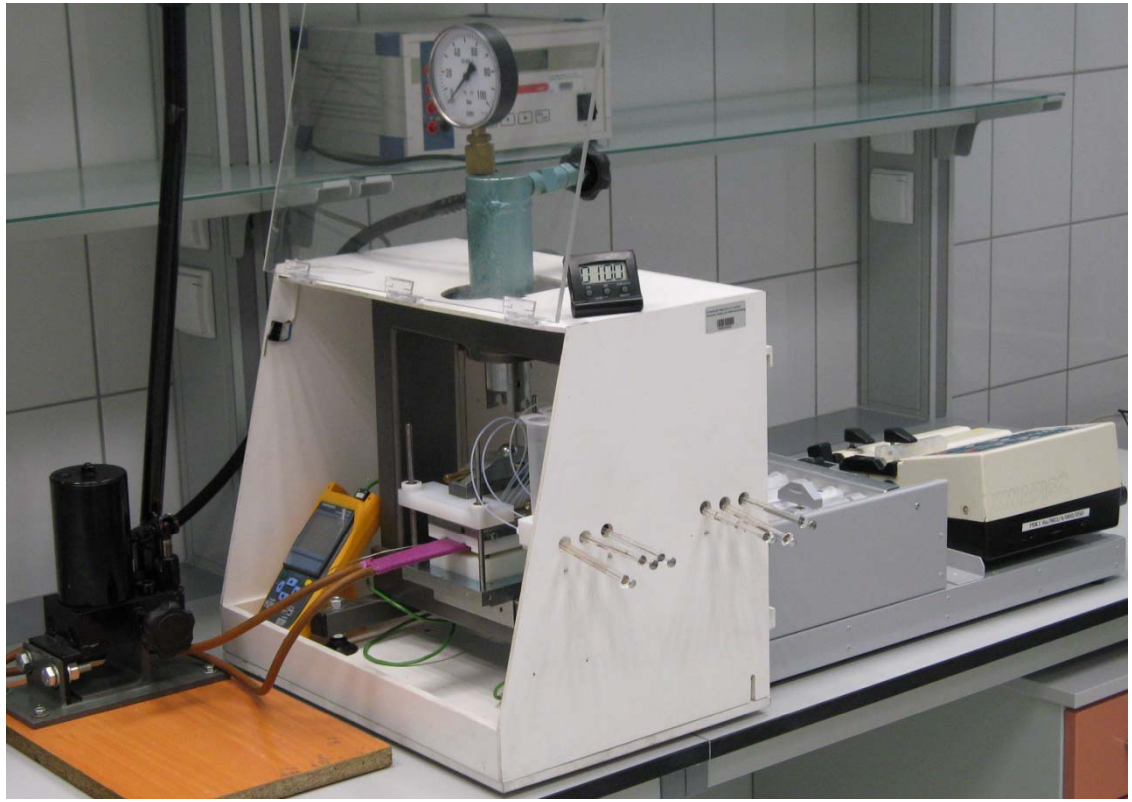
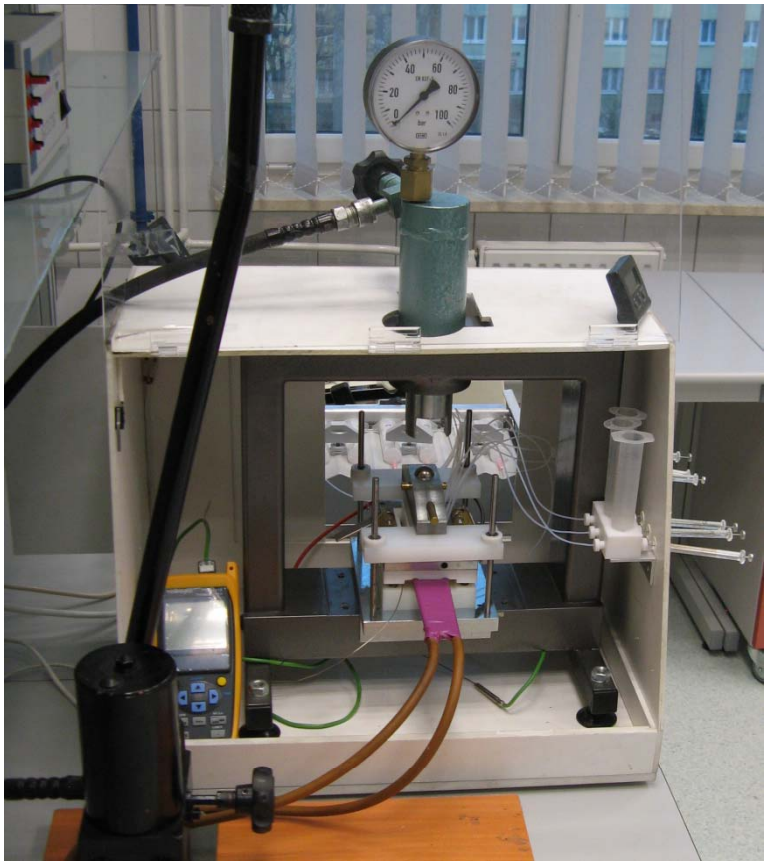
PPEC with on-line sample application



1- microsyringe, 2 – PTFE connector, 3 – PTFE tube,
4 – chromatographic plate, 5 – partition (in the PPEC chamber)

PPEC device with on-line sample application





Attributes of PPEC:

high kinetic performance,

short time of separation process,

change of separation selectivity.

High kinetic performance

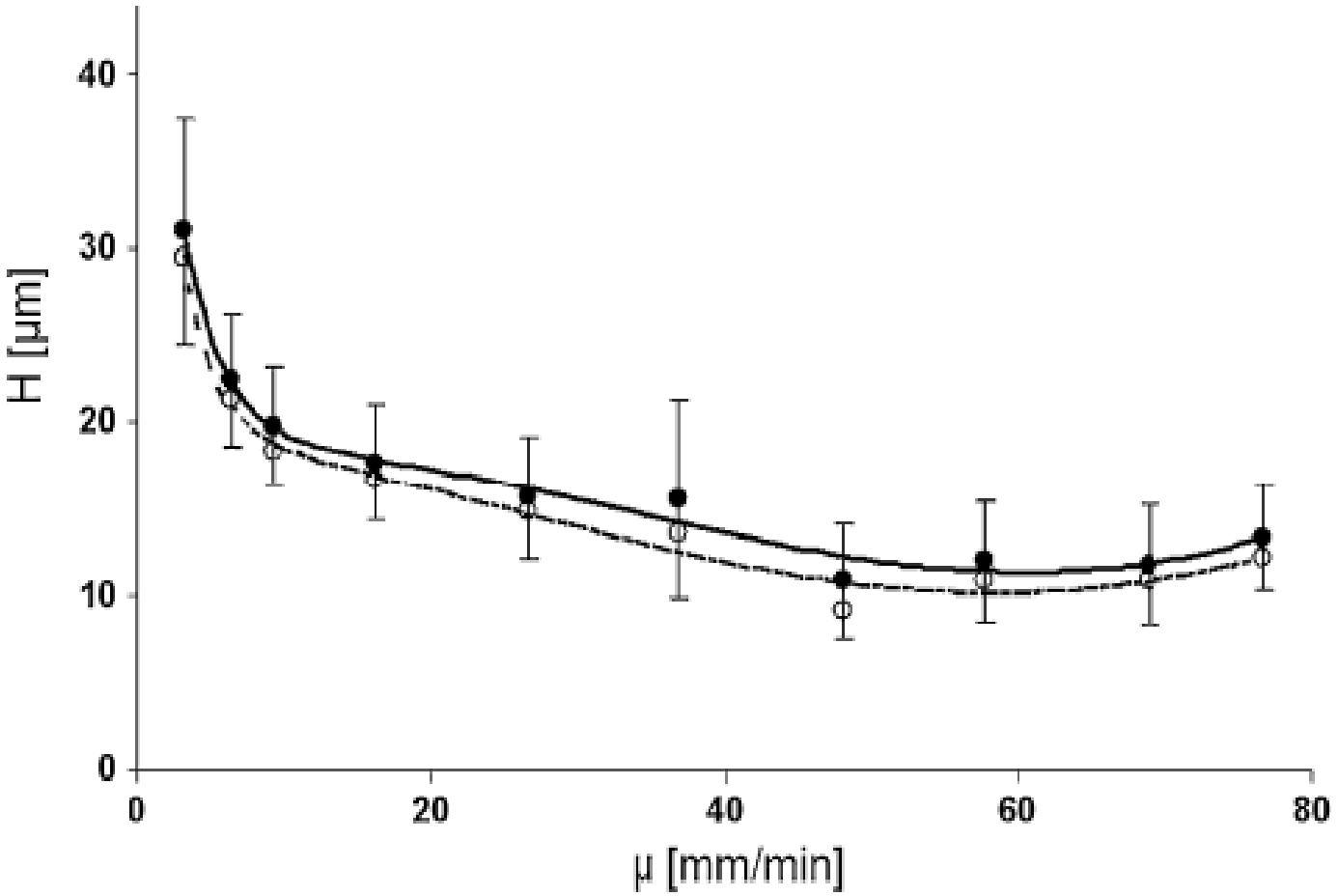


Plate height vs. flow velocity of the mobile phase for PPEC system with

(●) HPTLC RP-18W plate from Merck and

(∘) after subtracting variance concerned with starting spot width.

P. W. Płocharz, A. Klimek-Turek, T. H. Dzido, J. Chromatogr. A, 1217 (2010), 4868.

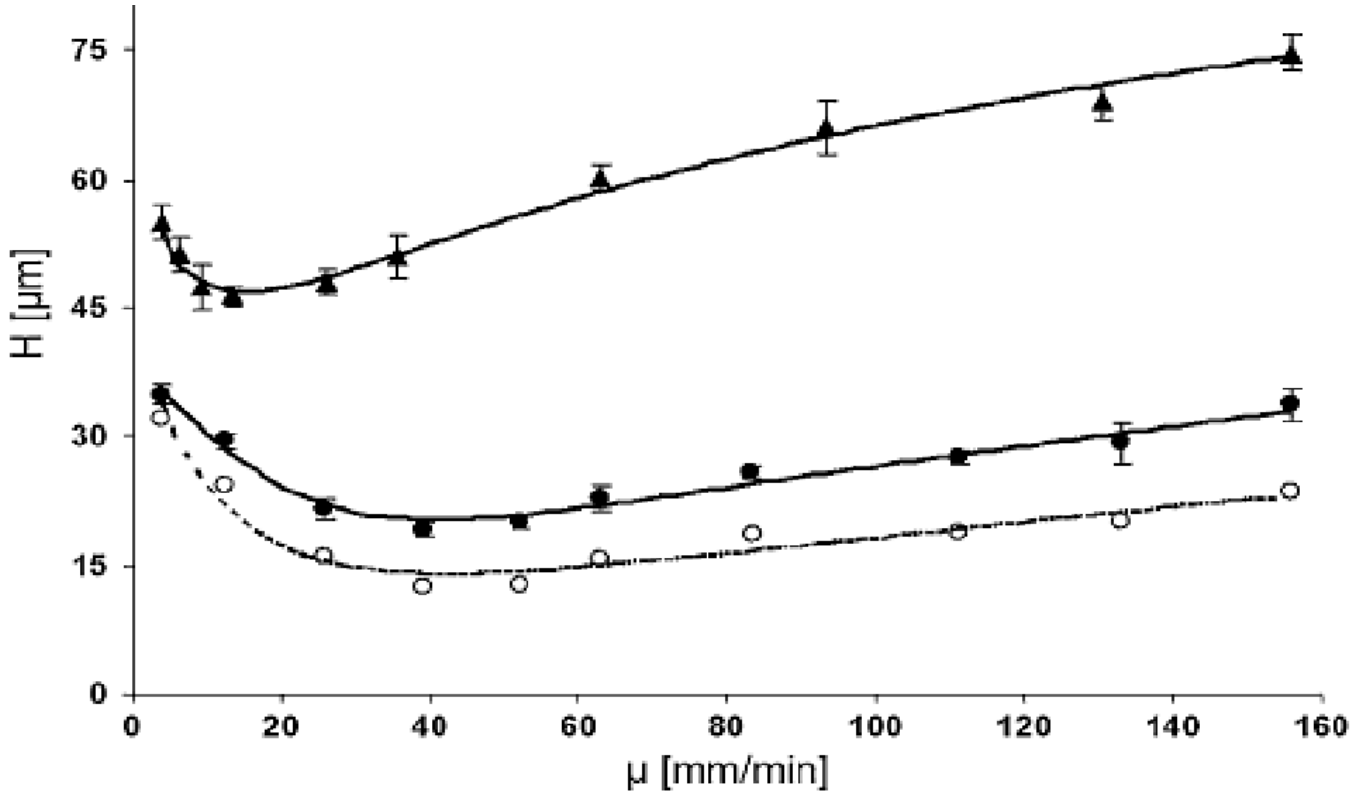


Plate height vs. flow velocity of the mobile phase for HPLC system with column packed with
(▲) adsorbent scraped from a chromatographic plate (HPTLC RP-18W, Merck),
(●) commercially available adsorbent with 5µm particles (LiChrosorb RP-18, Merck) and
(○) after subtracting variance concerned with external column volumes.

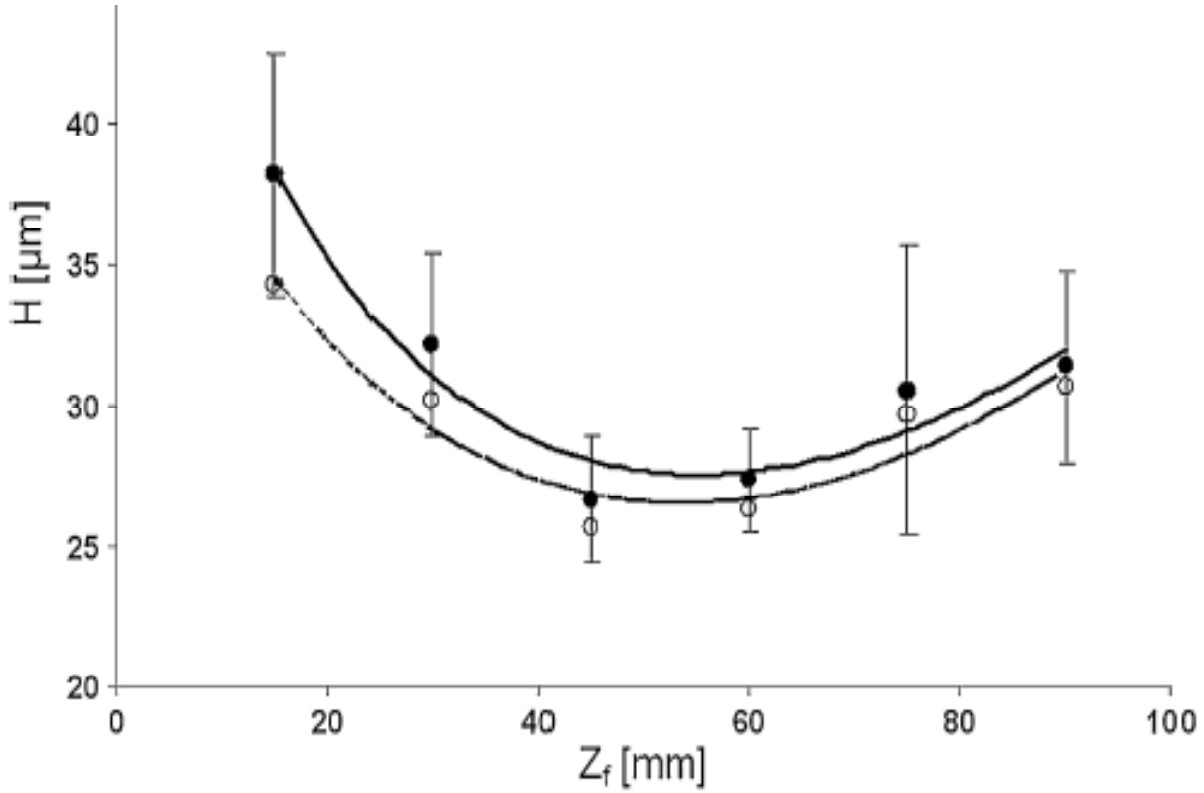
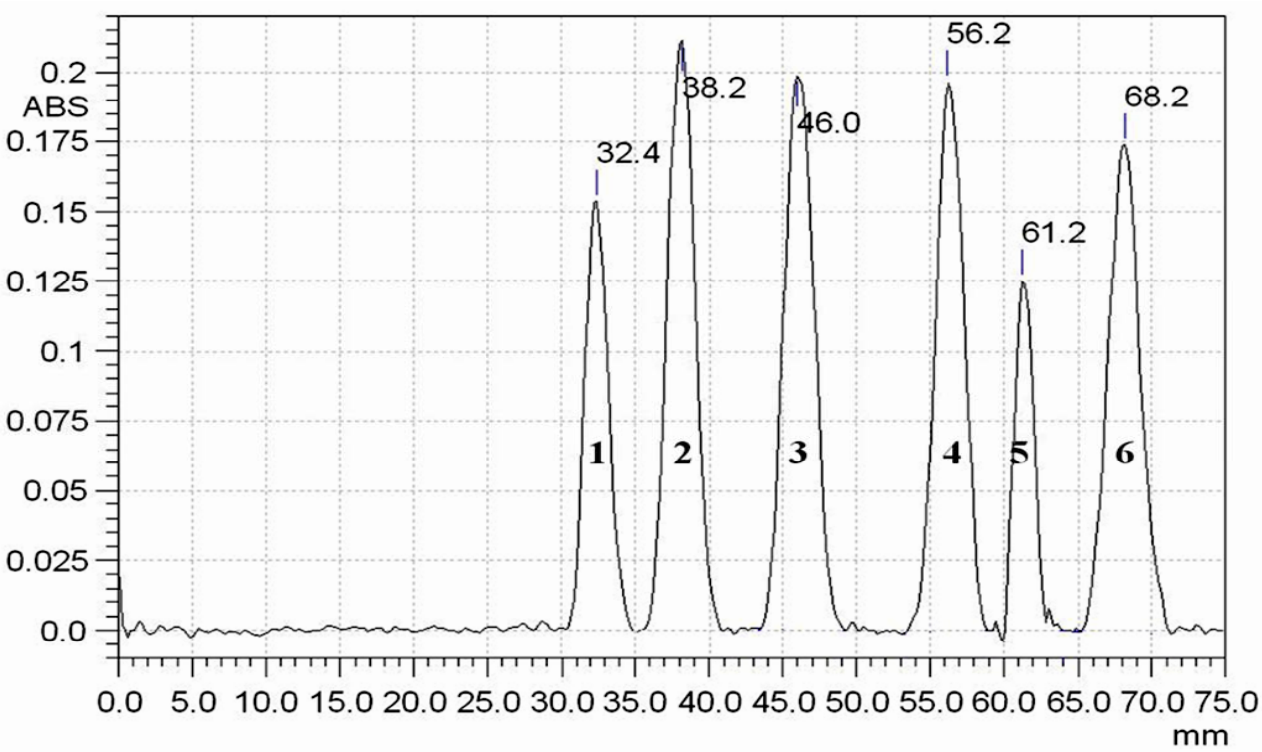


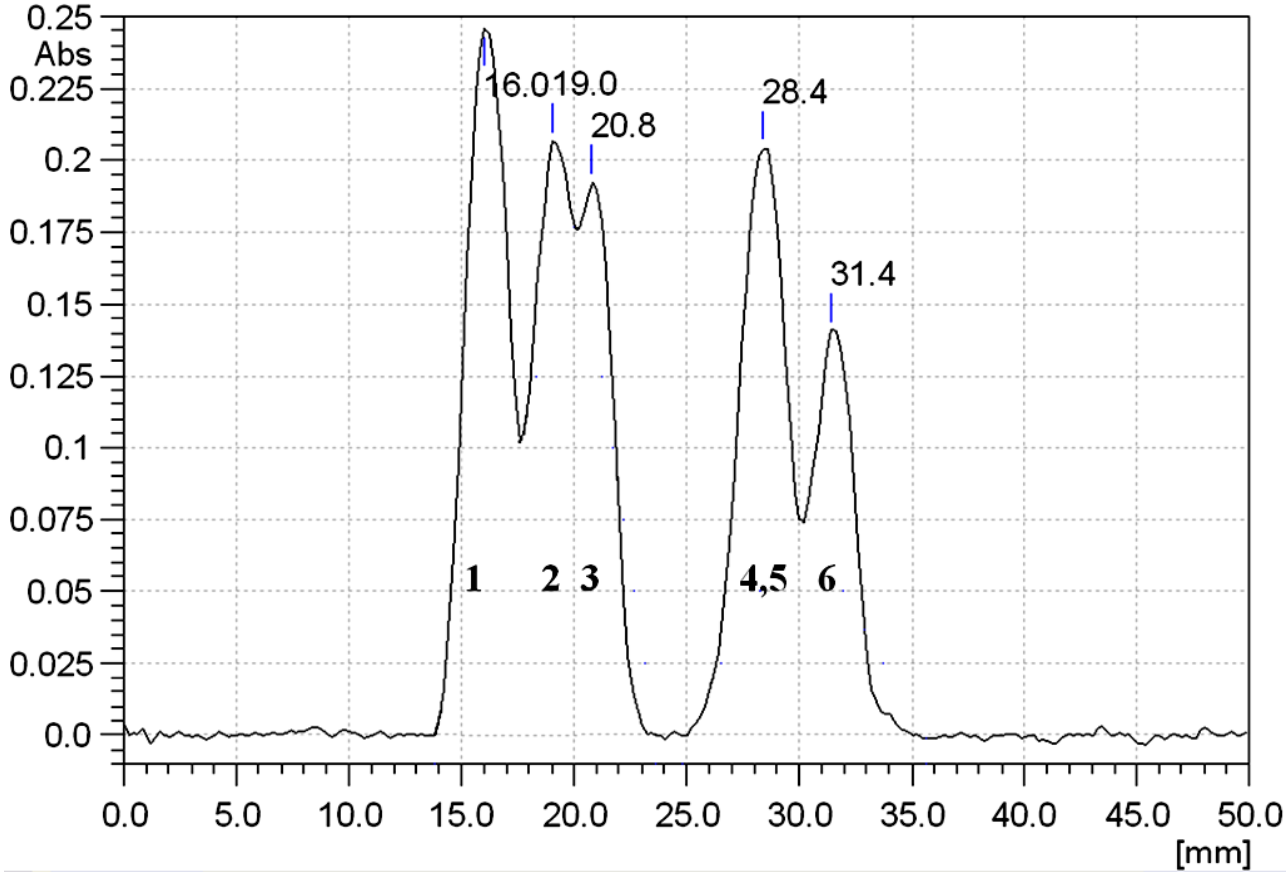
Plate height vs. migration distance of solvent front for HPTLC system. The TLC chamber (DS-II-5x10 from Chromdes) with (●) chromatographic plate HPTLC RP-18W from Merck and (∘) after subtracting variance concerned with starting spot width.

P. W. Płocharz, A. Klimek-Turek, T. H. Dzido, J. Chromatogr. A, 1217 (2010), 4868.



Electrochromatogram (PPEC) of the test mixture: (1) testosterone isobutyrate, (2) testosterone acetate, (3) methandienone, (4) hydrocortisone acetate, (5) 16-dehydropregnenolone acetate, (6) prednisolone succinate; RP-18W HPTLC plate from Merck, applied polarization voltage 2.5 kV, separation time 5 min, the mobile phase: 80% acetonitrile in buffer (pH= 5.0).

Płocharz, A. Klimek-Turek, T. H. Dzido, J. Chromatogr. A, 1217 (2010), 4868.



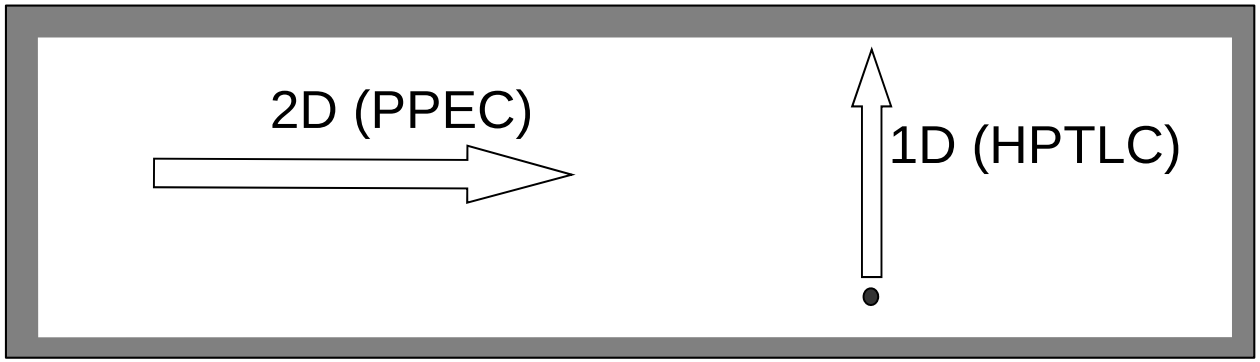
Separation of the test mixture by conventional high-performance planar chromatography with HPTLC RP-18W plate, separation time 6.5 min, the mobile phase and the test solute mixture as in previous slide.

Płocharz, A. Klimek-Turek, T. H. Dzido, J. Chromatogr. A, 1217 (2010), 4868.

Short time of separation process

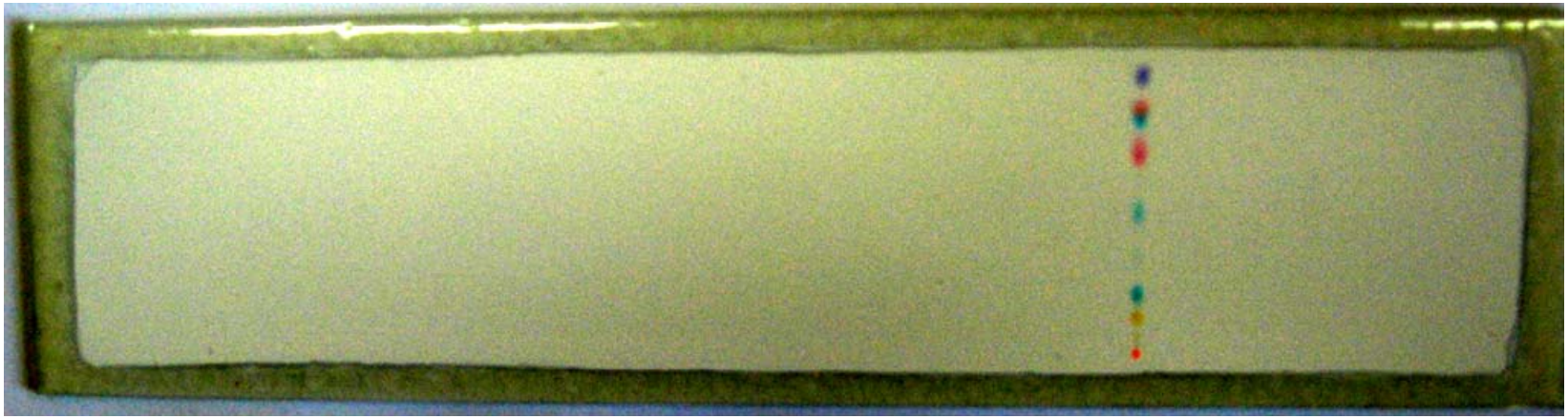
Change of separation selectivity

Two dimensional separation (2D HPTLC/PPEC)



Principle of two dimensional separation with HPTLC and PPEC (2D HPTLC/PPEC). Chromatographic plate with margins of silicone sealant on its whole periphery. Starting spot of the mixture to be separated is marked with a dot. Directions of the mobile phase migration in (1D) TLC and (2D) PPEC processes is marked with arrows.

1D



Digital picture of TLC chromatogram, HPTLC RP18W plate (Merck), mobile phase: 45% methanol in buffer pH 3.0;
(1) rhodamine 6G, (2) PAR, (3) patent blue, (4) green S, (5) azorubine, (6) brilliant blue, (7) allura red, (8) brilliant black.

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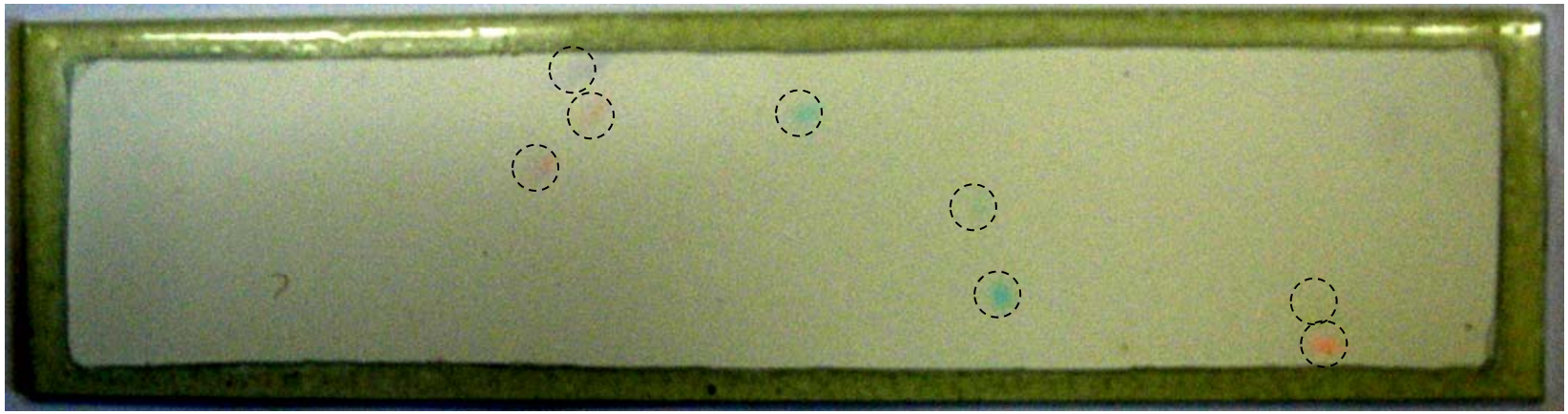
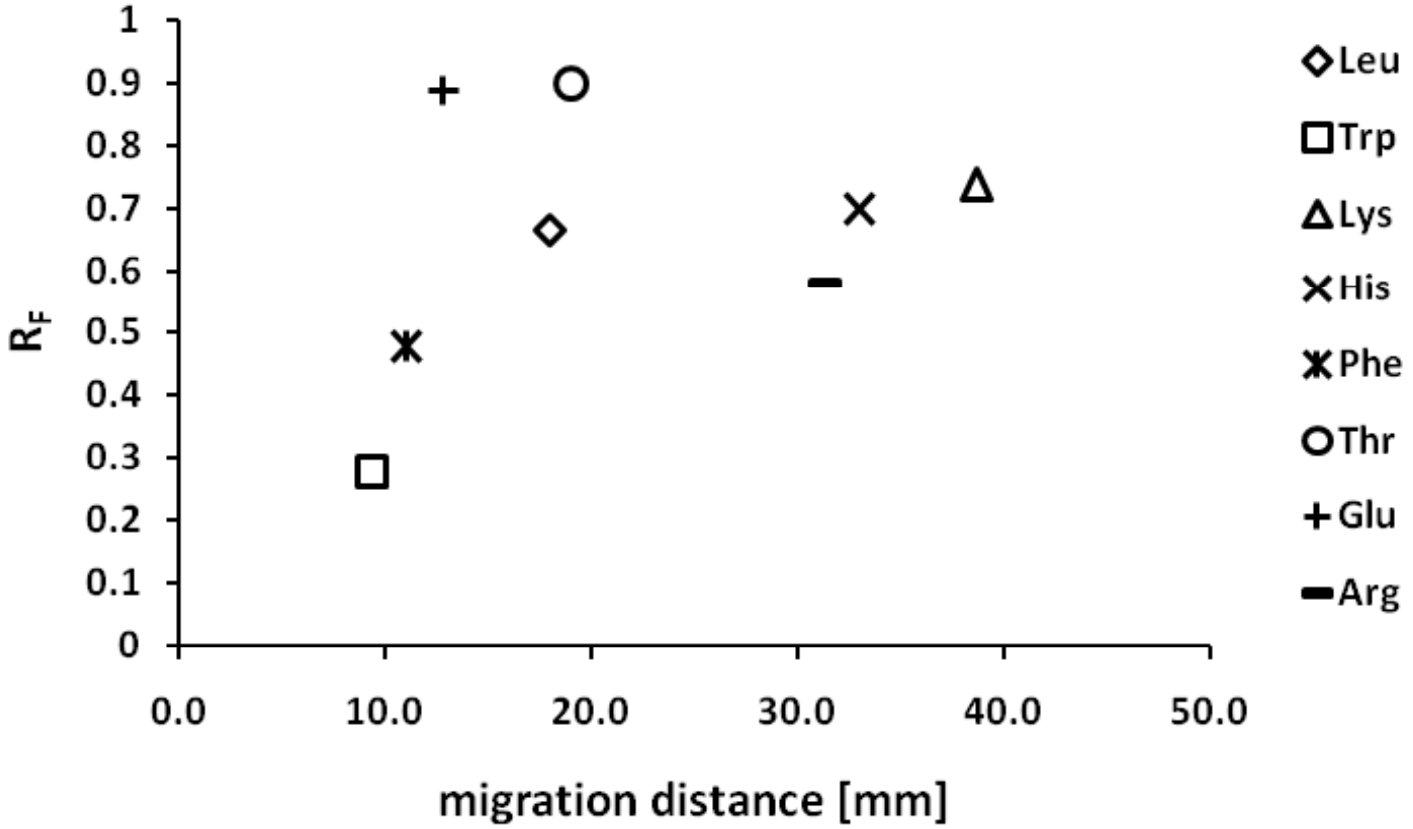
2D



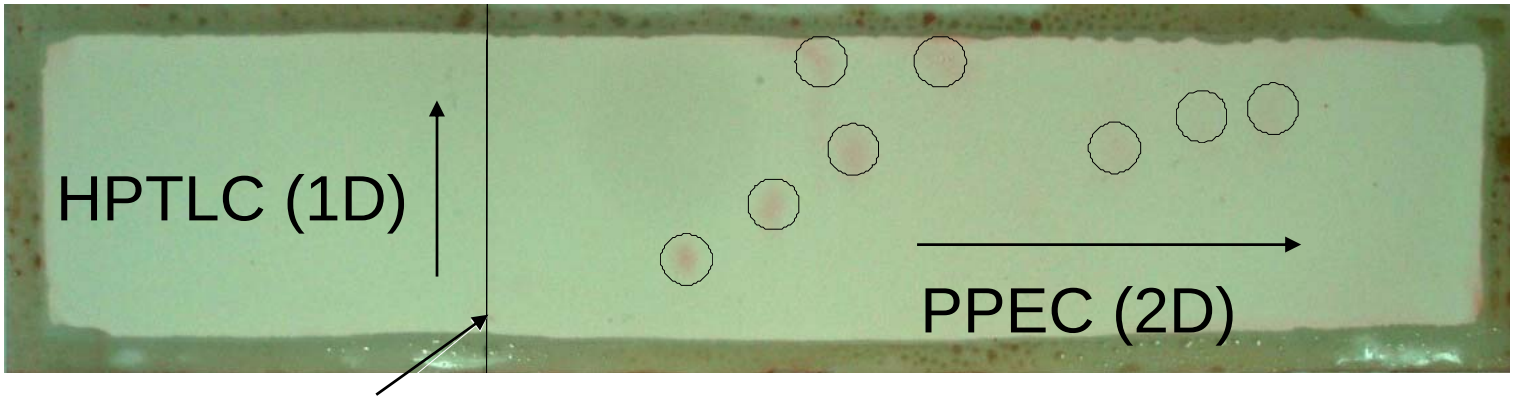
Digital picture of the chromatographic plate after 2-D HPTLC/PPEC separation, the mobile phase of the first dimension (HPTLC) as in previous slide, the mobile phase of the second dimension (PPEC): 75 % ACN in buffer pH 3.0, polarization voltage 2.5 kV.

A. Chomicki, P. Ślązak, T.H. Dzido Electrophoresis 30 (2009) 3718 - 3725.



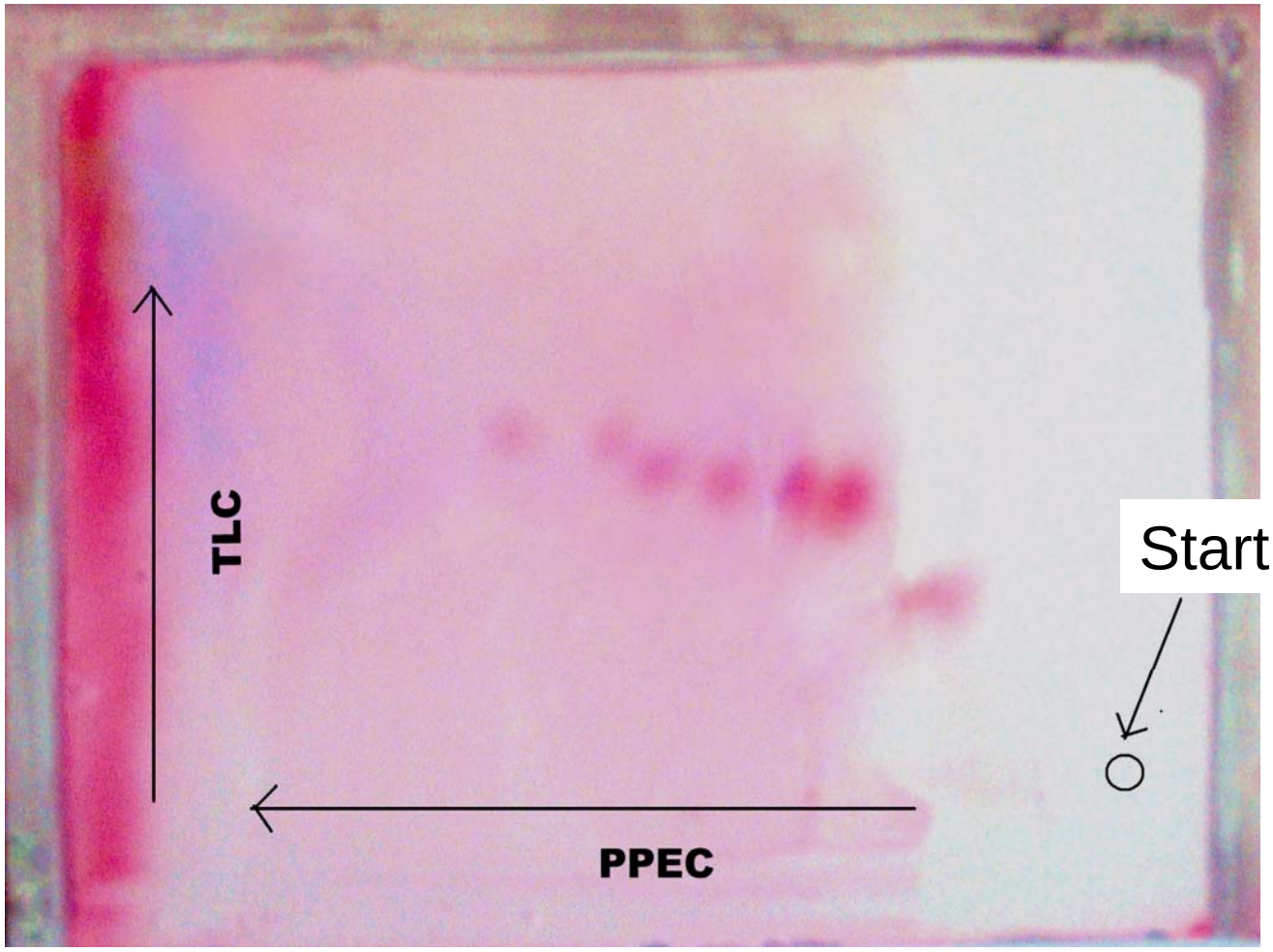
Comparison of R_F values (in HPTLC system, 10 % acetonitrile in buffer pH 3.2) and values of migration distance (in PPEC system, 10 % acetonitrile in buffer pH 3.2) of investigated amino acids, HPTLC RP 18W plate from Merck.

A. Chomicki, K. Kloc, T.H. Dzido, J. Planar Chromatogr. 24 (2011) 6 - 9.



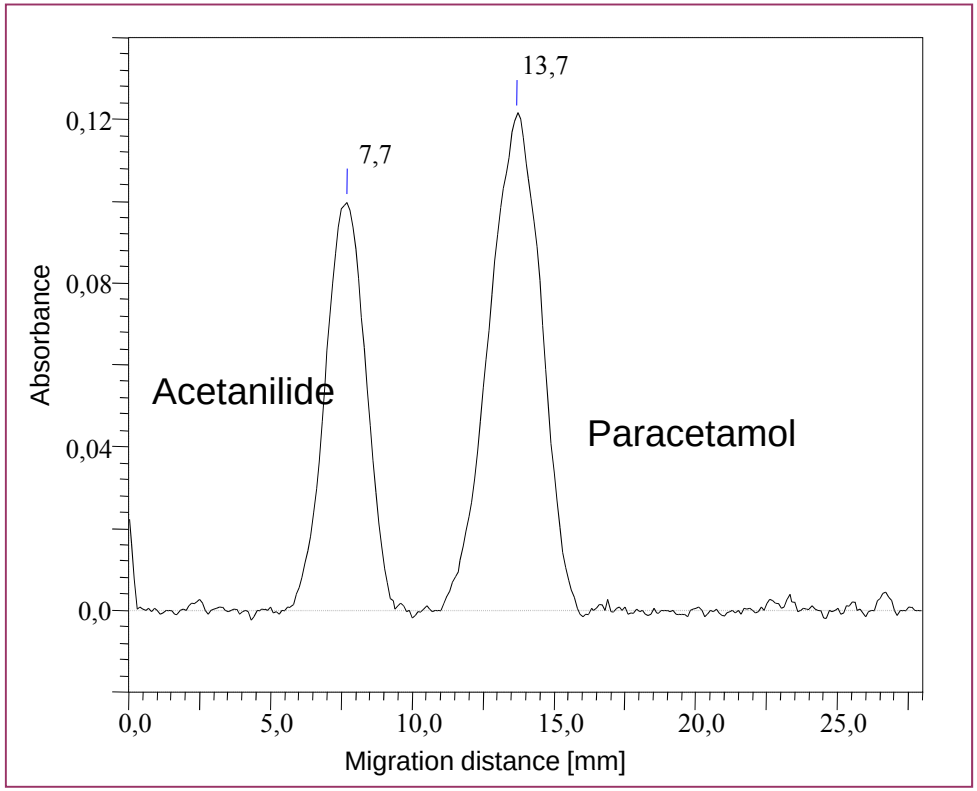
Digital picture of the chromatographic plate after 2D HPTLC/PPEC separation of eight amino acids, mobile phase in the first (HPTLC) and in the second dimension (PPEC): 10 % acetonitrile in buffer pH 3.2, HPTLC RP 18W plate (Merck); polarization voltage 2.5 kV.

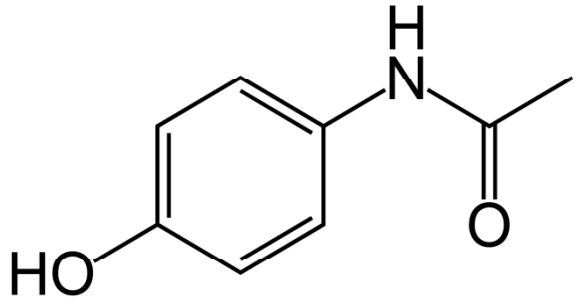
A. Chomicki, K. Kloc, T.H. Dzido, J. Planar Chromatogr. 24 (2011) 6 – 9.



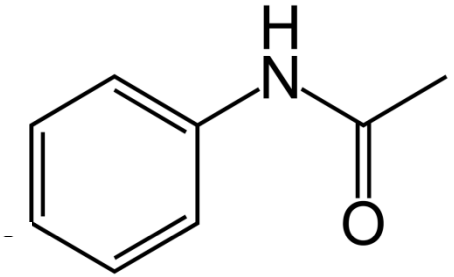
Digital picture of the chromatographic plate after
2D PPEC/HPTLC separation of seven amino acids (Trp, Tyr, Val, Ala, Gly, Cys, Lys); HPTLC Silica gel 60F $_{254}$s plate (Merck),
mobile phase in PPEC: acetonitrile + buffer pH 3.2 (5:1),
mobile phase in HPTLC: acetonitrile + buffer pH 3.2 (1:1).

Pharmaceutical analysis





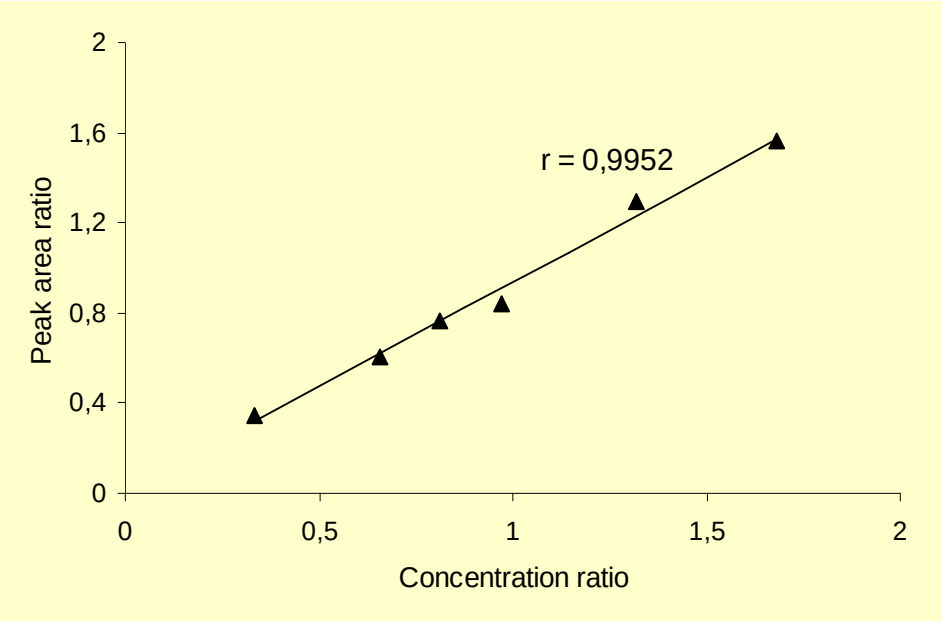
Paracetamol



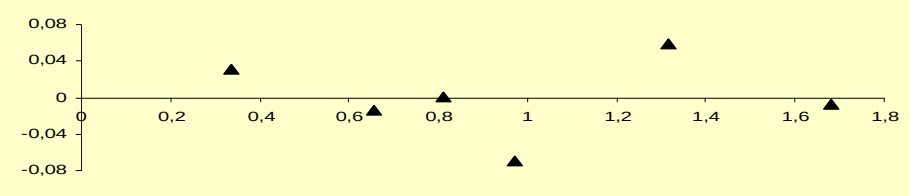
Acetanilide

PPEC, mobile phase: 20 %ACN + buffer, pH = 5.0, HPTLC RP-18W F_{254} plates (Merck), 1kV, 3 min, on-line application

Quantitation of paracetamol in tablets



Residuals



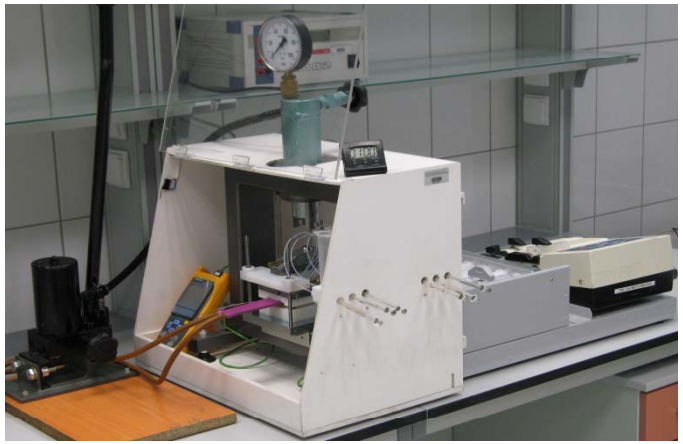


Table 9. Repeatability

Solute	Inter-day			Intra-day		
	Mean	SD	RSD[%]	Mean	SD	RSD[%]
Paracetamol	0.9392	0.0145	1.54	0.9335	0.0321	3.44

Separation time (min) of six samples by HPTLC and PPEC

Operation types	**HPTLC, aerosol application**	**PPEC, aerosol application**	**PPEC, on-line application**
Application	30	30	1
Conditioning	15	1	2
Separation	5	3	3
Registration	6	6	6
Additional operations	1	2	2
TOTAL	57	42	14

Conclusions

PPEC is attractive mode for application in laboratory practice with respect to its high kinetic performance, short time of separation process and different separation selectivity relative to liquid chromatography.

Application of the mode is especially attractive to two dimensional separations and pharmaceutical analysis.

Main limitations of PPEC (at present):

- equipment is not still commercially available,

- there are not chromatographic plates (stationary phases) dedicated to the mode.