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Flow rate dependent extra-column variance from injection in capillary liquid chromatography

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ABSTRACT

Efficiency and resolution in capillary liquid chromatography (LC) can be significantly affected by extra-column band broadening, especially for isocratic separations. This is particularly a concern in evaluating column bed structure using non-retained test compounds. The band broadening due to an injector supplied with a commercially available capillary LC system was characterized from experimental measurements. The extra-column variance from the injection valve was found to have an extra-column contribution independent of the injection volume, showing an exponential dependence on flow rate. The overall extra-column variance from the injection valve was found to vary from 34 to 23 nL. A new mathematical model was derived that explains this exponential contribution of extra-column variance on chromatographic performance. The chromatographic efficiency was compromised by ~130% for a non-retained analyte because of injection valve dead volume. The measured chromatographic efficiency was greatly improved when a new nano-flow pumping system with integrated injection valve was used.

1. Introduction

Liquid chromatography (LC) has become an indispensable analytical technique for characterizing mixtures of organic and biological compounds [1,2]. Analytical demands in these areas have only increased with time, necessitating further improvement in separating power [3,4]. Over the last 40 years, LC performance has improved significantly by optimizing both column selectivity and efficiency [5,6]. Improvements have been associated with evolution of stationary phase packing materials in areas such as particle synthesis and characterization [7–9], different bonding chemistries [10] and reduction in particle size [11–14]. Reduction in particle size has been accompanied by concomitant decrease in column diameter to alleviate consequences of heat generated in these columns by percolation of mobile phase at high flow rates [15–17]. Reductions in column and particle dimensions result in greatly reduced column volumes and low column permeability. Reduced column volume causes extra-column volumes associated with LC instrumentation to become significant contributors to analyte band dispersion [6,17]. Inherent extra-column band broadening of chromatographic peaks severely limits the separation potential of improved column packing materials. Moreover, the low column permeability of ultra-small particle columns restricts the maximum efficiency that can be achieved because of the pressure limit (~400 bar) of conventional LC systems [18,19].

The problem of delivering the mobile phase under high pressures has been solved with development of instruments capable of operating up to 1000–1500 bar [14,20]. However, the actual chromatographic performance of sub-2 μm packed columns has still not been realized to their full potential because of large extra-column volumes associated with these instruments. This issue of extra-column band broadening is well known, and considerable attention has been paid to reduce contributions arising from valves, connecting tubes, sampling devices (injectors), and detector cells [21–24]. The first notable study of extra-column volumes was conducted by Sternberg over 40 years ago, related to gas chromatography [25]. This study provided simple methods for calculating specifications that an instrument should meet, applicable to both GC and LC. Extra-column contributions were grouped into three different categories: (1) axial dispersion of the injection plug in the injection device [26], (2) axial dispersion of the injected band of analyte in any connecting tubing and detector cell [27], and (3) difference between the actual elution profile and the signal provided by the detector.

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Guiochon et al. [28–30] carried out studies to theoretically and experimentally characterize extra-column variances arising from different components of an instrument, and compared these contributions for two different commercially available systems. These studies included investigations of injection volume, injection time, sampling technique, diameter of connecting tubes, detector flow cell volume and detector response time. They provided a thorough investigation of all components with mathematical explanations for the observed phenomena. Most studies were conducted using 4.6–2.1 mm columns. Suggested methods to reduce this extra-column variance included reducing the sample volume, reducing the internal diameters of the sample loop and connecting capillary tubes, reducing the detector flow cell volume and optimizing the detector response rate [26]. In another study, Alexander et al. reported an interesting observation of increased extra-column variance associated with an automated injection system in contrast to manual injection [31]. These studies have proven to be useful in reducing extra-column variance; however, with the use of capillary LC columns, these contributions still prove to be significant.

In our recent research, we have been developing <150 μm i.d. monolithic capillary columns for LC [32,33]. In an effort to minimize extra-column variance, we have used on-column detection with no connecting tubing, thereby eliminating any extra-column variance associated with the second and third categories stated above. Extra-column variance due to the injection valve was minimized somewhat by optimizing a variety of factors as described in the literature; however, it could not be eliminated [22,26,34] In the past, extra-column variance due to the injector was described as a constant function of injection volume, with some contributions from valve geometry and mixing inside the valve [28]. However, this has never been fully characterized. Therefore, in this work, the injection valve contribution to band-broadening for a commercially available capillary LC system was characterized experimentally, and a new mathematical model was constructed from fundamental principles to explain the observed behavior. The effects of differences in extra-column variance on chromatographic performance for both retained and non-retained compounds were considered.

2. Experimental

2.1. Chemicals and samples

Poly(ethylene glycol) diacrylate (PEGDA, $M_n \sim 700$) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Analytical reagent grade dodecanol (Acros Organics, NJ, USA), decanol (Acros) and decane (Spectrum Chemical, New Burnswick, NJ, USA) were used as porogens. Tergitol 15-S-20, also used as a porogen, was obtained from Dow Chemical, Midland, MI, USA. UV transparent fused-silica capillary tubing was purchased from Polymicro Technologies (Phoenix, AZ, USA). All aqueous solutions and mixed mobile phases were prepared with HPLC-grade water and acetonitrile received from Fisher Scientific (Fair Lawn, NJ, USA). Test analytes included uracil, phenol, resorcinol, catechol and pyrogallol (Sigma–Aldrich). All samples were prepared in appropriate volumes of mobile phase to prevent the appearance of minor peak disturbances.

2.2. Instrumentation

The LC experiments were performed using an Ultimate 3000 high-pressure gradient LC system [Dionex (now Thermo Scientific), Sunnyville, CA] equipped with an FLM-3300 nanoflow manager (1:1000 split ratio). The injection system was a ten-port injection valve fitted with a zero dead-volume nanoViper (Thermo) sample loop having a volume of 1 μL. The injection valve had a 104 nL groove in the rotor and two connecting bore holes of 116 nL each, making a total swept volume of 336 nL. The swept volume is defined as the total volume in the injector, including the sample loop/groove and connecting bore holes, and is different from the actual volume selected by the sampling valve for introduction into the column. The sample volume injected in all experiments was 30 nL unless stated otherwise. Time-gated injections were carried out in all experiments with the injection valve being switched at different time intervals as a function of flow rate. On-column detection was accomplished immediately after the monolithic stationary phase at a detection wavelength of 214 nm using a Crystal 100 variable wavelength UV–vis absorbance detector (Thermo). The detector rise time was set at 1 s, corresponding to a time constant value of 0.45 s, and data were collected at a frequency of 10 Hz. Data acquisition was performed with Chrom Perfect software (Mountain View, CA, USA), and all peak analysis was done using Microsoft Excel. Every reported value represents the average of three repetitive measurements under the same conditions. All of the experiments were conducted at room temperature.

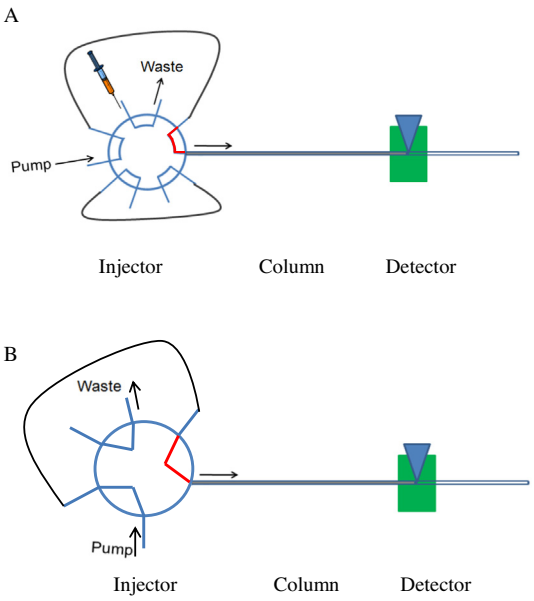

Fig. 1. Schematics of the LC systems used. (A) Commercial capillary LC system with injector valve having 336 nL swept volume, (B) nano-flow LC system with injector valve having 130 nL swept volume. The actual sample injection volumes for all measurements were 30 or 60 nL.

A second LC system designed and constructed by VICI Valco Instruments (Houston, TX, USA) was used to compare the differences in extra-column variance. This recently reported system consisted of a nano-flow pumping system with integrated injection valve [35]. The integrated 8-port injection valve had an internal V-shaped sample loop with a swept volume of 130 nL. Detection was performed using the same Crystal 100 variable wavelength UV detector described in the previous paragraph (Fig. 1).

2.3. Chromatographic column and conditions

The column used was a poly(ethylene glycol) diacrylate (PEGDA) monolithic capillary column fabricated using UV polymerization. Table 1 lists the column dimensions and reagent composition (i.e., amount of monomer, ratio of porogens, etc.) for the monolith. Details of the monolith fabrication have been published elsewhere [36,37]. The mobile phase composition used was 98% water in acetonitrile (v/v) for determining the extra-column variance of the injection valve using a non-retained analyte (uracil, 0.2 mg/mL). The mobile phase flow rates used in this study covered the range