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Critical comparison of performances of superficially porous particles and sub-2 μm particles under optimized ultra-high pressure conditions

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ABSTRACT

The performance of 2.7 μm superficially porous particles at 600 bar and sub-2 μm fully porous particles at 1000 bar were compared by the Poppe plot method. Theoretical Poppe plots were first constructed for each stationary phase to compare their kinetic performance at different analysis times. The theory was then verified by experiments under the optimized conditions identified from the Poppe plot calculation. We found that the 2.7 μm superficially porous particles at 600 bar can provide similar performance compared to the sub-2 μm fully porous particles at ultra-high pressure (1000 bar) when analysis times are very short (e.g. sub-minute). As analysis time increases, the superficially porous particles start to outperform the sub-2 μm particles and can give much higher efficiencies (e.g. > 2 times higher plate count) at very long analysis times (>3 h). The comparison was extended to gradient elution of a mixture of pharmaceutical interest by constructing gradient peak capacity Poppe plots and similar behavior was observed.

1. Introduction

Higher separation efficiency and faster speed have always been of great interest in HPLC and have become increasingly important in recent years mainly driven by the challenges of either more complex samples or growing numbers of samples [1]. Many approaches have been developed as potential solutions including high temperature [2,3], sub-2 μm particles at ultra-high pressure [4,5], monolithic columns [6] and the superficially porous stationary phases [7]. Among these techniques, superficially porous stationary phases have recently drawn a lot of attention. In fact, 5 μm superficially porous particles were developed more than a decade ago [8] and have been shown to provide excellent performance in various applications especially for peptides [9]. Interest in this type of particle design was greatly enhanced by the recent introduction of 2.7 μm particles with a 0.5 μm outer porous shell [7]. Due to the reduced diffusion length for analytes, superficially porous particles are expected to have superior mass transfer properties compared to the fully porous particles and therefore provide similar separation efficiencies compared to sub-2 μm particles but at much lower pressures. However, this is contingent upon the extent to which mass transfer resistance comes from inside the particle vs. the degree of external or film mass transfer resistance [10,11].

Attempts have been made to compare 2.7 μm superficially porous particles and the sub-2 μm fully porous particles [7,12]. Most studies focused on the mass transfer properties, backpressure of the columns and the separation efficiency under certain experimental conditions (mostly in isocratic elution). For instance, Cunliffe and Maloney [12] compared the 2.7 μm superficially porous particles to several sub-2 μm fully porous particles under isocratic conditions and found that superficially porous particles give slightly lower plate counts (~20%) but at much lower pressures (~50%). This feature allowed them to couple columns in series to obtain plate counts greater than 90,000. Guiochon and co-workers also showed that high peak capacities can be achieved with 2.7 μm superficially porous particles for biological separations [13].

Traditionally, the kinetic characteristics of different stationary phases and columns have been compared in terms of their van Deemter flow curves [14]. However, permeability considerations are missing from such plots and they do not tell one which particle design and what column format to choose for a particular separation [15]. To address this issue, Poppe proposed the "Poppe plot" wherein the plate time (t_0/N) is plotted against the plate count (N). This is an elegant tool for visualizing the compromise between separation efficiency and speed [16]. This concept was extended by Desmet and a family of "kinetic plots" was developed to meet the need of different applications [15]. Wang et al. have also extended

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the isocratic Poppe plot to gradient elution [17]. The biggest advantage of the "Poppe plot" or "kinetic plot" technique is that these plots allow one to find the optimal conditions (e.g. optimal column length and flow rate) under given separation conditions (e.g. fixed analysis time and maximum pressure). Therefore, different particle designs and columns can be compared under optimized conditions as opposed to some arbitrary conditions. This approach has provided many invaluable insights for the future development of chromatographic columns [16,18].

Poppe or kinetic plots are usually constructed by first measuring the flow curve of a stationary phase on a given column length. By assuming that the kinetic characteristics are independent of length, one can calculate the best achievable plate count for any column length. The accuracy of these methods was recently verified by measuring plate counts on a series of coupled columns under predicted conditions in the studies of Sandra and co-workers for Poppe plot [19] and of Desmet and co-workers for kinetic plot [20]. Most recently Cabooter et al. applied the kinetic plot method to design coupled column systems that can generate 100,000 plates in the shortest possible time on 1.7 μm fully porous particles at 1000 bar and 2.7 μm superficially porous particles at 600 bar. They found that both systems were able to produce theoretical plates close to 100,000 within approximately the same time [21]. They also achieved faster separations by elevating the column temperature from 30 to 80 °C.

The goal of the present study is to use the Poppe plot as a tool to make a critical comparison of the 2.7 μm superficially porous particles at pressures less than 600 bar (i.e. maximum pressure of the column hardware) and sub-2 μm fully porous particles at pressures less than 1000 bar (i.e. maximum pressure of the instrument). 2.7 μm Halo C18 and 1.7 μm BEH C18 were chosen in this study. Both theoretical calculations and experimental measurement were conducted under isocratic elution conditions. The comparison was then extended to gradient separation of a pharmaceutical mixture.

2. Theory

2.1. Isocratic Poppe plots

With isocratic Poppe plots, the goal is to calculate the best plate count that can be achieved at a specified maximum pressure. At a given column dead time (t_0), one computes the column length (L) and flow rate (F) so that the plate count (N) is maximized by simultaneously satisfying two constraints. First, the combination of column length and flow rate should give the desired column dead time:

$$L = ut_o = (\varepsilon_e/\varepsilon_{tot})u_e t_o \quad (1)$$

where u is the chromatographic linear velocity of an unretained solute, u_e is the interstitial linear velocity, ε_e and ε_{tot} are the interstitial porosity and total porosity respectively. Secondly, the column is operated at the desired pressure drop:

$$\Delta P = \Phi\eta\frac{u_e L}{d_p^2} \quad (2)$$

where ΔP is the pressure drop, Φ is the column flow resistance, η is the mobile phase viscosity, and d_p is the particle size. At any given t_0 and ΔP, flow rate and column length can be solved from Eqs. (1) and (2), and thus the optimal plate count (N) and plate count production (N/t_0) can be calculated. The value of t_0 is incremented from fast separation to long analysis to complete the Poppe plot. The calculations were done in Microsoft Excel.

An important aspect of isocratic Poppe plots is the asymptote of each curve (see Fig. 1). The vertical asymptote on the right represents the limiting plate count (N_{lim}) at very long analysis time. The horizontal asymptote at the bottom represents the limiting speed $(t_0/N)_{lim}$ at very short analysis time. The values of the two asymptotes can be calculated with the following two equations [22]:

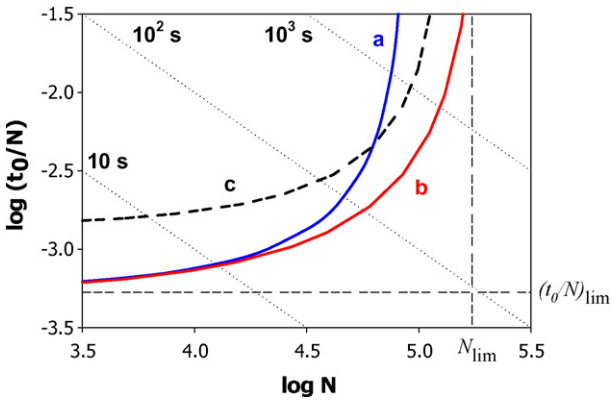

Fig. 1. Theoretical isocratic Poppe plots for packed bed columns. Each dotted line represents a constant column dead time. Case a: 1.7 μm BEH C_{18} at ΔP_{max} = 950 bar. Case b: 2.7 μm Halo C_{18} at ΔP_{max} = 570 bar. Case c (hypothetical): 2.7 μm BEH C_{18} at ΔP_{max} = 570 bar. Reduced van Deemter coefficients are listed in Table 1. Porosity and flow resistance are listed in Table 2. Other conditions: 21 °C; η = 0.64 cPoise; $D_m = 1.14 \times 10^{-5}$ cm²/s.

$$N_{lim} = \frac{\psi^2\lambda d_p^2}{BD_m} \quad (3)$$

$$\left(\frac{t_o}{N}\right)_{lim} = \frac{Cd_p^2}{\lambda D_m} \quad (4)$$

where ψ and λ are column property related constants, D_m is the diffusion coefficient of the analyte in the mobile phase, and B and C are the van Deemter flow curve coefficients. It is clear from Eq. (3) that a stationary phase of larger particle size and smaller B term (i.e. less longitudinal diffusion) can provide a higher maximum plate count. On the other hand, Eq. (4) suggests that a phase of smaller particle size and smaller C term (i.e. faster mass transfer) can provide faster separation.

To calculate the isocratic Poppe plot, several experimental variables were first determined. The mobile phase viscosity was estimated by Chen–Horvath equation [23]. The solute diffusion coefficient was calculated using the Li–Carr correlation [24]. The interstitial porosity (ε_e) of the Halo C18 was taken to be 0.423 from a study of Gritti and Guiochon [10]. The interstitial porosity (ε_e) of the BEH C18 was taken to be 0.353 from a study of Desmet and co-workers [25]. The total porosities (ε_{tot}) of both particles were measured by injecting uracil in 50/50 mixture of acetonitrile (ACN) and water. This in turn allowed the estimation of intra-particle porosity (ε_{in}) of both particles by:

$$\varepsilon_{in} = \frac{\varepsilon_{tot} - \varepsilon_e}{1 - \varepsilon_e} \quad (5)$$

Another important parameter needed for isocratic Poppe plots is the flow resistance of the column. Column backpressures were measured on both Halo C18 and BEH C18 at different flow rates during the flow studies. The value of flow resistance was calculated from the slope of the plot of column backpressure against interstitial linear velocity according to Eq. (2).

2.2. Gradient peak capacity Poppe plots

Since many practical separations are conducted by gradient elution, it is important to ensure that the conclusions from the isocratic Poppe plot comparison are applicable to gradient elution. Under gradient conditions, peak capacity is the most relevant measure of separation efficiency and it is of great interest to maximize the peak capacity and separation speed (i.e. peak capacity per unit time)