**UNIVERSITY OF PÉCS**

Doctoral School of Chemistry

# Mass transfer in high performance liquid chromatography

**PhD Thesis**

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# 1. Introduction

The chromatographic process is mostly described by equilibrium or kinetic models. The physico-chemical processes of the separation in a chromatographic bed can be described by differential mass balance equations. The equilibrium models assume very fast equilibrium between the mobile and the stationary phase. The kinetic models use rate constants for characterization of mass transfer. The stochastic model developed by Giddings and Eyring considers the random migration of molecules through the chromatographic bed. That model gives a direct view into chromatographic process.

The very fast mass transfer of small molecules in the stationary phase results in high resolution in HPLC. The mass transfer kinetics influences peak profiles, particularly the band broadening and asymmetry. For the correct description of the chromatographic process the determination of mass transfer kinetics in the particles of the packing material is required.

In chromatography, mass transfer refers to the movement of solute through the mobile and stationary phases. The band broadening is affected by the slow mass transfer, the diffusion in the chromatographic phases. The mass transfer resistances in the chromatographic bed have several sources: the axial dispersion in the stream of mobile phase and the external mass transfer resistance ($k_{ext}$) on the surface of particles and the diffusion in the pores of particles. These phenomena depend on particularly the molecular diffusivity and the kinetics of adsorption-desorption. In reversed phase chromatography the kinetics of the physical adsorption is very fast, the effect of adsorption/desorption kinetics on the band broadening can be negligible.

## 2. Aims

The aim of this study was to determine and compare the mass-transfer properties of neutral low-molecular-weight analytes and macromolecules on fully porous and fused core stationary phases.

The research goals were follows:

- Determination of the porosity of HPLC columns with inverse size-exclusion chromatography
- Determination of the distribution coefficient in SEC, $K_{SEC}$, using the stochastic model of size-exclusion chromatography
- Determination of molecular diffusivity of polystyrenes by peak parking method
- Comparison of mass transfer on totally porous and fused core stationary phases in size-exclusion chromatography
- Determination of mass transfer of neutral compound with small and macromolecules in reversed phase chromatography. Four different methods were used to calculate the coefficients: (i) the plate height equation originating from the general rate model of chromatography; (ii) the conventional van Deemter equation; (iii) the moment analysis; and (iv) the stochastic model of chromatography.
- Comparison of the mass transfer in totally porous and fused core stationary phases in reversed phase chromatography
- Comparison of the macroscopic and microscopic models by the mass transfer coefficients

## 3. Methods

An Agilent 1100 liquid chromatograph with a dual solvent delivery system, an auto sampler, a column thermostat, and a multi-wavelength UV detector was used for all measurements.

The following columns were used for this study:

a. totally porous Waters Symmetry C18 (150 mm × 4.6 mm, $d_p$ = 5 µm, 100 Å)
b. totally porous Waters Symmetry $C_{18}$ (75 mm × 4.6 mm, $d_p$ = 3.5 µm, 100 Å)
c. totally porous Waters SunFire $C_{18}$ (100 mm × 3.0 mm, $d_p$ = 3.5 µm, 94 Å)
d. fused core Halo $C_{18}$ (100 mm × 4.6 mm, $d_p$ = 2.7 µm, shell thickness of 0.5 µm, 90 Å)

*3.1. Determination of the porosity of HPLC columns:*

Inverse size-exclusion chromatography was applied for the determination of the porosity of the HPLC columns. The mobile phase flow rate was 1.0 and 0.5 mL/min for totally porous particles and superficially porous particles, respectively. Each measurement was executed with 100% tetrahydrofuran. 1 µL sample containing 0.5 mg/mL polystyrene standards (MW 580 – 3250000) was injected. The column thermostat was set at 20 $^{o}$C. The standards were detected at 205 nm. All measurement was repeated with a zero-volume connector instead of using chromatographic column.

*3.2. Determination of molecular diffusivity by peak parking method:*

The measurement was performed on column **b.** and **d.** A 1µL sample of a 0.5 mg/mL solution of polystyrene standard (MW = 10100, 31420, 70950) were injected at a flow rate of 0.5 mL/min until the concentration bandwidth reaches about half the length of the chromatographic column. The flow was stopped for 30, 60, 120, 180, 240, 480 min, as parking time. The column thermostat was set at 20 $^{o}$C. The standards were detected at 205 nm.

*3.3. Determination of mass transfer of neutral compound with low molecular weight:*

Separation of alkylbenzenes was performed on column **a.** The mobile phase flow rate was changed over the range 0.1 – 2.5 mL/min for separating a standard mixture. The column thermostat was set at the following temperature: 20 °C, 30 °C

and 40 °C. Each measurement was excited at 80 % methanol containing eluent. The standard mixture contained 0.4 µL/mL of toluene, ethylbenzene, n-propylbenzene, n-butylbenzene, n-pentylbenzene and $8 \cdot 10^{-4}$ mg/mL of thiourea. The hold-up time of this column was determined from the retention time of thiourea. 20 µL of the standard mixture was injected in all measurements. The components were detected at 254 nm.

A measurement series was done with $1.6 \cdot 10^{-3}$ mg/mL of thiourea, 1 µL was injected. The others parameter were the same as at the alkylbenzenes. The extra-column volume was derived from the retention volume the 0.4 µL/mL toluene measured with a zero-volume connector instead of using chromatographic column.

*3.4. Comparison of the mass transfer in totally porous and fused core stationary phases:*

The measurement series was performed on columns **c.** and **d.** The column thermostat was set at 20 $^{o}$C. The mobile phase flow rate was changed over the range 0.02–1.4 mL/min. Each measurement was executed with eluent containing 21 % acetonitrile and 79 % water (v/v), with 0.1 % trifluoroacetic acid. 1 µL sample containing 1 mg/mL human insulin ($M_w$ = 5.5 kD) was injected. The insulin was detected at 205 nm. The hold-up volume of columns was determined as before (3.3.).

*3.5. Determination of mass transfer of alkylbenzene using the stochastic model of chromatography:*

Separation of alkylbenzenes was performed on column **a.** Different flow rates were used, 0.25 mL/min, 0.50 mL/min, 1.00 mL/min and 1.50 mL/min. The column thermostat was set at the following temperature: 10 °C, 20 °C, 30 °C and 40 °C. The eluent contained 80 % methanol. The standard mixture contained 0.2 µL/mL of toluene, ethylbenzene, n-propylbenzene, n-butylbenzene, n-pentylbenzene and thiourea. The hold-up time of this column was determined from the retention time of thiourea. 20 µL of the standard mixture was injected in all measurements. The components were detected at 254 nm.

## 4. Results and discussion

Totally porous and fused core chromatographic particles were compared in this study. Mass transfer of low and high molecular weight components were determined and compared on two types of chromatographic bed.

Because the geometry of the chromatographic bed plays a crucial role in mass transfer, the determination of the porosity of the columns is needed. Inverse size-exclusion chromatography was applied to determine the porosities of columns used in this study.

The molecular diffusion has also an important function in mass transfer. This feature determines the movement in the particles and the axial dispersion and the external mass transfer depend on molecular diffusion respectively. The so called peak parking method was used to measure the molecular diffusivity of polystyrene standards. Our results agree with the numerical values calculated by empirical expressions for molecular diffusion.

The axial dispersion coefficient ($D_L$), external mass transfer rate constant ($k_{ext}$) and intraparticle diffusion coefficient ($D_p$) of polystyrenes were determined using the general rate model of chromatography. We have found that the band broadening of excluded polymers caused by axial dispersion is mostly irrespective of the inside-structure of particles. The reduced plate height of polystyrenes, which are capable getting into the pores is 4 to 6 times higher in totally porous, than in fused core particles. The contribution of the axial dispersion to the band broadening of small solute is infinitesimal and there is no difference between the investigated stationary phases from aspect of external mass transfer. We can conclude that the intraparticle diffusion has a significant role in the band broadening.

The mass transfer coefficients of alkylbenzenes on totally porous stationary phase were determined. Macroscopic and microscopic models were applied in the calculation.

Using the general rate model, the axial dispersion can be calculated for every experimental data point. When a plate height equation is fitted to the experimental data, or when moment analysis is used, one single value of axial dispersion coefficient is obtained over the entire range of mobile phase velocity. The axial dispersion strongly depends on mobile phase velocity due to eddy dispersion. In the range of conventional flow rates (F*v* = 0.5–1.0 mL/min) on 150 mm long columns we found very little difference between the various calculation methods.

The intraparticle diffusion coefficient should be constant regardless of flow rate. Our calculations did not confirm that, the contradiction may be due to model errors. There is no evidence that the Wilson–Geankoplis equation accurately estimates the external mass-transfer coefficient. Additionally, although the general rate model is thought to be the most detailed model of chromatography, it contains simplifications that may lead to inconsistent results. The most important constraint of the general rate model is that it assumes that mobile phase dispersion coefficient is independent of flow velocity. Experimental data clearly contradict to this assumption, hence it is worth considering the particle is not a singe world in the column, a slow flow can develop on the surface of pores, that can occurs flow rate dependent intraparticle diffusion. However, the difference between the various calculation methods is minor.

The stochastic model of chromatography offers a rather interesting view of the chromatographic process. The determination of the stationary phase sojourn times and the number of mass transfer events gives a practical view of the molecular process of separation.

In this study the typical time constants are as follows: the stationary phase residence time ranges between $\tau_m$ = 10–180 ms, and the mobile phase sojourn time is in the range of $\tau_s$ = 5–20 ms, depending on solute, temperature, and flow rate. The typical numbers of mass-transfer events is in the range of $n$ = 15000–25000, i.e. on the average, every molecule enters and leaves the stationary phase that many times during the migration along the chromatographic column. The enormous separation power of chromatography arises from the differences in the stationary phase sojourn time combined with the large number of mass-transfer events.

The stochastic analysis provides time constants for the stationary phase residence time, which are close to the ones calculated on the basis of the

macroscopic models. The typical stationary phase residence times are 12–25 ms calculated from the data of the applied macroscopic models. These values agree with the results calculated by using the stochastic model. Accordingly the stochastic model is a good alternative of macroscopic model of chromatography.

The mass transfer of insulin on totally porous and fused core particles was investigated. For the calculation, macroscopic and microscopic models of chromatography were applied again.

We noticed in case of insulin and other large molecules that the flow rate and the pressure strongly influence the retention. Using the general rate model, the axial dispersion ($D_L$), the external mass transfer coefficient ($k_{ext}$) and the internal diffusion coefficient ($D_p$) were determined on totally porous and fused core particles respectively. Since mobile phase flow rate strongly affects the retention factor, the fitting of the plate height equation, and accordingly the numerical values of the axial dispersion and the intraparticle diffusion coefficients are prone to error since both the fitting of the plate height equation and the moment analysis provide a single value of $D_L$ and $D_p$ for the applied range of flow rate.

In our experiments the solute has flow rate dependent retention factor, which means increasing the pore surface diffusion and effective diffusion also. Accordingly, the flow rate dependent intraparticle diffusion can be acceptable.

It was established that the average stationary phase residence times of insulin calculated by stochastic model is lower on the totally porous stationary phase (~100 ms), than on fused core stationary phase (~150 ms). The retention factor of insulin is two times lower for the totally porous stationary phase than for the fused core one. The stationary phase sojourn time is directly proportional to the retention factor, since $k' = \tau_s / \tau_m$. If the mobile phase composition were changed so that the retention factors are equal on the two columns, the average residence time in the fused core particle would be 75% of that in the totally porous one.

## 5. Thesispoints

1) Mass transfer coefficients of polystyrenes were determined on totally porous and fused core particles in size exclusion chromatography system:

   a) The axial dispersion is the main contributor (~80%) to the band broadening of the excluded polymers on both stationary phases. In case of small molecules, which can ingress to the particles, the intrapartilce diffusion contribute ~80% to the plate height.

   b) The reduced plate height of polystyrenes, which are capable getting into the pores, is 4 to 6 times lower in fused core, than in totally porous particles. The fused core inside of particles makes shorter diffusion patch.

2) Mass transfer coefficients of alkylbenzenes and insulin were determined on totally porous and fused core particles applying macroscopic and microscopic models of chromatography:

   a) In case of insulin and other large molecules the flow rate and the pressure strongly influence the retention. The fitting of the plate height equation, and accordingly the numerical values of mass transfer coefficients are prone to error since both the fitting of the plate height equation and the moment analysis provide a single value of $D_L$ and $D_p$ for the applied range of flow rate.

   b) In case of alkylbenzenes very little difference was founded between the various calculation methods at the determination of the axial dispersion in the range of conventional flow rates (F*v* = 0.5–1.0 mL/min).

   c) It can be assumed that there exists a slow flow at the surface of particles, because the general rate model gives a flow-rate dependent intraparticle diffusion in case of small molecules.

d) The flow-rate dependent retention of insulin resulted in flow-rate dependent surface and intraparticle diffusion, respectively.

3) The stationary phase residence time and the mobile phase sojourn time of alkylbenzenes and insulin were determined by the stochastic model of chromatography:

   a) In case of alkylbenzenes the typical stationary phase residence time $\tau_S$ = 5-20 ms,and the number of mass transfer steps are $n$ = 15000-25000.

   b) The stationary phase residence time of insulin on totally porous stationary phase is ca. 100 ms and on the fused core stationary phase ca. 150 ms. The differences of value of $\tau_S$ refer to the different retention factor of the solute on the columns in our experiment.

4) The stochastic analysis provides time constants for the stationary phase residence time, which are close to the ones calculated on the basis of the macroscopic models. The stationary phase residence time calculated by general rate model is $\tau_S$ = 12–25 ms, which agrees with correspond to the values obtained in stochastic analysis, $\tau_S$ = 5–20 ms.

## 6. Publications, posters

### Publications related to this Thesis

1. Ivett Bacskay, Attila Felinger

Macroscopic and microscopic analysis of mass transfer in reversed phase liquid chromatography

JOURNAL OF CHROMATOGRAPHY A 1216: (8)1253-1262 (2009) IF: 4.101

2. Ivett Bacskay, Attila Felinger

Rapid estimation of overall mass-transfer coefficients in liquid chromatography.

ANALYTICAL METHODS 2: (12)1989-1993 (2010) IF: 1.036

3. Ibolya Kiss, Ivett Bacskay, Ferenc Kilár, Attila Felinger

Comparison of the mass transfer in totally porous and superficially porous stationary phases in liquid chromatography

ANALYTICAL AND BIOANALYTICAL CHEMISTRY 397: (3)1307-1314 (2010) IF: 3.841

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Mass transfer kinetics from macroscopic and microscopic approaches in reversed phase liquid chromatography. International CEEPUS II Summer School, Torun-Gdansk-Bialystok, Poland. September 7-13, 2006, p. 212

2. Bacskay I., Felinger A.

Az anyagátadás kinetikája a fordított fázisú folyadékkromatográfiában makroszkopikus és mikroszkopikus megközelítésben. XII. Nemzetközi Vegyészkonferencia. Csíkszereda, Románia. 2006. október 3-8. 11. o.

3. I. Bacskay, A. Felinger

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4. A. Feinger, I. Bacskay
Molecular dynamic analysis of the retention mechanism in reversed phase liquid chromatography. 31thInternational Symposium on high Performance Liquid Phase Separations and Related Techniques. Genth, Belgium. June, 17-21, 2007
5. I. Bacskay, A. Felinger
Determination of Mass Transfer Coefficients with Macroscopic and Microscopic Approaches in Reversed Phase Liquid Chromatography. 7th Balaton Symposium on High-Performance Separation Methods. Siófok, Hungary. September 5-7, 2007, P-5
6. A. Felinger, I. Bacskay
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7. I. Kiss, I. Bacskay and A. Felinger
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9. Bacskay I., Felinger A.
Porózus és héj szerkezetű kromatográfiás állófázisokon kialakuló anyagátadási kinetika összehasonlítása nagy molekulatömegű komponensek esetében. Elválasztástudományi vádorgyűlés, Sárvár, 2008 november 5-7, P-1
10. Kiss I., Bacskay I., Felinger A.
Az anyagátadási együtthatók vizsgálata különböző geometriájú állófázisok esetében. Elválasztástudományi vádorgyűlés, Sárvár, 2008 november 5-7, P-24
11. I. Kiss, I Bacskay, F. Kilár, A. Felinger
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12. I. Kiss, I. Bacskay, F. Kilár, A. Felinger

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**Publications not related to this Thesis**

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2. Ivett Bacskay, Robert Gora, Zoltán Szabó Z, Ibolya Kiss, Václav Kasicka, Gabrial Peltre, FerencKilar

Seasonal Variations of Polycyclic Aromatic Hydrocarbons in Air Particulate Extracts

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3. Borbála Boros, Ágnes Farkas, Silvia Jakabova, Ivett Bacskay, Ferenc Kilar, Attila Felinger

LC-MS Quantitative Determination of Atropine and Scopolamine in the Floral Nectar of Datura Species

CHROMATOGRAPHIA 71: S43-S49 (2010) IF: 1.075

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2. S. Hjertén, Á. Végvári, I. Bacskay, A. Takátsy, F.Kilár, O. Kornysova, A. Maruska, J. Sedzik. Methological Advances in Microelectrophoresis, including Studies of the „Artificial Antibodies" against a given Protein, Virus or. Bacterium. APCE2002. The Fourth Asia-Pacific International Symposium on Microscale Separations and Analysis. Shanghai, China. October 11-14, 2002

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7. F. Kilár, I. Bacskay, A. Takátsy, A. Ballagi, S. Hjertén
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