Effect of Pressure and Temperature Gradient on Separation of Macromolecules

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In High Performance Liquid Chromatography (HPLC), temperature affects the retention factors of compounds separated significantly. Due to the frictional heating, temperature can vary both radially and axially in an HPLC column. The temperature gradient is more significant under ultra high pressure conditions (>700 bar). In addition, if molecular volume changes during adsorption, the pressure gradient affects the solute retention as well [1]. As a result, the migration velocities of chromatographic bands vary spatially through the column. This velocity gradient affects the widths of chromatographic peaks through peak compression or peak expansion [2]. Accordingly, both the increase and the decrease of separation efficiencies may be obtained as a result of pressure- and/or temperature-induced gradients of migration velocities.

In this work, the effect of axial temperature and pressure gradients on the retention behavior of macromolecules are studied by a modeling approach in ultrahigh pressure liquid chromatography. The local retention factors, zone velocities and retention times of compounds are estimated from the changes of Gibbs free energy alongside the column. The changes of molar volume ($\Delta V_m$) and internal energy ($\Delta E$) during adsorption are varied between 0-100 cm$^3$/mole and 5-10 kJ/mole, respectively. In order to calculate the effect of $\Delta T$ and $\Delta P$ on separation efficiency, the equilibrium-dispersive model of chromatography were extended by taking into account the axial temperature and pressure gradients. The model is solved by the Martin-Synge algorithm. The change of retention times, physical bandwidths, release velocities and peak shapes are determined by the calculation of chromatograms. The effect of axial temperature and pressure gradients on the separation efficiencies are presented and discussed.

By applying the presented modeling approach, the effect of pressure and temperature gradients on chromatographic efficiency can be studied and understood. It can also be used in method development and optimization.

References

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