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Reequilibration time of superficially porous silica based columns in gradient elution reversed phase liquid chromatography

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ABSTRACT

Between repetitive analyses using gradient elution liquid chromatography the column must be reequilibrated to the initial conditions, extending run times. We studied the reequilibration time of three superficially porous silica columns compared to one fully porous silica column on a chromatograph with a reduced flush-out volume. Post-gradient acetone injections made at the interface of the pure organic-highly aqueous phase show anomalous, pressure-related band focusing, and increased retention compared to injections on either side of the interface. These anomalies are explained by applying the Buckley–Leverett theory of oil displacement in sands to column reequilibration. Reequilibration was shown to occur quickly, with less than three column volumes of conditioning solvent, and depends on the reproducibility as required by the application. Offline LC–GC was used to quantitate the percent acetonitrile eluting from each column post-gradient. After an initial, large expulsion of acetonitrile, a steady small amount (~0.03%) of acetonitrile is detected long after the column is considered equilibrated. The limiting variable with column equilibration is not the desorption of organic modifier from the stationary phase, but rather the pressure required to force the aqueous phase into the pores.

1. Introduction

In liquid chromatography, gradient elution has two main advantages over isocratic elution: sharper peak shape and reduced run time. With sharper peak shape, peak overlap is reduced and the limit of detection and the limit of quantitation are lowered (improved). Shorter run times allow for more analyses in a set time frame, which is of particular interest within comprehensive two-dimensional chromatography where the speed of the second dimension is generally the limiting factor for the total analysis time [1].

The disadvantage of gradient elution is the required time post-gradient to flush initial mobile phase composition through the column to ensure reproducible retention times of analytes in the subsequent injection [2]. The lack of reproducibility of retention times post-gradient is due to the extent of solvation of the alkyl chains of the stationary phase by the organic modifier component in the mobile phase. In order for retention to be equivalent run-to-run, the alkyl chains must be returned to the initial solvation pre-gradient. Ideally, the initial mobile phase would remove the residual gradient solvent and after some reequilibration time the eluent exiting the column would be of the same composition as the mobile phase entering the column. In 1982, Gilpin et al. [3,4] used offline LC–GC to quantitate the release of alcohol solvents from a C-10 phase when flushed with pure water. It was shown that a 1–10 ppm concentration of organic modifier was still detectable after flushing the column with ~600 mL of pure water, approximately ~850 column volumes, with a column dimension of 25 cm × 2.4 mm i.d. and assuming a porosity of 0.6. Ideal conditions for gradient elution cannot be attained with real world analysis times, though a minimal amount of organic modifier can remain on column and still achieve reasonable run-to-run reproducibility. This is analogous to the proposed definition of Schellinger et al. [5] that full equilibration occurs when a column provides reproducible retention times for all solutes independent of the reequilibration time. However, most chromatographic analyses do not require this state of full equilibration; rather there only needs to be an acceptable run-to-run reproducibility. Run-to-run reproducibility is of particular importance to comprehensive two-dimensional chromatography when aligning the sampling phase [6] of sequential chromatograms to visualize a contour plot of the separation space.

To determine a necessary reequilibration time, two methods have been devised. Cole and Dorsey [7] injected a weakly retained analyte (acetone, $0 < k' < 1$) each minute, post-gradient, once the mobile phase composition was adjusted at the proportioning valve. Because acetone is weakly retained, its retention time should vary measurably with stationary phase solvation. Once the retention of acetone reached a constant value the column was considered equilibrated. "Constant value" or "equilibrated value" was defined

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later by Coym and Roe [8] as within 0.01 min of the average value of retention times for injections where the column is assumed to be equilibrated. Cole and Dorsey found that the inclusion of a set amount of *n*-propanol (3%) significantly reduced the reequilibration time by robustly solvating the stationary phase.

The second method of determining necessary reequilibration time was described by Schellinger et al. [5] wherein a series of gradient runs were performed sequentially with varying reequilibration times interspersed. The retention times of the analyte mixture were compared to a control run with a 15 min reequilibration time. With the use of a second switching valve before the injection valve, the dwell volume of the pump could be bypassed, ensuring that the variation of the retention times was a function of stationary phase solvation and not flushing out the volume of the pump. It was shown that a very small amount of mobile phase (1–2 column volumes) was necessary to produce acceptable reproducibility of retention time (<±0.002 min) for neutral analytes on the fully porous columns studied [5]. Notably, acetone was the mixture component that consistently had the greatest variability with reequilibration time. For basic analytes, it was later found that 5 column volumes of initial buffered eluent were enough for full equilibration [9]. It was suggested that a new rule-of-thumb for reequilibration volume is the sum of dwell volume of the system and 1–2 column volumes, or 5 column volumes if using buffered mobile phase.

Recently, columns packed with superficially porous silica with a particle diameter of <3 μm have become commercially available and provide significant performance within the pressure limitations of conventional instrumentation (400 bar) [10–13]. The media within these columns have a solid, non-porous silica core with a particle diameter of 1.7 μm (Agilent Poroshell, AMT Halo) or 1.9 μm (Phenomenex Kinetex). Surrounding the solid core is a porous silica layer with a thickness of 0.5 μm (Agilent Poroshell, AMT Halo) or 0.35 μm (Phenomenex Kinetex). The enhanced performance is due to decreased resistance to mass transfer kinetics from the lesser diffusion distance into the particle as well as a narrower particle size distribution when compared to fully porous silica [14,15].

In this work, we use the Cole–Dorsey method to determine necessary reequilibration time of superficially porous silica with a reduced dwell volume system. Given the smaller diffusion distance for residual acetonitrile (MeCN) sorbed to the stationary phase, we would expect the columns to reequilibrate faster than fully porous columns if the rate-limiting step is the diffusion of acetonitrile from the stationary phase. With offline LC–GC, we quantify the acetonitrile content of the eluent and compare superficially porous with fully porous silica.

2. Experimental

2.1. Reagents

All water used was purified to a resistance of approximately 18 MΩ cm using a Barnstead (Dubuque, IA, USA) NANOPure Diamond water purification system. HPLC grade acetonitrile (MeCN) and reagent grade 1-propanol (1-PrOH) were obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade acetone and sodium nitrate were obtained from Fisher Chemicals (Fair Lawn, NJ). Mobile phases were prepared by mixing the appropriate volumes of MeCN or 1-PrOH, and H_2O then vacuum filtered through 0.45 μm filters prior to use. 0.5% (v/v) acetone samples were prepared by mixing 5 mL with 1 L of H_2O then filtered. Sodium nitrate samples were prepared mixing an amount with 300 mL of water, then diluted until the detector signal was ~70 mV without a column. All retention times obtained were the average of three series of repetitive acetone injections. System peaks are identified from a blank run, where no acetone injections were made.

2.2. Liquid chromatograph instrumentation

Three Shimadzu pumps (Kyoto, Japan) were used for this study: two LC-10ATVP isocratic pumps and one LC-10ADVP gradient pump outfitted with a DGU-14A inline degasser and an FCV-10ALVP quaternary proportioning valve. One Valco (Houston, TX, USA) E90 four-port automated switching valve and a Valco E60 six-port automated switching valve were used to reduce system dwell volume and make well-timed, repetitive injections. System volumes measured were Valve A to detector (96.0 ± 0.0 μL, $n = 6$), injection loop to detector (76.2 ± 0.4 μL, $n = 6$), and injection loop to column (14.40 ± 0.02 μL, $n = 3$). Measurement of the injection loop to column volume was done by acetic acid titration. First, the volume was filled with glacial acetic acid using a 60 mL plastic syringe. Then, with air from another, empty syringe, the acetic acid was expelled from the volume into 10 mL of water. This solution was then titrated to endpoint with 0.010 M NaOH. Acetic acid volume was calculated from the inflection point of the titration curve, the density of glacial acetic acid (1.049 g/mL), and the molar mass (60.05 g/mol).

Pumps and valves were synchronized by an SCL-10AVP System controller. A Waters (Milford, MA, USA) 486 tunable wavelength UV–Vis detector was used with a wavelength of 254 nm for acetone or 190 nm for acetonitrile. All data were collected using a Perkin Elmer Nelson (Waltham, MA, USA) 970A integrator set to 25 Hz per channel and TotalChrom 6.2.1 software for analysis. Four stationary phases were evaluated: Halo C18 2.7 μm 90 Å (AMT, Inc.), Kinetex C18 2.6 μm 100 Å (Phenomenex, Inc.), Poroshell EC-C18 2.7 μm 120 Å (Agilent Technologies), and Zorbax 300Extend-C18 3.5 μm 300 Å (Agilent Technologies). The first three columns listed are superficially porous, whereas the last column is fully porous. Column dimensions were each 4.6 mm i.d. × 100 mm length. Column void volumes are reported in Table 1. Each method of void volume measurement has advantages and disadvantages, as outlined and discussed in reference [16]. Static void volume was determined by pycnometry using acetonitrile and chloroform as the solvents. Kinetic void volumes were determined by the average of triplicate injections of uracil. For 100% H_2O runs, the column was conditioned for 4 h at 1.00 mL/min prior to injection. For all other solvents, the column was conditioned for 1 h at 1.00 mL/min. With exception for the Kinetex column, the static void volumes agree well with the 100% MeCN kinetic void volumes, but the high

Table 1
Measured void volumes of the columns studied. Acetonitrile and chloroform were used for pycnometry. Kinetic voids were calculated from the elution time of the peak maximum for an injection of uracil. For each standard error, $n = 3$.

	Static void		Kinetic void					
	Pycnometry		100% H_2O		90%:10% H_2O:MeCN		100% MeCN	
	V_0 (mL)	σ (mL)	V_0 (mL)	σ (mL)	V_0 (mL)	σ (mL)	V_0 (mL)	σ (mL)
Zorbax 300Extend-C18	1.025	0.016	1.468	0.008	1.065	0.018	0.953	0.006
Poroshell 120 EC-C18	0.997	0.018	1.653	0.010	1.084	0.011	1.001	0.010
Kinetex C18 2.6 μm	0.907	0.020	3.227	0.012	1.767	0.006	1.802	0.015
Halo C18	0.848	0.025	1.367	0.007	1.091	0.011	0.831	0.007