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Unexpected retention and efficiency behaviors in supercritical fluid chromatography: A thermodynamic interpretation

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ABSTRACT

Experimental conditions leading to unexpected shift in retention, band compression, and to band enlargement of small molecules in supercritical fluid chromatography are reported. The stationary phase is a 3.0 mm × 150 mm column packed with 1.8 μm fully porous high strength silica (HSS) StableBond (SB) C_{18} particles. The mobile phase is pure carbon dioxide preheated at 107 °C and the column back pressure is set at 100 bar. The column was thermally insulated in a vacuum chamber at a pressure of 10^{-5} Torr in order to maintain the integrity of the peak symmetry. The sample solution was prepared by dissolving seven *n*-alkylbenzenes (from benzene to dodecylbenzene) in pure acetonitrile. The injected sample volume (1 μL) was three orders of magnitude smaller than the column volume. Remarkably, the retention time of octylbenzene is found 15% smaller than that expected for this series of homologous compounds. Most strikingly, the plate counts change from about 20 000 for the three least retained analytes (benzene, ethylbenzene, and butylbenzene) to 60 000 for hexylbenzene and to only 5000 for the three most retained compounds (octylbenzene, decylbenzene, and dodecylbenzene). These unexpectedly high (reduced plate height of 1.3) and low (reduced plate height of 15) column efficiencies observed for closely related compounds are consistent with the overlap between the spatial concentration zone of the sample solvent (acetonitrile, Langmuir isotherm, $k \simeq 2$) and those of the analytes (competitive linear isotherms, $0 < k < 10$). The present observations are fully supported by chromatogram simulations which assume that the Henry's constants of the infinitely diluted analytes are strongly dependent on the concentration of the sample solvent in the mobile phase.

1. Introduction

Supercritical fluid chromatography (SFC) possesses three main advantages over liquid chromatography (LC) and very high-pressure liquid chromatography (vHPLC): (1) it is environment-friendly (inertness of gaseous carbon dioxide), (2) it can be operated at very high linear velocities under conventional system pressures (<600 bar) due to the very low viscosity of SFC mobile phases [1–3], and (3) the strength of SFC mobile phases can be tuned over a large range of solvent strength by adjusting independently temperature, pressure, and content of organic modifier. For these reasons, its interest has been continuously growing over the last decade and supported by the manufacture of new high-performance SFC systems [1]. Among other applications, SFC is extensively used as both a high-throughput purification process and a high-resolution analytical screening tool for the preparation and discovery a new pharmaceutical drugs [4–6].

The practice of SFC differs from that of LC due to the specific properties of mixtures of carbon dioxide with organic solvents. The proper optimization and operation of SFC-based units involves some additional knowledge with respect to those required in LC. The impact of temperature and pressure on SFC retention behavior is not similar to that observed in LC [7,8]. The efficiency of SFC columns may also be very sensitive to the surrounding thermal environment [9–11]. The reproducibility of SFC data depends strongly on the control of the inlet mass flow rate [12], the pressure set by the active back pressure regulator (BPR), the oven temperature, the nature (methanol, acetonitrile, ethanol,...) and concentration (0–40% in volume) of the organic modifier [13–15], the nature of the sample solvent [16,17], and the thermal environment in which the SFC column is placed [9–11]. SFC instruments, columns, and methods should then be well controlled for the sake of data robustness.

One important difference between the separation mechanism taking place in SFC with respect to LC is that the local eluent density, its linear velocity, and the equilibrium constants of the analyte may be subject to changes during the band elution [12,14,18] as pressure

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and temperature may vary along and across the column. First, as a general rule, the retention of the analytes is primarily controlled by the average eluent density along the column: in practice, isopycnic (constant density) conditions are then critical in SFC for the proper scale-up of purification methods. Isopycnic plots for pure carbon dioxide and its mixtures with organic modifiers are extremely useful for the experimenter [19]. Secondly, regarding the efficiency of a SFC column and similarly to what has been intensively reported in vHPLC [20–22], the experimenter has to make sure that the steepness of the radial density gradients across the column diameter are kept as small as possible. This was clearly revealed by Poe et al. [9,10,23] when low-density SFC mobile phases are used (carbon dioxide at elevated temperatures and low pressures). The heat exchanged between the column wall and the external environment should be minimized. The ultimate solution consists in placing the column in a strict adiabatic environment. This was recently achieved for air pressure below 10^{-4} Torr [24,25] (high vacuum). Maintaining the integrity of column efficiency in SFC is closely tied to the knowledge of the isenthalpic plots of the mobile phase because any adiabatic decompression of a fluid is performed at constant enthalpy [25,26]. These plots inform the experimenters about the required pressure and temperature that will minimize enthalpy changes during the mobile phase decompression. These adjustments are especially critical when the expansion coefficient of the SFC mobile phase is large [9].

In this work, the effect (either positive or negative) of an additional physico-chemical phenomenon on the retention and efficiency of a SFC column is revealed experimentally and explained theoretically. The efficiencies of a series of seven homologous compounds (*n*-alkylbenzenes from benzene to dodecylbenzene) are recorded on a 3.0 × 150 mm column packed with 1.8 μm fully porous HSS-SB-C_{18} particles. The mobile phase (100% carbon dioxide) was preheated at 107 °C and the BPR pressure was set at 100 bar. The column is fully insulated from the external thermal environment by applying a high air vacuum (10^{-5} Torr) in order to preserve integrity of the peak shape for such a highly expansible mobile phase. Unexpected and repeatable changes in the retention and efficiencies of the seven *n*-alkylbenzenes are reported. The main goal of the paper is to identify and quantify the physical origins of such behavior. Finally, simple calculations based on the equilibrium-dispersive model of chromatography accounting for the proposed relevant physical phenomena was performed in order to predict the observed retention and efficiency anomalies and confirm the separation mechanism of the seven *n*-alkylbenzenes.

2. Theory

The adsorption system is composed of a series of homologous compounds (*n*-alkylbenzenes present at infinitely diluted concentrations in the mobile phase, carbon dioxide), of one organic modifier (small injection volume of the sample solvent, acetonitrile), and of carbon dioxide as the mobile phase. The next section present empirical models for the adsorption isotherms of the analytes and of the organic modifier from pure carbon dioxide onto the HSS-SB-C_{18} stationary phase.

2.1. Adsorption isotherms

The adsorption isotherm of the organic modifier (subscript A) was assumed to be the non-competitive (the amount of analytes injected is infinitely small) Langmuir isotherm. Accordingly,

$$q_A = q_S \frac{K x_A}{1 + K x_A} \tag{1}$$

where q_A is the amount of organic modifier adsorbed at equilibrium onto the stationary phase, q_S is the monolayer saturation capacity, x_A is the volume fraction of the organic modifier in the mobile phase, and K is the adsorption–desorption equilibrium constant. The best isotherm parameters q_S and b were determined unambiguously from the retention time method [27,28]. Accordingly, $q_S = 0.125$ and $K = 100$.

The adsorption isotherms of the seven *n*-alkylbenzenes is assumed to be linear (infinitesimally small amount injected) and competitive (with respect to the acetonitrile concentration C_A). The retention factor, k_n, of the homologous compound C_n (n is the number of carbon atoms in the alkyl chain), is best described by a curved non-linear solvation model [13,29]:

$$\ln k_n(x_A) = \ln k_{0,n} + \frac{\alpha_n x_A}{1 + \beta_n x_A} \tag{2}$$

where $\ln k_{0,n}$, α_n, and β_n are empirical parameters. In this work, the simulation of the chromatograms was performed for 8 homologous compounds. $\ln k_{0,n}$ is increasing regularly from 0.9 to 1.2, 1.5, 1.8, 2.1, 2.4, 2.7, and to 3.0 (one methylene group is adding 0.3 to the intensity of $\ln k_{0,n}$) with increasing n from 0 to 2, 4, 6, 8, 10, 12, and to 14. The parameters α_n decreases from −5, to −10, −15, −20, −25, −30, −35, and to −40 (one single methylene group is adding −5 to the intensity of α_n). The parameters β_n are barely increasing from 1.5 to 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, and to 2.2, respectively (one single methylene group is adding 0.1 to the intensity of β_n). These parameters were determined so that the predicted retention times agree qualitatively well with the observed retention times of the *n*-alkylbenzenes.

2.2. Simulation of band profiles

The calculations of the concentration profiles of the organic modifier and of the analytes at the column outlet was performed using the equilibrium-dispersive (ED) model of chromatography [27]. This apparent model was chosen for our SFC purpose because it is relatively simple to use and requires only moderate computing time. By no means, it reflects on the exact local physical properties (density, linear velocity, equilibrium constant) along and across the SFC bed. For instance, it does not account for the compressibility of the mobile phase and the non-linear change of the flow rate as a function of position along the column. The axial non-uniformity of the column affects essentially the retention of compounds, they do not have a significant impact on the column efficiency [22]. By essence, it will reveal on the importance of the band overlap (competition for adsorption) of the analyte and sample solvent during their propagation of the chromatographic zone. This model assumes instantaneous equilibrium between the mobile and the stationary phases and a finite column efficiency characterized by an apparent axial dispersion coefficient, D_a. The apparent axial dispersion coefficient is related to the apparent column efficiency through:

$$D_a = \frac{u_0 L}{2N} \tag{3}$$

where u_0 is the chromatographic linear velocity of the mobile phase, $L = 15$ cm is the column length, and N is the number of theoretical plates or apparent efficiency of the column. In this model, the mass balance for any compounds (analytes and organic modifier) is written:

$$\frac{\partial c}{\partial t} + u_0 \frac{\partial c}{\partial z} + \frac{1 - \epsilon_t}{\epsilon_t} \frac{\partial q}{\partial t} - D_a \frac{\partial^2 q}{\partial z^2} = 0 \tag{4}$$

where t is the time and z the distance along the column. q and C are the adsorbed and bulk concentrations of the organic solvent