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Investigation of the validity of the kinetic plot method to predict the performance of coupled column systems operated at very high pressures under different thermal conditions

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ABSTRACT

The present study investigates how strong the kinetic plot method is influenced by the changes in plate height, retention factor and apparent column permeability that arise under conditions of very high pressure. More precisely, the study investigates how well a set of performance measurements conducted on a single short column can be used to predict the performance of a long sequence of coupled columns. This has been investigated for the two practically most relevant thermal conditions, i.e., that of a forced-air oven and that of a still-air oven. Measuring column performance data for acetophenone and benzene on a series of coupled 3.5 μm columns that could be operated up to 1000 bar, it was found that the kinetic plot method provides accurate predictions of time versus efficiency for the still-air oven systems, over the entire range of investigated pressures and column lengths (up to 60 cm), provided k' and K_{v0} are evaluated at the maximal pressure. For the forced-air oven which leads to worse performances than the still-air oven, the kinetic plot prediction is less accurate, partly because the thermal conditions (near-isothermal) tend to vary if the number of coupled columns increases. The fact that the thermal conditions of the column wall might vary with the column length is an additional complexity making very-high pressure separations less predictable and harder to interpret and model.

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1. Introduction

In recent years the main focus in the field of liquid chromatography has been on the development of faster and better separations [1–7]. Reducing the particle size has become one of the most popular ways to pursue this. Very high efficiencies can be obtained using small particle columns [5–7], because the efficiency of a column scales with its particle size:

$$H_{min} = \beta d_p \tag{1}$$

However, there is a downside to working with these ultra-small particles because the pressure drop over the column scales in inverse proportion to the square of the particle size:

$$\Delta P = \frac{\phi \eta L u}{d_p^2} \tag{2}$$

This implies that for a certain column length, the use of smaller particles requires higher pressures.

Facing the engineering problems associated with working at high pressures, several manufacturers have developed equipment able to deliver pressures up to 1000 bar [8–11]. But the mechanical strength of the valves, pumps, tubing and column hardware is not the only issue of concern. High pressures also influence the physicochemical characteristics of the mobile phase [12] and have a direct effect on chromatographic parameters such as the plate height and the retention factor.

Moreover, when working at high inlet pressures, severe frictional heating can occur at high flow rates. The amount of heat generated equals the product of flow rate F and pressure drop over the column ΔP [13,14]:

$$\text{Power} = F\Delta P \tag{3}$$

The generated heat leads to a number of unwanted effects when it is poorly dissipated, as is the case when using large-bore columns: the temperature of the mobile phase increases, leading to axial and radial temperature gradients inside the column [15,16]. These

temperature gradients affect the viscosity of the mobile phase and the retention factor of the analytes, and lead to changes in band broadening [17–19].

Several authors have investigated the effect of high pressure operations on chromatographic parameters in liquid-chromatography. Below is a summary of the most important changes in retention factor, apparent permeability (K_{v0}) and performance one can expect as a result of high pressure alone and when the effect of high pressure and the generated heat inside the column are combined. The notion "apparent permeability" is used here to denote the effect of the pressure- and temperature dependence of the mobile phase viscosity on the observed permeability.

Jorgenson and co-workers studied the effect of high pressure on the retention factor of benzene-derivates using fused silica capillaries with a diameter of 33 μm and packed with 1.0 μm nonporous particles. In such a thin capillary, the viscous friction heat can be efficiently removed and the observed effects are due to the pressure alone. The authors observed a linear increase in retention factor of 21–53% for an increase in applied pressure from 1 to 5000 bar [6], meaning that the expected increase in retention factor under the currently available ultra-high pressure conditions in the laboratory (up to 1000 bar) is 4–10%. Another study of the pure high pressure effect on the retention was performed by McCalley and co-workers, covering a broad series of low molecular weight analytes with different functionalities. They used a column with conventional dimensions (50 × 2.1 mm) and studied the effect of pressure without the interference of frictional heating by working at a constant, low flow rate and increasing the pressure by using restriction capillaries of different lengths at the column outlet [20]. The authors found that the retention factor of small, neutral compounds increased linearly with maximum 12% for a column pressure increase of 1000 bar when using a mobile phase consisting of water and acetonitrile or methanol. These results agree very well with the findings of Jorgenson and co-workers [6]. However, for a mixture of strong bases and a mixture of strong acids a linear increase in retention factor of 35–50% and 25–35% was respectively observed for a column pressure increase of 1000 bar. The authors attributed the larger increase in retention of ionisable compounds to larger changes in molar volume of the solute as it is transferred from the mobile to the stationary phase. In an earlier study by Ohmacht and Boros [21], the dependency of retention on pressure was studied in a similar way: a constant, low flow rate was applied and the pressure was increased using a backpressure regulator installed behind the column. Using a 4.6 mm i.d. column the authors found a linear relationship between pressure and retention factor increase. The authors also studied the combined effect of pressure and temperature on retention by increasing the pressure in the normal way, i.e. by increasing the flow rate. The relationship was no longer linear, but the retention factor increased initially with increasing pressure and decreased again at higher pressures. The latter decrease was attributed to the poor dissipation of the heat generated in the column, causing the formation of a longitudinal temperature gradient inside the column, leading to a larger over-all temperature and hence to a lower retention equilibrium.

With respect to the band broadening, new insights on the influence of high pressures on the theoretical plate height were given in a recent theoretical paper by Neue and Kele [17] for the idealized cases of radially uniform isothermal and adiabatic columns. When considering the effect of pressure alone, the authors could demonstrate that the theoretical plate height increases faster with increasing flow rate than predicted by the classical van Deemter equation. This leads to an upward curvature of the C-term, which can be entirely attributed to the influence of pressure on the diffusion coefficient of the analytes (high pressure leads to a lower diffusivity). The authors showed that at a pressure of 1000 bar, the C-term of the van Deemter equation increases with roughly 30%.

However, the band broadening in columns that are operated under adiabatic or close-to-adiabatic conditions is never subjected to the pure pressure effect alone, but is also always influenced by the longitudinal temperature gradient along the column. The latter has an opposite effect to that of the pressure, and it was concluded that for pressures up to 1000 bar the departure of the C-term from the low-pressure van Deemter equation remains negligible under adiabatic conditions.

Another important parameter determining the performance of a column is its permeability. The column permeability can be expressed with the Kozeny–Carman law [22,23]:

$$K_{v0} = \frac{u_0 \eta L}{\Delta P} \qquad (4)$$

In conventional HPLC, the back pressure of a column increases linearly with the chromatographic velocity (u_0), making the apparent permeability independent of the flow rate. Mellors and Jorgenson [5], however, observed a nonlinearity when plotting the chromatographic velocity measured on a 30 μm i.d. capillary versus pressure. At 4600 bar, the linear velocity was 31% lower than predicted, resulting in a lower than expected apparent permeability. This effect could be entirely explained by the increase of the mobile phase viscosity at higher pressures. Using 2.1 mm i.d. columns on the other hand, Gritti and Guiochon observed the opposite trend [24]. In their case, the linear velocity was slightly higher than expected at high inlet pressures. This was attributed to the temperature increase due to the heat generated in the column, resulting in a viscosity decrease of the mobile phase. As the influences of pressure and temperature nearly compensated each other the deviation was, however, very small. This observation is not in contradiction with that of Mellors and Jorgenson [5], for the latter used capillary columns, where the heat is removed much easier than in a 2.1 mm column, so that the mobile phase is only subjected to an increased pressure and does not undergo any compensating effect from an increased mobile phase temperature.

The above described effects show that the use of very high pressure makes important chromatographic parameters such as retention factors or plate heights less constant than they are at conventional pressures. Whereas for pressures up to 400 bar retention factors are generally considered as being independent of the flow or the column length and whereas the plate height value at a given flow rate is usually assumed to be independent of the column length, this is no longer the case when entering the very high-pressure realm. This now also has implications for the kinetic plot method [25–28], which is based on the assumption that plate heights and retention factors are column-length independent. To investigate this in detail, the present study was set up to measure the variation of efficiency, apparent permeability and retention factors in coupled column sequences with increasing length at different flow rates and hence column pressures. The two practically most relevant thermal conditions were considered. The first condition was that of a still-air oven, where the column is placed inside a still-air compartment that is heated to a certain set-point value by a heating element situated near the column. Because the heat transfer between still air and the column wall is quite poor [29], this condition will be referred to as "near-adiabatic". The second investigated thermal condition is encountered in a forced-air oven, in which the temperature is precisely controlled by high velocity air flow. The more favorable heat transfer between the column and the environment leads to a better radial heat transport and will further on be referred to as "near-isothermal". A third possible thermal condition, obtained if one would be using a thermo-stated water bath, was not considered as this condition anyways leads to the poorest performance [29], because of the formation of a strong radial velocity gradient.