UNIVERSITY OF PÉCS

Doctoral School of Chemistry

The effect of the geometric variability of the stationary phase on the peak shape in liquid chromatography

PhD thesis

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1. Introduction

In chromatography the sample components become distributed over the separation path as discrete zones, and these zones expand continuously as the separation process advances. The result of the separation depends on whether the zones are narrow enough so that we can keep avoiding overlap and cross contamination with adjacent zones. Therefore, the major aim in chromatography is the limitation of zone spreading. To fulfill this, one needs to understand the processes underlying the zone formation and spreading.

The efficiency of the chromatographic separations is decisively affected by the physical parameters of the column and the sample molecules, such as the pore size and distribution of the stationary phase and the molecule size and distribution of the sample. The most preferred method to investigate the relative size of the sample molecules to the pores is Size Exclusion Chromatography (SEC). It allows to get a more accurate picture of the impact of the distributions on the separation because in an ideal case there is no interaction between the sample molecules and the stationary phase, the separation is only based on the size of the molecule relative to the pore size. To consider these effects, however, a proper model is needed which comprises the above-mentioned distributions.

The stochastic theory describes the chromatographic process at the molecular level so it is obvious to introduce the pore size distribution (PSD) and polymer polydispersity into that in order to obtain more relevant information about the retention properties.

2. Motivation and problem statement

The main aims of this work were:

- integration of the pore size distribution into the stochastic theory of chromatography to obtain the characteristic function and the main moments describing the chromatographic peak;
- investigation of the effects of the parameters influencing the separation by taking into account the possible pore geometries;
- determination of the porous structure (pore size distribution and pore geometry) of commercially available columns filled with totally porous and superficially porous particles using the novel model;
- investigation of the difference in the porous structure of commercially available silica-based monolithic columns of the first and second generations using the novel model;
- integration of the molecule size distribution (polydispersity) into the stochastic theory of chromatography to investigate the effect of that on the separation and its efficiency for various pore geometries.

3. Experimental and methods

To derive the models for stochastic theories containing the effects of pore size distribution and polydispersity, all calculations were carried out with the software package Mathematica 9 (Wolfram Research). The elution profiles were obtained via numerical inverse Fourier transform using 1024 points. The moments were obtained using the derived equations and validated via integration of the peak profiles.

Monte Carlo simulation by generating one million normally distributed random numbers was carried out in Mathematica 9 to show the relationship between the breadth of the molecule size distribution and the polydispersity.

Inverse size exclusion measurements were carried out using an Agilent 1100 liquid chromatograph. Different type of C18 columns were used to obtain the pore size distribution of the stationary phase: 4 of them were filled with superficially porous particles, 3 were filled with totally porous particles and 2 of them were monolithic columns.

Polystyrene standards were purchased from Varian, (Varian, Inc. USA). The molecular weight of the standards was in a range of 580 - 3 250 000 Da.

HPLC grade tetrahydrofuran (THF), purchased from Fisher Scientific (Fisher Scientific, Ltd. UK) was used as mobile phase and solvent. The flow rate was kept at 0.50 cm^3/min. Each measurement was executed with 100% THF containing eluent. The column thermostat was set at 293.15 K. The concentration of the samples was 0.50 mg/cm^3 dissolved in THF. The injected volume was 1.00 μL. The components were detected at 254 nm. The retention volumes of polystyrene standards were corrected for the extra-column volume measured at the same condition for each polymer.

4. Results

The stochastic theory describes the chromatographic process at the molecular level so it is obvious to introduce the pore size distribution (PSD) into that in order to obtain more relevant information about the retention properties. The stochastic theory of Size Exclusion Chromatography (SEC) was extended for log-normal PSD. By assuming a pore geometry (slit shaped, cylindrical, and conical or spherical), the statistical moments of the peak profiles can easily be calculated using the derived equations and the representation of the chromatograms is feasible by inverse Fourier transform of the characteristic functions.

The results presented for the calculated chromatograms verify previous observations and experiences the PSD has strong influence on the retention properties (retention time, peak width, and peak shape) of macromolecules. We can conclude that for the separation of macromolecules, the wide PSD will increase retention and efficiency. Therefore, in all modes of liquid chromatography, the efficient separation of macromolecules calls for a broad PSD.

The novel model accounting for PSD is usable to develop SEC measurements and so to obtain relevant information from the pore structure by a nondestructive way in basis of containing information about both the pore geometry and the distribution of the pore sizes.

By using our newly developed model, Inverse Size Exclusion Chromatography (ISEC) has become a more accurate method to investigate the structure of a porous HPLC packing material (pore size and its distribution) without destroying the column than it was before. The stochastic theory of SEC with PSD was used to calculate the pore sizes and PSD of various commercially available HPLC columns; four of them were packed with superficially porous particles and three were packed with totally porous particles. The novel model of SEC fits significantly better to the experimental results than the monopore model for all the columns investigated. By the fittings, we can get a realistic view of the structure of stationary phases and choose the most appropriate column to separate a complex sample.

The stochastic theory of SEC extended for log-normal PSD was also used to estimate the pore size and its distribution of the first generation (1G) Chromolith Performance and the second generation (2G) Chromolith High Resolution monolithic columns via the same ISEC procedure. One can observe a significant difference between the SEC calibration curves of these two monolithic columns. The slopes of the curves are entirely different in the hydrodynamic range, i.e. for the molecules excluded from the mesopores. From the hydrodynamic effect, the sizes of the macropores were determined. The macropore sizes were

found to be 1.55 and 0.95 μm in case of 1G and 2G monoliths, respectively. The 22.7% increase of mesopore volume can be attributed to the highly structured macropore and skeleton structure of 2G monoliths. Although the distribution of the mesopore sizes of the 1G and 2G stationary phases differ, the real mean radii of the mesopores are similar: 8.8 and 8.9 nm, respectively.

The stochastic theory of Size Exclusion Chromatography (SEC) was extended for polydisperse samples where the molecule size is described by a normal and log-normal distribution. By assuming a pore geometry, the statistical moments of the peak profiles can be calculated using the derived equations and the representation of the chromatograms is feasible by inverse Fourier transform of the characteristic functions. By the novel models it was confirmed that we cannot distinguish between the effect of the PSD and that of the polydispersity neither experimentally nor using models.

5. Thesis points

1. The stochastic theory of Size Exclusion Chromatography was extended for log-normal pore size distribution. Chromatograms and their statistical moments were calculated by the novel model assuming certain pore geometries.
2. The parameters affecting the retention properties were changed individually to investigate their effect. It was shown that the effect of the model parameters studied (ρ, σ, α) and that of the quantities characterizing the separation (N, relative resolution) are the most intensive in case of conical pore geometry. The pore size distribution has minor influence on the retention properties of small molecules compared to the larger molecules.
3. The novel model was used to ascertain the pore size distribution and the pore geometry of various porous, spherical HPLC packing materials. The fitting of our model is in better concordance with the experimental data of non-destructive ISEC measurements compared to the model where no pore size distribution was assumed. It was confirmed that the pore size distribution of superficially porous particles packed columns is always broader compared to the totally porous particles packed columns which arise from the synthesis method.
4. The mesopore size and the pore size distribution of the two silica-based monolith generations were determined with ISEC measurements of polystyrene standards on 1G Chromolith Performance and the 2G Chromolith High Resolution monolithic columns. It was concluded that although the pore size distribution of the 2G monolith is narrower compared to the 1G monolith, there is no difference in the average mesopore size of the two generations.
5. The stochastic theory of Size Exclusion Chromatography was extended for polydisperse samples (where the molecule size is described by a distribution). Chromatograms and their statistical moments were calculated for several pore geometries to investigate the effect of this distribution on the retention properties. It was concluded that the increase in polydispersity leads to a shift of retention time and causes band-broadening.

Publications related to this thesis

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