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Performance of the same column in supercritical fluid chromatography and in liquid chromatography

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ABSTRACT

We have studied the chromatographic behavior of the homologous series of alkylbenzenes (ranging from octylbenzene to octadecylbenzene) on the same C_{18} reversed-phase column in supercritical fluid chromatography (SFC) and reversed phase liquid chromatography (RPLC) at various experimental conditions, such as different eluent compositions, flow-rates, and mobile phase densities.

The first and the second moments of the peaks were used to estimate the overall mass-transfer processes in both chromatographic modes using the stochastic model of chromatography.

The results confirm that in SFC – as the density of the mobile phase is influenced by the flow-rate – there is a broader variation of mass-transfer properties than in liquid chromatography.

As expected, the optimum mobile phase velocity is higher in SFC, but there is no real difference in the minimum value of plate height, i.e. in the optimum efficiency.

1. Introduction

Due to the latest technological improvement in instrumentation, supercritical fluid chromatography (SFC) has recently gained increased attention in the fields of applications and theoretical investigations too [1–14]. SFC offers faster separations than HPLC, and SFC is often suggested as an alternative to HPLC. A number of studies investigated the dependence of retention factors in SFC on temperature, pressure, or the density of the mobile phase. Since HPLC columns can be installed and used in SFC, a direct comparison of the two modes of operation can be obtained.

A number of equilibrium and kinetic macroscopic models are used to understand the chromatographic processes in liquid chromatography. The use of the models, however, is complicated by the fact that in supercritical fluid chromatography the physicochemical properties of the mobile phase change along the column with the density of the mobile phase.

The characterization at molecular level of the movement of the sample band through the chromatographic column gives another possibility for the description and comparison of the mass transfer in the different chromatographic modes. The stochastic model of chromatography describes the separation process as a random migration, and randomly occurring adsorption–desorption of the molecules in the chromatographic column [15]. According to this model, the number of adsorption and desorption steps and the sojourn time that a molecule will spend in the stationary phase during the adsorption are random variables. Using the first and the second central moments of the chromatographic peaks, the overall mass-transfer coefficients can be calculated [16].

Another advantage of the use of this model in supercritical fluid chromatography is that it simplifies the correction for the mobile phase band spreading, which is in this case strongly affected by the eluent composition and by the pressure and temperature conditions.

The aim of this study was to determine the mass-transfer coefficients of a homologous series of alkylbenzene molecules in supercritical fluid chromatography and in liquid chromatography for the comparison of the two chromatographic modes. The moments of the chromatographic peaks were analyzed according to the stochastic model of chromatography.

2. Theory

2.1. Stochastic analysis

The molecular dynamic model introduced by Giddings and Eyring [15] describes the movement of chromatographic bands

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with random migration of molecules along the column with randomly occurring adsorption–desorption events. When we apply the stochastic theory to reversed phase separations, the model will rather characterize the mass-transfer process – including the external mass-transfer resistance, the diffusion in the pore of the particle and the adsorption kinetics – than the adsorption process itself [17].

According to the stochastic model, the chromatogram is determined as the probability density function of the residence times of the molecules in the column. The number of adsorption and desorption steps (n) is a random variable characterized by a Poisson-distribution [18]. Each molecule spends in average the same time in the mobile phase (τ_m) and a random time bound to the stationary phase. The average sojourn time that the molecules spend adsorbed in a single step is represented by τ_s.

The void time (t_0) spent by an unretained compound in the column originates from the average number of mass-transfer steps and the sojourn times of molecules in the mobile phase, while the adjusted retention time (t'_R) is determined by the number of mass-transfer events and the average sojourn times in the stationary phase:

$$t_0 = n\tau_m \tag{1}$$

$$t'_R = n\tau_s \tag{2}$$

where

$$t'_R = t_R - t_0. \tag{3}$$

The variance of the peak due to the stationary phase process can be given as:

$$\sigma^2 = 2n\tau_s^2 \tag{4}$$

3. Calculations

To apply the stochastic model of chromatography, the stationary phase induced sample band broadening needs to be characterized. The first moments (μ_1) and the second central moments (μ'_2) of the sample peaks were determined by fitting an exponentially modified Gaussian (EMG) function. The effects of the mobile phase dispersion and the extra column effects on band spreading have to be eliminated by the subtraction of the corresponding moments of an unretained compound from the moments of the sample peaks. However, the correct determination of the μ'_2 of an unretained compound can be difficult, because of asymmetric or tailing peak shapes.

Therefore the following calculation is suggested and was used. The moments of the measured peaks were corrected with the moments of the unretained compound peak. According to Eqs. (1)–(4), the following equations can be written:

$$\Delta\mu_1 = t_R - t_0 = \mu_1 - \mu_{1,0} \tag{5}$$

$$\Delta\mu'_2 = \sigma^2 - \sigma_0^2 = \mu'_2 - \mu'_{2,0} \tag{6}$$

$$\Delta\mu_1 = n\tau_s \tag{7}$$

$$\Delta\mu'_2 = 2n\tau_s^2 \tag{8}$$

where $\Delta\mu_1$ is the corrected first moment of a peak and it is equal to the adjusted retention time; $\Delta\mu'_2$ is the corrected second central moment of the peak and represents the variance of the peak due to the stationary phase process.

We observed, however, that the plate heights of the members of a homologous series slightly increase with retention, the equations above rearranged to the following equation, give a linear relationship between the square of $\Delta\mu_1$ and $\Delta\mu'_2$ for the members of the homologous series of alkylbenzenes (see Fig. 1):

$$\mu'_2 = \mu'_{2,0} + 2n\tau_s^2 = \frac{2}{n}\Delta\mu_1^2 + \mu'_{2,0} \tag{9}$$

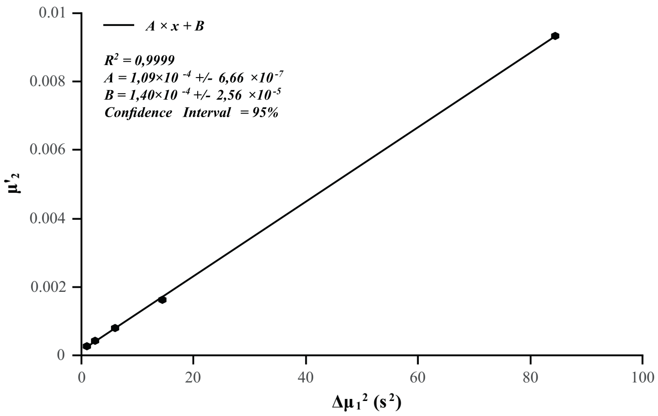

Fig. 1. The plot of μ'_2 against the square of $\Delta\mu_1$ for the series of alkylbenzenes measured in the UHPLC experiments.

The slope of the linear equation gives information about the number of the mass-transfer events, and the intercept is equal to the band spreading of an unretained compound in the mobile phase.

The interstitial velocities (u_h) were calculated using the porosity values determined for the column in use by Bacskay et al. [19], and from the void time determined using a non-retained compound in both chromatographic modes.

4. Experimental

The experiments were carried out on an Agilent 1290 Infinity ultra-high pressure liquid chromatograph (UHPLC) (Agilent Technologies, Palo Alto, CA, USA) and on a Waters Acquity UPC^2 (Waters Corporation, Milford, MA, USA) supercritical fluid chromatograph (SFC). The instruments were equipped with a diode-array detector. The same reversed phase (C_{18}) chromatographic column was installed in the two instruments, an Agilent Zorbax (100 × 4.6 mm with the particle size of 3.5 μm) purchased from Agilent Technologies. Agilent Chemstation software was applied on the UHPLC system while the SFC instrument was controlled by the Empower 3 software. The injection volume was 2 μL, and the flow-rate was set over the range 0.2–2.5 mL/min in each experiment. All measurements were carried out at constant 35°C column temperature.

4.1. Liquid chromatography conditions

In case of the UHPLC measurements, the injected sample was a mixture of the homologous series of alkylbenzenes: octylbenzene, decylbenzene, dodecylbenzene, tetradecylbenzene, octadecylbenzene and thiourea. The sample was prepared in 0.2 mg/mL concentration dissolved in methanol. The standards were purchased from Sigma-Aldrich, (Germany, Schnelldorf). The mobile phase was mixture of methanol and water (Sigma-Aldrich, Germany, Schnelldorf). Two mobile phase compositions were used during the experiments: one was a mixture of methanol and water 97.5:2.5% (v/v) and the other was pure methanol. The retention factor (k) of the most retained compound (octadecylbenzene) was found around 7.2 and 14 at the different mobile phase compositions, respectively (see Table 1).

4.2. Supercritical fluid chromatography conditions

The same mixture of octylbenzene, decylbenzene, dodecylbenzene, tetradecylbenzene, octadecylbenzene, in 1 mg/mL concentration, dissolved in a mixture of methanol and heptane 30:70% (v/v) was injected during SFC experiments. The standards