

# **Pressurized Planar Electrochromatography – Challenges and Perspectives**

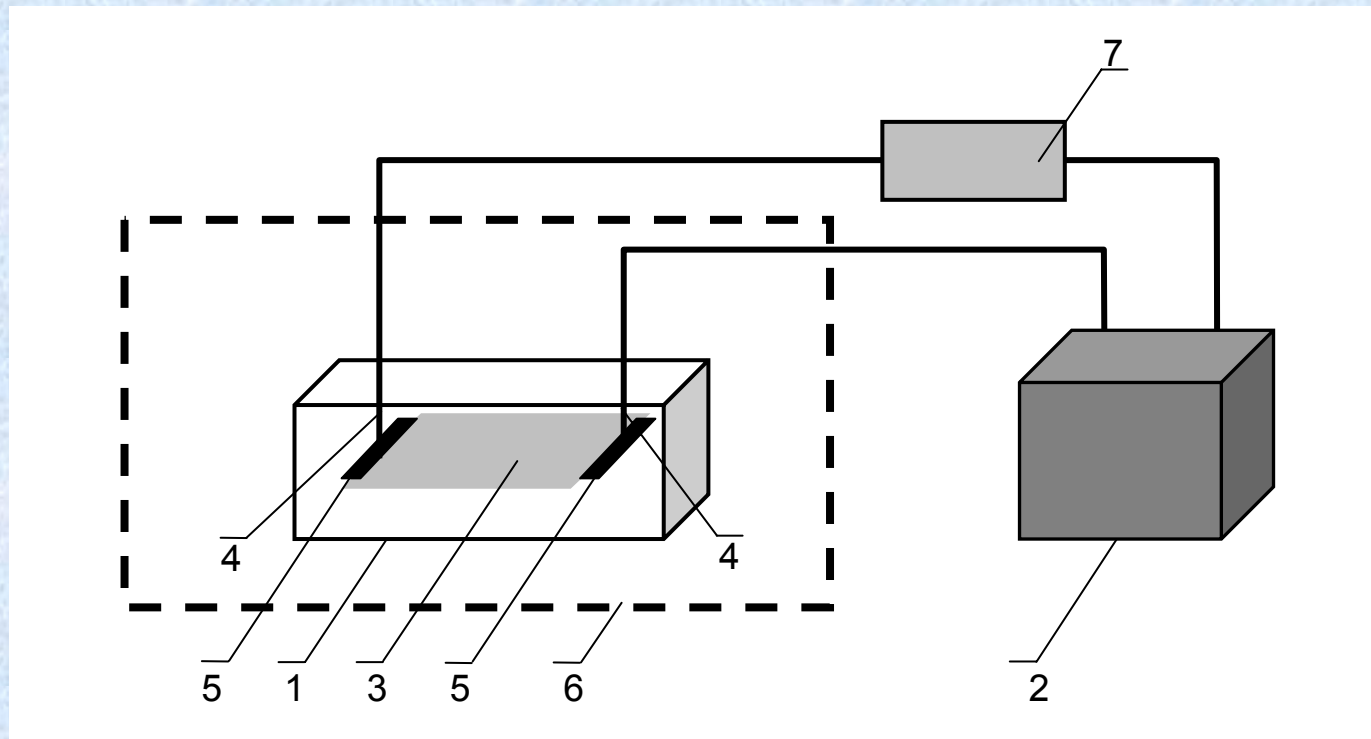
**T. H. Dzido, P. W. Płocharz, P. Ślązak, B. Polak,  
A. Hałka and A. Chomicki**

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Lublin, Poland**

**HPTLC 2008, HELSINKI: 11<sup>th</sup> – 13<sup>th</sup> June 2008**

Planar electrochromatography 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

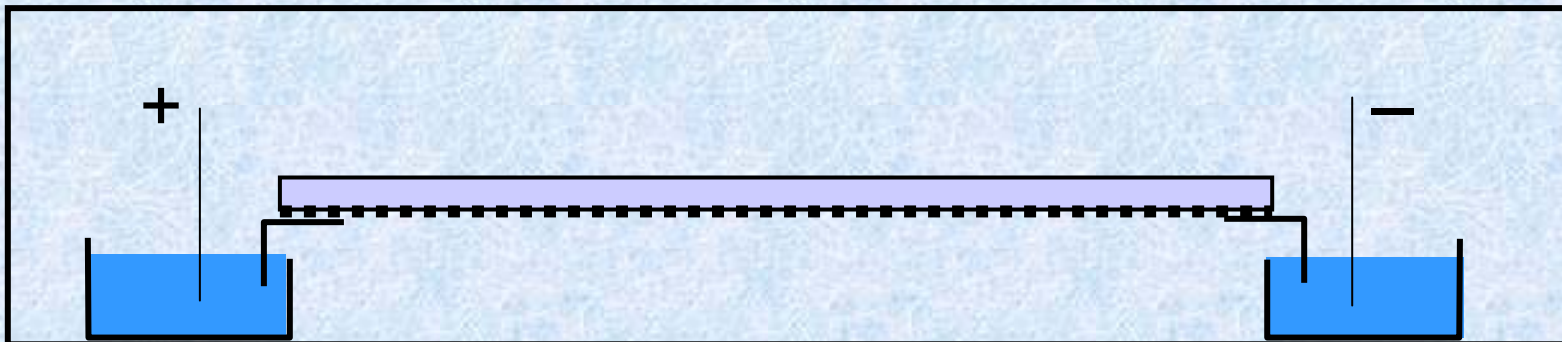


(1) chamber for PPEC, (2) high voltage power supply, (3) chromatographic plate, (4) electrodes, (5) reservoir of the mobile phase, (6) cabinet for PPEC chamber, (7) ammeter.

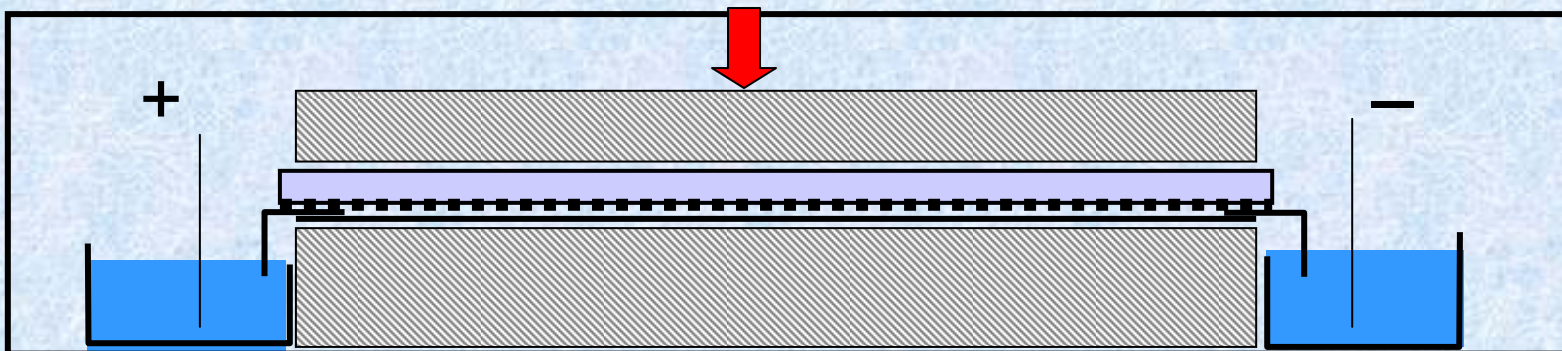
## Two modes of planar electrochromatography:

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

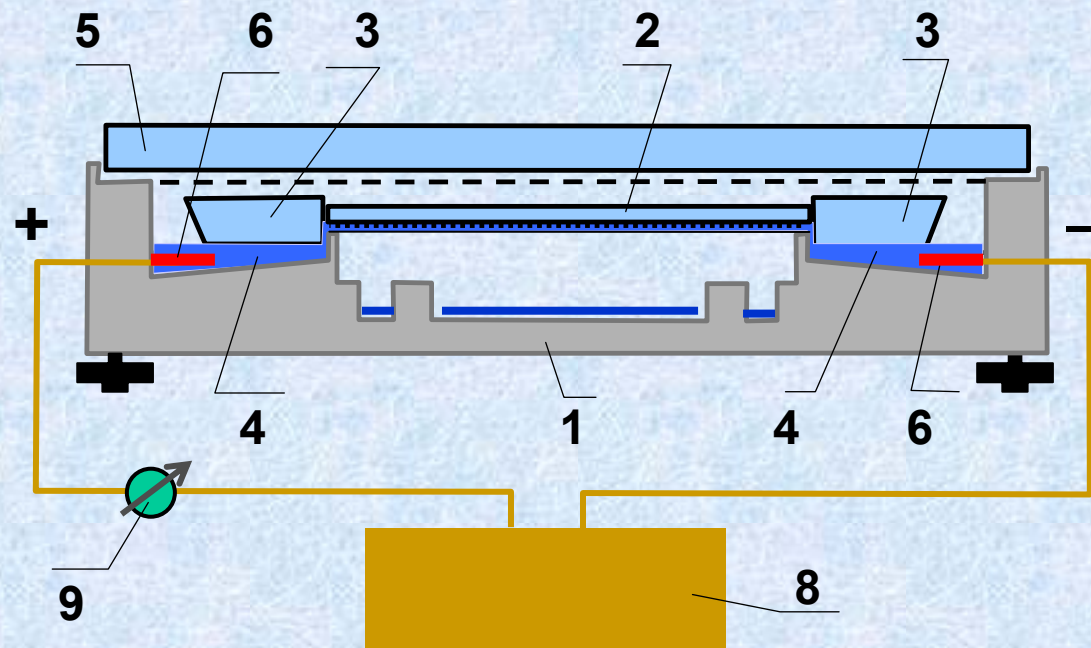
# Planar electrochromatography in open system - **PEC**



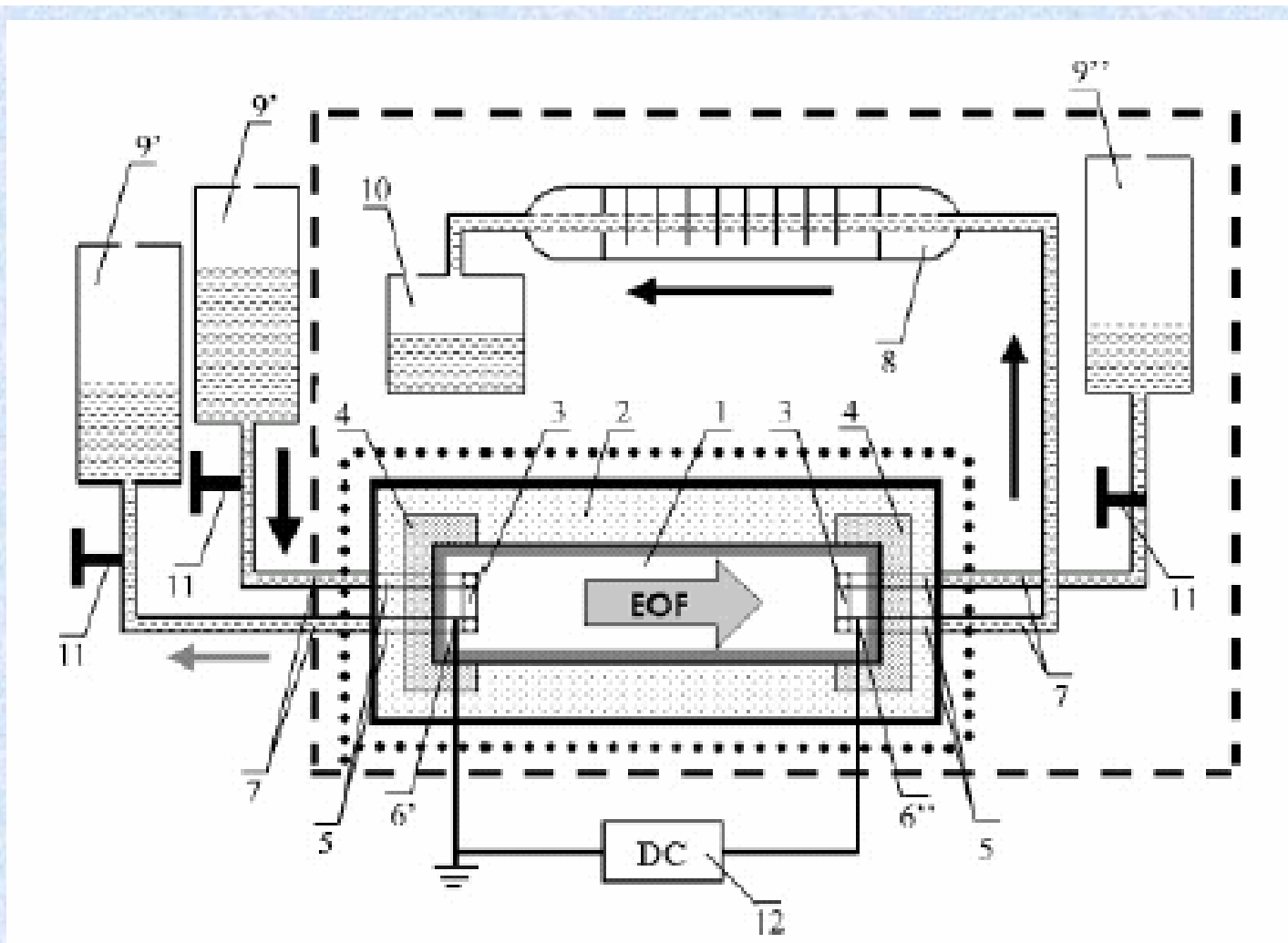
# Planar electrochromatography in closed system under pressure - **PPEC**



# Horizontal DS chamber adapted for planar electrochromatography (PEC)

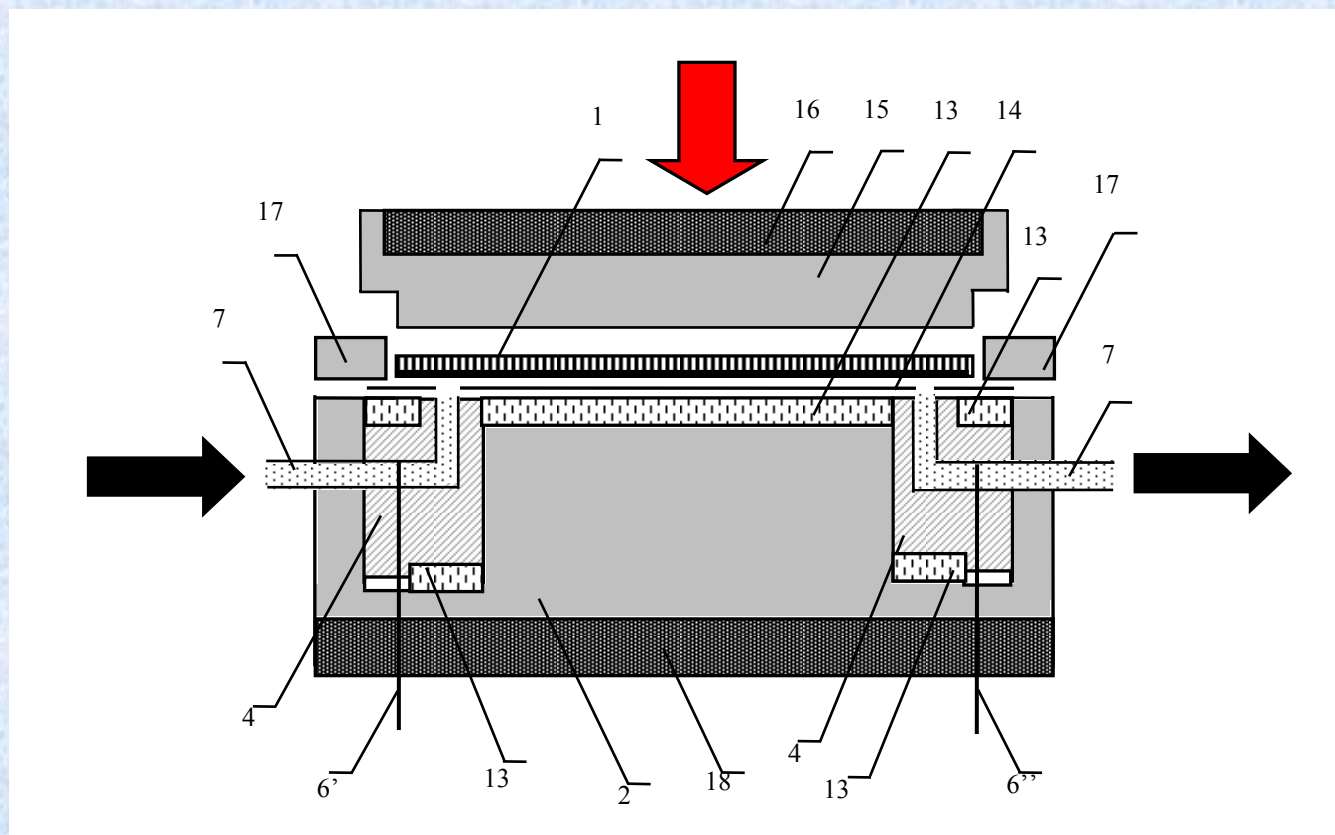


T.H. Dzido, R. Majewski, B. Polak, W. Gołkiewicz, E. Soczewiński, J. Planar Chromatogr. , 16 (2003) 176.



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

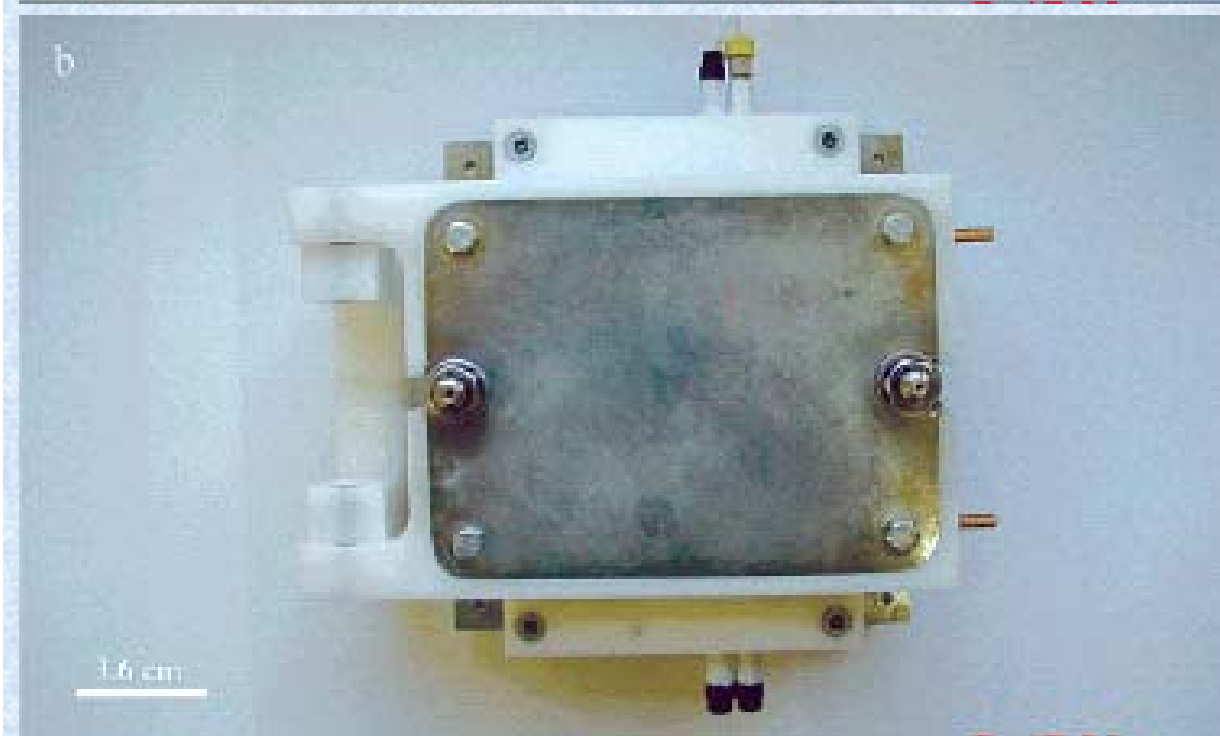
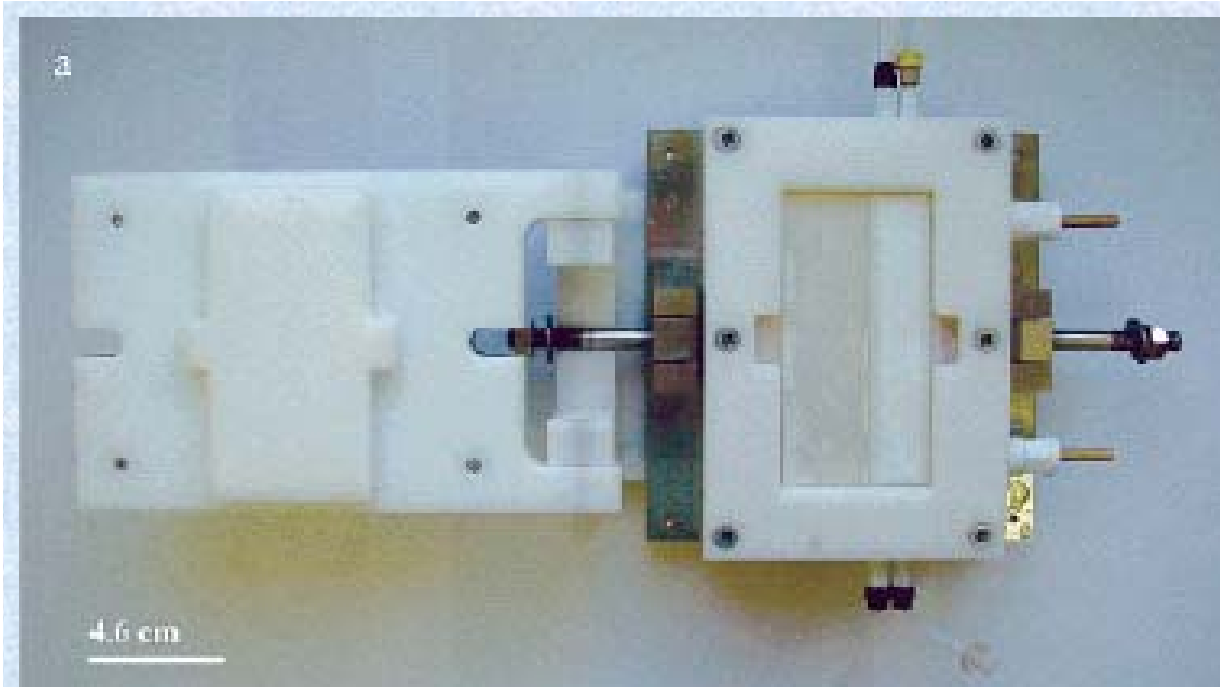
T.H. Dzido, P.W. Płocharz, P. Ślązak, Anal Chem. 78 (2006) 4713 – 4721.



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

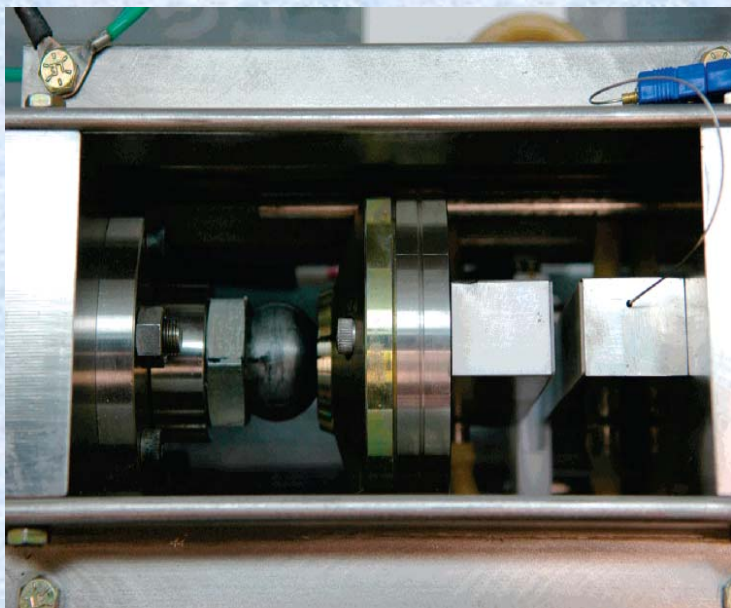
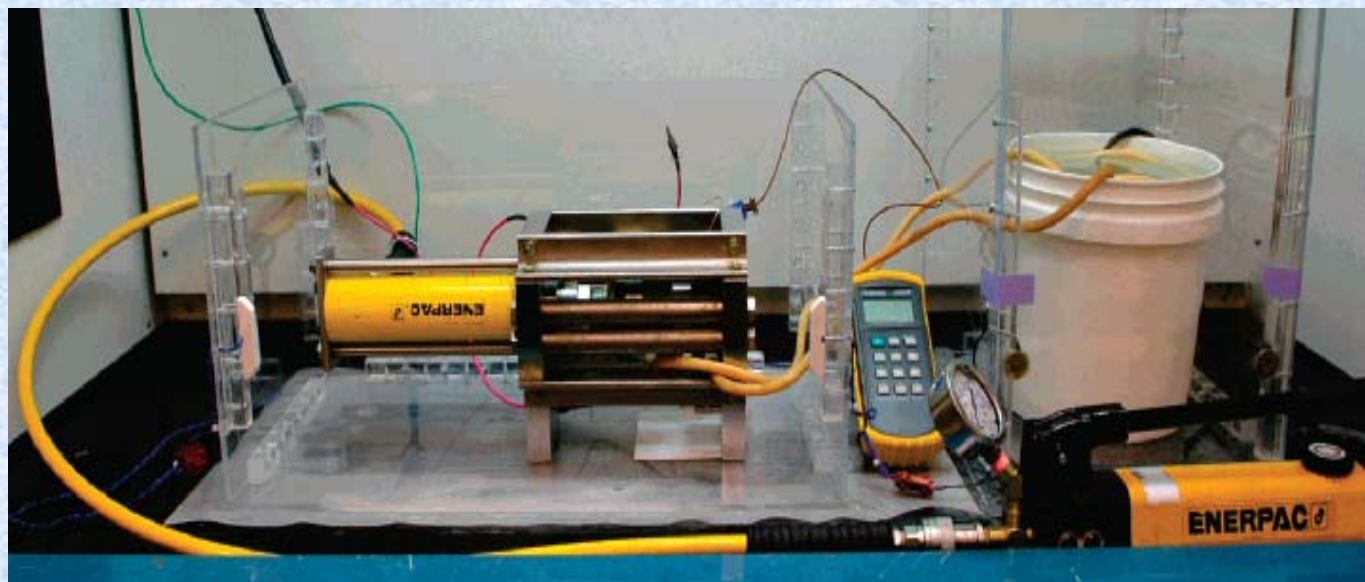
T. H. Dzido, P. W. Płocharz, J. Liq. Chromatogr. 30 (2007) 2651–2667.





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.

T.H. Dzido, P.W. Płocharz, P. Ślązak, *Anal. Chem.* 78 (2006) 4713 – 4721.



Apparatus for performing PPEC.

D. Nurok, et al., Anal. Chem., 78  
(2006) 2823 – 2831.



1. A kísérlet célja az elektrolízis során a kadmium és az ólom ionok oxidációjának megfigyelése és az elektrolízis során keletkező gázok azonosítása.  
2. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
3. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
4. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
5. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
6. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
7. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
8. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
9. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
10. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
11. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
12. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
13. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
14. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
15. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
16. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
17. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
18. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
19. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.  
20. A kísérlet célja az elektrolízis során keletkező gázok azonosítása.

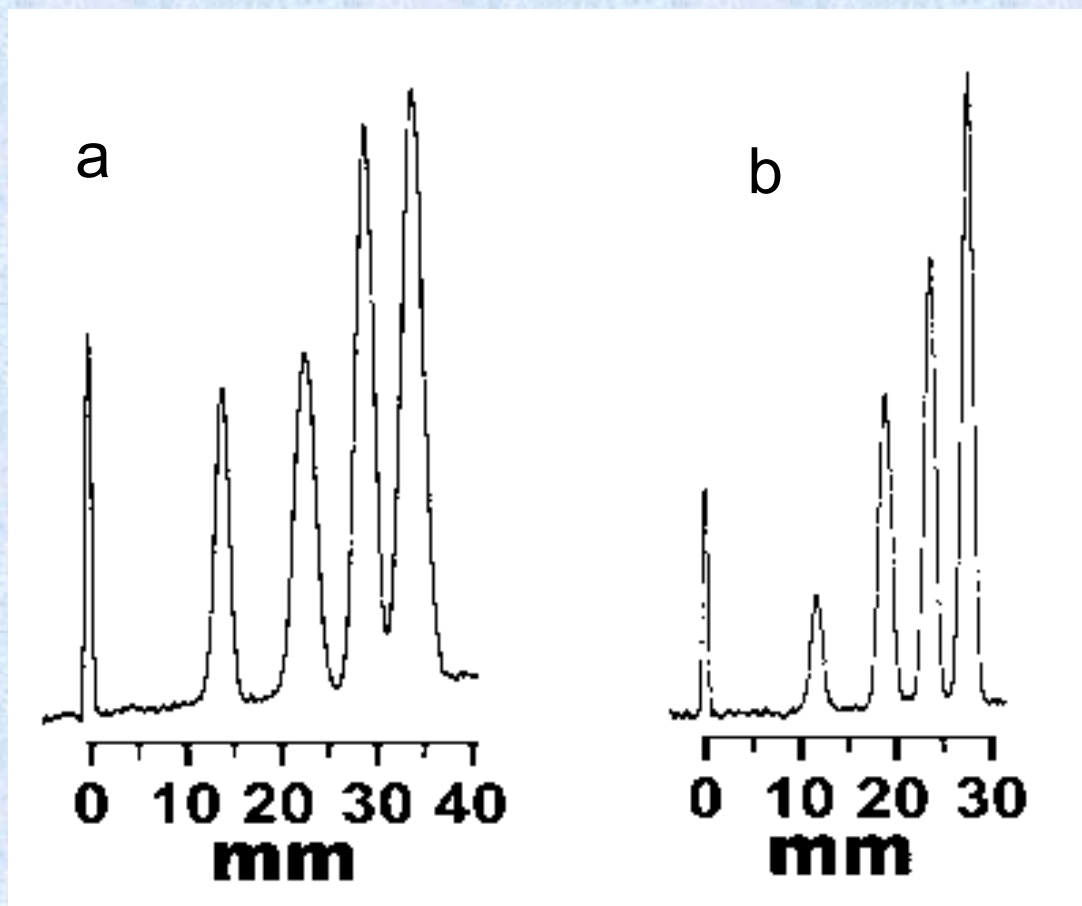
**Elektrolízis Híg zsva**

Árnyék	2,0%
Ólom	2,0%
Vas	2,0%
Árnyék	2,0%
Árnyék	2,0%
Árnyék	2,0%
Árnyék	2,0%

MAGYAR KÖZÖSSÉG KÉMIAI FÜZETEK

## **Advantages of PPEC:**

- short time,
- high performance,
- change of separation selectivity.

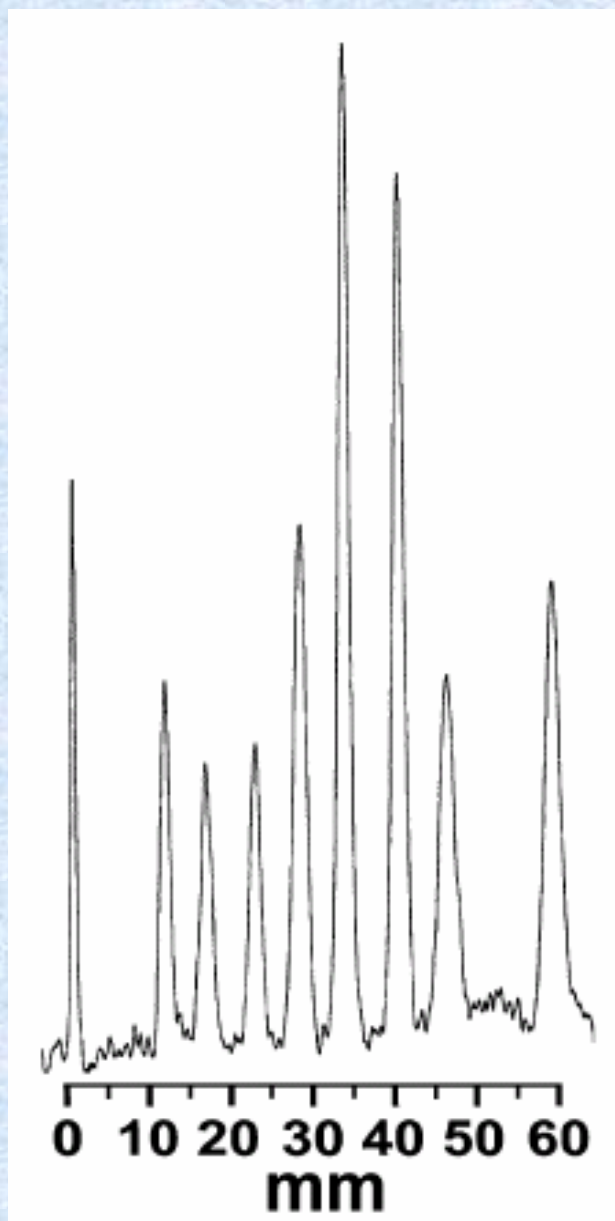


A separation of test solutes on a LiChrospher C18 plate,

(a) TLC, **24 min**,

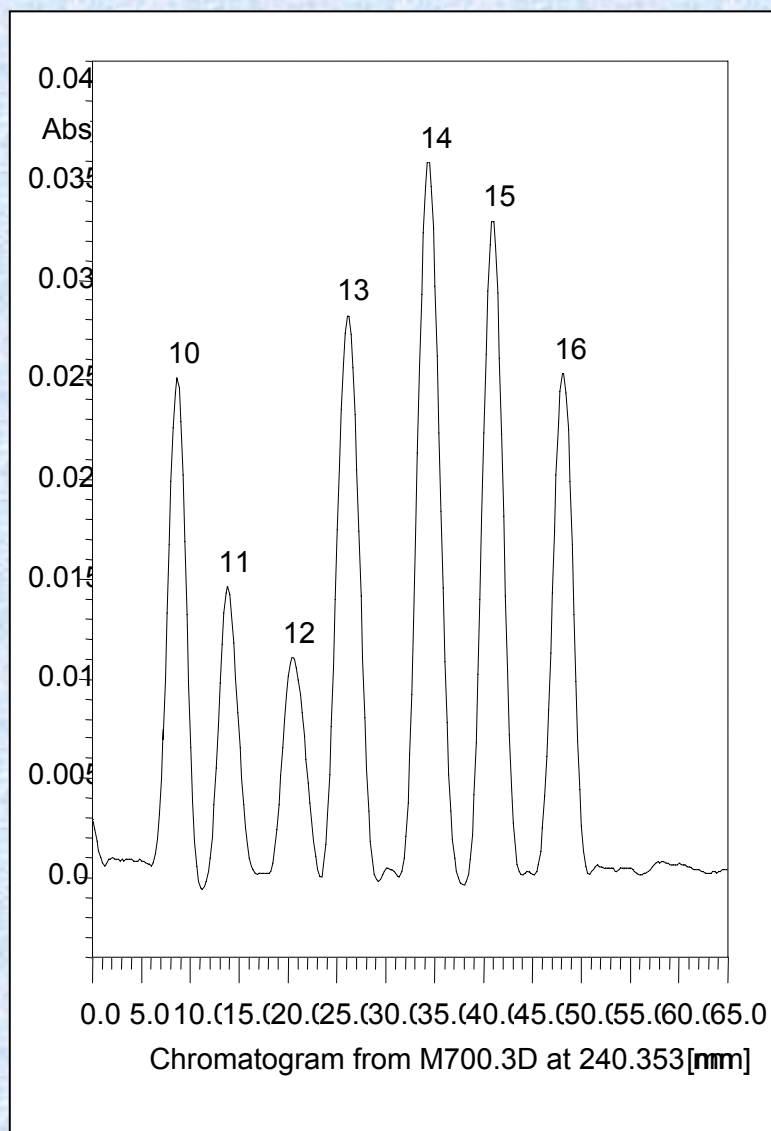
(b) PPEC, 9 kV; 55% ACN in acetate buffer pH 4.7; **1-min**.

D. Nurok, J. M. Koers, A. L. Novotny, M. A. Carmichael, J. J. Kosiba, R. Santini, G. L. Hawkins, and R. W. Replogle, *Anal. Chem.* 2004, 76, 1690-1695



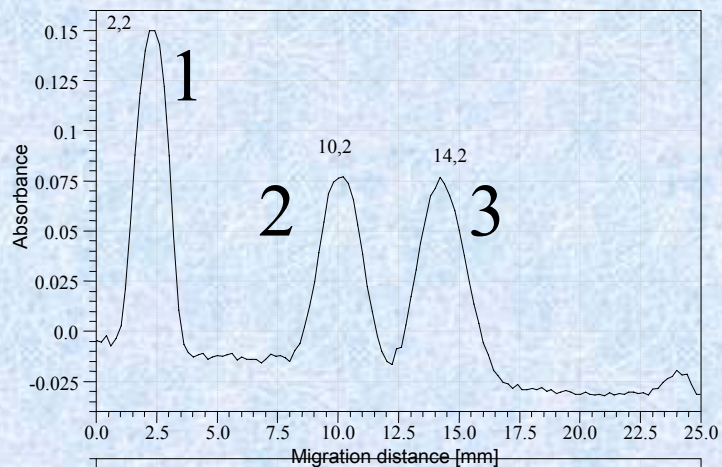
Separation of a nine-component mixture in 2 min. PPEC was performed at 9 kV and 63 atm using of 55% aqueous acetonitrile containing 5 mM acetate buffer at a pH of 4.7 on a high-performance layer.

D. Nurok, et al., *Anal. Chem.*, 78 (2006) 2823 – 2831.

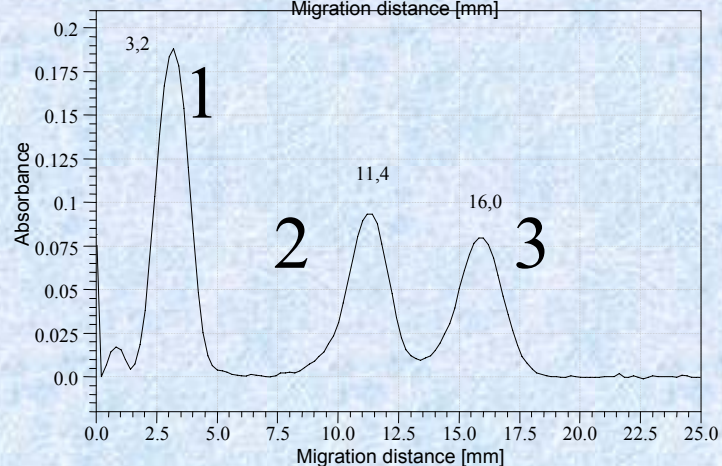


Electrochromatogram of pesticide mixture, 60% acetonitrile in water, HPTLC RP18W F254s plate (Merck).

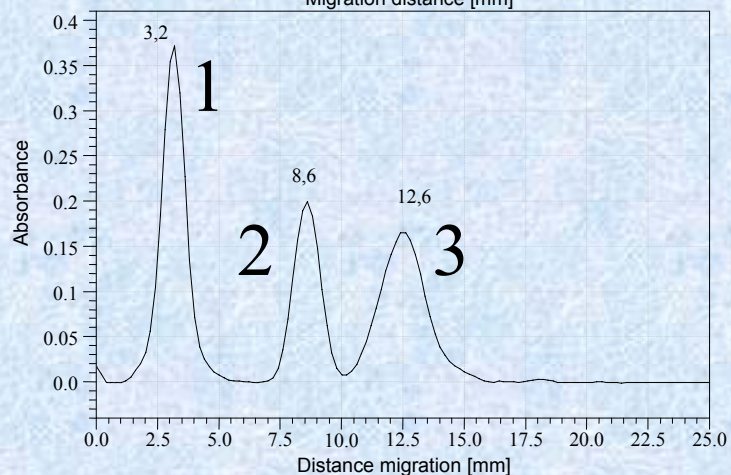
P. Boguta, MA thesis, Medical University, Lublin 2006.



**TLC**, 1 - propyphenasone,  
2 – caffeine, 3- paracetamol  
HPTLC RP 18W (Merck),  
20% ACN in buffer (pH 4,1),  
time **7.5 min**

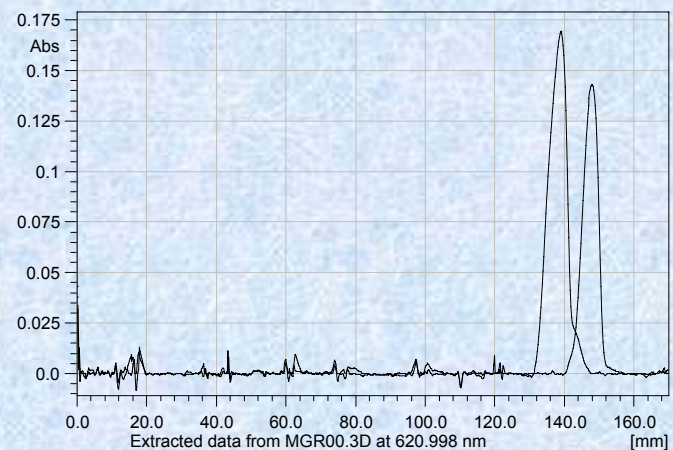
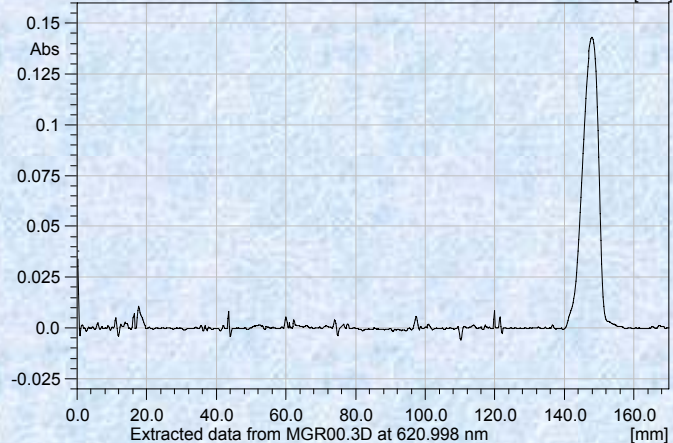
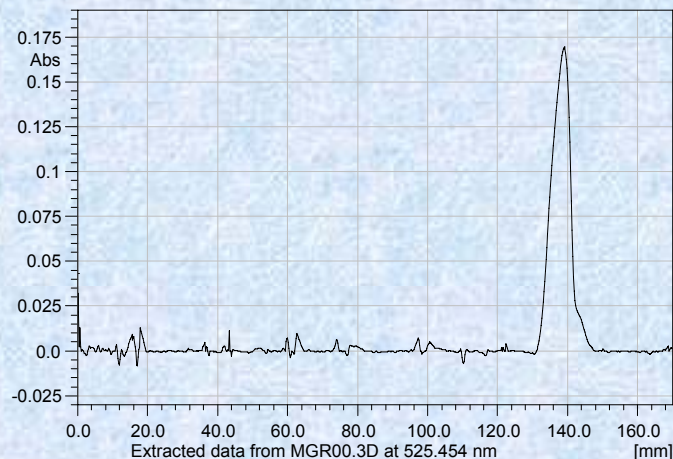


**PPEC**  
HPTLC RP 18W (Merck),  
25% ACN in buffer (pH 4,9),  
0.5 kV, time **8 min.**



**PPEC**  
HPTLC RP 18W (Merck),  
20% ACN in buffer (pH=4,1)  
3.5 kV, time **30 s.**



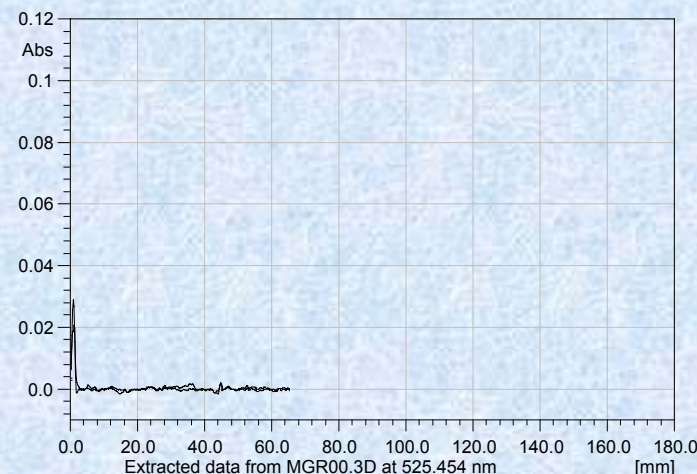


PPEC separation of (1) rhodamine,  
(2) malachite green, using 20 cm  
long plate, HPTLC RP 18W  
(Merck),

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

Migration distance of the first zone  
15 cm

6 kV, **11 min**



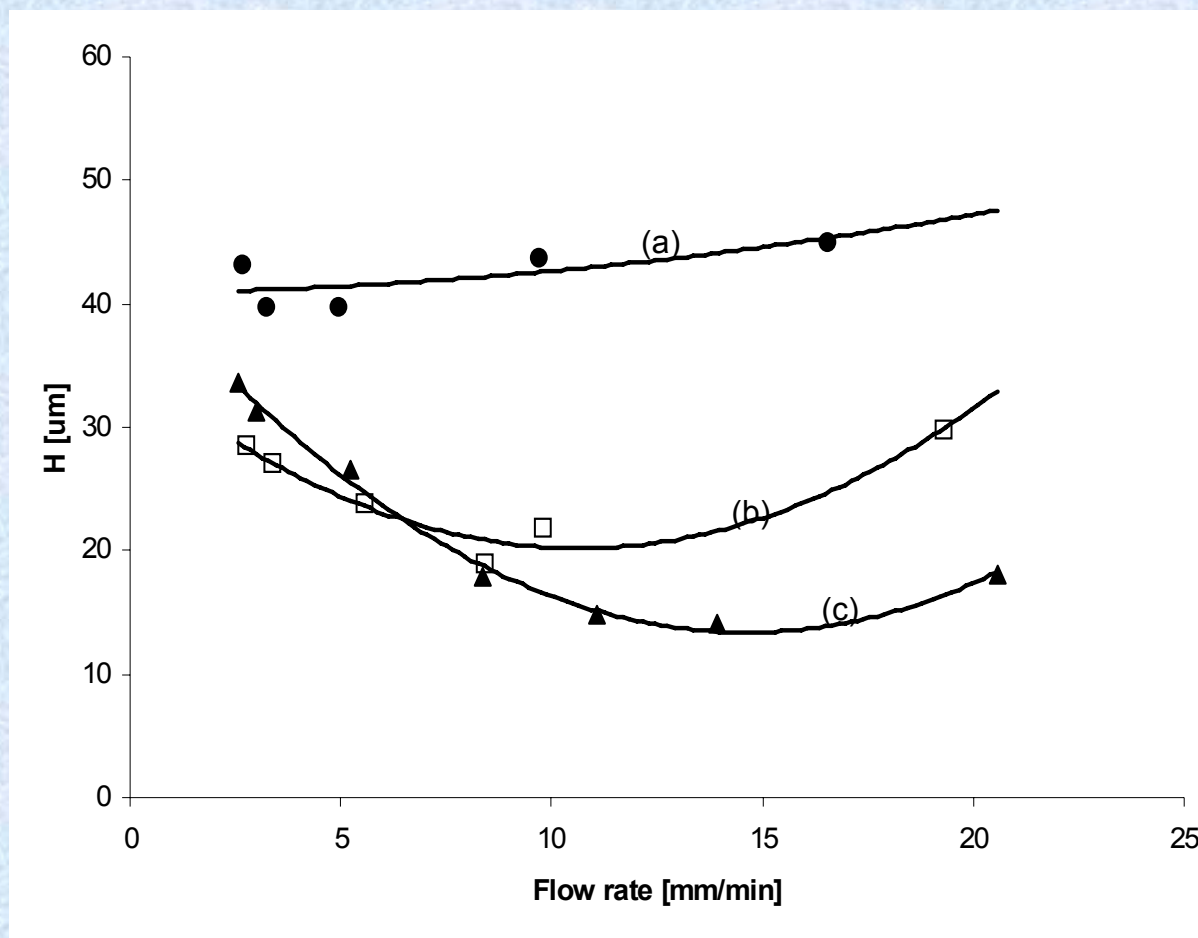
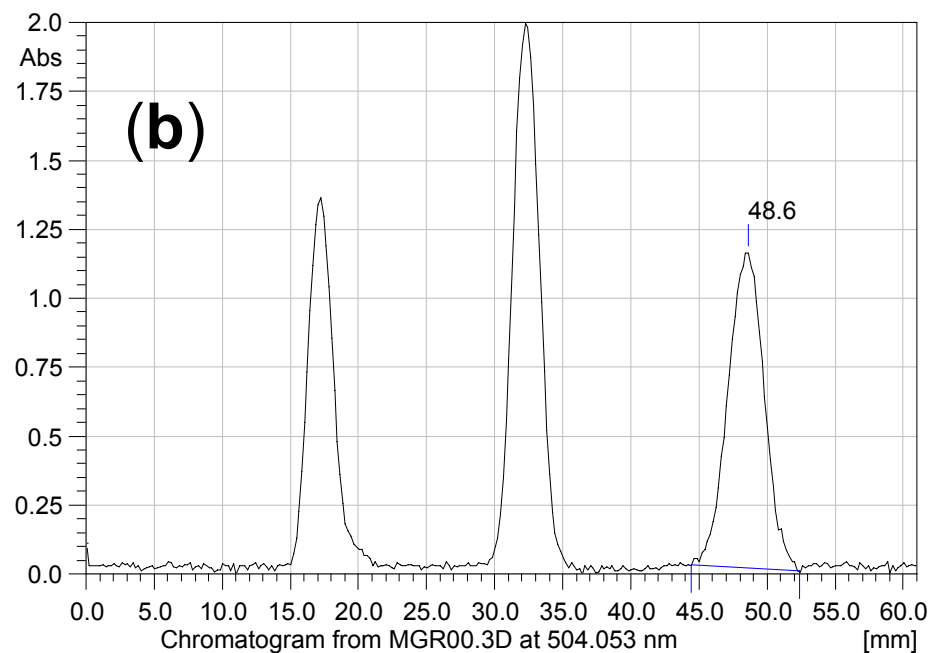
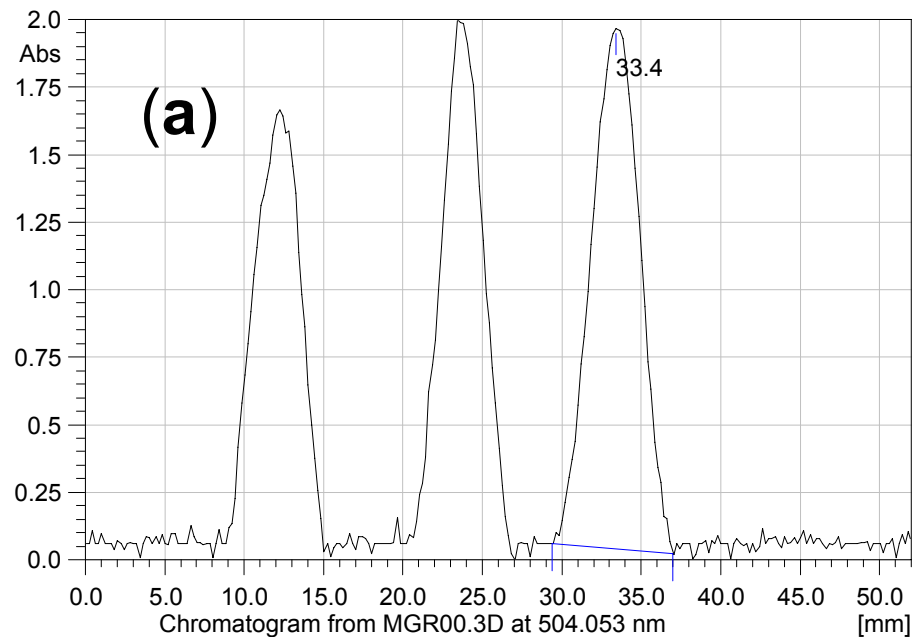


Plate height vs. flow rate of the mobile phase for PPEC systems with RP18 F254s HPTLC plate (Merck). The various modes of sample application: (a) – application with hand operated 5  $\mu$ L micro-syringe, (b) – application of the sample as a scrap of adsorbent layer, (c) – application with aerosol applicator.

P.W. Płocharz, T.H. Dzido, P. Ślązak, G. W. Józwiak, A.Torbicz, J. Chromatogr. A, 1170 (2007) 91.

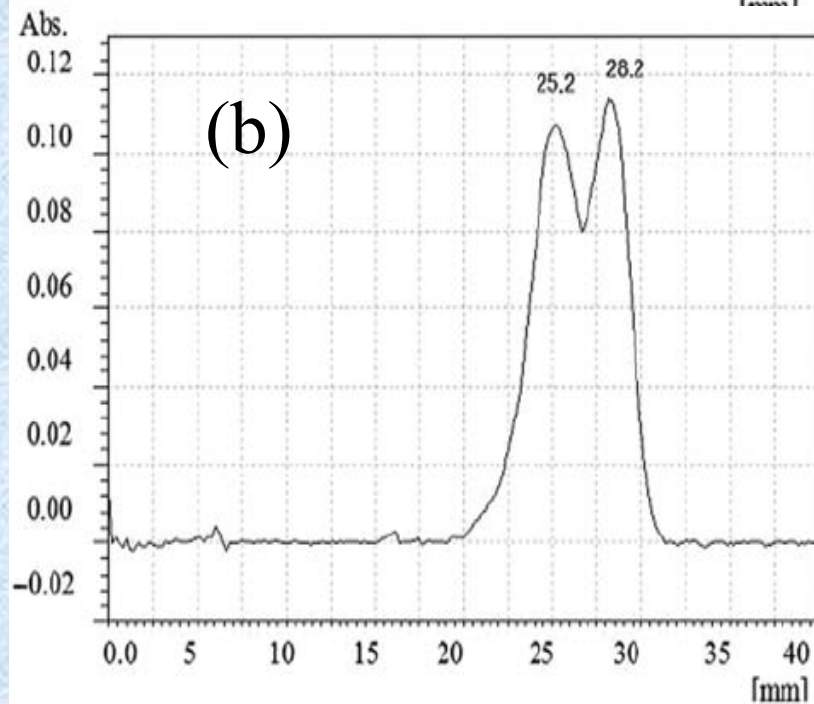
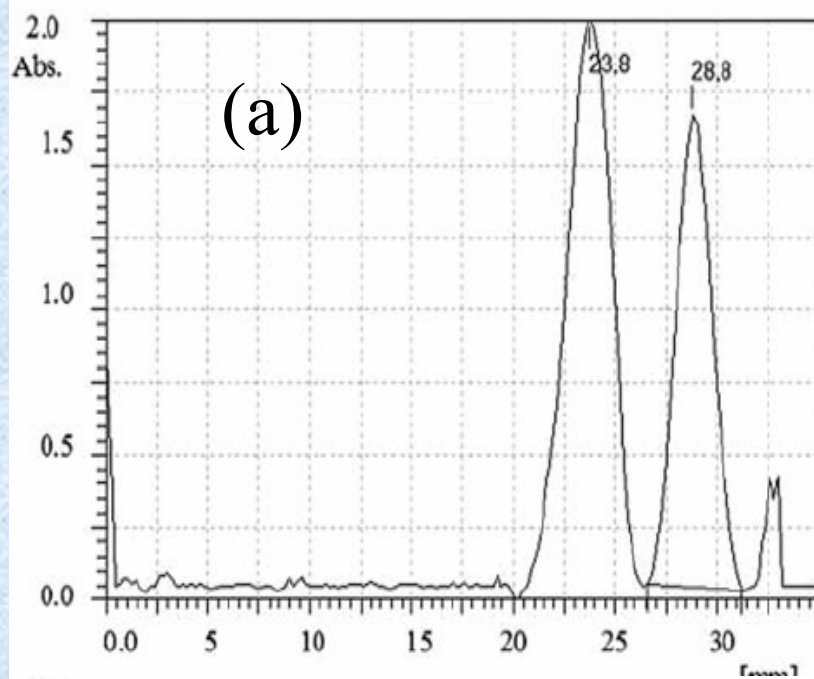


Electrochromatograms of test mixture

(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.

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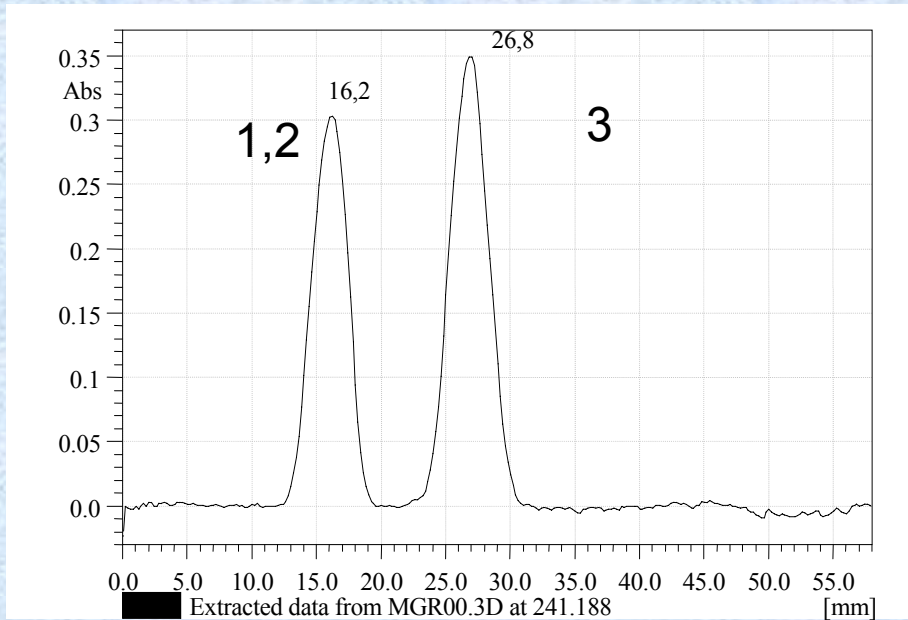
## Separation of the enantiomers of tryptophan

(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

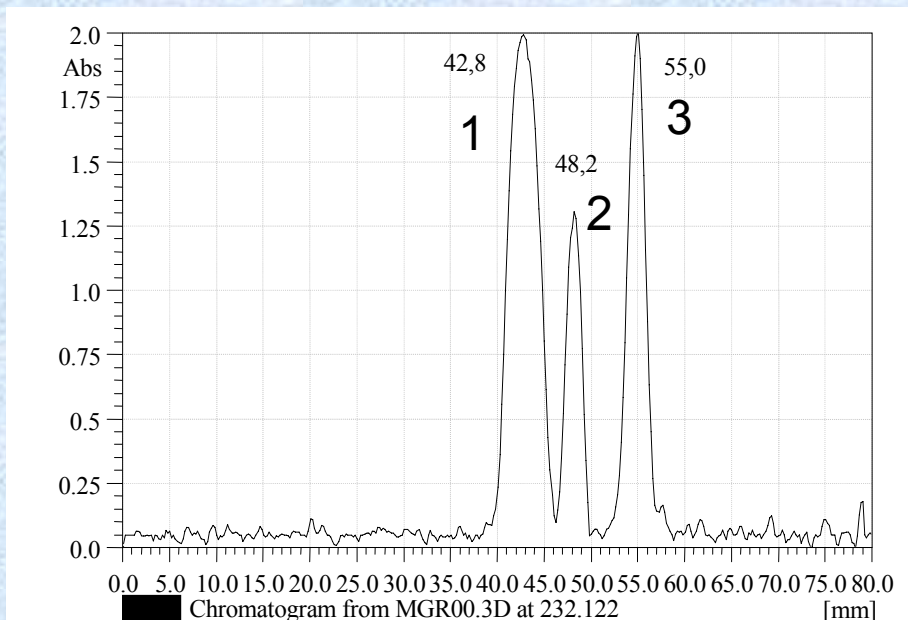
B. Polak, A. Hałka, T.H. Dzido, J. Planar Chromatogr. 21 (2008) 33.



## 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.

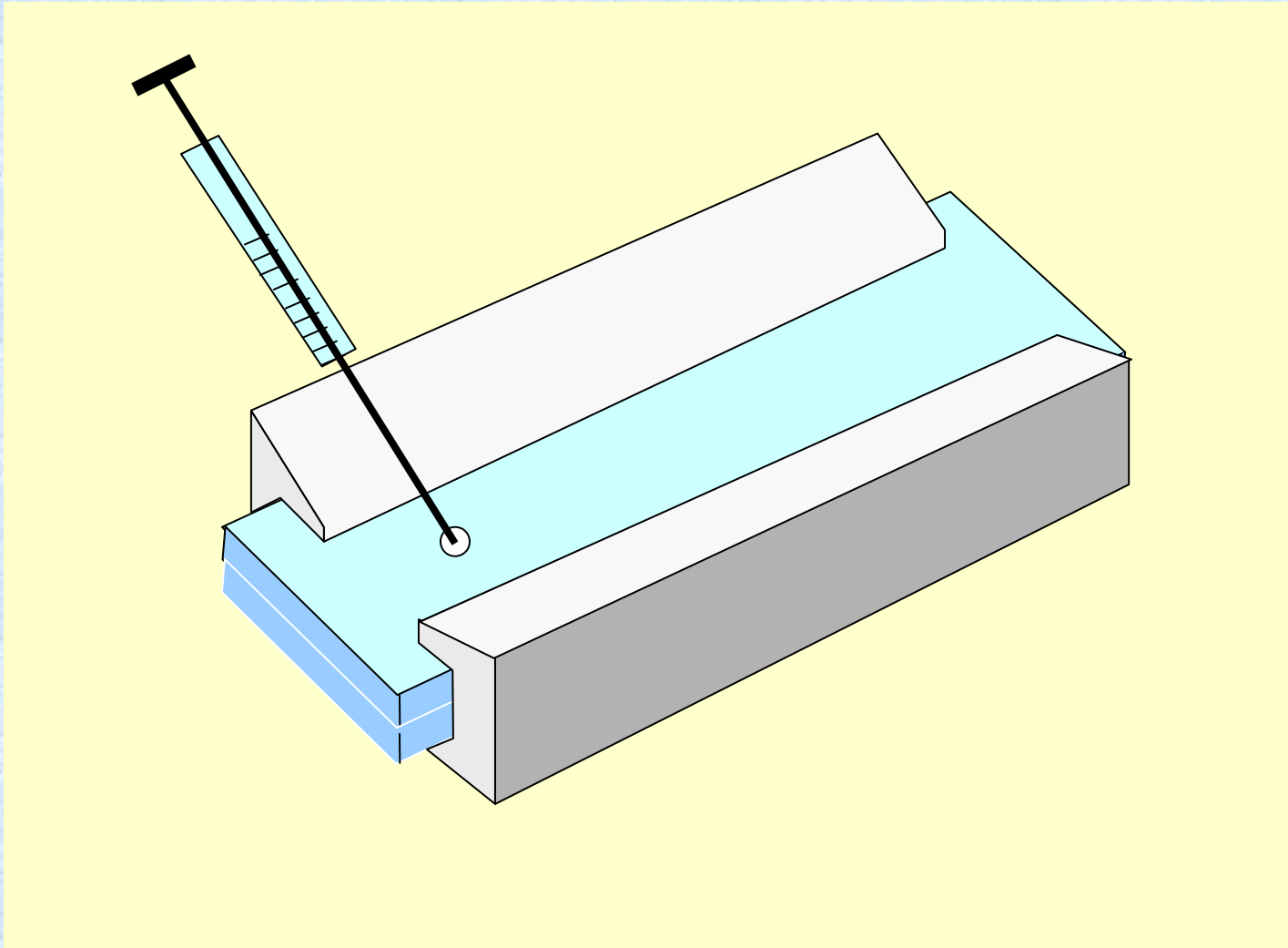
## **The problems:**

- 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:

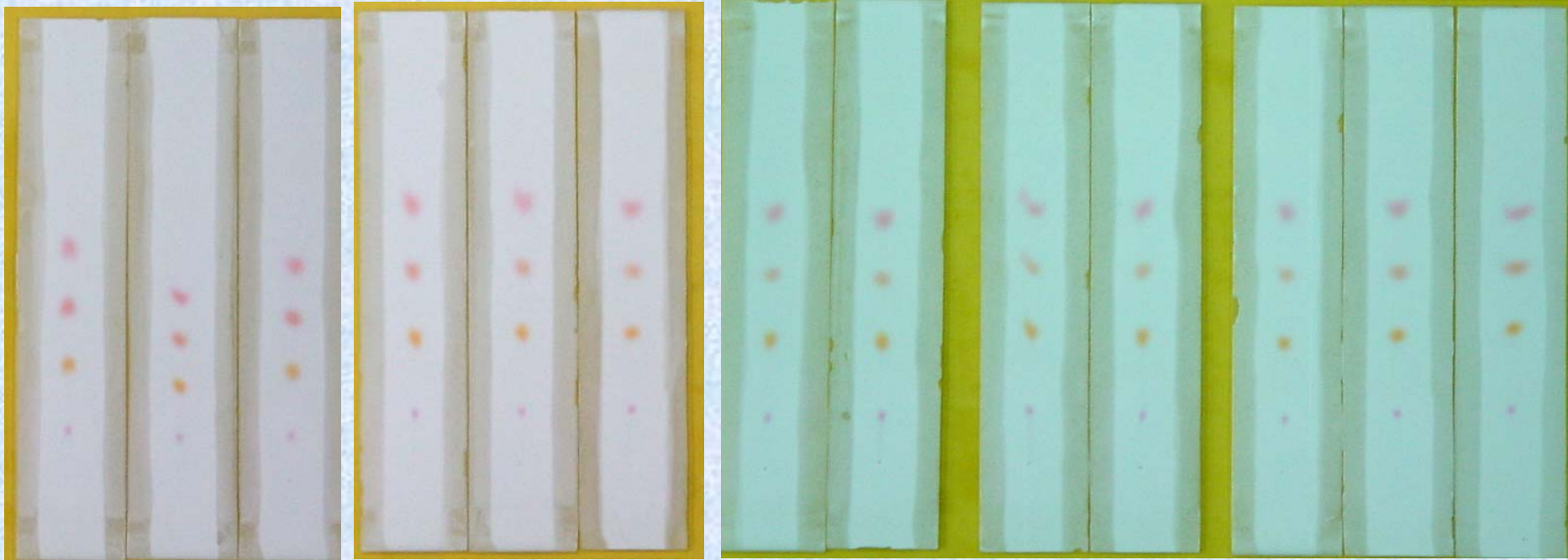
**3 sec**

**1 min**

**5 min**

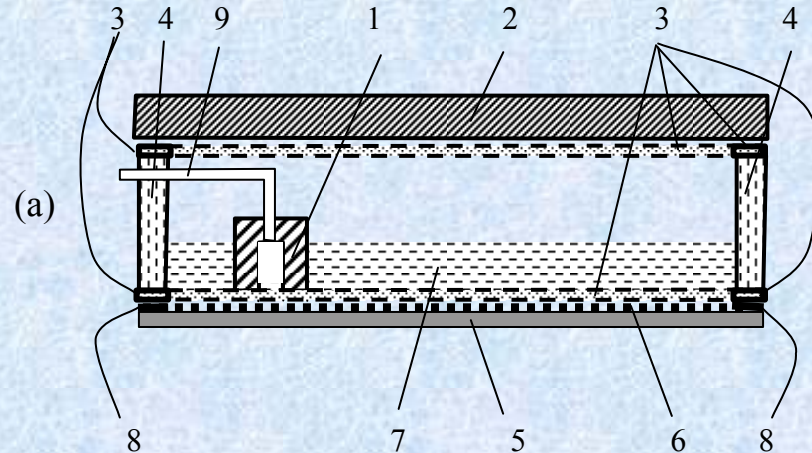
**10 min**

**30 min**



T. H. Dzido, J. Mróz, G. Józwiak, J. Planar Chromatogr. 17 (2004) 404 – 410.

# 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;

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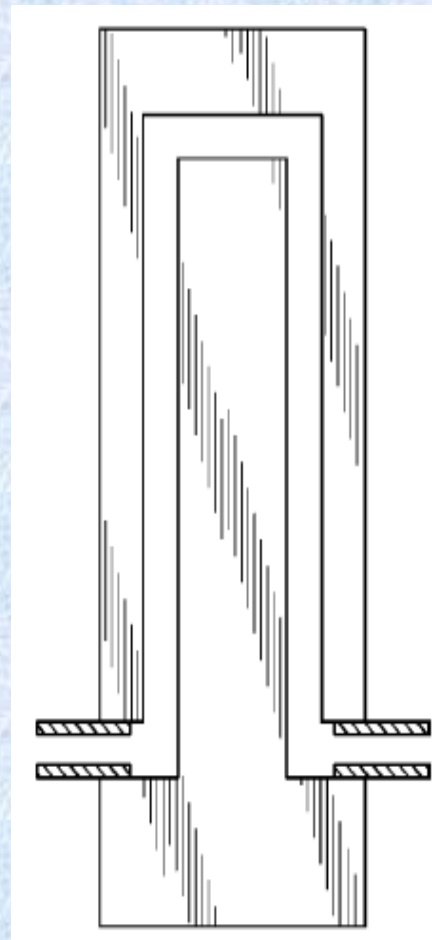
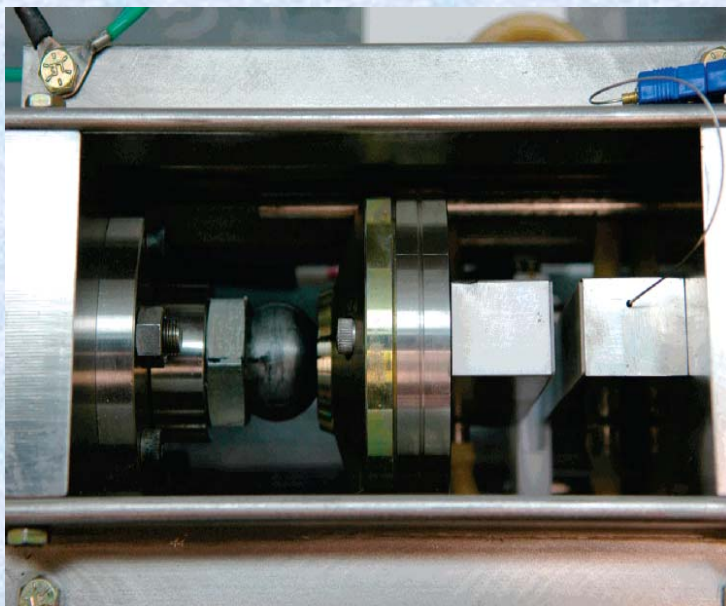
**Repeatability of migration distance,  $Z_s$ , of the solutes in PPEC systems, 80% acetonitrile in buffer, pH 6, HPTLC RP18 plates (MERCK), time 5 min, polarization voltage 2.5 kV.**

Solute	<i>N</i>	Prewetting the chromatographic plate followed by sample application with hand operated microsyringe		Prewetting the chromatographic plate followed sample application with aerosol applicator	
		$Z_s$ (mm)	RSD	$Z_s$ (mm)	RSD
1-(2-pyridylazo)-2-naphthol	7	8.88	0.078	11.57	0.037
4-(diethyloamino)-azobenzene		20.89	0.049	27.73	0.032
1-(4-hydroxyphenyl azo)-2-naphthol		34.86	0.041	46.30	0.034

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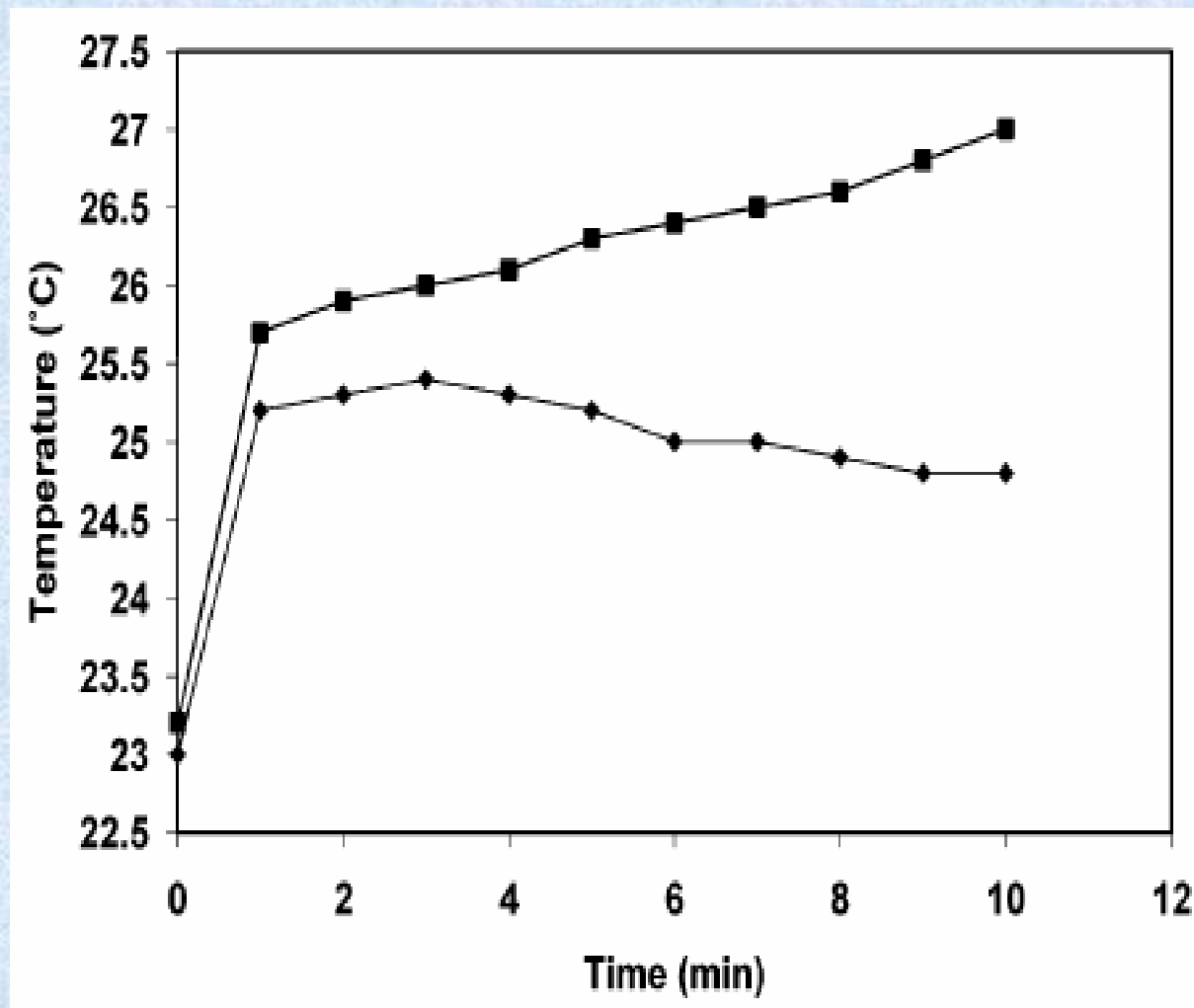
## **Joule heat generation:**

- cooling the electrochromatographic system,



Passages for flow of water in die block.

D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.



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).

D. Nurok, et al., Anal. Chem., 78 (2006) 2823 – 2831.

## **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.



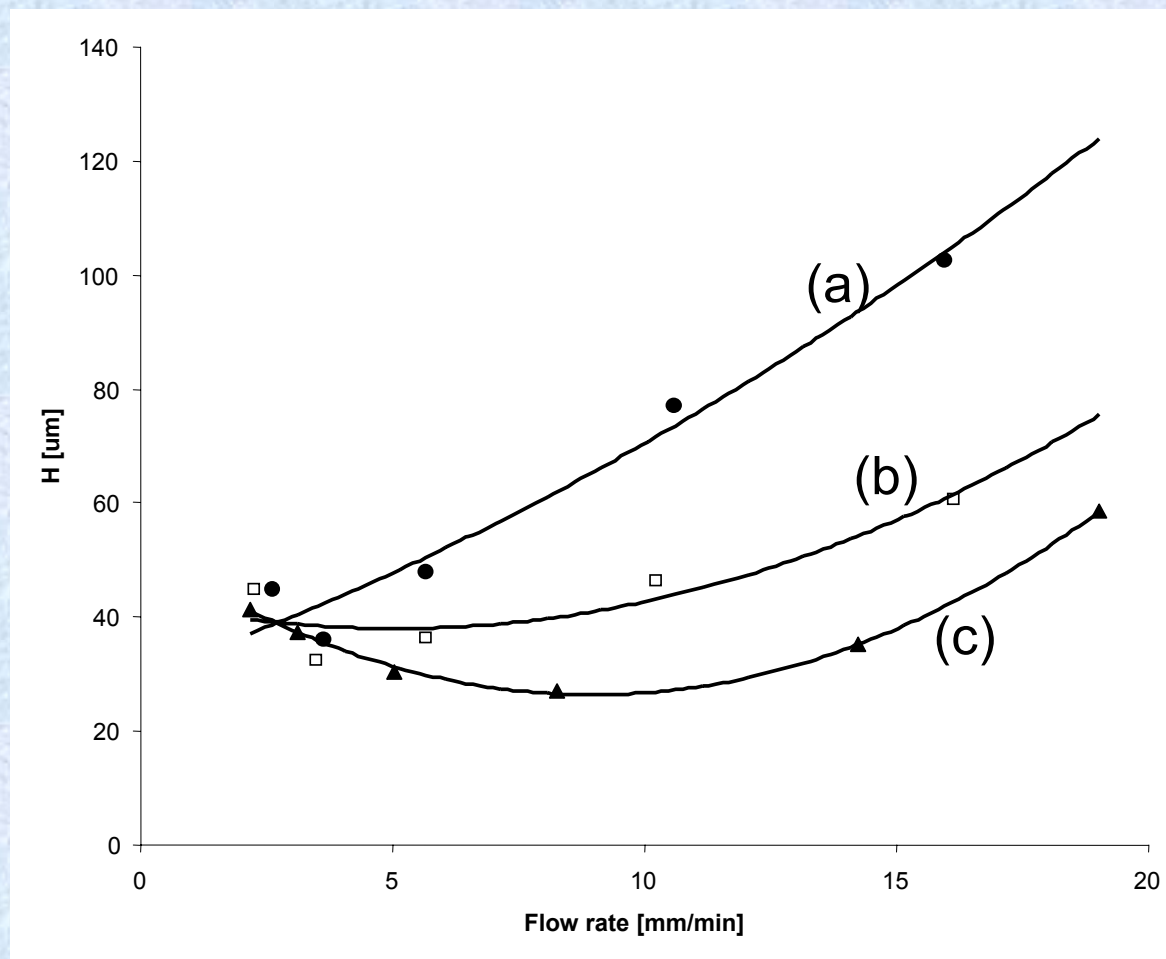


Plate height vs. flow rate of the mobile phase for PPEC systems with TLC RP18 F254s plates (Merck). The various modes of sample application: (a) – application with hand operated 5  $\mu$ L micro-syringe, (b) – application of the sample as a scrap of adsorbent layer, (c) – application with aerosol applicator.

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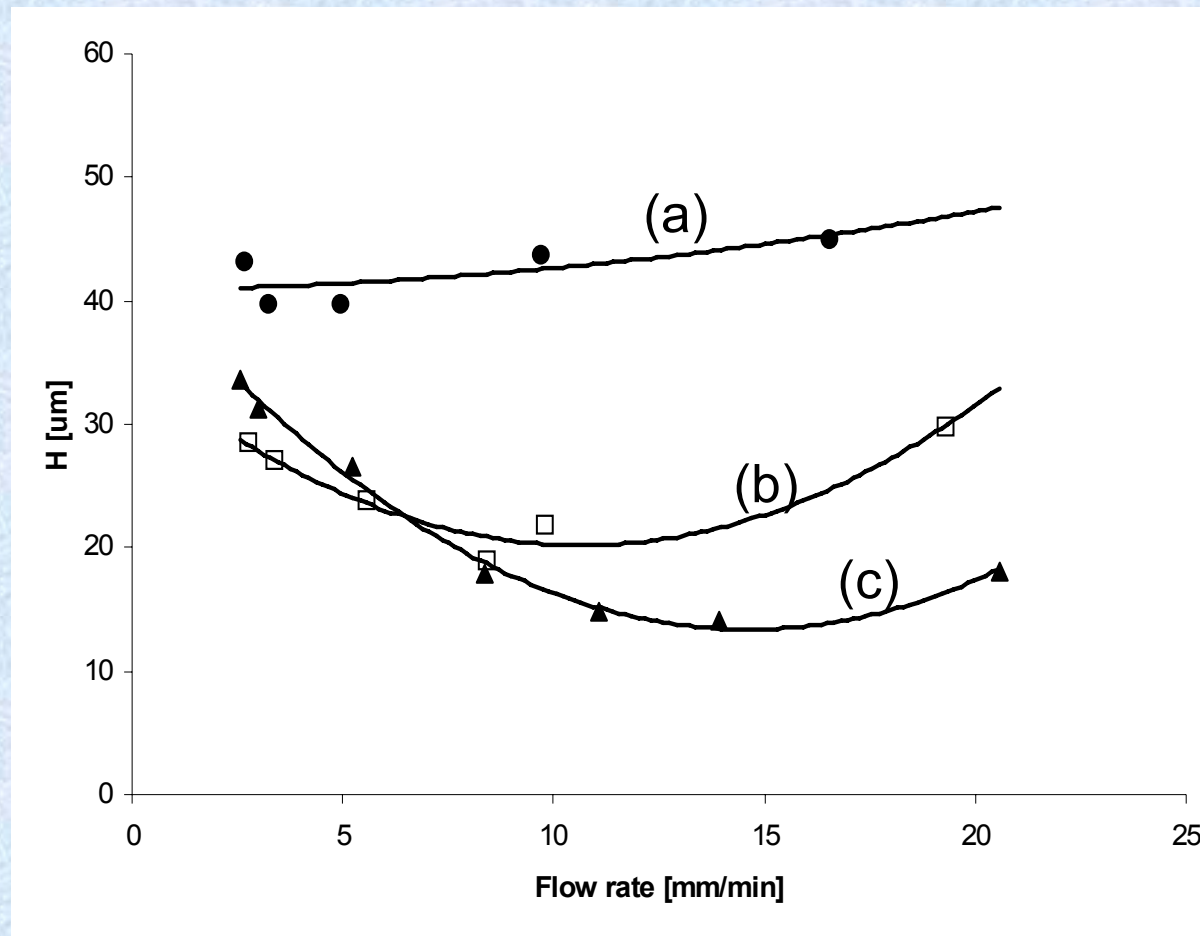
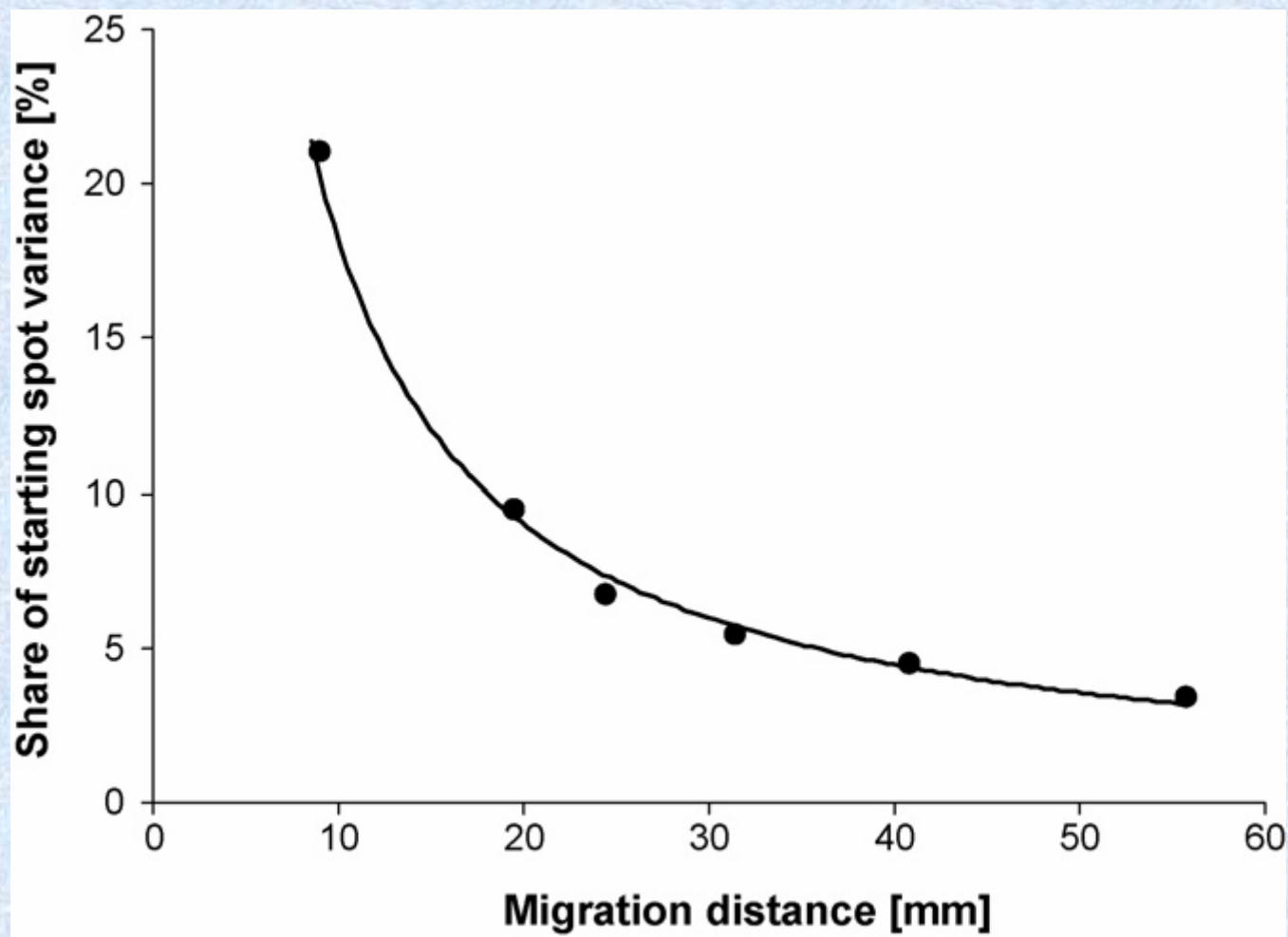


Plate height vs. flow rate of the mobile phase for PPEC systems with RP18 F254s HPTLC plate (Merck). The various modes of sample application: (a) – application with hand operated 5  $\mu$ L micro-syringe, (b) – application of the sample as a scrap of adsorbent layer, (c) – application with aerosol applicator.

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Share of starting spot variance in total variance of the solute zone vs. its migration distance, TLC RP18, diameter of starting spot 1.0 mm.

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