# Study of retention processes in linear and nonlinear liquid chromatography

PhD thesis

Author
Szabolcs Horváth
chemist

supervisor
Dr. Krisztián Horváth
associate professor

Chemical Engineering and Material Sciences Doctoral School

Veszprém
2023

# 1. Introduction and aims

For the design, development and optimisation of analytical and preparative liquid chromatographic separations, a thorough understanding of separation theory and the physical and chemical processes that take place in the column is essential. The slow kinetics of the separation, the surface heterogeneity of the stationary phase and the shape of the equilibrium isotherm describing the interactions between the sample and the stationary phase are among the factors that lead to broadening and distortion of chromatographic peaks and, consequently, to a reduction in the efficiency of separations.

The aim of this research is to investigate the physical and chemical processes in the column in linear and nonlinear liquid chromatography.

The intention of my work is to further develop the general rate model of liquid chromatography to characterise the retention properties and efficiency of bi-layered solid-core stationary phases, taking into account the kinetic and equilibrium processes in the column, and to apply the developed model to investigate the design and application of new types of chromatographic packings. A potential option for accelerating the mass transfer kinetics of large molecules (e.g. proteins) is the use of a column filled with bi-layered core-shell particles with a second large pore diameter shell.

Although frontal analysis is a highly accurate method for the determination of isotherms, it requires large amounts of sample and solvent. The inverse method, on the other hand, requires a small sample volume but for its successful application requires knowledge of the isotherm equation, i.e. the method is model-based. Therefore, my aim was to present an isotherm equation-free inverse method for the determination of adsorption isotherms with the same accuracy as the frontal analysis, but with the small sample requirement of inverse methods.

I also aimed to theoretically investigate the potential of separation efficiency increase of liquid chromatography column packings with gradient properties (particle size, ion exchange capacity) .

# 2. Experimental

### Software used for numerical calculations

The numerical calculations were carried out with a software written in house in Python programming language (v. 3.6, Anaconda Python Distribution), using the NumPy and SciPy packages.

### Theoretical analysis of efficiency of bi-layer core-shell stationary pases in the high performance liquid chromatography of large biomolecules

The separation power of the multi-layer particles was investigated for a typical large peptide (e.g., insulin: Molecular weight, $M$, ∼6 kDa, molecular diffusion coefficient, $D_m$,$\sim 6\times10^{-5}\frac{\mathrm{cm}^2}{\mathrm{min}}$) The values of the numerical parameters necessary for the numerical calculations, such as the column parameters, the particle size, and so on, are listed in Table 1.

Table 1: Numerical parameter values used in the study of efficiency of bi-layer core-shell stationary phases

| **Parameter** | **Value** |
|---|---|
| column length ($L$) | 10 cm |
| column diameter ($d_c$) | 0.3 cm |
| particle diameter ($d_p$) | 2.7 $\mu$m |
| external porosity ($\varepsilon_e$) | 0.4 |
| interstitial mobile phase velocity ($u_e$) | 5 $\frac{\mathrm{cm}}{\mathrm{min}}$ |

### Theoretical study of the efficiency of liquid chromatography columns with particle size gradient

The values of the numerical parameters necessary for the numerical calculations, such as the column and the molecular parameters are listed in Table 2. Note that the retention sensitivity parameter, $S$, has a quite high value. That large value was deliberately chosen in order to represent large polymers and proteins.

Table 2: Numerical parameter values used to study the efficiency of liquid chromatography columns with particle size gradient

| **Parameter** | **Value** |
|---|---|
| column length ($L$) | 0.05 m |
| parameters of the Knox equation ($A, B, C$) | 1, 1.5, 0.5 |
| molecular diffusion coefficient ($D_m$) | $10^{-8}$ m$^2$/s |
| column resistance factor ($\phi$) | 1000 |
| linear mobile phase velocity ($u_0$) | 0.0111 m/s |
| eluent viscosity ($\eta$) | $10^{-3}$ Pa s |
| initial molar fraction of the stronger eluent ($\varphi_0$) | 0.05 |
| change of the eluent composition ($\Delta\varphi$) | 0.7 |
| retention factor for $\varphi = 0$ ($k_0$) | $10^4$ |
| retention sensitivity ($S$) | 30 |
| gradient time ($t_G$) | 600 s |

## Unbiased determination of adsorption isotherms by inverse method in liquid chromatography

*Instrumentation and materials*

The chromatographic experiments were carried out using an Agilent 1100 Series liquid 111 chromatograph (Agilent Technologies, Palo Alto, CA), equipped with a multisolvent delivery system, an automatic injector, a column thermostat a DAD detector, and an HP Chemstation data aquisition system. Band profiles of butyl benzoate (Sigma-Aldrich) were recorded at 290 nm. The column used during the experiments was a 50 mm x 2.1 mm Waters Cortecs C18 column packed with 5 $\mu$m particles. 65:35 methanol-water mixture was used as the eluent. The eluent flow rate was 0.2 ml/min, the column temperature was 30°C and the concentration of the butyl benzoate sample was 7.25 g/l.

*Calculations*

In the developed isotherm-equation-free method, the derivative of the isotherm is replaced by the derivative of the spline (a special function defined piecewise by polynomials) fitted to each isotherm point. For the fitting, cubic spline was used, where the successive points are connected by polynomials of degree three. The sum of squares of the difference between the measured and calculated peak profiles was minimized by optimizing the position of each isotherm point with Nelder-Mead simplex method [1]. The determination of the initial value of the isotherm points was based on the classical inverse method, assuming Langmuir isotherm. The number and distribution of isotherm points depends on the shape of the measured band profile.

### Study of efficiency of capacity gradient ion-exchange stationary phases

The numerical parameters required for these calculations, such as the column and molecular parameters, are listed in Table 3.

Table 3: Numerical parameter values used to study the efficiency of liquid chromatography columns with ion exchange capacity gradient

| Parameter | Value |
|---|---|
| column length ($L$) | 25 cm |
| mobile phase velocity ($u$) | 25 cm/min |
| initial eluent concentration ($[\mathrm{OH}^-]_0$) | 1 mM |
| rate of change in eluent concentration ($\beta$) | 10 mM/min |

Model ions and their ion-exchange selectivity coefficients [2] used for the calculations are listed in Table 4.

Table 4: The values of the ion-exchange selectivity coefficients of the anions used in this work.

| Anion | $K_{\mathrm{A/OH}}$ |
|---|---|
| chlorid | 1.51 |
| nitrete | 5.04 |
| szulfate | 29.05 |
| fumarate | 66.21 |

# 3. New scientific results: thesis points

The results of my scientific work are summarised in the following thesis points:

1. Bi-layer liquid chromatography stationary phase particles provide higher separation efficiency compared to conventional shell phases when the retention or pore diffusion of the outer layer is higher than that of the inner layer. In this way, the double layer grain structure can increase the column efficiency by 15-20%.

2. In gradient elution mode, columns with a negative - i.e. decreasing - particle size gradient towards the end of the column show an efficiency increase of ∼1-4% compared to columns with constant particle size and the same permeability, due to the extra band compression effect, depending on the particle size gradient profile. By optimizing the boundary particle sizes, this advantage can be further increased up to ∼5-8%.

3. The developed isotherm-free inverse method for determining isotherms, in which the derivative of spline fitted to the isotherm points is used instead of the isotherm equation in the mass balance equation of the Martin-Synge algorithm, can be used to isotherm determination with an accuracy comparable to that of the frontal analysis.

4. By combining an ion exchange capacity gradient with an eluent concentration gradient, an increase in efficiency can be achieved compared to a column with a constant ion exchange capacity for the same analysis time. This increase in efficiency is manifested through an increase in the difference in retention times and a decrease in bandwidths.

# 4. The author's scientific activity

## 4.1. Publications related to the dissertation

1. **Szabolcs Horváth**, Fabrice Gritti, Róbert Kormány, Krisztián Horváth: Theoretical analysis of efficiency of multi-layer core-shell stationary phases in the high performance liquid chromatography of large biomolecules, Molecules, 2019, 24 (15), 2849.
   `DOI:10.3390/molecules24152849`

2. **Szabolcs Horváth**, Fabrice Gritti, Krisztián Horváth: Theoretical study of the efficiency of liquid chromatography columns with particle size gradient, J. Chromatogr. A, 2021, 1651, 462331.
   `DOI:10.1016/j.chroma.2021.462331`

3. **Szabolcs Horváth**, Diána Lukács, Evelin Farsang, Krisztián Horváth: Study of efficiency of capacity-gradient ion exchange stationary phases, *Separations*, 2023, 10(1),
   `DOI:10.3390/separations10010014`

4. **Szabolcs Horváth**, Diána Lukács, Evelin Farsang, , Krisztián Horváth: Unbiased Determination of Adsorption Isotherms by Inverse Method in Liquid Chromatography , *Molecules*, 2023, 28(3),
   `DOI:10.3390/molecules28031031`

## 4.2. Conference presentations and posters related to the dissertation

### Presentations and posters at international conferences

1. **Szabolcs Horváth**, Fabrice Gritti, Krisztián Horváth: Effect of multi porous layer on the efficiency of core-shell particles, 24TH International Symposium on Separation Sciences : ISSS (2018), 17-20 June 2018, Jasná, Slovakia

2. **Szabolcs Horváth**, Evelin Farsang, Krisztián Horváth : Theoretical study of the efficiency of HPLC columns with particle size gradient, 48th International Symposium on High-Performance Liquid Phase Separations and Related Techniques, 16-20 June 2019, Milan, Italy

3. Krisztián Horváth, **Szabolcs Horváth** : Applicability and Efficiency of Gradient Stationary Phases in HPLC, 12th Balaton Symposium on High-Performance Separation Methods, 11-13 September 2019, Siófok, Hungary

4. Krisztián Horváth, **Szabolcs Horváth** : Design and Optimization of Capacity Gradient Stationary Phases, 33rd International Symposium on Chromatography: ISC 2022, 18-22 September 2022, Budapest, Hungary

## 4.3. Publications not related to the dissertation

1. Krisztián Horváth, **Szabolcs Horváth**, Diána Lukács: Effect of axial temperature gradient on chromatographic efficiency under adiabatic conditions, J. Chromatogr. A, 2017, 1483, 80-85. `DOI:10.1016/j.chroma.2016.12.063`

## 4.4. Conference presentations and posters not related to the dissertation

**Other presentations and posters at international conferences**

1. Evelin Farsang, **Szabolcs Horváth**, Krisztián Horváth: Study of retention behavior of bioactive macromolecules in ultrahigh pressure liquid chromatography, 45th International Symposium on High Performance Liquid Phase Separations and Related Techniques: HPLC 2017, 18-22 June 2017, Prague, Czech Republic

2. Evelin Farsang, **Szabolcs Horváth**, Krisztián Horváth: The effect of axial temperature and pressure gradients on the separation efficiencies

of macromolecules, 24th International Symposium on Separation Sciences : ISSS (2018), 17-20 June 2018, Jasná, Slovakia

3. Evelin Farsang, **Szabolcs Horváth**, Regina Törőcsik, Márton Szigeti, András Guttman, Krisztián Horváth: Development of HILIC Method for Liquid Chromatographic Separation of Maltodextrins with UV Detection, 9th International Symposium on the Separation and Characterization of Natural and Synthetic Macromolecules, 30 January-1 February 2019, Amsterdam, Netherlands

4. **Szabolcs Horváth**, Krisztián Horváth: Qualitative interpretation of retention behaviour of proteins under UHPLC condition, 12th Balaton Symposium on High-Performance Separation Methods, 11-13 September 2019, Siófok, Hungary

**Other presentations and posters at national conferences**

1. Horváth Krisztián, **Horváth Szabolcs**, Farsang Evelin, Surguta Miklós, Lukács Diána: Axiális hőmérséklet gradiens hatása kromatográfiás elválasztások hatékonyságára, Elválasztástudományi Vándorgyűlés 2016, 2016 november 9-11, Kecskemét, Magyarország

2. **Horváth Szabolcs**, Törőcsik Regina, Szigeti Márton, Guttman András, Horváth Krisztián: HILIC módszer fejlesztése maltodextrinek folyadékkromatográfiás elválasztására UV detektálás alkalmazásával, Elválasztástudományi Vándorgyűlés 2018, 2018 november 8-10, Tapolca, Magyarország

# References

[1] J. A. Nelder, R. Mead, A Simplex Method for Function Minimization, The Computer Journal 7 (1965) 308–313. `doi:10.1093/comjnl/7.4.308`.

[2] K. Horváth, P. Hajós, Retention profiles and mechanism of anion separation on latex-based pellicular ion exchanger in ion chromatography, J. Chromatogr. A 1104 (1) (2006) 75 – 81. `doi:https://doi.org/10.1016/j.chroma.2005.11.064`.