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Performance of columns packed with the new shell Kinetex-C_{18} particles in gradient elution chromatography

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ABSTRACT

The performance of columns packed with the new 2.6 μm Kinetex-C_{18} shell particles was investigated in gradient elution chromatography and compared with those of the 2.7 μm Halo-C_{18} shell particles and the 1.7 μm BEH-C_{18} totally porous particles. The peak capacities P_c of these columns were derived from the resolution of the components of a peptide mixture (β-Lactoglobulin digest) and of a mixture of two biomolecules (insulin and lyzozyme).The three columns exhibit the same peak capacities for the peptides at low linear velocity ($u_0 < 0.05$ cm/s) and at any gradient steepness ($0.8 < G < 10$). When the linear velocity is increased 10-fold, the peak capacity of the Kinetex column remains nearly unchanged while those of the Halo-C_{18} and the BEH-C_{18} columns decrease by 20%, approximately. This result confirms the very flat HETP curve, the very low C term of the Kinetex column and its ability to successfully operate at high flow rates while experiencing less efficiency loss than other columns. Despite its smaller average mesopore size (96 Å *versus* 130 Å), the column packed with 2.6 μm shell Kinetex-C_{18} particles gives an equivalent or even slightly better separation of biomolecules having a size and a mass around 40 Å and 15 kDa, respectively, than the column packed with 1.7 μm BEH-C_{18} totally porous particles. This result demonstrates the advantages of the shell *versus* the conventional particle technology when it comes to resolve mixtures of large and slow diffusive biomolecules.

1. Introduction

The rapid evolution of packed column technology over the last 10 years was marked by the successive appearance of the silica monolithic rods [1–3], the sub-2 μm particles [4,5], and the shell particles [6–9]. Currently, efficiencies of more than 100,000 plates per meter can consistently be achieved by monolithic columns [10] while efficiencies of at least 300,000 plates per meter are readily achieved with columns packed with sub-2 μm particles, with only a small efficiency loss due to heat friction and to the formation of radial temperature gradient when these columns are operated under nearly adiabatic conditions [11]. In both cases and for different reasons (the extremely high permeability of monolithic columns and the small hold-up volume of sub-2 μm particle packed columns), analysis times were reduced by nearly an order of magnitude compared to those achieved with columns packed with conventional 5 μm particles, which dominated the field one decade ago. Nevertheless, serious difficulties remain. The acceptance by the analyst community of columns packed of sub-2 μm particles is hampered by the heavy cost required to switch from conventional HPLC systems (which can operate at a maximum inlet pressure of 400 bar) to chromatographs of the new generation that are able to operate at inlet pressures up to 1200 bar. Conversely, the efficiency of the monolithic columns that are now available is low, due to their radial heterogeneity. Progress would require either improved column manufacturing or the development of a dedicated injection procedure placing the sample at the very center of the monolithic rod, where its bed is homogeneous [10]. However, such improvements come but slowly.

In order to overcome the limitations of the sub-2 μm particles and of monolithic silica rods, some manufacturers have focused on the development of very efficient columns that could supply separations exhibiting minimum plate heights around 3 μm (i.e., efficiencies in excess of 300,000 plates per meter) with a specific permeability comparable to that of columns packed with 3 μm particles ($k_0 \simeq 9 \times 10^{-11}$ cm^2). These columns would deliver analyses comparable to those achieved with the best columns packed with sub-2 μm particles but could be operated with the same instruments as those used for conventional columns. At the same time, these manufacturers propose adjustments of the injection/connector/detection systems of conventional HPLC instruments that would minimize the contributions to band

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broadening due to the extra-column volumes of the instruments. These contributions that have negligible influence on the effective efficiency of conventional columns cause an excessive decrease of the intrinsic performance of highly efficient columns [12]. This last aspect of column technology is suddenly becoming very important because the ability of using the most advanced columns on conventional instruments permits large savings.

To keep the column back-pressures moderate (i.e., below 400 bar) and still operate columns at velocities significantly larger than the optimum velocity for maximum column efficiency, the particle size should range between 2.5 and 3.0 μm [7]. In order to improve the column efficiency, researchers have focused on the development of either shell or superficially porous particles. In theory, decreasing the thickness of the porous layer of porous material should cause a decrease of the *C* term in the van Deemter plot, because the length along which molecules should diffuse decreases [13]. First, Advanced Material Technology, ended up with the 2.7 μm superficially porous or shell Halo-C_{18} particles [6,7,14–17]. This exceptionally performing material was made of 1.7 μm solid silica core covered by a 0.5 μm porous silica shell. It provided minimum reduced HETPs of ca. 1.4±0.17 for low molecular weight compounds [18], a significant improvement in packed column technology since the minimum reduced HETP of totally porous particle is usually of the order of 2.2. More recently, Phenomenex developed columns packed with a new type of shell particle (2.6 μm Kinetex particles) that are made of a 1.9 μm solid silica core and a 0.35 μm thick layer of porous silica. The lowest value of the reduced HETP measured on this column was only 1.15 ± 0.14 for the low molecular weight anthracene in pure acetonitrile [12], an unprecedented record in HPLC column technology. Most strikingly, the *C* term or overall coefficient of mass transfer resistance between mobile and stationary phases measured for a Kinetex-C_{18} column for compounds having low diffusion coefficients, like insulin and lyzozyme, was nearly eight times smaller than for the Halo-C_{18} column. No definitive reason has yet been suggested to explain such a large difference between the performance of columns packed with these two particles, except the difference in the roughness of the external surface and the porosity of the shell of these two particles.

Besides their structure, made of a shell around a solid core particle, the interesting feature of these superficially porous particles is their extremely narrow size distribution (PSD). Their $d_{90/10}$ size ratio is typically 1.13 ± 0.02 [12] while it is usually within the range between 1.5 and 2.0 for conventional totally porous particles. This characteristic feature of shell particles was unexpected; it is not understood why building porous shells would eventually lead to extremely narrow PSDs, unless the production process of solid core particles leads readily to a narrow PSD. It is suspected that this narrow PSD is the key for their success at separating small molecules [6,7,14], although the rational behind this assertion is unclear. It is inconsistent with previous experimental results [19]. Carta and Bauer [20] calculated elution profiles for columns having beds made of particles of the same average size but different size distributions. They showed that, if the distribution was symmetrical, its variance had little influence on the profile. Strongly skewed distributions only may affect elution profiles. Recent measurements have shown that columns packed with shell particles are not more radially homogeneous than those packed with traditional totally porous particles [21]. This suggests that the higher performance of columns packed with shell particles does not result from a decrease of their transcolumn structure heterogeneity. The exceptionally low reduced HETPs measured with shell particles seems to be better explained by a diminution of their short-range interchannel velocity biases, biases that take place over average distances of one particle diameter [12]. Additional measurements of the transcolumn velocity biases in the Kinetex column are needed in order to assess its contribution to the eddy dispersion term in the Kinetex column.

The goal of this work is a further characterization of the kinetic performance of columns packed with Kinetex-C_{18} shell particles, in the gradient elution mode. For a sake of a comparison with some of the best performing conventional columns available, we measured and compared the peak capacities for mixtures of peptides and proteins of three high performance columns: (1) a 100 mm × 4.6 mm column packed with 2.6 μm Kinetex-C_{18} shell particles; (2) a 150 mm × 4.6 mm column packed with 2.7 μm Halo-C_{18} particles; and (3) a 100 mm × 3.0 mm column packed with 1.7 μm BEH-C_{18}. The peak capacities were measured with the same samples on each column, at constant chromatographic linear velocity and intrinsic gradient steepness, in order to generate comparable retention windows for the least and the most retained compounds. A model accounting for the compression factor in gradient elution is used to predict the experimental peak capacity and serve as a reference for the comparison between the performance of the three columns [22,23].

2. Theory

2.1. Theoretical peak capacity

The definition and the general expression of the peak capacity P_c in chromatography, assuming a resolution of unity between the successively eluted peaks is written [24]:

$$P_c = 1 + \int_{t_I}^{t_F} \frac{1}{4\sigma} dt \tag{1}$$

where t_I is the retention time of the first eluted peak (usually that of a non-retained compound), t_F is the retention time of the last eluted peak, dt is a dummy time variable, and σ is the time standard deviation of a peak.

In this work, we assume that the plate height H of the column remains independent of the mobile phase composition. Accordingly, the time band variance σ of an eluted peak is [25]:

$$\sigma^2 = G_{12}^2 HL \left(\frac{1 + k'_E}{u_0} \right)^2 \tag{2}$$

where u_0 is the chromatographic linear chromatographic velocity (related to the hold-up time t_0), k'_E is the retention factor of the sample at the column outlet, L is the column length, and G_{12}^2 is the band compression factor. With the linear solvent strength (LSS) retention model and for linear, non-retained, and non-distorted gradients, the band compression factor was derived by Poppe et al. [26]:

$$G_{12}^2 = \frac{1 + p + (1/3)p^2}{(1+p)^2} \tag{3}$$

where p is defined as [26]:

$$p = S \frac{\Delta\varphi}{t_g} t_0 \frac{k'_0}{1 + k'_0} = G \frac{k'_0}{1 + k'_0} \tag{4}$$

where $\Delta\varphi$ is the change in solvent composition during the gradient, t_g is the gradient run time (with $\beta = \Delta\varphi / t_g$ the gradient slope), t_0 is the column hold-up time, S is the slope of the relationship between the natural logarithm of the retention factor measured under isocratic conditions and the organic solvent concentration in the case of the LSS model, k'_0 is the retention factor of the compound at the beginning of the gradient, and $G = S\beta t_0$ is the intrinsic gradient steepness [27]. The LSS model is written [27]:

$$\ln k' = \ln k'_0 - S(\varphi - \varphi_0) \tag{5}$$

where φ_0 is the volumetric fraction of the strong eluent at the beginning of the gradient.