Packed and monolithic capillary columns for LC

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Summary

In this thesis the preparation and characterization of microparticulate and monolithic capillary columns for high-performance liquid chromatography (HPLC) and capillary electrochromatography (CEC) are described. An introduction is given in Chapter 1. Methods that describe the performance limits of columns in LC are discussed. Also, the existing column formats, i.e. microparticulate, monolithic, and open-tubular columns, are described. Furthermore, some new trends in LC, i.e. aspects of miniaturization and the use of ultra-high-pressure liquid chromatography, are pointed out.

Chapter 2 describes the influence of the aspect ratio, the ratio of the column diameter to the particle diameter, of packed capillary columns on column parameters, such as efficiency, retention factors and flow resistance. In order to compare the true efficiencies of different columns, a procedure to account for external band broadening was applied. High efficiencies were obtained with capillary columns with internal diameters of 150, 100, and 75 µm, packed with 10-µm particles. In contrast to previous reports in the literature, no significant improvements in efficiency or flow resistance were observed when the aspect ratio of such columns was decreased. Our observations suggest that the wall effect in these columns is not significant. When the aspect ratio was decreased by increasing the particle size, a decrease in the reduced plate height was observed. However, the results of flow-resistance measurements showed that the latter effect might just as well be attributed to differences in packing and particle-batch quality as to differences in the aspect ratio.
In Chapter 3 the preparation and characterization of monolithic stationary phases with different morphologies is described. Methacrylate-ester-based monolithic stationary phases were prepared *in-situ* in fused-silica capillaries and simultaneously in vials. The influence of the composition of the polymerization mixture on the morphology was studied with mercury-intrusion porosimetry, scanning electron microscopy, and nitrogen-adsorption measurements. A high-density porous polymeric material with a unimodal pore-size distribution was prepared with 40 wt% monomers and 60 wt% solvent in the mixture. A low-density material, prepared with a 20:80 ratio of monomers vs. pore-forming solvent, showed a bimodal pore-size distribution and a much finer structure than the high-density monolith. The characteristic pore size could be controlled by changing the ratio of pore-forming solvents. With increasing solvent polarity, both the pore size and the dimension of the globules increased. The best efficiency in the CEC mode was obtained with an average pore size of 600 nm. Low-density monoliths exhibited lower $A$- and $C$-terms than high-density monoliths. With the optimal monolithic material a minimum plate height of 5 μm could be obtained. The low-density monolith also performed better in the HPLC mode, giving a minimum plate height of 15 μm and a much higher flow permeability than that of the high-density material.

A method for the comprehensive characterization and comparison of columns in the HPLC and CEC modes is proposed in Chapter 4. Using this approach, column parameters such as the number of plates, the eddy-diffusion and mass-transfer contributions to peak broadening, the permeability, and the analysis time are incorporated in a single graph and a comparison in terms of efficiency and speed is obtained. The chromatographic performance of silica-based and polymer-based monolithic capillary columns is discussed and a comparison is made with the performance of packed columns. Also, the potential of ultra-high-pressure liquid chromatography is discussed in this context. In the HPLC mode, the best results
were obtained with silica-monoliths; in the CEC mode, the low-density methacrylate monoliths showed the best performance.

Chapter 5 describes the preparation of methacrylate-ester-based monolithic capillary columns and the characterization of their chromatographic properties in CEC. Methacrylate-ester-based monolithic capillary columns containing quaternary ammonium groups were prepared in-situ and in simultaneous experiments in vials employing thermal initiation. Good repeatability in pore-size could be obtained with the monolithic materials, as were studied with mercury-intrusion porosimetry. A satisfactory column-to-column and run-to-run repeatability was obtained for the electro-osmotic mobility, the retention factors ($k$ values) and the column efficiency in the CEC mode. The retention factors obtained on the monolithic columns showed a behavior similar to that observed with columns packed with C$_{18}$-modified silica particles. The electro-osmotic mobility was found to be influenced only marginally by mobile-phase parameters such as the pH, ionic strength, and acetonitrile content.

Different photografting approaches were applied for the functionalization of the surface chemistry of monolithic stationary phases in Chapter 6. A monolithic matrix was prepared of poly(butylmethacrylate-co-ethylenedimethacrylate) using 1-decanol and cyclohexanol as the pore-forming solvents. Positively and negatively charged monomers were photografted onto the monolithic matrix, which allowed control over the direction of the EOF. The magnitude of the EOF was influenced by changing the concentration of charged monomers in the grafting solution keeping the photografting time constant. With increasing monomer concentration an increasing number of ionizable monomers were grafted on the pore surface, thereby increasing the magnitude of the electro-osmotic flow. By increasing the photografting time, keeping the monomer concentration in the grafting solution constant, the magnitude of the EOF increased until complete coverage of the matrix with charged was achieved.