A New Semi-Automatic Device with Horizontal Developing Chamber for Gradient Thin-Layer Chromatography

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Outline

- Theoretical basis
- The current state of technique in analytical laboratories
- Objective of the research
- Results and disscussion
- Conclusions

Isocratic and Gradient Elution

Isocratic

- the conditions of separation are **not changed** throughout the time required for the sample separation

- When?

- simple separations

Gradient

- „a chromatographic technique using within the separation area locally **different** separation conditions" Niederwieser

- When?

- general elution problem

Types of Gradient

Mobile-phase gradients

Composition

pH

Ionic strength

Stationary-phase gradients

Composition

Impregnation

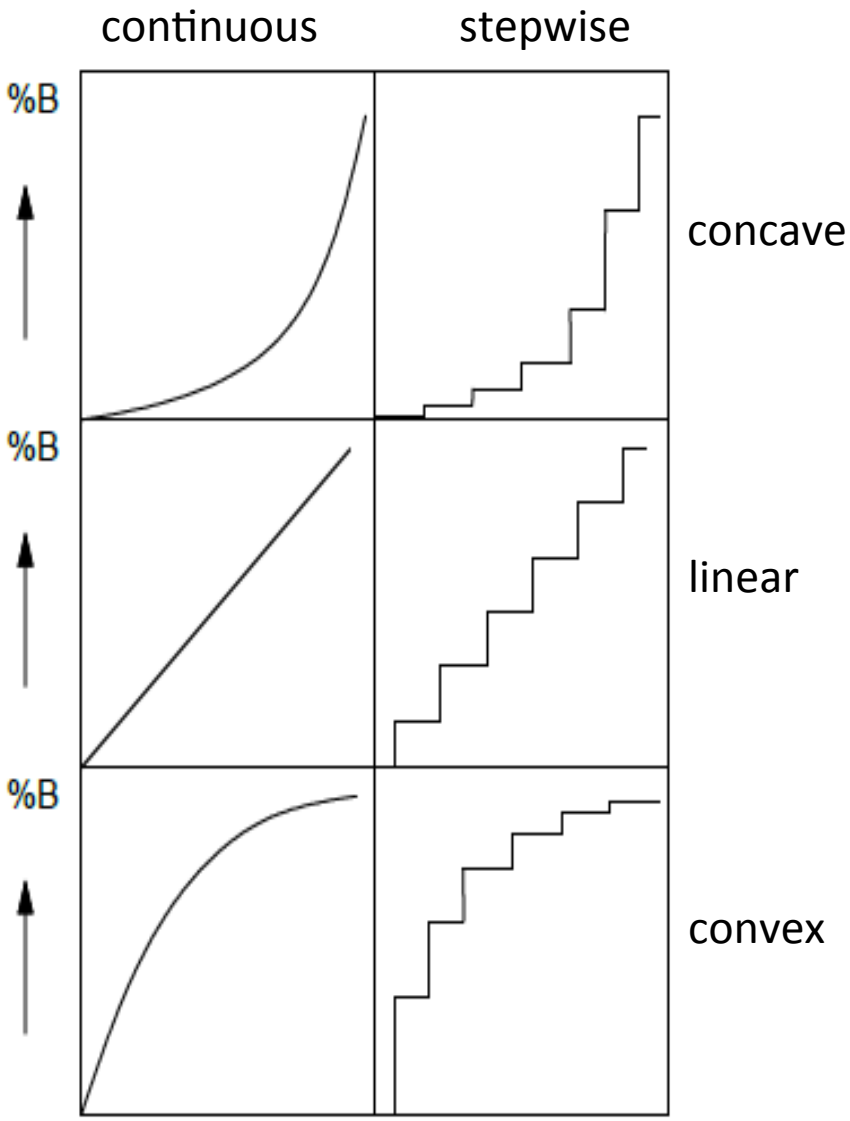
Activity

Gradients concerned with change of

Temperature

Flow rate

Vapor pressure



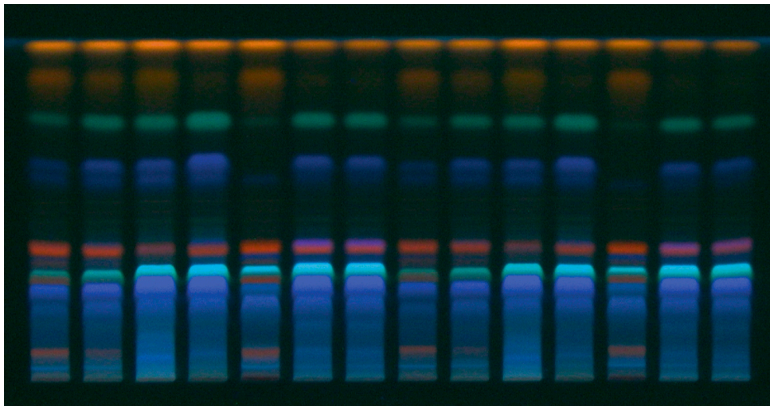
The Current State of Technique in Analytical Laboratories

AMD 2

- multiple development of the chromatographic plate in the same direction
- each successive run extends over a longer distance
- between runs, the eluent is completely removed from the chamber and the layer is dried under vacuum
- each successive run uses a solvent of lower elution strength
- focusing effect and gradient elution results in extremely narrow bands

[1] www.camag.com

AMD 2



Advantages

- good performance
- good reproducibility
- separation can be carried out under a nitrogen atmosphere
- can work without operator supervision

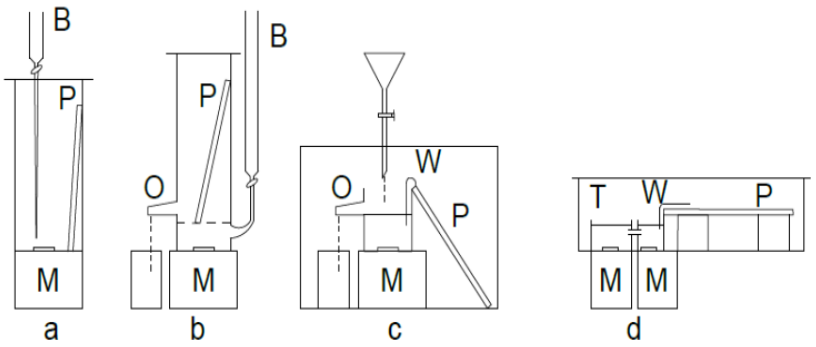
Disadvantages

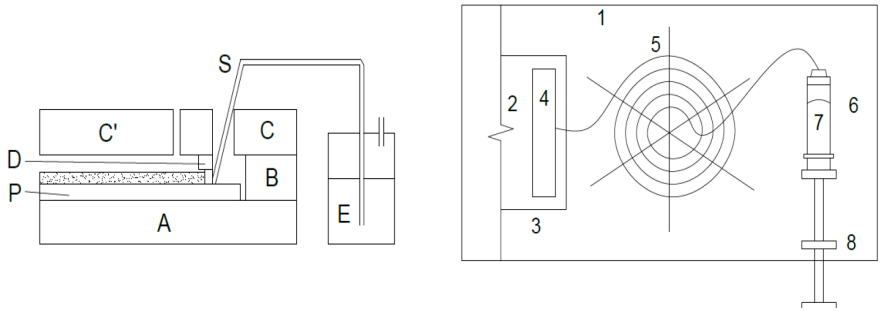
- a long time of development
- stability testing of samples should be pefrormed (decomposition of the analyzed components is possible)
- limited number of suitable solvents
- lack of adequate and simple model method development and optimization
- normal-phase systems only

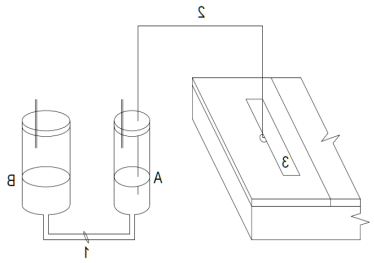
[1] www.camag.com

Other Eqipment for Mobile Phase Gradient

- Rybicka
- Wieland and Determan
- Luzatto and Okoye
- Strickland
- Niederwieser and coworkers
- Sander and Feld
- Soczewiński and Matysik
- Burger and Jaenchen
- Vajda et al.
- Markowski, Wróblewski and Dzido





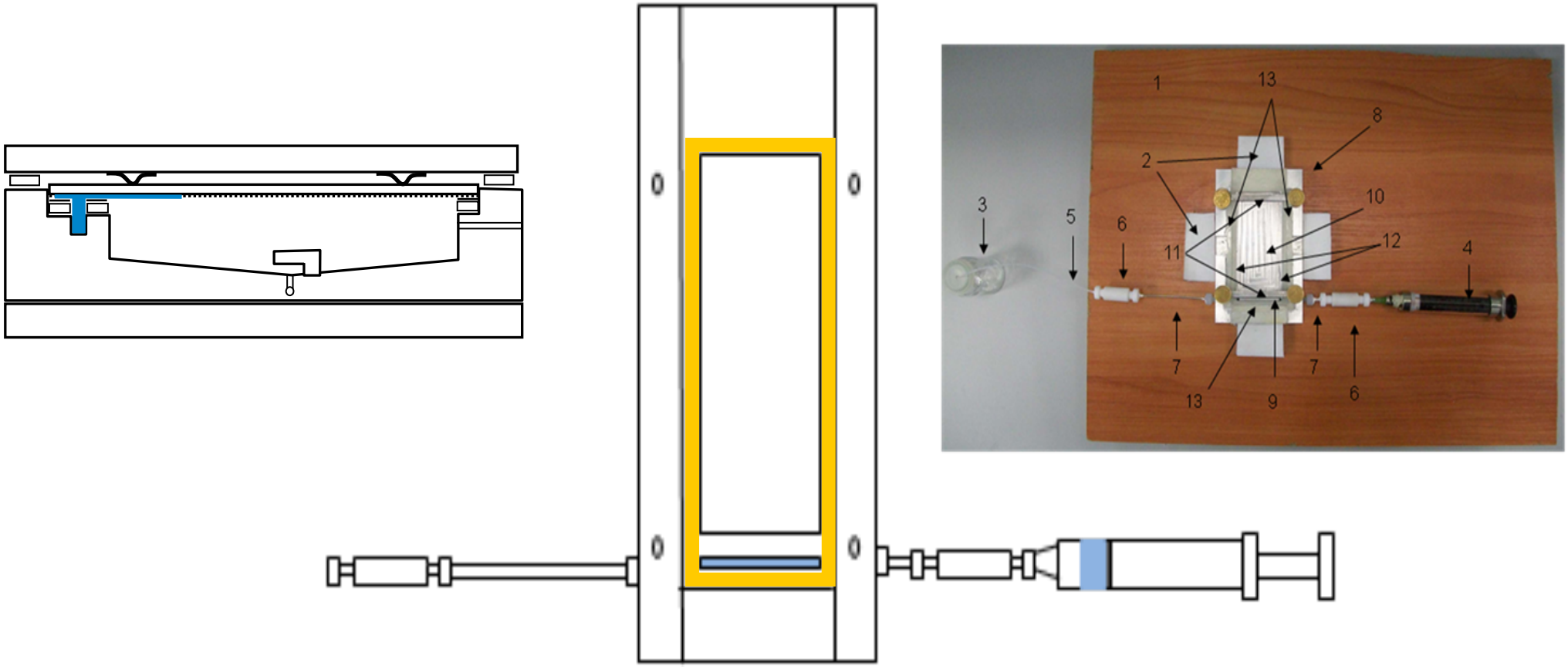


Objective of the Work

Developing a device which:

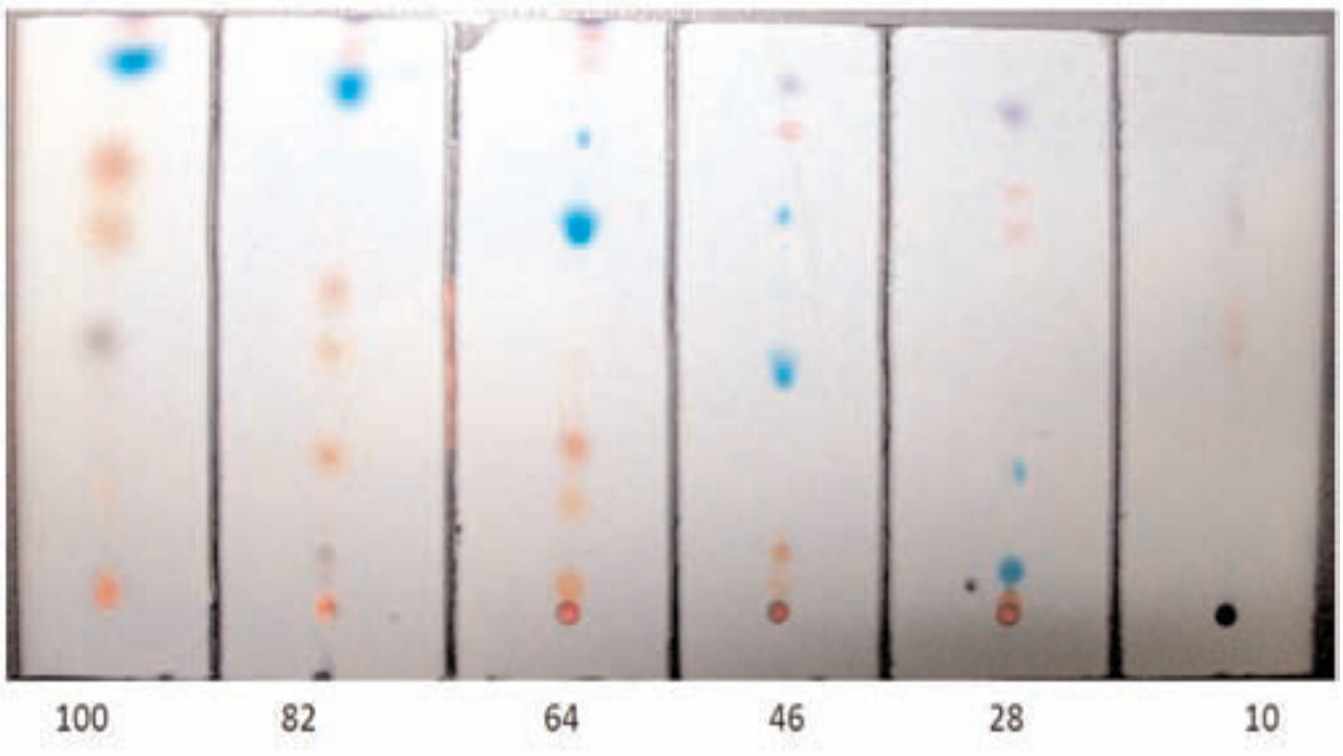
- enables to perform gradient elution **with one void volume of the mobile phase**
- enables to perform gradient elution **in normal and reverse-phase** thin layer chromatography
- can be easily **automated**

Horizontal Developing Chamber for Stepwise Gradient Elution



[2] Markowski W., Wróblewski K., Dzido T.H., J. Planar Chromatogr. 25 (2012) 3, 200–207

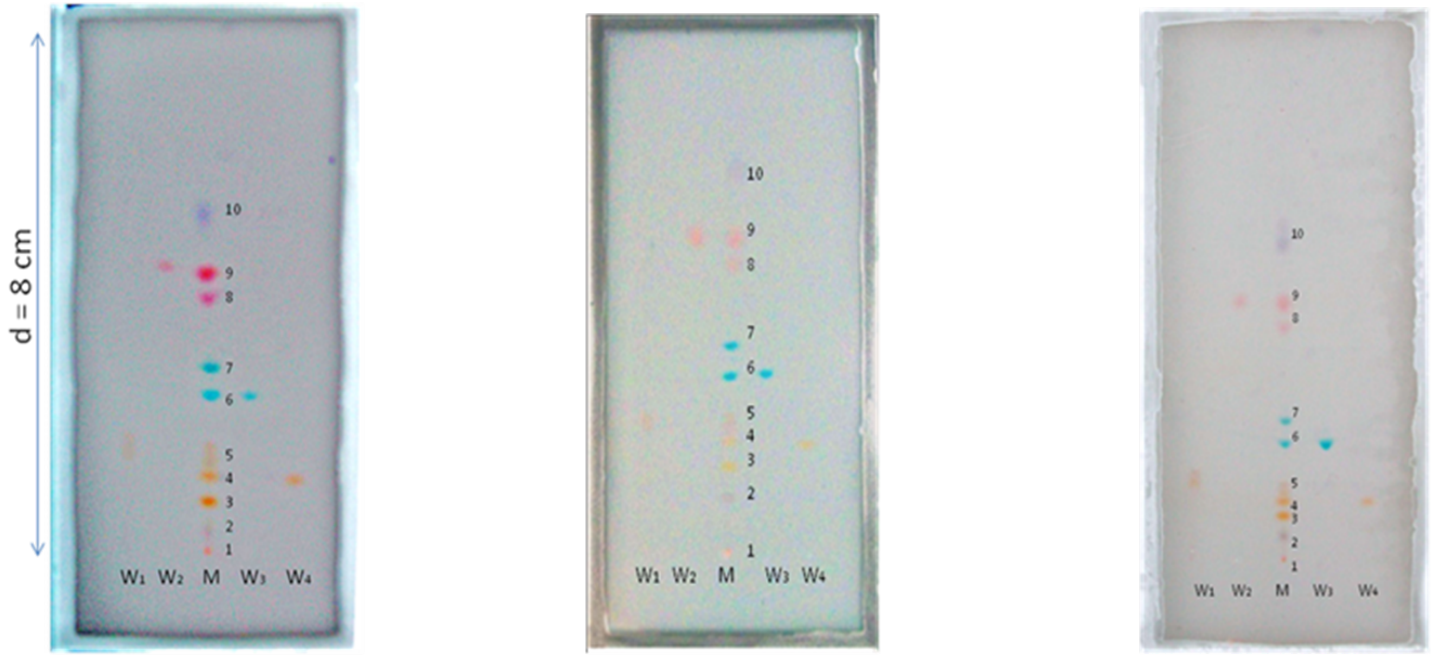
Isocratic Elution of the Mixture of Ten Dyes



The chromatograms of the mixture of ten test dyes, RP-18 W HPTLC plate from Merck, eluent: methanol + water, chromatograms developed in DS-M Chamber (Chromdes)

[2] Markowski W., Wróblewski K., Dzido T.H., J. Planar Chromatogr. 25 (2012) 3, 200–207

Gradient Elution of the Mixture of Ten Dyes with Horizontal Developing Chamber



linear **convex** **concave**

The chromatograms of test dye mixture, RP-18 W HPTLC plates from Merck, eluent: gradient of methanol + water ; the development distances are 8 cm

[2] Markowski W., Wróblewski K., Dzido T.H., J. Planar Chromatogr. 25 (2012) 3, 200–207

Horizontal Developing Chamber for Stepwise Gradient Elution

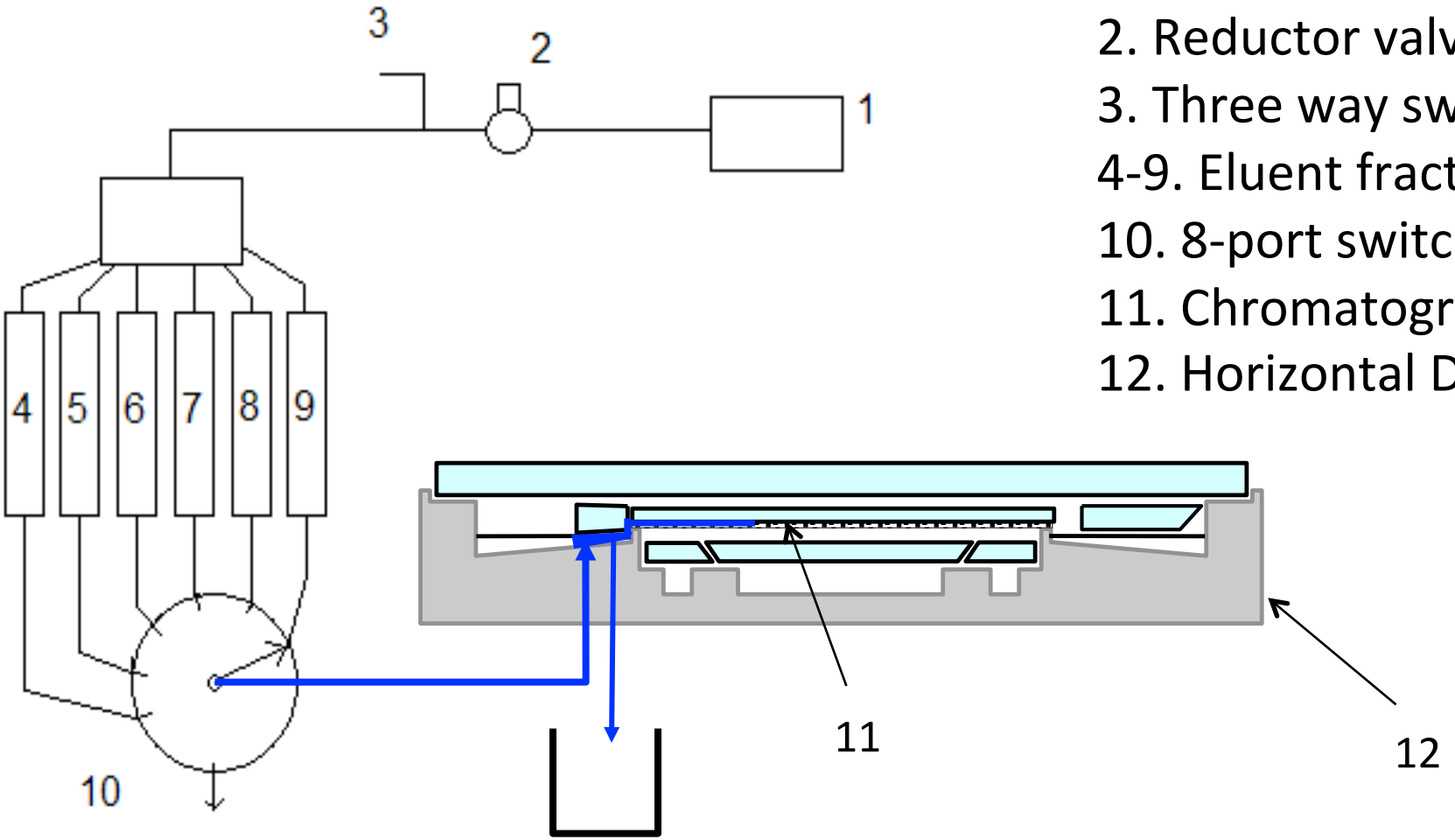
Advantages

- **reasonable time** of gradient chromatogram development (eg 20-30 min. for 4–6 cm)
- gradient elution in **reversed-phase systems** (bioanalysis)
- **good agreement** of the calculated and experimental values of retention of separated solutes
- satisfactory repeatability

Disadvantages

- a lot of manual operations and operator supervision required
- margins production of 4 mm width of silicone sealant on the whole periphery of the plates is necessary

The New Semi-Automatic Horizontal Developing Chamber for Stepwise Gradient Elution



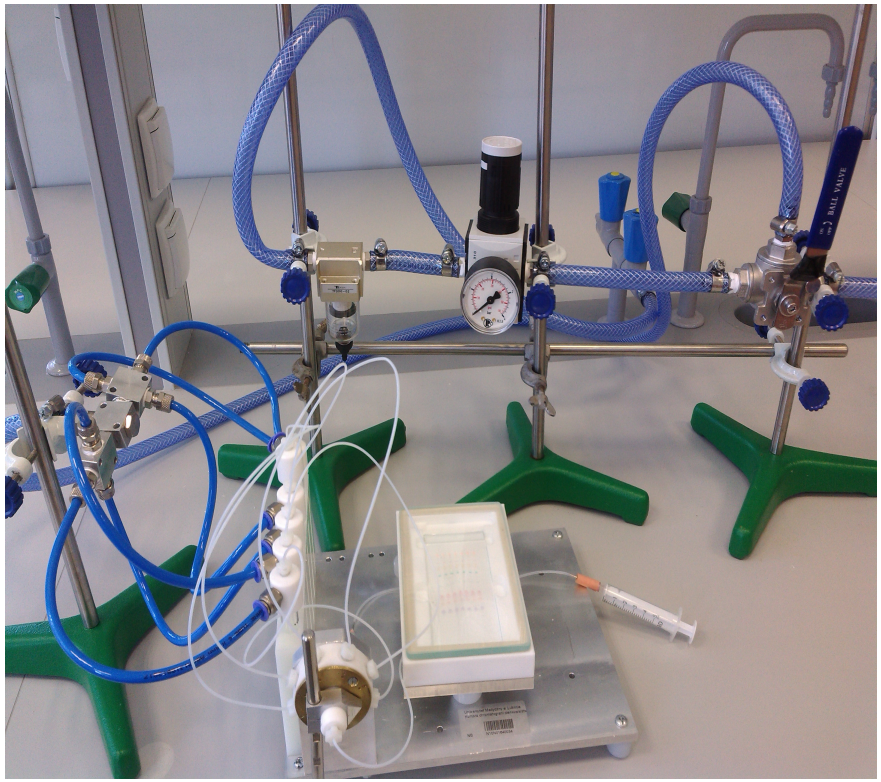
1. Gas reservoir
2. Reductor valve
3. Three way switching valve

4-9. Eluent fractions

10. 8-port switching valve
11. Chromatographic plate
12. Horizontal DS chamber

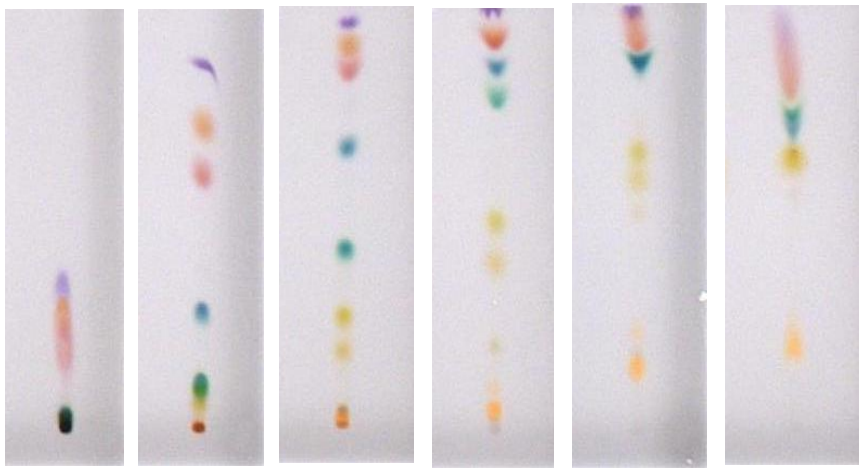
Picture of the New Semi-Automatic Horizontal Developing Chamber for Stepwise Gradient Elution

With pneumatic delivery solvent system

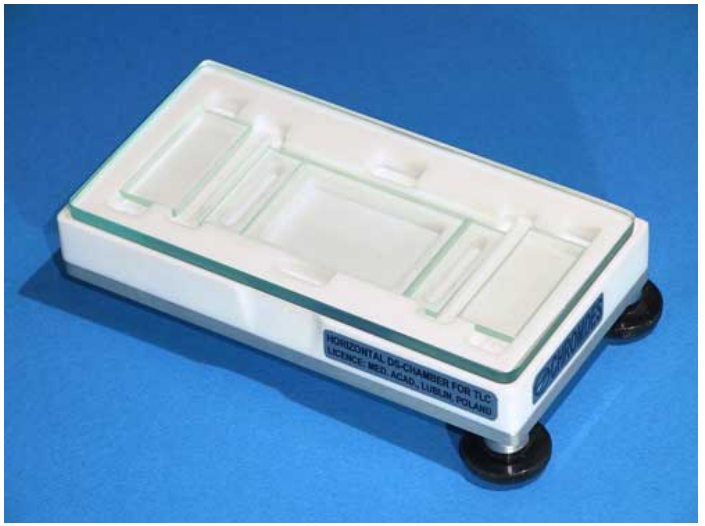


With gradient pump (HPLC)

Isocratic Elution of the Mixture of Ten Dyes

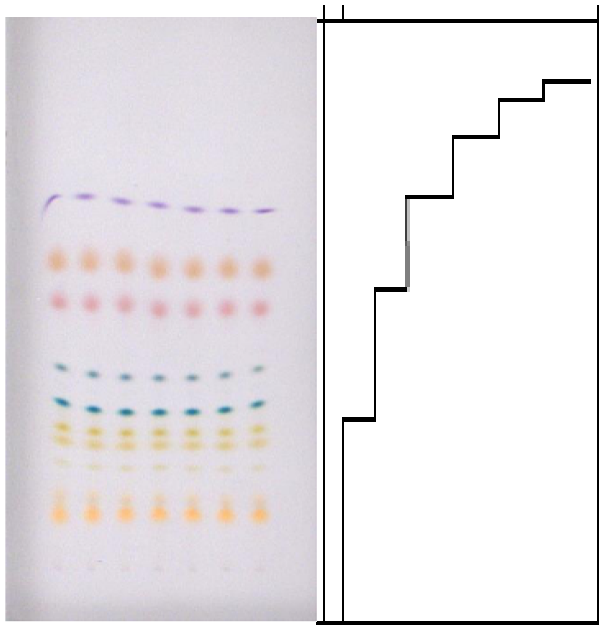


20 35 50 65 80 100 % of Methanol (v/v)

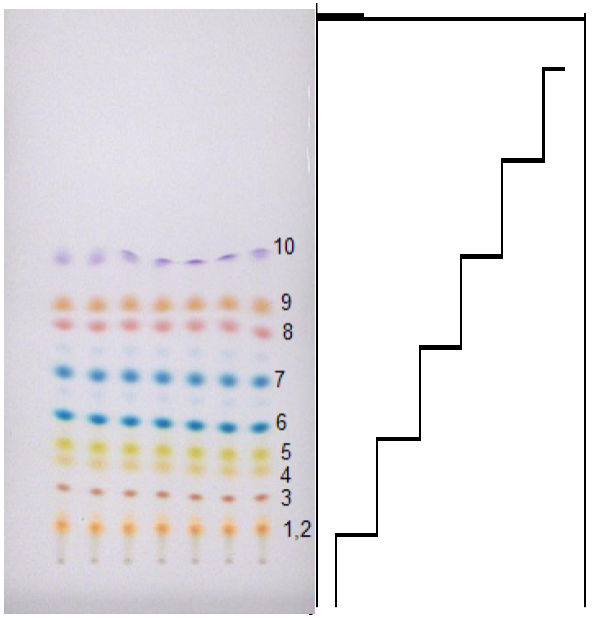


The chromatograms of the mixture of ten test dyes, RP-18 W HPTLC plate from Merck, eluent: methanol in buffer pH 3.0, chromatogram developed in Horizontal DS, 5 x 10, Chamber (Chromdes)

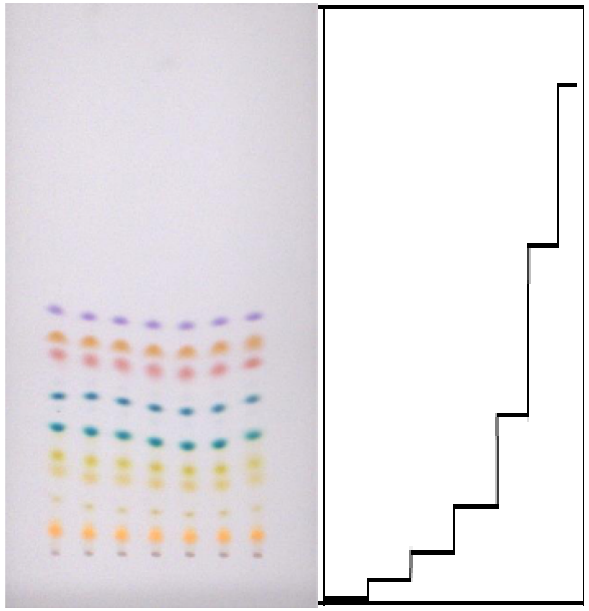
Gradient Elution of the Mixture of Ten Dyes with the New Semi-Automatic Horizontal Developing Chamber



convex



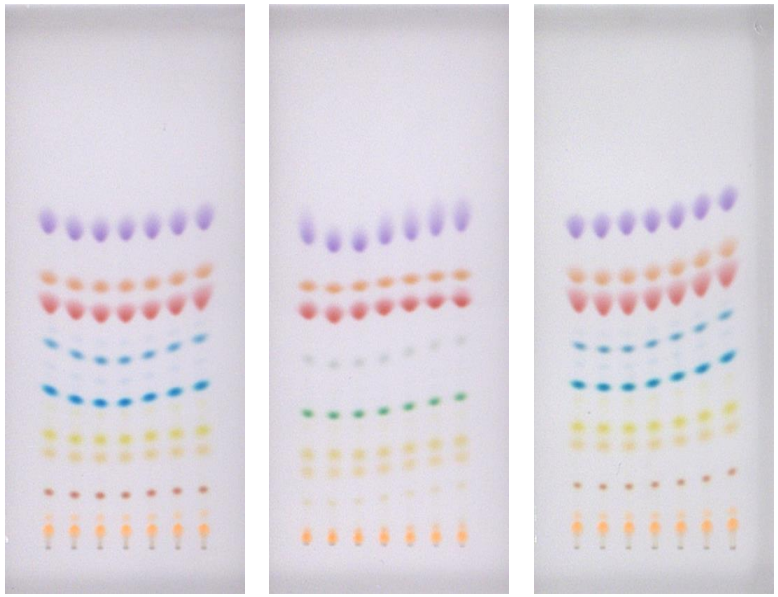
linear



concave

The chromatograms of the mixture of ten test dyes, RP-18 W HPTLC plate from Merck, eluent: 4 step gradient of methanol in buffer pH 3.0, development distance 8 cm

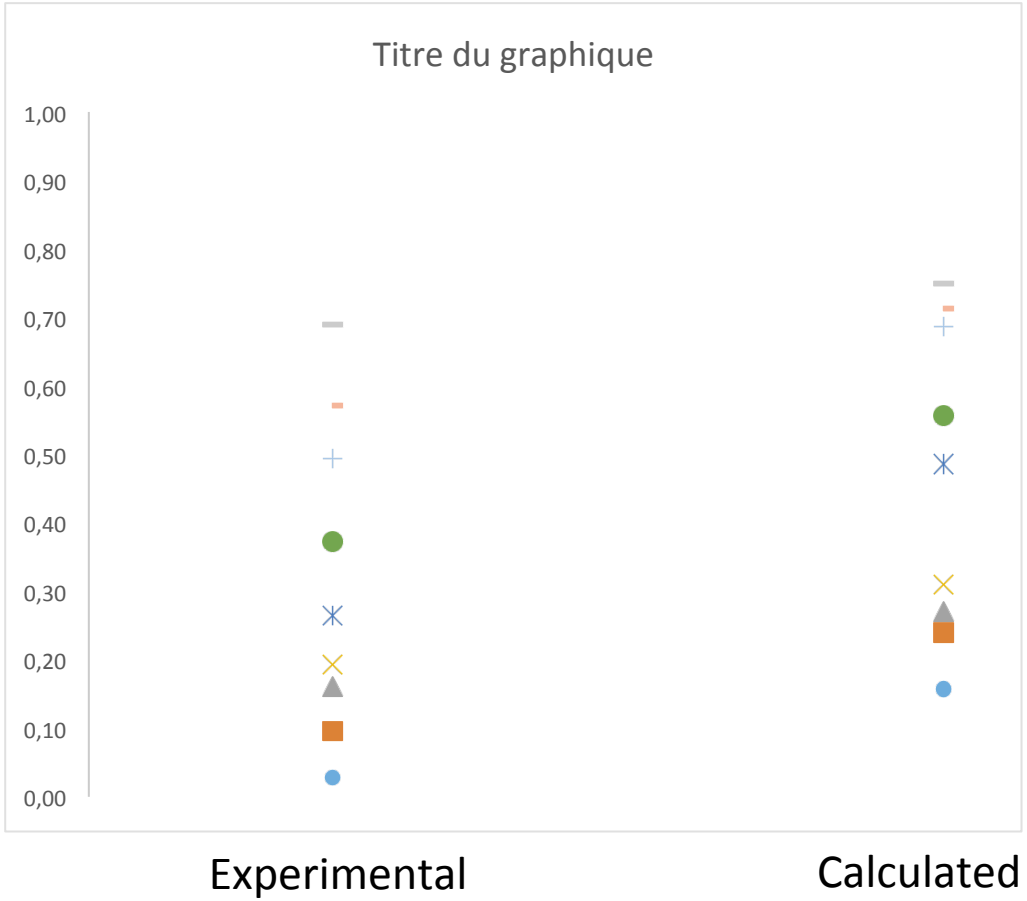
Reproducibility of Results Obtained with the New Semi-Automatic Horizontal Developing Chamber



Substance	Migration distance	Standard deviation	RSD [%]
1	1.65	0.25	15.25
2	6.07	0.60	9.94
3	12.43	0.38	3.04
4	16.43	0.41	2.50
5	18.70	0.59	3.18
6	24.83	0,99	3.98
7	29,23	0,61	2.08
8	35.53	2.01	3.99
9	38.93	2.24	5.76
10	46.03	4.57	9.94

The chromatograms of the mixture of ten test dyes, RP-18 W HPTLC plate from Merck, eluent: linear gradient of methanol in buffer pH 3.0, development distance 8 cm

The Prediction of Retention by a Computer Program



The New Semi-Automatic Horizontal Developing Chamber

Advantages

- limitation of manual operations
- no need for margins production
- resonable time of chromatogram development

Disadvantages

- reproducibility is not satisfactory
- too high difference in migration distance of solute zones

Conclusions

- the procedures for the preparation of chromatographic plates and the process of development of chromatograms were significantly simplified
- eqipment could be easly automated
- the reproducibility is still not satisfactory and requires modifications of this new equipment

Thank you for your kind attention