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Retention and bandwidths prediction in fast gradient liquid chromatography. Part 2—Core–shell columns

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ABSTRACT

Recently, we confirmed that the well-established theory of gradient elution can be employed for prediction of retention in gradient elution from the isocratic data, method development and optimization in fast gradient chromatography employing short packed fully porous and monolithic columns and gradient times in between 1 and 2 min, or even less. In the present work, we extended this study to short core–shell reversed-phase columns. We investigated the effects of the specification of the stationary phase in the core–shell structure on the prediction of gradient retention data. Two simple retention models describing the effects of the mobile phase on the retention by two-parameter equations yield comparable accuracy and can be used for prediction of elution times. The log–log model provides improved prediction of gradient bandwidths, especially for less retained compounds. A more sophisticated three-parameter model did not offer significant improvement of prediction. We compared the efficiency, selectivity and peak capacity of fast gradient separations of alkylbenzenes, phenolic acids and flavones on seven core shell columns with different lengths and chemistry of bonded shell stationary phase. Within the limits dictated by a fixed short separation time, appropriate adjustment of the range of the composition of mobile phase during gradient elution is the most efficient means to optimize the gradient separation. The gradient range affects sample bandwidths equally or even more significantly than the column length. Both 5-cm and 3-cm core–shell columns may provide comparable peak capacity in a fixed short gradient time.

1. Introduction

Speed of separation is crucial issue in improving the productivity of an analytical laboratory. Fast generic gradient methods are required for high throughput in food safety control, in environmental analysis and especially in pharmaceutical laboratories throughout the whole drug analysis process, including drug discovery screening, raw material analysis, impurity profiling, pharmacokinetic studies and final product stability tests. In two-dimensional comprehensive LC × LC, fast second-dimension separations (preferably using gradient elution) are especially important because of the limited second-dimension separation time [1,2].

Fast gradient separations can be achieved by using short columns packed with small particles. Reduction in the particle size improves separation efficiency, but at a cost of increased pressure drop across the column, proportional to second power of decreasing particle size. Clearly, the speed of separation increases at a higher flow rate of the mobile phase, but is traded for either decreased resolution or increased operation pressure. Extremely fast efficient separations can be achieved on columns packed with sub-2 μm particles in so-called ultra high performance liquid chromatography (UHPLC) setup, at very high operation pressures over 100 MPa [3]. Alternative solutions enabling fast efficient separations using a conventional HPLC instrumentation are possible using new column formats. One approach relies on using monolithic columns, which consist of a single-piece continuous separation media (rod), allowing approximately three-times faster analyses than the particulate packed columns of the same length at the same operating pressure [4].

Columns packed with non-porous or superficially porous (core–shell) particles offer increased efficiency at a cost of weaker retention and lower sample load capacity [5]. Superficially porous fused-core (core–shell) 2.7 μm silica-based particles with sub-1 μm active porous layer [6] are prepared by depositing silica-sol particles onto a spherical non-porous solid core. Core–shell materials provide reduced band broadening and outstanding efficiency (height equivalent to a theoretical plate) lower than monolithic columns even at high mobile phase velocities, while sufficient outer particle size allows moderate permeability to accomplish very fast

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separations without significant loss in efficiency at relatively moderate operation pressure, in contrast to the columns packed with sub-2 μm fully porous particles [7–9].

A short diffusion path in a thin surface layer offers a very flat *C* term of the van Deemter plot due to fast mass transfer kinetics in the shallow pores. Recently, it was suggested that the actual advantage of core–shell column lies mainly in the diminution of the longitudinal diffusion, *B*, and eddy diffusion, *A*, terms rather than in a smaller *C* term [10,11]. Typical 2.6–2.7 μm core–shell particles contain a 1.9 μm nonporous core surrounded by a 0.35–0.5 μm porous shell. Narrow-bore columns packed with 1.7 μm [12,13] and 1.3 μm [14] core–shell particles provide peaks that have a variance of 2.1 μm. Recently introduced columns packed with 5 μm core–shell particles with 0.5–0.7 μm thick porous shell provide faster separations [15] and were claimed to provide reduced extra-column broadening effects in comparison to smaller particles [9].

Mass-transfer kinetics in chromatographic columns packed with core–shell particles was studied in detail by Guiochon and co-workers [7,16,17]. In fast gradient chromatography, columns packed with fused-core columns generally perform better than monolithic columns for separations of low-molecular compounds [18]. The reason is that retention factors of the last eluted compounds largely decrease during gradient elution as the analytes migrate along the column, and the superficial flow velocities imposed to the monolithic columns are twice or thrice larger than optimum [19].

Various 3 and 5 cm commercial core–shell columns can be used for fast gradients in the second dimension. Besides the type of the shell stationary phase, also the column length, hold-up volume, mobile phase flow-rate and the gradient retention range affect the quality of fast gradient separation. Recently, we studied the effects of the injected sample volume and solvent, of the gradient ramp, gradient range and mobile phase flow-rate on the fast second-dimension gradient separations of model alkylbenzenes and of phenolic and flavonoid antioxidants on several short commercial columns [20,21] and developed a new approach to the optimization of second-dimension gradient in two-dimensional LC × LC [22].

In the present work, we extended our previous study of fast gradients on short packed fully porous and monolithic columns to short core–shell columns. We investigated the effects of the solid core volume on the determination of retention factors and we compared two simple retention models describing the effects of the mobile phase on the accuracy of prediction of elution volumes and bandwidths in fast gradients. Finally, we studied the effects of the column length, chemistry of the stationary shell phase and of the gradient composition range on the bandwidths, efficiency, selectivity and peak capacity of seven commercial core–shell columns.

2. Theory

2.1. Retention factors and phase ratio

The column phase ratio, Φ, i.e., the ratio of the volume of the stationary phase, V_S, and of the mobile phase, V_m, in the column, relates the distribution constant, K_D, (the concentration ratio of the analyte in the stationary phase, c_S, and in the mobile phase, c_M) and the retention factor, k, (the ratio of the sample molar masses in the two phases, n_S and n_M), which can be evaluated from the experimental retention times, t_R, measured under isocratic conditions at a constant mobile phase composition and flow rate, F_m:

$$k = \frac{t_R F_m - V_m}{V_m} = \frac{n_S}{n_M} = \frac{c_S V_S}{c_M V_m} = K_D \frac{V_S}{V_m} = K_D \Phi \quad (1)$$

To relate the chromatographic retention data to the distribution constant, the phase ratio in the column, Φ, should be known. Usually a more or less thick liquid layer of different composition than the bulk mobile phase is immobilized by occlusion (adsorption) at the solid adsorbent surface and forms thus a part of the stationary phase, which may participate in the sample distribution process by partition mechanism. During gradient elution, the volume of the liquid immobilized by adsorption may change, depending on the actual composition of the mobile phase. Hence, it is difficult to fix the boundary (dividing surface) between the stationary and the mobile phase [23].

The practical solution to this problem is by adopting a convention, defining the volume of the stationary phase, V_S, as the part of the column, which is inaccessible to a non-retained marker compound ("nothing is adsorbed in terms of volume"). The volume of stationary phase, $V_S = V_C - V_m = V_C\,(1 - \varepsilon_T)$, is given by the difference of the volume of the empty column V_C, and the volume of the mobile phase in the column ($V_m = V_C \varepsilon_T$), where ε_T is the total column porosity.

Molecules differing in size may penetrate into different proportion of pores; hence every specific sample compound theoretically has its own (thermodynamic) dead volume [24]—and consequently its own column phase ratio. However, practical comparison of the retention of various solutes requires a single hold-up volume marker compound, which is neither retained nor excluded from the column (kinetic) dead volume [25]. Various methods were proposed for the determination of the column dead volume using various markers [26,27]: marked components of the mobile phase (^2H_2O, needing refractometric detection), or inorganic salts (KBr or KNO_3, which may be subject to ion exclusion from absorbent pores), or small neutral compounds (uracil or thiourea), which are most frequently used. It has been noted that a small error in the determination of the column phase ratio and hold-up volume may lead to wrong conclusions about the retention mechanism [28]. However, there is still the issue of the correct phase boundaries in the column. Anyway, the selection of the dead volume seems less critical issue, if the main task is the prediction of gradient data from the experimental isocratic data, as in this study. The most important in practice is using consequently the same dead volume marker both for the data acquisition and for the prediction of retention. The *vNA* convention does not imply any concrete physical model of retention mechanism, neither adsorption nor partition.

In core–shell columns, the solid core is impervious to both the sample and the mobile phase and does not participate in the chromatographic distribution process. If we do not include the core volume, V_{core}, into the corrected volume of the stationary phase, $V_{S,cor} = V_C - V_m - V_{core} = V_S f_{cor}$. and assume that the sample distribution is controlled by the same thermodynamics as with fully porous particles, we can re-write the definition equation for the retention factor as:

$$k_{cor} = K_D \frac{V_S f_{cor}}{V_m} = K_D \frac{V_C - V_m - V_{core}}{V_m} \quad (2)$$

The core correction factor, f_{cor}, accounts for the ratio of the thickness of the shell layer, d_{shell} and the mean particle radius, r_{partic}, $\rho = d_{shell}/r_{partic}$:

$$f_{cor} = \left[1 - \left(1 - \frac{d_{shell}}{r_{partic}}\right)^3\right] \quad (3)$$

The non-porous inner core represents between 25% and 36% of the core–shell particle volume; however the loading capacity was found comparable to that of fully porous sub-2 μm particles and better correlated to the pore volume or surface coverage than to the shell thickness [28]. In the present work, we studied the effects of the corrections for the core volume on the accuracy of prediction of the gradient retention data.