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Rapid development of core–shell column technology: Accurate measurements of the intrinsic column efficiency of narrow-bore columns packed with 4.6 down to 1.3 μm superficially porous particles

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ABSTRACT

The intrinsic heights equivalent to a theoretical plate (HETPs) of 31 narrow-bore and wide-bore columns packed with four different brands of core–shell particles were accurately measured on an optimized vHPLC instrument (1290 Infinity system) that has an extra-column volume variance of $13.6 \pm 0.3\ \mu L^2$. These results were derived from the slopes of the linear plots of the apparent plate heights of each column *versus* the reciprocal of $(1+k')^2$ for seven homologous compounds with a linear regression coefficient larger than 0.999. The results show that the kinetic performance of narrow-bore columns packed with core–shell particles increases almost linearly with decreasing particle diameter. The optimum reduced plate heights increase slightly from 1.6 to 1.9 with decreasing particle sizes from 4.6 to 1.3 μm. This confirms that wide-bore columns provide better efficiencies than narrow-bore columns.

1. Introduction

Columns packed with sub-3 μm shell particles, also called pellicular or superficially porous particles, are now solidly established in HPLC column technology since 2007. Their introduction provided large improvements in the separation of small molecules [1–6]. No fewer than ten manufacturers have developed their own brands of core–shell particles, which are available in sizes between 5 and 1.3 μm. Several advanced theoretical [7–13] and experimental [3,4,6,14–21] investigations have allowed the elucidation of the reasons that explain their advantages in the separation of molecules of widely different sizes. To summarize, the A, B, and C terms of the van Deemter HETP equation decrease when fully porous particles are replaced with superficially porous particles. The decrease of the A term at high flow rates is the main explanation for this success. For small molecules, the efficiencies of narrow-bore columns packed with 2.6 or 2.7 μm particles is similar to that of those packed with sub-2 μm fully porous particles but they require a much lower back pressure [22,23]. They can be used with conventional instruments provided that the extra-column band broadening contribution is minimized by replacing standard tubings with similar ones of smaller volumes and/or by focusing the injected sample band at the column inlet [24–27]. For large molecules, the van Deemter curves of these columns are flatter at high flow rates than those measured for fully porous particles [3,9,28,29], provided that the structure of the mesoporous volumes is similar in both types of columns.

Most columns packed with core–shell particles are now available in a narrow-bore diameter (2.1 mm) that allows savings on solvents and samples. However, the contribution to band broadening of even advanced vHPLC instruments is large compared to that of these columns. Measuring meaningful column efficiencies is challenging because the contributions of instruments to band broadening differ from an instrument to the next. This is a source of endless discussions and arguments between column manufacturers, claiming exceptionally high column performance, and customers, complaining about the relatively poor column performance that they measure. Furthermore, determining the true intrinsic efficiencies of narrow-bore columns is made difficult by the current measurement process in which the volume variances of the system estimated by using a zero dead volume (ZDV) union connector are subtracted from the total recorded volume variance. It was recently shown [30,31] that the mere replacement of the chromatographic column with a ZDV union connector introduces a systematic error, of up to 40%. A non-invasive method should

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be better. To solve these problems, a measurement process based on plotting the apparent HETP (not corrected HETP for the system variance) *versus* the reciprocal of $(1+k')^2$ for a series of homologous compounds was recently proposed [31]. If all the data points form a straight line, the extrapolation to an infinite retention factor provides the true intrinsic HETP of the column for this class of compounds.

The other result of this method is that it was proved to work well with standard, non-optimized HPLC and vHPLC system with system volume variances as large as 40 μL^2. Accurate estimates of the true efficiency of a new column can be made without minimizing the system volume [25,26] or without developing new injection techniques [27] to correct for most of the pre-column band broadening contributions. However, reducing as much as possible the contribution of the instrument used to band spreading is necessary. Analyst who seek to achieve the best performance from the modern columns available must understand this new requirement. Another advantage of this method is that it provides the true system volume variance provided that the column hold-up volume be accurately known [31,32]. This method was validated by showing that it provided the same intrinsic efficiency for a given narrow-bore column, independently of the instrument with which it was used to acquire the apparent column efficiency and the retention factors of the probe compounds [31].

The goal of this work was to determine the intrinsic kinetic performance of a large collection of columns packed with early (2.7 μm Halo and 2.6 μm Kinetex of 2007–2009) and recent core–shell particles (1.6 μm CORTECS or 1.3 μm Kinetex in 2013). Thirty one columns were tested including, 23 narrow-bore (2.1 mm i.d.) columns. Four different brands of particles, Advanced material technologies, Phenomenex, Agilent Technologies, and Waters Technologies were studied. The alternative, non-invasive method described above was applied. It permits accurate measurements of the extra-column volume variances of HPLC instruments in the presence of a chromatographic column and that of the intrinsic efficiencies of modern narrow-bore columns. The justifications and experimental conditions for the application of this protocol are discussed in details. The impact of the column diameter and of the particle size on the true column performance is finally discussed.

2. Theory

2.1. Band variance under isocratic and quasi-isocratic conditions

The total volume variance of a chromatographic peak includes the contributions of the instrument (its injector, valve, connecting tubes, detector, electronics) and of the column to the recorded band width. Under isocratic conditions, it is given by [26,33,34]:

$$\sigma^2_{\mathrm{v,Exp.}} = \sigma^2_{\mathrm{v,ex}} + \frac{V_0^2}{N_{\mathrm{intrinsic}}}(1+k')^2 \qquad (1)$$

where $\sigma^2_{\mathrm{v,Exp.}}$ is the total volume variance measured, $\sigma^2_{\mathrm{v,ex}}$ is the extra-column volume variance, V_0 is the column void volume, $N_{\mathrm{intrinsic}}$ is the intrinsic column efficiency, and k' is the retention factor given by:

$$k' = \frac{V_{\mathrm{R}} - V_{\mathrm{ex}} - V_0}{V_0} \qquad (2)$$

where V_{R} is the elution volume and V_{ex} is the extra-column volume. Note that Eq. (1) assumes that the sample is dissolved in the same eluent as the one used as the mobile phase.

2.2. Relationship between the apparent and the intrinsic HETP

By definition, the HETP. H, (*e.g.*, the overall apparent performance of the column and instrument as measured by the analyst) is written:

$$H = L\frac{\sigma^2_{\mathrm{v,Exp.}}}{V_{\mathrm{R}}^2} \qquad (3)$$

Assuming that $V_{\mathrm{ex}} \ll V_0$, substitution of Eq. (1) into Eq. (3) provides the relationship between H, $H_{\mathrm{intrinsic}} = L/N_{\mathrm{intrinsic}}$, the column dimensions (V_0 and L), the system variance in volume unit ($\sigma^2_{\mathrm{v,ex}}$), and the retention factor k':

$$H(k') = H_{\mathrm{intrinsic}} + L\frac{\sigma^2_{\mathrm{v,ex}}}{V_0^2}\frac{1}{(1+k')^2} \qquad (4)$$

This result shows that, if under certain conditions of flow rate, temperature, and mobile phase composition, the intrinsic HETP depends weakly on k' for a series of closely related compounds (same class of compounds such as a series of homologous analytes), the plot of the apparent HETP *versus* the reciprocal of $(1+k')^2$ is expected to be linear. Its y-axis intercept should provide $H_{\mathrm{intrinsic}}$ and its slope an estimate of the true extra-column volume variance $\sigma^2_{\mathrm{v,ex}}$. The main advantage of this approach for the determination of $H_{\mathrm{intrinsic}}$ and $\sigma^2_{\mathrm{v,ex}}$ is that the column does not need to be replaced with a zero dead volume (ZDV) connector. This method is particularly recommended when the system volume variance is much larger than the column volume variance. This concerns standard HPLC and vHPLC instruments used with narrow-bore columns. The main disadvantage is that the conditions mentioned above are not systematically met under certain RPLC conditions.

3. Experimental

3.1. Chemicals

The mobile phase used was a binary mixture of acetonitrile and water (65/35, v/v). Small volumes of tetrahydrofuran (THF) were used for the ISEC measurements of the 2.1 mm × 50 mm column packed with 1.3 μm Kinetex column. For the other columns used, we used results obtained and published earlier. All the solvents used were HPLC grade from Fisher Scientific (Fair Lawn, NJ, USA). Acetonitrile was filtered before use on a surfactant-free cellulose acetate filter membrane, 0.2 μm pore size (Suwannee, GA, USA). The eleven polystyrene standards (MW = 590, 1100, 3680, 6400, 13,200, 31,600, 90,000, 171,000, 560,900, 900,000, and 1,870,000) were purchased from Phenomenex (Torrance, CA, USA). The standard RPLC checkout sample (1 mL ampoule) was purchased from Agilent technologies. It contains 100.3 μg/mL (± 0.5%) of acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, benzophenone, and acetanilide. The solvent of this sample is a mixture of acetonitrile and water (35/65, v/v, same composition as that of the mobile phase). Acetophenone, valerophenone, and octanophenone (all >99% purity) were separately purchased from Sigma–Aldrich (Suwannee, GA, USA).

The choice of these probe compounds was justified in an earlier publication [31].

3.2. Instruments

A very high pressure liquid chromatograph (vHPLC) was used in this work. This standard 1290 Infinity system (Agilent, Waldbroen, Germany) was slightly optimized. It includes a 1290 Infinity Binary Pump with solvent selection valves and a programmable autosampler. The injection volume (0.2 or 0.5 μL) is drawn into one end