Theoretical study of incomplete sampling of the first dimension in comprehensive two-dimensional chromatography

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Abstract

The process of regularly transferring material from the primary column to the secondary column is critical in producing comprehensive two-dimensional separations. A series of calculations have been performed to determine how sampling period, duty cycle, and sampling phase affect (1) the fraction of material transferred from the primary column to the secondary column, (2) the accuracy of primary retention time determination, and (3) the effective peak width along the primary retention axis. The results demonstrate that comprehensive two-dimensional separations can be produced without a substantial loss in quantitative precision and with only a moderate loss in primary column resolution if the sampling period is less than 1.5 times the primary peak standard deviation. The quantitative precision of total peak areas (for duty cycles less than 1.0) and primary retention time determination are rapidly reduced as the sampling period is increased above 1.5 times the primary peak standard deviation. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

The comprehensive two-dimensional chromatography approach allows two-dimensional separations to be generated with standard chromatographic columns and a modulator [1,2]. Modulators are devices that transfer primary column effluent to a secondary column as a series of sharp pulses. Each pulse is subjected to a high-speed separation in the secondary column. Sampling of primary column effluent is performed at regular intervals throughout the chromatographic run. Some modulators collect primary effluent throughout the entire sampling period. Such

devices have duty cycles of 1.0. Examples include thermal modulators used in gas chromatography [2–6] and dual-loop eight-port valves used in liquid chromatography [1]. In contrast, some modulators sample the primary effluent for less than 100% of the sampling period (i.e. have duty cycles less than 1.0). Examples include single-loop six-port valves used in liquid [7] and gas chromatography [8,9], direct diverting valves used in gas chromatography [10], and flow gating methods used in liquid chromatography–capillary zone electrophoresis [11,12].

Ideally, secondary separations can be generated without diminishing the separation produced by the primary column. This goal, however, places a constraint on the maximum sampling period. Sampling periods substantially greater than the widths of the peaks emerging from the primary column will lead to

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a loss in primary column resolution. On the other hand, small sampling periods decrease the time available for secondary separations and thereby reduce secondary resolution. It is also desirable that modulators transfer a reproducible fraction of each component from the primary column to the secondary column. This may not be the case if the sampling period is excessive and the duty cycle is less than 1.0. At large periods, the transferred fraction is expected to be dependent upon the relative position of the peak within the succession of sampling events.

It is important to develop a quantitative understanding of the effect that sampling period has on key separation properties such as the size of two-dimensional peaks, the accuracy of retention times, and overall separation efficiency. Several investigators [13–15] have determined that a noise-free Gaussian distribution is fully characterized when samples are acquired at least once every standard deviation. Gaussian peaks corrupted with noise require even greater sampling frequencies to completely reconstruct the underlying signal [16]. However, the goal of a typical comprehensive two-dimensional chromatography experiment is not to perfectly reconstruct the peaks emerging from the primary column, but to produce an accurate representation of the lowest modes of each peak: i.e., the total area, the retention time, and the width. Murphy and co-workers [17] have studied the relationship between modulator sampling period and the widths of peaks along the primary retention axis. They only considered modulators with a duty cycle of 1.0. They found that peak width increased by 30% or less if the primary effluent was sampled at least once every two primary peak standard deviations.

Here we report the results of computer calculations that further examine the effects of modulator sampling rate and duty cycle on two-dimensional separations. Specifically, we investigate how these sampling parameters affect the fraction of material passed from the primary to the secondary column, the accuracy of primary retention time determination, and the effective primary peak widths. Our calculations do not consider the impact of modulator settings on the performance of the secondary separation.

2. Model description

We consider the case of a primary peak that is well described by a Gaussian distribution:

$$C(t) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(t-t_1)^2}{2\sigma^2}\right)$$

where t_1 and σ are the retention time and standard deviation of the primary peak, respectively. For increased simplicity, the peak function is transformed into standard-normal coordinates:

$$C(z) = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{z^2}{2}\right)$$

where $z = (t-t_1)/\sigma$.

Peak sampling is modeled with three parameters: (1) the dimensionless sampling period τ_z, (2) the phase of sampling ϕ, and (3) the duty cycle d. We define the dimensionless sampling period τ_z as the ratio of the modulator sampling period τ to the standard deviation of the primary peak, $\tau_z = \tau/\sigma$. Fig. 1a shows a peak sampled with $\tau_z = 1.5$, whereas Fig. 1b shows a peak sampled with $\tau_z = 1.0$. Our study considered values of τ_z ranging from 0.5 to 3.0 in increments of 0.1.

The phase of sampling ϕ adjusts the position of the peak maximum relative to the sampling events. We define ϕ as the time difference between the center of the sampling cycle nearest to the peak maximum and the peak maximum, with this difference divided by the sampling period. Fig. 1a shows a peak sampled with $\phi = 0$, whereas Fig. 1c shows a peak sampled with $\phi = 0.5$. Our study considered values of ϕ ranging from -0.5 to 0.5 in increments of 0.025.

The duty cycle d represents the fraction of a sampling period when primary column effluent is collected. The effect of duty cycle on peak sampling can be seen by comparing Fig. 1a where $d = 0.8$ and Fig. 1d where $d = 0.5$. Our study considered d ranging from 0.1 to 1.0 in increments of 0.1.

The mean dimensionless sampling time of a collection cycle, z_i, is given by

$$z_i = \tau_z(i + \phi)$$
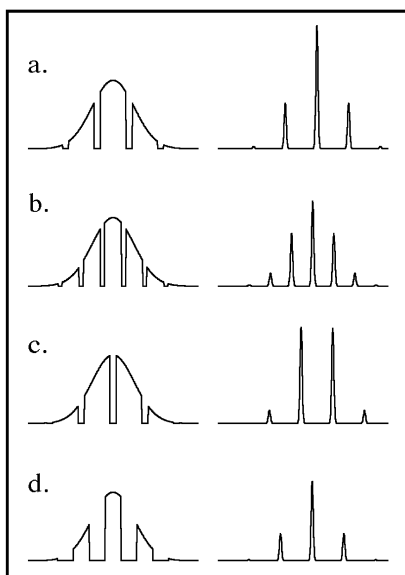
where i is an integer index. The range of indices

Fig. 1. A primary peak sampled with four different combinations of dimensionless sampling period (τ_z), phase (ϕ), and duty cycle (d). The left column shows the portions of the peak collected by the modulator. The missing parts of the Gaussian distributions correspond to portions of the peak not sampled by the modulator. The right column shows the pulses that leave the modulator. The plotted intensity of the pulses has been attenuated by a factor of 5 so that the primary peaks and the pulses can be shown with comparable height. The simulated conditions are as follows: (a) $\tau_z = 1.5$, $\phi = 0$, $d = 0.8$; (b) $\tau_z = 1.0$, $\phi = 0$, $d = 0.8$; (c) $\tau_z = 1.5$, $\phi = 0.5$, $d = 0.8$; (d) $\tau_z = 1.5$, $\phi = 0$, $d = 0.5$.

considered was chosen so that $z_{i_{\min}} < -4.0$ and $z_{i_{\max}} > 4.0$. The starting time of the sampling portion of a given modulator cycle, $z_{i,s}$, is

$$z_{i,s} = z_i - \frac{\tau_z d}{2}$$

The finishing time of the sampling portion of a modulator cycle, $z_{i,f}$, is

$$z_{i,f} = z_i + \frac{\tau_z d}{2}$$

The total quantity of material collected in a single sampling cycle is proportional to the area of the primary peak segment, A_i, that lies between $z_{i,s}$ and $z_{i,f}$:

$$A_i = \int_{z_{i,s}}^{z_{i,f}} C(z)\, \mathrm{d}z$$

This integral is evaluated numerically using a standard algorithm for calculating the error function [18]. A_i is proportional to the quantity of material injected into the secondary column at the completion of a sampling cycle and hence proportional to the area of the secondary peak observed by the detector. The right column of Fig. 1 shows the pulse sequences that would exit the modulator. The total areas of the pulse peaks are plotted as one-fifth of their respective A_i's. This was done so that the pulses could be shown on the same scale as the primary peaks.

3. Results and discussion

3.1. Component transfer

In a typical comprehensive two-dimensional chromatography experiment, the quantity of a single component of a mixture is determined by summing the areas of the constituent peaks emerging from the secondary column. In mathematical terms, the sum of the secondary column peak areas is given by A_{tot}:

$$A_{tot} = \sum_{i=i_{\min}}^{i_{\max}} A_i$$

In the case of ideal sampling, the value of A_{tot} equals the area of the peak exiting the primary column (A_{prim}) multiplied by the duty cycle (d). We define the transfer coefficient A^* as the ratio of A_{tot} to the ideal total area:

$$A^* = \frac{A_{tot}}{A_{prim} d}$$

For the case of the standard-normal peak function, $A_{prim} = 1$. Therefore, A^* is given by

$$A^* = \frac{A_{tot}}{d}$$

The value of A^* is indicative of the effect that peak sampling has on the fraction of a component transferred from the primary column to the sec-

ondary column. When A^* is greater than unity, the fraction of the peak sampled is greater than the duty cycle and the sum of the secondary column peak areas produces an overestimation of the actual primary peak area. When A^* is less than unity, the fraction of the peak sampled is less than the duty cycle and the sum of the secondary column peak areas produces an underestimation of the actual primary peak area.

The effect of sampling phase on A^* is shown in Fig. 2 for the case of $\tau_z = 2.0$ and four different duty cycles. When the duty cycle is 1.0, the value of A^* is unity and independent of ϕ. This result can also be deduced from simple mass conservation principles. For duty cycles less than 1.0, the value of A^* depends on ϕ. The largest values of A^* are obtained when $\phi = 0$ because the central portion of the peak is sampled (see Fig. 1a). The smallest values of A^* are obtained when $\phi = 0.5$ because the central portion of the peak is not sampled (see Fig. 1c). The mean of A^* over the entire phase range (i.e., $\phi = -0.5$ to 0.5) is 1.0 for each duty cycle considered. The magnitude of the phase-dependent deviations of A^* from unity for a given value of τ_z increases with decreasing duty cycle.

As the value of τ_z is varied, the dependence of A^* on ϕ remains similar to that shown in Fig. 2. However, the magnitude of the variation of A^* increases with increasing τ_z. The standard deviation of A^* over the entire phase range is plotted in Fig. 3 as a function of τ_z. The results show that when

Fig. 3. The standard deviation of the transfer coefficient, A^*, plotted as a function of the dimensionless sampling period, τ_z, for four different values of duty cycle ($d = 0.1, 0.5, 0.8$, and 1.0). The standard deviations were determined from calculations of 41 different values of sampling phase, ϕ, ranging from -0.5 to 0.5 in increments of 0.025. The standard deviation of A^* represents the fluctuations in the fraction of a component transferred from the primary to secondary column caused by slight changes in primary retention time.

$\tau_z < 1.5$, the standard deviation of A^* is less than 0.001 for all values of duty cycle. The standard deviation of A^* increases as τ_z becomes larger than 1.5 (except for the case of $d = 1$, where the standard deviation is always zero). The increase is especially pronounced for small duty cycles.

In an experimental setting, small changes in the primary retention time lead to changes in sampling phase. The standard deviation of A^* over the phase range represents the fluctuations in mass transferred from the primary column to the secondary column due to changes in sampling phase. The calculation results show that if $\tau_z < 1.5$, the fraction of a component transferred from the primary column to the secondary column is constant to within 0.1%.

3.2. Primary retention time determination

In a typical comprehensive two-dimensional chromatography experiment, only the effluent leaving the secondary column is monitored. However, the primary retention time of a component can be estimated from the area-weighted average of the mean modulator sampling time of each secondary peak [9]. We have used our model to determine how sampling
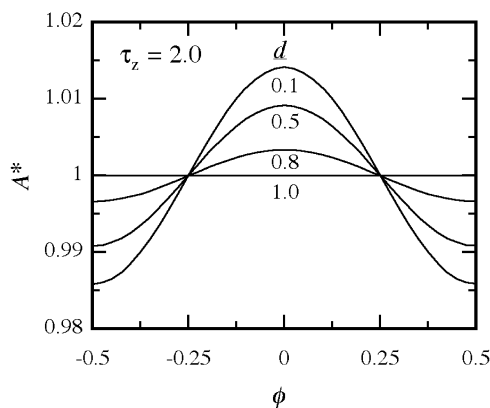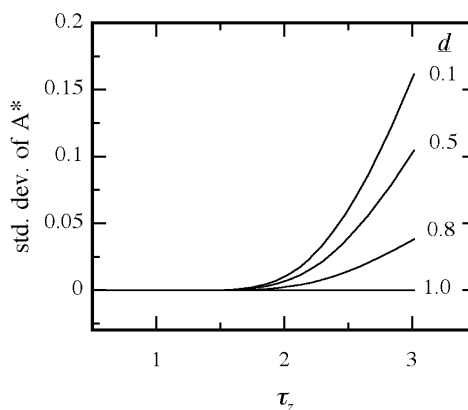

Fig. 2. The transfer coefficient, A^*, plotted as a function of sampling phase, ϕ, for calculation conditions of $\tau_z = 2.0$ and $d = 0.1, 0.5, 0.8$, and 1.0.

conditions influence the accuracy of primary retention time estimation. The apparent dimensionless primary retention time, z^*, is defined as the area-weighted average of the mean dimensionless sampling times of the peak segments:

$$z^* = \frac{\sum_{i=i_{\min}}^{i_{\max}} z_i A_i}{A_{\text{tot}}}$$

The value of z^* corresponds to the difference of an experimentally determined primary retention time from the actual primary retention time, with this difference expressed relative to the standard deviation of the primary peak. Since the standard-normal peak function is symmetric and centered at $z = 0$, ideal sampling should produce $z^* = 0$. Positive values of z^* result from sampling conditions that lead to an overestimation of the primary retention time, whereas negative values of z^* result from sampling conditions that lead to an underestimation of the primary retention time.

The calculated value of z^* is shown in Fig. 4 as a function of sampling phase for four different duty cycles with $\tau_z = 2.0$. When the peak is sampled symmetrically about the center (i.e., when $\phi = 0$ and/or when $\phi = \pm 0.5$) the value of z^* equals 0. Positive deviations of z^* are observed for $0 < \phi < 0.5$, while negative deviations are observed for $-0.5 < \phi < 0$. The magnitudes of the deviations increase with decreasing duty cycle. The average of z^* over the entire phase range is 0.0 for values of τ_z
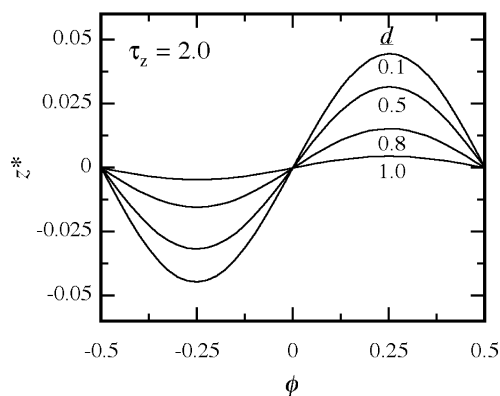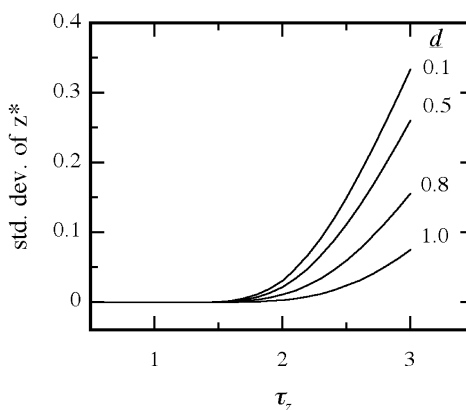

Fig. 5. The standard deviation of the apparent dimensionless primary retention time, z^*, plotted as a function of the dimensionless sampling period, τ_z, for four different values of duty cycle ($d = 0.1$, 0.5, 0.8, and 1.0). The standard deviations were determined from calculations at 41 different values of sampling phase, ϕ, ranging from -0.5 to 0.5 in increments of 0.025. The standard deviation of z^* represents the error in estimating the primary retention time.

and d considered. The standard deviation of z^* over the phase range is shown in Fig. 5 as a function of τ_z. When $\tau_z < 1.5$, the fluctuations are negligible for all values of d. However, the standard deviations of z^* increase sharply as τ_z increases above 2.0.

The calculated values of z^* indicate that primary retention times can be estimated with high accuracy when $\tau_z < 1.5$. For longer sampling periods, the estimated primary retention time will exhibit substantial phase-dependent deviations from the actual retention time. This effect is most pronounced when the sampling duty cycle is near zero.

3.3. Peak broadening along the primary axis

The width of a two-dimensional peak along the primary retention axis is estimated by calculating the standard deviation of the area-weighted mean sampling times of each constituent peak [9,17]. In dimensionless coordinates, the standard deviation along the primary retention axis, σ^*, is defined as

$$\sigma^* = \left(\frac{\sum_{i=i_{\min}}^{i_{\max}} z_i^2 A_i}{A_{\text{tot}}} - (z^*)^2 + \frac{\tau_z^2}{12} \right)^{1/2}$$

The $\tau_z^2/12$ term represents the resolution limit

Fig. 4. The apparent dimensionless primary retention time, z^*, plotted as a function of sampling phase, ϕ, for calculation conditions of $\tau_z = 2.0$ and $d = 0.1$, 0.5, 0.8, and 1.0.

imposed by the sampling period (i.e., if only one secondary peak is observed, the value of σ^* is equal to the standard deviation of a rectangular peak with width τ_z). The $\tau_z^2/12$ term is only significant when τ_z is greater than 2. The standard deviation of the standard normal distribution used to model the primary peak is equal to 1.0. Thus, ideally sampled peaks should have σ^* near 1.0. Values of σ^* greater than 1.0 represent broadening of the peak along the primary retention axis caused by sampling.

The dependence of σ^* on the sampling phase is shown in Fig. 6 for four different sampling duty cycles with $\tau_z = 2.0$. The value of σ^* is greatest when $\phi = \pm 0.5$ because the center of the peak is split into two secondary peaks (see Fig. 1c). The magnitude of σ^* increases with increasing duty cycle. The values of σ^* obtained with $d = 1$ are in excellent agreement with the results of Murphy et al. [17]. The averages of σ^* obtained over the entire phase range, $\langle\sigma^*\rangle$, are shown in Fig. 7. The values of $\langle\sigma^*\rangle$ are essentially 1.0 when τ_z is less than 0.5. As τ_z increases, $\langle\sigma^*\rangle$ steadily increases.

The calculation results show that the sampling process leads to primary peak broadening unless very small sampling periods are employed. However, the average broadening is less than 30% (i.e., $\langle\sigma^*\rangle$ is less than 1.30) if the peaks are sampled at least once every two primary peak standard deviations.

3.4. Application of calculation results
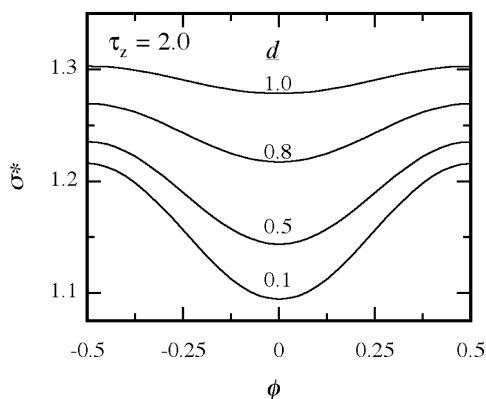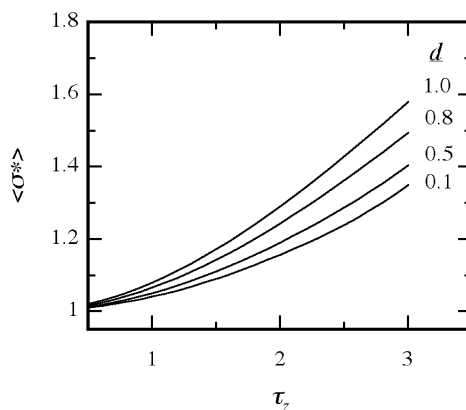
The calculation results can be used to determine

Fig. 7. The average of the broadening factor, σ^*, plotted as a function of the sampling period, τ_z, for four different values of duty cycle ($d = 0.1$, 0.5, 0.8, and 1.0). The averages were determined from calculations of 41 different values of sampling phase, ϕ, ranging from -0.5 to 0.5 in increments of 0.025. The average of σ^* represents the mean relative increase of the peak width along the primary retention axis resulting from the sampling process.

the limitations that sampling conditions place on the accuracy of compound quantitation, the accuracy of primary retention time determination, and on the performance of the primary separation. We used the model to examine the sampling conditions employed in our GC×GC studies [8]. Our differential flow modulator is operated with a sampling period of 1.0 s and a duty cycle of 0.9. The widths of peaks at half maximum emerging from the primary column are approximately 1.5 s. Under such conditions $\tau_z = 1.57$. The calculations produce a standard deviation of A^* that is less than 0.001. Thus, in the absence of experimental irregularities, the sampling process should contribute less than 0.1% to the relative standard deviations of the total peak area. The standard deviation of z^* is calculated to be less than 0.001. This result indicates that sampling contributes to the uncertainty of primary retention determination by less than 0.1% of the standard deviation of the primary peak. Finally, the average value of σ^* is 1.17. Thus, the two-dimensional chromatogram should have peaks that are broadened along the primary retention axis by 17%.

We have also examined the flow gating interface of Jorgenson et al. [12]. Their experiments operate under conditions where peaks are sampled at least three times across a width of 4σ with a duty cycle of

Fig. 6. The broadening factor, σ^*, plotted as a function of sampling phase, ϕ, for calculation conditions of $\tau_z = 2.0$ and $d = 0.1$, 0.5, 0.8, and 1.0.

0.1. Thus, calculations were performed with $\tau_z =$ 1.33 and $d = 0.1$. Both the standard deviation of A^* and the standard deviation of z^* are less than $1.0 \cdot 10^{-4}$. The sampling process is therefore predicted to contribute negligibly to the uncertainty of compound quantitation and primary retention time determination. Finally, the average value of σ^* is 1.07, indicating that sampling will broaden the peaks along the primary retention axis by 7%.

4. Summary

The calculations show that when the sampling technique has a duty cycle of 1.0, the fraction of a peak sampled by the sampling period is constant and equal to unity. When the duty cycle is less than 1.0, the fraction of material passed to the secondary column depends on the sampling period and the position of the peak relative to the sampling events (i.e., the sampling phase). The calculations demonstrate that the sampled fraction of a Gaussian peak is constant to within 0.1% and given by the duty cycle when the sampling period is less than the standard deviation of the primary peak multiplied by a factor of 1.5. As the sampling period becomes larger, the fraction transferred becomes increasingly sensitive to sampling phase, especially for duty cycles near zero. The calculations also show that the primary retention time of a Gaussian peak can be determined with high accuracy when the sampling period is less than the peak standard deviation multiplied by a factor of 1.5. The accuracy of the estimated primary retention time decreases with increasing sampling period, especially when the sampling duty cycle is near zero. Finally, the calculations demonstrate that the effective width of a two-dimensional peak along the primary retention axis steadily increases with increasing sampling period. However, peak broadening remains below 30% if the sampling period is less than the standard deviation of the primary peak multiplied by a factor of 2.0.

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