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Simple method for the quantitative examination of extra column band broadening in microchromatographic systems

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Abstract

In recent years capillary chromatography has gained popularity for trace analyses. Most often UV or electrochemical detection is employed because the small peak volumes make post-column derivatization challenging. We have developed a simple method based on flow injection for determining contributions to peak broadening from post-column reactors. The only requirement for application of our methodology is that diffusion be in the Taylor regime so that radial concentration gradients are relaxed enabling mixing purely by diffusion.

Keywords: Band broadening; Post-column reactors

1. Introduction

In the past several years the use of capillary chromatography has attracted a great deal of attention. Small HPLC columns with diameters ranging from 25 to 500 μm are advantageous in trace analysis because of the increased mass sensitivity that can be achieved with a smaller column volume. Typically, these analyses are carried out with UV or electrochemical detection [1–7] with derivatization (if necessary) achieved pre-column. Post-column derivatization is challenging considering the small peak volumes associated with these analyses. Nonetheless, post-column derivatization is useful when the derivatization agent is not compatible with commercial columns or when the separation of the underivatized analytes is well understood. As post-column reactor volumes must be small, the determination of their contribution to band broadening is challenging. To determine small peak variances, a simple and reliable method is required.

We have developed such a method. In the method, we have abandoned the idea of using very small injection volumes in order to visualize band broadening from other sources. For this work, a large sample loop has been used to produce a steady-state signal that can be differentiated to yield two peaks. According to linear response theory [8], the differentiation of the response from a step function yields the response to a delta function input. The spreading of the delta function in a simple reactor can then be determined by standard methods. A capillary or channel operating in the Taylor regime [9] is the simplest reactor without special flow path geometries

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[10]. In such a reactor two fluid steams joined in a single channel will mix by diffusion only. Thus, we have applied the method to determine band broadening in a simple capillary.

Though a significant literature exists detailing the mathematical equations for peak shape and band broadening in traditional flow injection techniques, these experiments are not performed in the Taylor regime [11,12]. As a result that theory is not applicable to the problem.

By using the *time* equivalent to a theoretical plate certain experimental and computational simplifications result. From plate height theory [13,14] we know that the plate height in units of time, H_t, is related to the length-dimensioned plate height, H_L, as shown in Eq. (1) where σ^2 is the similarly dimensioned standard deviation of the zone squared (second central moment or variance), the distance that the band has traveled is L, and v is the average solute velocity.

$$H_L = \sigma_l^2/L = (\sigma_t^2/t)\cdot v \quad (1)$$

We also know that the plate height is related to the solute dispersion coefficient, D, as shown in Eq. (2).

$$H_L = 2D/v \quad (2)$$

From Taylor's theory of dispersion in an open tube of radius a for a solute with a molecular diffusion coefficient D_{mol}, the dispersion coefficient is shown as Eq. (3).

$$D = v^2a^2/48D_{\text{mol}} \quad (3)$$

Therefore, Eq. (4) expresses the plate height in units of time, which will be referred to as the ''plate time'' in this paper.

$$H_t = 2D/v^2 = a^2/24D_{\text{mol}} \quad (4)$$

The use of time units has several advantages. In applications using flow splitting, it can be difficult to maintain strictly constant fluid velocity while changing tubing lengths and diameters. The plate time is not dependent on the individual values of tubing length or fluid velocity, so the determination of an experimental plate time is straightforward, as both parameters, i.e., σ_t^2 and t (second central and first moments) are represented in the data and require no physical measurements of length or volume. Also, the estimation of a theoretical plate time from Eq. (4) is uncomplicated.

2. Experimental

2.1. Reagents

Trifluoroacetic acid (TFA), sodium perchlorate and 1-propanol were purchased from Sigma (St. Louis, MO, USA). Ruthenium hexaminetrichloride was purchased from K&K Laboratories (Cleveland, OH, USA). All solutions were made with Milli-Q house-deionized water.

2.2. Instrumentation

The aqueous flow solution containing 0.1% TFA, 3% 1-propanol and 0.1 *M* sodium perchlorate was pumped with a Waters 600 E quaternary pump at a rate of 100 μL/min. A splitter tee carried 95–98 μL/min flow to waste and the remainder to a 32 cm×50 μm (ID) capillary in which dispersion was studied. In total, three different waste line dimensions were used: 43.8 cm×127 μm, 45.4 cm×178 μm plus 43.8 cm×127 μm and 77.8 cm×127 μm. An Upchurch microinjector was used for sample injection. All injection volumes were 1.01 μL. The $Ru(NH_3)_6^{3+}$ was detected at −250 mV versus Ag/AgCl at a 10 μm carbon fiber electrode 400 μm in length placed inside the lumen of the reactor capillary. Potential was controlled with a BAS (W. Lafayette, IN, USA) CV-27 potentiostat, and current was measured with a Keithly (Cleveland, OH, USA) 427 Picoammeter. Signals from the ammeter were collected at 50 Hz by EZChrom (Scientific Software, San Ramon, CA, USA).

For chronoamperometry a three-electrode system consisting of a Ag/AgCl reference electrode, a platinum wire auxiliary electrode and a 4.40-mm diameter glassy carbon disk electrode were placed in a water jacketed cell. The contents of the electrochemical cell were temperature controlled to 22.8 °C using a circulator. This matched the temperature at which the flow injection experiments were carried out. A Model CS-1090 Cypress Systems computer controlled potentiostat (Lawrence, KS, USA) was used to control potential and measure the current

response. The initial and final potentials for the oxidation of ruthenium hexaminetrichloride were −400 and 400 mV, respectively. The solution containing 1.07 m*M* ruthenium hexaminetrichloride and 0.1 *M* sodium perchlorate was sparged with argon to remove oxygen. The current was sampled at 100 Hz and the potential step duration was 15 s. The electrode was shielded to prevent diffusion from the sides and a blank subtracted signal was used for the determination of the diffusion coefficient.

2.3. Data treatment

Data were imported into Mathcad (Mathsoft, Inc.) for filtering and differentiation using the function ''ksmooth'', then exported to PeakFit version 4 (AISN Software, Inc.) for determination of the first and second central statistical moments. The filtering was necessary as differentiation amplified the noise considerably. The statistical moments were determined by fitting the 5-parameter GEMG function to the data based on the least squares criterion. The 5-parameter GEMG function was produced by convolving a gaussian with a hybrid response function consisting of a half-gaussian multiplied by an exponential decay. The GEMG function was chosen because it provided the best fit, determined by the residual sum of squares, to the experimental data.

3. Results and discussion

We assess whether or not the experiment is in the Taylor regime from a consideration of the Peclet number and the capillary diameter and length [9]. The Peclet number ($\mathrm{Pe} = v \cdot a/D$) is a dimensionless variable relating the rates of radial diffusion and axial convection. If radial diffusion is fast compared to axial convection then $L/a \gg \mathrm{Pe}$ [9]. For our system Pe ranges from 1000 to 3000 and L/a is equal to 12 550.

Fig. 1A shows the signal response at three different split ratios for a system consisting of a 1.01-μL sample loop and a 32 cm (628 nL) capillary. Differentiation of the signal in Fig. 1A yields a peak from the leading edge that would have resulted from the injection of a delta function. Fig. 1B shows a data trace and the first derivative of the trace. The

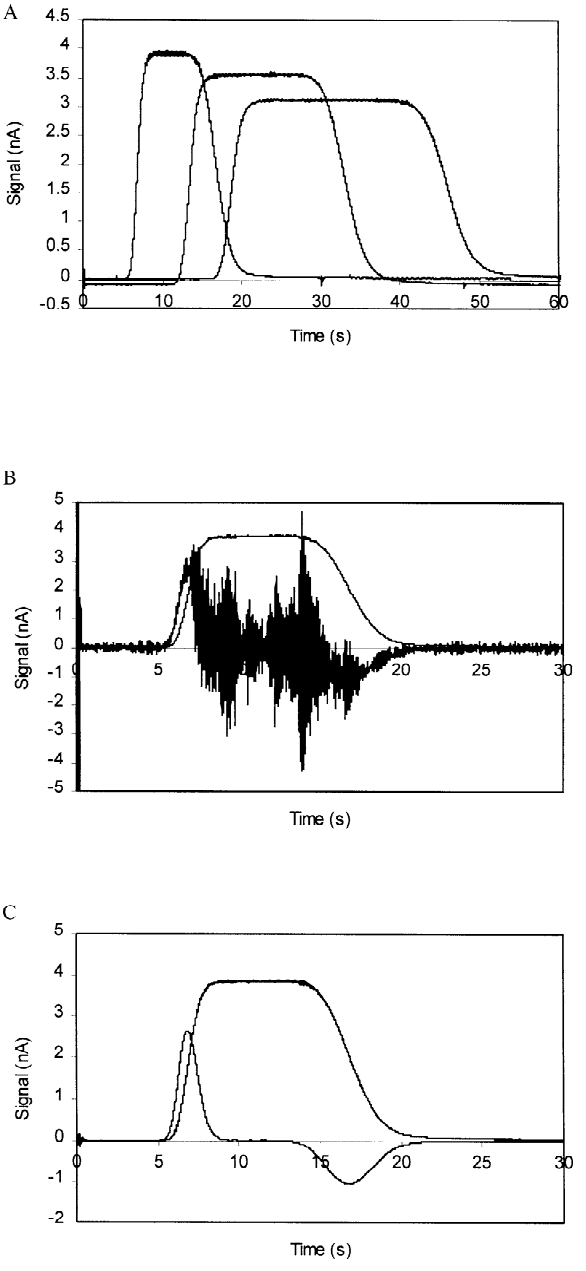

Fig. 1. (A) Flow injection analysis of 10 μ*M* ruthenium hexamine trichloride. Flow-rates ranging from 2.1 to 5.6 μL/min were achieved by changing the split ratio. A 10-μm carbon fiber electrode 400 μm in length at a potential of −250 mV versus Ag/AgCl was used for detection. The aqueous flow solution contained 0.1% TFA, 3% 1-propanol and 0.1 *M* sodium perchlorate. (B) A data trace and the first derivative obtained in Mathcad. (C) The same data trace as in B overlaid with the filtered version of the raw data (0.21 s) and the derivative obtained from the filtered data.

first derivative is determined (Mathcad) based on a cubic spline interpolation of data points. Clearly, the derivative adds considerable noise. As a result, the noise in the data must be removed before differentiation. Fig. 1C shows that the filtering operation described in the data treatment section is successful and has virtually no effect on the original data. The smoothing function used has a parameter like a time constant to control the extent of filtering. We determined empirically with real data that when this parameter was set from 0.14 to 0.21 s the data were well filtered and the filtered data overlapped the original data very well. Synthetic data were generated that ranged in width from being similar to actual data to having sharper transitions. The filtering operation does not change the first moment and adds a constant 0.0031 s^2 to the second central moment (peak variance) when the filter width is 0.14 s.

To validate the approach, we used the electrochemically well-behaved solute ruthenium hexaminetrichloride to assess the broadening occurring in the capillary connecting the injector to the detector. The diffusion coefficient of ruthenium hexaminetrichloride was determined experimentally using chronoamperometry to be $3.89 \cdot 10^{-6} \pm 8 \cdot 10^{-8}$ cm^2/s. Using this value and the capillary radius of 25.0 μm, a slope ($a^2/24D_{mol}$) of 0.0669 ± 0.0014 s is calculated. Fig. 2 shows the results of the experimental data. Included in the figure are data from different days and flow splits. The slope of 0.0658 ± 0.0012 s agrees well with that predicted by theory.

While there is no evidence of systematic error, contributions to band spreading from other causes must be discussed. Mismatches in tube diameters and connection of tubing to the injector may cause volumes where mixing can occur. As a result, a good connection to the injection should be made. The capillary should be cut square, be burr-free and be pushed snugly to the bottom of the port. If the electrode is approximated as a rectangular function, its effect on peak shape can be determined. The 400 μm carbon fiber electrode employed in our experiments contributes 0.22 nL to the peak standard deviation. Data treatment, i.e., filtering of the data can also increase the peak width. The filtering process described in the data treatment section contributes 1.9 nL to the total peak standard deviation. Both of these will prove to be negligible in most cases since the largest contribution to post-column peak broadening arises from dispersion occurring in the reactor.

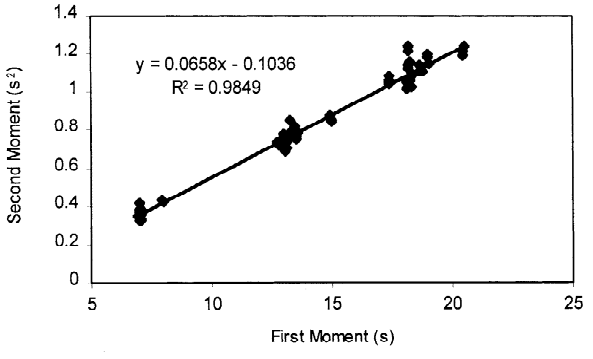

Fig. 2. (A) Relationship between second central moment and first central moment for analytes that have passed only through the capillary tubing from the injector to the detector.

The slope determined from the data in Fig. 2 can be used to calculate the maximum length of capillary that can be implemented without significant broadening of chromatographic peaks. By the addition of variance rule, the standard deviation added to a chromatographic peak post column can be no more than 0.3 times the standard deviation of the peak to suffer less than 10% increase in peak width.

A peak eluting from a 180 μm ID column would have a standard deviation of approximately 150 nL. The standard deviation added to this peak by passage through a 32 cm × 50 μm capillary is 32 nL. This is considerably smaller than the actual volume of the tube which is 628 nL. Summing all contributions to the peak variance arising post column, approximately 32 nL is added to the standard deviation of the peak. In this system a chromatographic peak would not be excessively broadened (32^2 is approximately 4.5% of 150^2). This reactor would, however, contribute appreciably to the width of a peak eluting from a 50-μm ID column which would have a standard deviation of approximately 63 nL.

4. Conclusions

We have developed a method for determining extra column band broadening based on flow injection experiments. If diffusion is in the Taylor

regime a simple mathematical model can be used to predict the effect of a post-column reactor on peak shape. This will be particularly useful for developing and evaluating capillary HPLC reactors because of the small peak volumes associated with these systems.

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