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Short Communication

Comparison of the effects of extra-column aerosol and liquid-phase volumes on high-performance liquid chromatographic separations with inductively coupled plasma detection

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ABSTRACT

This communication demonstrates that the extra-column, laminar-flow, aerosol volume for HPLC detection methods employing typical aerosol interfaces has a small effect on peak variance compared to extra-column volume in the liquid phase. The higher velocities used with aerosol systems accommodate larger extra-column volumes before band broadening becomes significant. The reported results are limited to situations where a laminar flow profile is established at atmospheric pressure.

INTRODUCTION

Liquid chromatography is often coupled via an aerosol interface to detectors such as mass spectrometry (LC–MS), inductively coupled plasma atomic emission or mass spectrometry (ICP-AES or ICP-MS), or light scattering [1–4]. With the large surface-area-to-volume ratio of aerosols, relatively non-volatile analytes are readily enriched in the droplet phase by the evaporation of the typically more volatile solvents. However, the use of an aerosol interface results in the addition of hundreds of milliliters of extra-column volume which is often used to condition the aerosol (evaporate and remove solvent vapors, modify the particle size distribution, etc.) prior to the detector. Commonly, this volume is in the laminar flow regime where band broadening as a result of convection and axial dispersion is expected to occur.

One report concerning the effects of extra-column aerosol-phase volume on chromatography detection by ICP-AES was published in 1982 [5]. This report compared internal *versus* external placement of the aerosol spray chamber with respect to the torch box. Internal placement resulted in additional liquid-phase extra-column volume while external placement added to the aerosol volume and minimized the extra-column liquid-phase volume. External placement resulted in lower peak broadening and demonstrated a lower sensitivity dependence on the mobile phase flow-rate as compared to internal

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placement. This hinted that dispersion effects in the liquid volume are more significant than in the aerosol phase on a direct volume-to-volume basis and to minimize the loss of chromatographic resolution, placement of the spray chamber as close as possible to the column is preferred.

Perhaps more impressive was the interfacing of LC to MS with the counter-flow gas diffusion cell (CFGDC) by Vestec Corporation [6]. The CFGDC is one part of the universal interface and is used to remove solvent vapors from analyte transport system while at atmospheric pressure. As vapor removal is accomplished through evaporation and subsequent diffusion through a permeable membrane, the required processing volume is quite large. Nonetheless, Vestec has demonstrated that dispersion and particle losses are negligible.

More recently, we have published a report describing the influence of the aerosol volume on discrete signals with an ICP detector [7]. In brief, the aerosol-based laminar-flow volume between the spray chamber and the torch box was varied. The most dramatic condition tested for desolvated particles was the addition of 308 ml of aerosol volume (10 m of tubing with an internal diameter of 6.4 mm) which required a transport time of 37 s. This caused the peak width at half-maximum to increase by only 35%. Through computer simulation, band broadening for the conditions tested was shown to be the result of convective dispersion. An important parameter affecting convective dispersion is the peak residence time within the flow system [7]; lower residence times reduce band-broadening and minimize the loss in peak intensity. As aerosol interface systems generally operate at relatively high volumetric flows (l/min), peak residence time within hundreds of ml of flow volume can be relatively short. An additional factor, unique to aerosols that reduces the apparent band-broadening, is the irreversible nature of aerosol particle-transport wall collisions that occur because of either dispersion, centripetal or gravitational forces. Particles that are lost in this fashion may have otherwise caused the signal shape to broaden.

The influence of flame atomic absorption spectrometry (FAAS) on flow injection analysis signals has been reported by Fang *et al.* [8]. As with ICP-AES, FAAS utilizes a nebulizer and spray chamber to modify the aerosol prior to the flame. Unlike ICP-AES, the gas and liquid introduction velocities are higher. At a typical sample uptake flow-rate of 4.2 ml/min, dispersion was reported to be negligible with sample volumes as-low-as 50 μl. By analogy, they concluded that their detection system including readout was comparable to a 10 cm liquid-phase capillary tube with an internal diameter of 0.5 mm. For extremely small sample volumes (<10 μl), dispersion was independent of the liquid uptake flow-rate over a range of 1 to 6 ml/min because the analyte pulse is instantaneously distributed within the spray chamber.

The effects of liquid-phase flow volume on dispersion are well-known from the liquid chromatography and flow injection analysis literature [9,10]. In general as either the length or internal diameter of extraneous liquid-phase tubing increases, dispersion increases. This results from the convective flow profile. Coiling or knotting the transport path acts to minimize convective dispersion by improving radial mixing through the development of secondary flow streamlines.

The aim of this communication is to demonstrate the relative effects of the aerosol-phase extra-column volume *versus* liquid-phase volume for liquid chromatographic separations coupled with ICP-AES detection, although these effects should hold true with most other detectors using aerosol interfacing. To do this, we will compare liquid chromatograms for the separation of chromium species obtained with ICP-AES detection where: (a) aerosol and liquid-phase volumes are minimized, (b) liquid-phase volume is increased dramatically, and (c) aerosol-phase volume is increased dramatically. This is intended to show that with aerosol interfaces relatively large extra-column aerosol volumes can be tolerated without serious degradation of chromatographic profiles, unlike the situation with liquid-phase extra-column volumes.

EXPERIMENTAL

Instrumental

Fig. 1 represents an overview of the experimental system. The dual piston pump util-

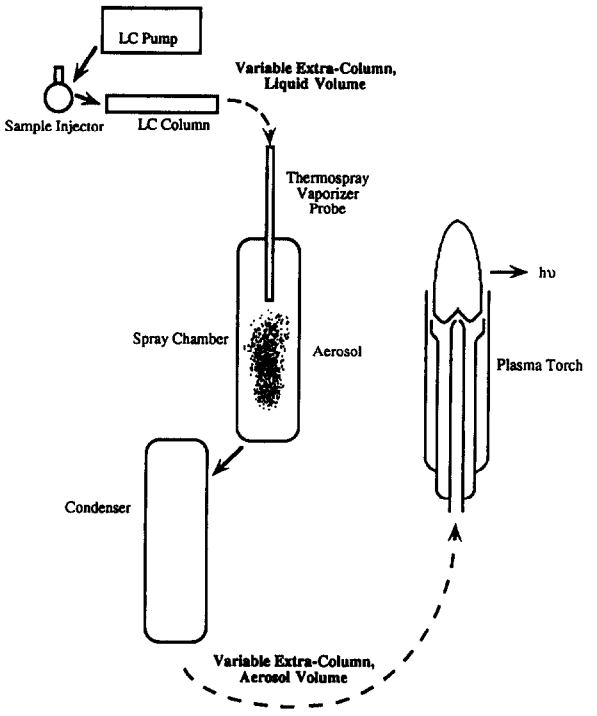

Fig. 1. Overview of experimental design. The dashed lines represent the location for the extra-column volumes.

ized in the separation was an Autochrom (Milford, MA, USA) Model 111. The flow-rate was 1.0 ml/min. The injector was a Rheodyne Model 7125 (Cotati, CA, USA) and was equipped with a 50-μl sample loop. The aerosol was generated by an aperture-based thermospray system. Thermospray sample introduction was chosen over pneumatic-based sample introduction because the particles produced are desolvated and hence of smaller size. This enriches the analyte concentration and results in improved mass transport efficiency for these experiments by reducing gravitational loss. Details surrounding the construction of the thermospray varporizer probe and optimization can be found elsewhere [11]. The exit aperture was 50 μm in diameter and the tip temperature was maintained at 176°C. The power to the probe was supplied by a Vestec (Houston, TX, USA) temperature controller. The spray chamber was heated to approximately 140°C and the condenser used to remove the solvent vapors was at 0°C. The combined spray chamber and condenser volume was 649 ml.

The ICP system was a Perkin-Elmer (Norwalk, CT, USA) Model 5500 operating at a forward power of 1.25 kW. The plasma gas flow-rate was 16 l/min with an auxiliary rate of 1.4 l/min. The carrier gas rate was 0.70 l/min and metered by a Tylan (Torrance, CA, USA) Model FC 260 mass flow controller. The chromium line used for detection was 205.5 nm. Wavelength modulation for background correction was accomplished by the oscillation of a quartz refractor plate positioned in front of the exit slit of the monochromator. The function generator used to drive the oscillation was a Wavetek (San Diego, CA, USA) Model 114 and the signal was tracked by a Stanford Research Systems (Sunnyvale, CA, USA) Model SR510 Lock-in-Amplifier. The viewing height was 7 mm above the load coil.

The signal was processed with Asystant + software (Macmillan Software, New York, NY, USA) via a MetraByte DAS-8 A/D (Keithley, Taunton, MA, USA) board and stored on an Epson Equity 1+ (Torrance, CA, USA) computer. Further signal processing was completed by converting the signal to an ASCII file and importing as a KaleidaGraph 2.0 (Synergy Software, Reading, PA, USA) file on a Macintosh Classic computer (Apple Computer, Cupertino, CA, USA).

Chromatographic separation

The separation chosen was the mobile-phase ion pairing speciation of chromium(VI) and chromium(III) which has been employed by this laboratory for thermospray sample introduction to ICP-AES [11]. The mobile phase composition was 5 m*M* sodium pentanesulfonic acid, 0.01 *M* magnesium acetate, 1% (v/v) acetic acid and 10% (v/v) methanol. The final pH was adjusted to approximately 3.5 with acetic acid. An Adsorbosphere HS C_{18} (Alltech, Deerfield, IL, USA) column was used. The size of the column packing was 7 μm and the column dimensions were 250 mm × 4.6 mm I.D. The Cr(III) concentration was 10 μg/ml and was prepared fresh from chromium nitrate to minimize the formation of additional complexes. The Cr(VI) concentration was 2 μg/ml and was prepared from potassium dichromate.

Equations

The following equations were used throughout this paper. The liquid-phase and aerosol-phase Reynold's flow numbers, R_e, were calculated as:

$$R_e = \frac{\rho v d}{\eta}$$

where ρ is the fluid density, v is the fluid velocity, d is the tube diameter and η represents the fluid viscosity [12]. The values chosen for both the fluid density and viscosity for the liquid flow number were that of water at room temperature.

The equation used for the calculation of resolution, R_s, was:

$$R_s = \frac{2\Delta Z}{W_x + W_y}$$

where ΔZ represents the difference in the retention times of the two species and W_x, W_y represents the baseline widths for the two components [12]. The baseline widths were estimated by assuming a Gaussian profile and hence, that the baseline width is 1.7 times greater than the full-width at half-maximum (FWHM). In a similar fashion, peak area was calculated as 1.25 times the product of the baseline width and peak height.

The reduced time constant (τ) was calculated as:

$$\tau = \frac{Dt}{a^2}$$

where D is the diffusion coefficient, t is time and a is the tube radius [13]. For the aerosol phase, a diffusion coefficient of $5.3 \cdot 10^{-6}$ cm^2 s^{-1} was used and 10^{-5} cm^2 s^{-1} for the liquid phase. A reduced constant of less than 0.01 indicates that convective dispersion is dominating whereas a value greater than 0.1 indicates diffusion controlled band-broadening.

Extra-column volumes

The goal of this study was to demonstrate the relative effects of liquid-phase and aerosol-phase volume by comparing three extremes: (a) a case where both liquid-phase and aerosol-phase volumes are minimized, (b) a case where the aerosol volume was kept the same as in (a), but the liquid-phase volume was increased dramatically, and (c) a case where the liquid-phase volume was minimized as in (a) but the aerosol volume was increased dramatically.

The dashed lines in Fig. 1 illustrate the experimental location for the added extra-column volumes. A chromatogram minimizing both the liquid- and aerosol-phase extra-column volume was acquired and will subsequently be called the *reference chromatogram*. In this case, the liquid volume between the column outlet and the thermospray probe was 2.74 μl [5.4 cm of polyether ether ketone (PEEK) tubing with an internal diameter of 254 μm]. The aerosol volume, excluding the spray chamber and condenser, was 31.7 ml (98.5 cm of tygon tubing with an internal diameter of 6.4 mm) and connected the condenser to the ICP torch.

In a second chromatogram, the liquid-phase volume between the end of the column and the thermospray vaporizer was increased to approximately 2.0 ml, using 2.2 m of PTFE tubing with an internal diameter of 1.09 mm for connection. This tubing was substituted for the PEEK tubing and coiled with a circumference of approximately 30 cm. The calculated Reynold's flow number was 84 which indicates laminar flow. The aerosol volume was the same as the reference chromatogram.

In the third chromatogram, the liquid volume was the same as the reference chromatogram. However, the aerosol volume between the exit of the aerosol condenser and the plasma torch was increased to 240 ml using 7.45 m of Tygon tubing with an internal diameter of 6.4 mm. This tubing was joined to the existing transport tube resulting in a final aerosol volume of 272 ml. The Tygon transport tubing was horizontal and straight except for four 90° bends. In this instance, the Reynold's number was 187 which is also in the laminar regime.

Reagents

All chemicals used were either HPLC or reagent grade. All solution containing glassware was scrupulously cleaned, acid soaked and rinsed with deionized/distilled water prior to use.

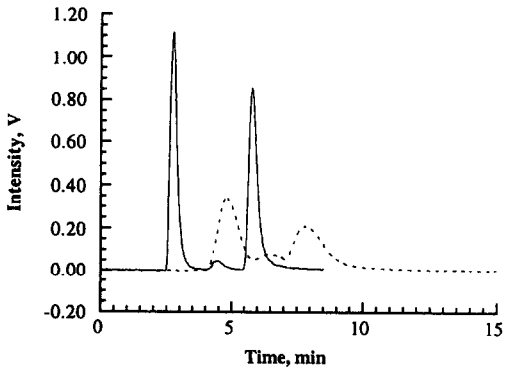

Fig. 2. Chromatogram (dashed line) demonstrating the influence of 2.0 ml of extra-column liquid-phase volume *versus* the reference chromatogram (solid line).

RESULTS AND DISCUSSION

Figs. 2 and 3, respectively, indicate the addition of extra-column liquid and aerosol volume on the chromatographic separation. The reference chromatogram represented by the solid line is the same in both figures. Each chromatogram consists of three peaks, the first of these is for Cr(VI) and the latter eluting component is for Cr(III). Although the exact identity of the interim peak is not known, it may be the result of Cr(III) complex formation with water [11]. The peak shapes are non-Gaussian which may reflect a slight over-loading of the column. Table I summarizes the figures-of-merit for each chromatogram. The values indicated in the data table correspond directly with the chromatograms in Figs. 2 and 3. The numbers indicated in parentheses express the percent relative standard deviation as calculated from two trials. With the exception of the residence time for the additional aerosol, the relative standard deviation is less than 10%. The higher deviation with the added aerosol is the result of using a manual trigger for the data acquisition process. Residence time was measured as the difference in elution times, determined at peak maximum intensity, for each respective component in the chromatograms. In this report, the differences between the reference and each of the other chromatograms are of interest.

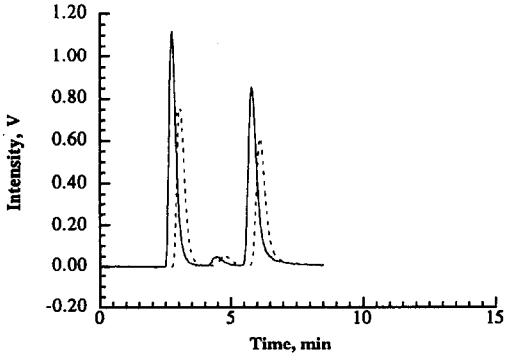

Fig. 3. Influence of 240 ml of extra-column aerosol volume on the chromatographic separation (dashed line). The solid line represents the reference chromatogram.

Table I shows that resolution was diminished substantially by the addition of 2.0 ml of liquid-phase, extra-column volume. In contrast, little change in resolution occurred with the addition of 240 ml of aerosol-phase volume. From the data table, the residence time for the Cr(VI) peak was only 17 s within the added aerosol volume, but was 125 s within the added liquid volume. Likewise for the added aerosol and liquid volumes, the residence times for the Cr(III) peak were 20 and 125 s, respectively. This is in good agreement with the anticipated times based on the added volume and the volumetric flow-rate. Hence, the integrity of the separation is better maintained in the larger volume of the aerosol-phase because of the lower residence time. The reduced time constant for the aerosol phase was 0.009 whereas the value was 0.42 for the liquid phase. This indicates that the aerosol phase was under the influence of convective dispersion but that the liquid-phase band-broadening was diffusion controlled. To realize a 125 s residence time in the aerosol phase with a tube of 6.4 mm I.D. at a volumetric rate of 0.7 l/min (as used in this work) would require a length of 45.3 m which has a reduced time constant of 0.006 which is still indicative of convective dispersion.

Also evident from the data table and Figs. 2 and 3, the peak heights are reduced to a greater extent by the addition of liquid-phase volume; this effect results from the differences in dispersion for the two cases, as outlined above. In contrast, the peak areas are influenced more by the added aerosol volume. For the added liquid-

TABLE I

COMPARISON OF THE FIGURES-OF-MERIT

FWHM represents the full-width at half-maximum intensity. Values in parentheses represent the percent relative standard deviation.

	Reference	Added liquid volume	Added aerosol volume
Intensity (V)			
Cr(VI)	1.12 (4.56%)	0.346 (8.22%)	0.758 (0.09%)
Cr(III)	0.85 (1.31%)	0.211 (6.27%)	0.614 (2.10%)
FWHM (s)			
Cr(VI)	16 (0.0%)	53 (6.4%)	18 (0.0%)
Cr(III)	20 (3.6%)	77 (0.9%)	22 (0.0%)
Residence time (s)			
Cr(VI)		125 (7.0%)	17 (16%)
Cr(III)		125 (6.9%)	20 (14%)
Area (V s)			
Cr(VI)	19.1	19.5	14.5
Cr(III)	18.1	17.3	14.4
Resolution	5.98	1.65	5.47

phase volume, one would anticipate a loss in signal intensity but that the area would remain constant. This trend is supported by the areas corresponding to the Cr(VI) and Cr(III) signals differing by only 2 and 4%, respectively.

In considering the added aerosol volume, a reduction in peak area results due to particle collisions and loss at the transport wall. Such collisions generally result in the irreversible adherence of the particle to the wall, and therefore loss of part of the total signal contained within the peak. However, particles most likely to be lost are those in the slow-velocity lamina closest to the wall. These particles represent the signal within the peak tail [7]. Therefore, loss of these particles leads to a reduction in peak tailing, counteracting dispersion effects on peak width and resolution to some extent.

CONCLUSIONS

This report compared dispersion and the loss of chromatographic resolution resulting from the addition of 2.0 ml of extra-column liquid volume to that resulting from the addition of 240 ml of aerosol volume. The effect of the aerosol volume on resolution was lower because the peak residence time within the added aerosol volume was significantly lower. In contrast, signal areas were reduced with the added aerosol volume because of particle losses at the wall. This latter effect also acts to counteract the detrimental effects of dispersion on band widths. These results demonstrate that relatively large extra-column aerosol-phase volumes can be tolerated when aerosol techniques are employed to interface liquid chromatography with aerosol-based detectors, compared to the effects of extra-column liquid-phase volume. Of course, the level of band-broadening and the absolute value of aerosol-phase volume that can be tolerated will depend on the bandwidths provided by the separation technique. Although these results are only demonstrated with ICP-AES detection, it is likely that these observations are also applicable to ICP-MS detection, to LC–MS with particle beam interfacing and to other neutral particle atmospheric pressure aerosol-based detectors.

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