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High performance liquid chromatography column packings with deliberately broadened particle size distribution: Relation between column performance and packing structure

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ABSTRACT

The effect of the addition of 25%, 50% and 75% (weight percent, wt%) of larger particles (resp. 3 and 5 μm) to a commercial batch of 1.9 μm particles has been investigated as an academic exercise to study the effects of particle size distribution on the kinetic performance of packed bed columns in a magnified way. Comparing the performance of the different mixtures in a kinetic plot, it could be irrefutably shown that the addition of larger particles to a commercial batch of small particles cannot be expected to lead to an improved kinetic performance. Whereas the addition of 25 wt% of larger particles still only has a minor negative effect, a significantly deteriorated performance is obtained when 50 or 75 wt% of larger particles are added. In this case, separation impedance number increases up to 200% were observed. Studying the packing structure through computational packing simulations, together with the experimental determination of the external porosity, helped in understanding the obtained results. This showed that small particles tend to settle in the flow-through pores surrounding the larger particles, leading to very high packing densities (external porosities as low as 32% were observed) and also negatively influencing the column permeability as well as the band broadening (because of the broadened flow-through pore size range).

1. Introduction

Porous silica particles with diameters ranging between 1.5 and 5 μm currently are the most widely used packed bed materials in HPLC. The manufacturing processes [1–4] that are used to produce these particles usually yield particles with a very broad size range. Most commercial column production processes therefore involve a step wherein the particle size distribution (PSD) of the produced particle batches is narrowed through a series of sizing steps using for example sedimentation, field flow fractionation or air classifiers [5–8]. The motivation to do so is that it is the general experience that particle batches with a narrower PSD lead to higher quality column packings and consequently to better chromatographic performances [4,9,10]. Particle classification is also necessary to remove the smallest particles, the so-called fines, as these negatively affect the kinetic performance [11–13]. On the other hand, literature also reports on the difficulty of packing very narrow, monodisperse particles [14].

There are only a few studies in literature [11,12,15–17] that try to put a number on how narrow exactly the PSD of a column packing has to be to produce a high quality column packing. Most of this research is done in-house, in the labs of the column manufacturers, who need to make a balance between column performance and process economics. Indeed, each additional sieving step is time-consuming, and consequently comes at an extra cost in the column production process, not to mention the losses of valuable silica material [9].

Commercial columns packed with fully porous particles nowadays typically have PSDs with an RSD of 25% or smaller and have a ratio between their d_{90}-value (average diameter value at the 90%-point of the cumulative distribution function) and their d_{10}-value (average value at 10% of the cumulative distribution) not exceeding 1.5–2.0 [7,18]. The recently introduced porous-shell particle columns however have markedly narrower PSDs with an RSD of 4–5% and a d_{90}/d_{10}-value of 1.1 [10,19–24].

The debate on how narrow the PSD should precisely be to generate the most stable packing and the highest column performance has become timely again. On the one hand, the recently observed superior performance of porous-shell particle columns has been linked to the much narrower PSD with which porous-shell particles can be produced compared to fully porous particles [10,23,25].

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On the other hand, an unambiguous link between PSD and column performance has not been established yet [12,26–29]. Furthermore, claims have been made that it would be advantageous to increase the PSD again slightly beyond that of the current commercially employed PSD, i.e., by adding up to 30% of 3.5 μm particles to a batch of 1.8 μm particles [30]. Doing so, the band broadening would only be minimally affected whereas the permeability would be significantly enlarged, thus leading to an overall improvement of the kinetic performance.

The inspiration for the latter most probably originates from a frequently cited study on the effect of the PSD of column packings: the famous 1983 study of Dewaele and Verzele [12]. They investigated the column efficiency of a series of columns packed with mixtures of different compositions. They mixed 3 μm and 8 μm particles in two different weighted proportions (3:7 and 6:4, m:m) and compared the chromatographic behaviour in RPLC mode with that of columns consisting purely of 3 μm or 8 μm particles. Using an effective particle diameter based on the observed column permeability to calculate the reduced plate heights and velocities for their different particle mixtures, the main conclusion drawn by Verzele and Dewaele was that a larger PSD did not influence the column efficiency when operated around its optimal velocity. A negative effect, though small, was observed at higher velocities. There is however no theoretical foundation for the equivalent-sphere-diameter approach, which is based on the assumption that the flow resistance is independent of the PSD.

Given the above, it was considered worthwhile to make an update to the Dewaele and Verzele experiments, using a set of modern HPLC particles, and analyzing the obtained results using the kinetic plot method [31–43], which allows to assess the packing quality without the need to define or measure an equivalent particle size.

The first part of the present contribution therefore reports on a series of column performance measurements conducted on different reversed-phase HPLC columns packed with different particle batches obtained by mixing commercial 1.9 μm, 3 μm and 5 μm particle batches (further on referred to as the "pure" batches) in different mass proportions. The "pure" particle batches hence have a PSD that is typical of that encountered in commercial columns, while the mixtures have a PSD that is considerably enlarged with respect to the batches used in the current commercial columns.

In the second part of the paper, it is attempted to relate the packing configuration resulting from the packing of the different particle mixtures to the experimentally measured dispersion and permeability values. Although recent breakthroughs in X-ray tomography [44] are promising, it is currently impossible to look directly inside the columns. An indirect approach has therefore been followed, investigating the experimentally studied column packings via computer-generated packings generated using a commercially available packing program and imposing similar types of particle mixtures as those studied experimentally.

2. Experimental

2.1. Production of particle mixtures and columns

Hypersil Gold particles (Thermo Fisher Scientific) of 1.9, 3 and 5 μm diameter (primary or "pure" batches) were used as base material. They had a standard deviation of respectively 0.25, 0.35 and 0.5 μm. All particles had an average pore size of 175 Å and were coated with a C18 layer. Six different particle mixtures were created by blending the 1.9 μm particles with 3 or 5 μm particles in mass proportions of 25%, 50% and 75 wt%.

The pure batches and the particle mixtures were packed in columns (2.1 × 50 mm) using the same in-house developed standard procedure for every column and mixture. The employed packing method was fully similar to that used for the preparation of commercial Hypersil Gold columns. In this method, a known mass of powder was evenly dispersed with ultrasonication in a proprietary solvent mixture of specific volume. The packing pressure profile was the same for each column. To investigate the repeatability of the experiments, three columns were tested for each of the primary batches and particle mixtures.

2.2. Chemicals and columns

Uracil and methyl-, ethyl-, propyl- and butylparaben were provided from Sigma–Aldrich (Steinheim, Germany). Acetonitrile was of HPLC grade from Sigma–Aldrich (Steinheim, Germany). HPLC grade water was prepared in house using a Milli-Q Purification System (Millipore, Billerica, MA, USA). Columns used in this study were provided by Thermo Ficher Scientific (Runcorn, UK).

2.3. Apparatus

Chromatographic data were acquired with an HPLC Agilent 1200 system (Agilent Technologies, Waldbronn, Germany) which can withstand pressures up to 600 bar. This instrument includes an auto-sampler with a 2 μL loop, an UV–Vis detector with a 2 μL flow cell, and a column oven set at 30 °C. Data acquisition, data handling, and instrument control were performed by Chemstation (Agilent Technologies). Samples consisting of 50 μg/ml uracil and parabens were dissolved in a water/acetonitrile (70/30%, v/v) mixture and the injection volume was reduced to 0.5 μl. Absorbances were measured at 254 nm, using a time constant of 25 ms and a sampling rate of 40 Hz.

2.4. Particle size measurements

To determine the particle size distribution of the studied 'pure' batches, the columns were opened after having performed the experiments and the particles were removed by flushing the columns with methanol. The particles were subsequently dried by evaporating the methanol and prepared for SEM (scanning electron microscopy) measurements. The SEM pictures were recorded with a JSM6400 (JEOL) instrument which had a Tungsten filament and was operated at an accelerating voltage of 25 keV and a 3500 times magnification. The particles were conductive enough to omit the use of a carbon coating. At least 10 pictures of each sample were taken and more than 1000 particle diameters were measured for every column. To determine the particle sizes, the SEM pictures were uploaded into a drawing program (Windows Paint) and straight lines corresponding to the diameter of the particles were manually drawn over the particles. This manual procedure was preferred because it allowed determining the position of the particle circumference with the highest possible degree of precision and certainty. Subsequently, the length of the straight lines was determined in an automated way using an in-house written script in Imaq Vision Builder (National Instruments Corporation, Austin, TX, USA).

2.5. Chromatographic experiments

All experiments were conducted in the isocratic mode with a water/acetonitrile (70/30%, v/v) mobile phase, to obtain appropriate retention factors. For further column evaluation and analysis of the obtained results, butylparaben was selected, because of its symmetric peak shape and its high retention factor ($k' = 14$), which