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Variance contributed by pressure induced injection in capillary electrophoresis

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Abstract

The contribution of variance from a pressure injection to the final analyte peak in capillary electrophoresis (CE) is studied quantitatively. The band broadening process of CE can be described by the product of the Laplace transformed contributing factors; the inverse transform of the product gives the final peak shape which is the convolution of the original functions. The CE process is, therefore, similar to the chromatographic processes described by Sternberg. Based on the additivity of the variance, the injection induced variance is transferred directly to the final peak. The differences between the concepts of band broadening and peak broadening are discussed. Because the injection length from a pressure induced injection is the same for all analytes, and the migration rates during the CE separation are different for these analytes, the variance contributed by injection is larger for slower migrating analytes. The pressure forced flow generates a parabolic component in an injected sample plug, and this should also be considered in calculating the total injection length (τ). The variance is $(1/16)\tau^2$ for a sample plug with a Gaussian concentration profile, and $(1/12)\tau^2$ for one with a rectangular profile. It is demonstrated that the total variance of an analyte peak increases at the same rate as the injection variance. The difference between the total variance and the injection variance is contributed by longitudinal diffusion and other factors.

Keywords: Peak shape; Band broadening; Peak broadening; Injection methods; Nucleosides; Nucleotides

1. Introduction

Both the column used in chromatography and the capillary used in CE act as Gaussian operators during a separation, but the capacity of the Gaussian operator for the capillary in CE is much smaller. Thus, the injection length in CE may contribute to sample band width much more significantly. The theory describing the relationship between the injection variance and the total variance of the analyte peak in gas chromatography has been discussed by Sternberg [1].

Sternberg's theory describes how an ideal chromatographic column, that acts as a perfect Gaussian operator, blends the effect of a sample plug into the final analyte peak. The variance resulting from different shaped plugs is obtained by using the second derivative of a function that describes the plug shape [2]. If a cylindrical plug (rectangular in concentration profile) with a length, τ, is injected onto the column, the resulting contribution to the final peak variance is equal to $\tau^2/12$. If a plug with a Gaussian concentration distribution around its center of gravity (Gaussian profile) is injected, the variance contributed to the final peak is $\tau^2/16$.

The effect of electrokinetic injection in CE has

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been studied previously [3–7]. Some authors have assumed that the injection plug is perfectly cylindrical, and used the constant 1/12 to test other results when electrokinetic injection is used [3–6]; others have shown that when plotting the total peak variance against the injection variance, the slope is usually somewhere between 12 and 16 when optical gated injection was employed [7].

Pressure injection, or sometimes hydrodynamic injection, is the most common injection method in commercial automated CE systems. However, the effect of injection length on the final analyte peak shape has not been systematically studied. This paper demonstrates that pressure injection and electrokinetic injection not only differ in mechanism, but also in the amount of variance contributed to the final analyte peak.

2. Theory

2.1. The variance of an analyte peak

The efficiency of a separation column, or the number of theoretical plates, N, is obtained by:

$$N = \frac{L^2}{\sigma^2_{\mathrm{Tot,B}}} \tag{1}$$

where L is the effective length of the capillary, $\sigma_{\mathrm{Tot,B}}$ is the total standard deviation of a band on the column, which is 1/4 of the baseline band width if the concentration profile of the band is Gaussian, and $\sigma^2_{\mathrm{Tot,B}}$ is the variance of the band. It should be noted that the units of standard deviation in Eq. (1) have to be units of length. When the analyte band passes through the detection window, the band width is translated to peak width, and the band variance is transformed to peak variance. The total variance of a peak is defined as:

$$\sigma^2_{\mathrm{Tot}} = \sum_{k=1}^{n} \sigma^2_k \tag{2}$$

where σ^2_{Tot} is the total variance of the peak, and k represents the different factors that contribute to peak broadening. Eq. (2) is called the additivity of variance. If injection volume and longitudinal diffusion are the major factors that contribute to peak broadening, the total variance will be:

$$\sigma^2_{\mathrm{Tot}} = \sigma^2_{\mathrm{Inj}} + \sigma^2_{\mathrm{LD}} + \sigma^2_{\mathrm{Other}} \tag{3}$$

where σ^2_{Inj}, σ^2_{LD} and $\sigma^2_{\mathrm{Other}}$ are the variances caused by injection, longitudinal diffusion and other factors, respectively. The variance caused by other factors has been discussed by Hjertén [8] and Cheng et al. [9]. The σ_{Tot} in Eq. (3) is often measured from the analyte peak, thus, has units of time. All terms on the right hand side of Eq. (3) have to be converted to time as well before the additivity of variance can be used. Therefore, the variance contributed by an identical sample plug length for different analytes can contribute to the analyte peak very differently if the migration rates of the analytes are different. It should be noted that band broadening is described in the distance domain and peak broadening is described in the time domain.

2.2. Laplace transform and its first and second moments

When a sample is injected onto the capillary column, the concentration profile of the sample can be described by a certain mathematical function, $I(t)$, such as a rectangular function or a Gaussian function. The Laplace transform of the input function is:

$$L\{I(t)\} = \int_0^\infty \mathrm{e}^{-st} I(t)\mathrm{d}t = i(s) \tag{4}$$

where e^{-st} is called the kernel function for the Laplace transform. One should note that the transformed function $i(s)$ is the image of the function $I(t)$ in the domain of a new variable, s. Two useful applications of Laplace transforms in separation science are the first and second moments of the response function. The first and second derivatives of the response function are:

$$i'(s) = L\{-tI(t)\} = \int_0^\infty \mathrm{e}^{-st}(-t)I(t)\mathrm{d}t \tag{5}$$

$$i''(s) = L\{(-t)^2 I(t)\} = \int_0^\infty \mathrm{e}^{-st} t^2 I(t)\mathrm{d}t \tag{6}$$

The first and second moments, M_1 and M_2, about the origin of the transformed function are obtained from the first and second derivatives to the original transform function while the s in the kernel function approaches zero. As $s \rightarrow 0$,

$$i(0) = \int_0^\infty I(t)\mathrm{d}t \tag{7}$$

$$M_1 = i'(0) = \int_0^\infty (-t)I(t)\mathrm{d}t \tag{8}$$

$$M_2 = i''(0) = \int_0^\infty (-t)^2 I(t)\mathrm{d}t \tag{9}$$

If the t is evaluated relative to the center of gravity, $\bar{t}$, the integration of all the t values of Eq. (8) (which is the first moment, M_1) should be equal to zero, and $\bar{t}$ can be obtained by solving the resulting equation.

$$\int_{\text{all } t \text{ values}} (t - \bar{t})I(t)\mathrm{d}t = 0 \tag{10}$$

For a normalized Gaussian input function:

$$I(t) = \frac{C_t}{C_0} = \frac{1}{\sigma\sqrt{2\pi}} \cdot \exp\left[-\frac{1}{2}\left(\frac{t - t_0}{\sigma}\right)^2 \right] \tag{11a}$$

where C_t is a Gaussian function, C_0 is the value at the center of gravity (the maximum) of the function, the center of gravity is $\bar{t} = t_0$.

For a normalized plug injection, the input function is $C_t = C_0 = 1$, where $0 \le t \le \tau$, and the center of gravity is $\bar{t} = \tau/2$.

The second moment, or the variance, σ^2, is obtained by:

$$M_2 = \sigma^2 = \int_{\text{all } t \text{ values}} (t - \bar{t})^2 I(t)\mathrm{d}t \tag{11b}$$

For a Gaussian input function, the variance is the same as that of the input function. Therefore, if we define the injection length of a Gaussian shaped plug as $\tau = 4\sigma$, the resulting variance, σ^2, should be $(1/16)\tau^2$.

In Sternberg's paper, the σ^2 for a Gaussian input function is said to be $(1/36)\tau^2$. This arises from a rather unconventional definition of τ, which was defined as 6σ. Sternberg defined the τ as such only to match the results from a then widely quoted paper by Guiochon [10]. As can be seen in Table 3 of Guiochon's paper, however, the results were far from conclusive. The peak width of a Gaussian function was defined as 4σ, so the effective injection length, τ, should be defined as 4σ rather than the 6σ used in Sternberg's paper.

For a rectangular input function $C_t = C_0$, $0 \le t \le \tau$, where τ is the plug length, the variance is calculated to be $(1/12)\tau^2$ based on Eq. (9).

2.3. The convoluted peak shape in CE

Because of the additivity of variances (Eq. (2)), the variance contributed by injection will be transferred directly to the final analyte peak. Therefore, the final peak shape is a simple convolution of the different factors that cause band broadening.

Another useful feature of the Laplace Transform is the product of two or more transformed functions. The inverse transform of the product gives the convolution of the original functions. This property is of primary importance in operational mathematics [11].

Assuming there are only two contributing factors in an ideal CE system, σ^2_{Inj} and σ^2_{LD}, the convolution of the two functions is:

$$L^{-1}\{i(s)f(s)\} = I(t)*F(t) = \int_0^t I(\tau)F(t - \tau)\mathrm{d}\tau \tag{12}$$

where $I(t)$ is the injection function, $F(t)$ is the function for longitudinal diffusion, $i(s)$ and $f(s)$ are their Laplace transforms respectively, and the * between two functions represents a convolution operation. The properties of convolution, the commutativity, the distributivity and the associativity, are shown in Eqs. (13–15).

$$I(t)*F(t) = F(t)*I(t) \tag{13}$$

$$I(t)*[F(t) + O(t)] = I(t)*F(t) + I(t)*O(t) \tag{14}$$

$$[I(t)*F(t)]*O(t) = F(t)*[I(t)*O(t)] \tag{15}$$

The $O(t)$ in Eqs. (14,15) is the function for other factors, assuming the other factors that contribute to the band broadening can be represented by one function. These properties are extremely important because they allow us to study each individual contributing factor to the band broadening process by approximating the other factors as one convoluted function.

Fig. 1 demonstrates some hypothetical situations in a CE system when injection and longitudinal diffusion are the only contributing factors to the final peak variance. The longitudinal diffusion term can be considered a convoluted function for all other factors in real cases. In Fig. 1, the injection plugs are represented by the rectangular functions in a(1), b(1) and c(1), and the diffusion factors are represented by the Gaussian functions. The peak width is defined as the distance between the two points on the baseline where tangents are drawn to the inflection points of a Gaussian peak. The standard deviation is defined as 1/4 of the baseline peak width except for the rectangular functions; the standard deviation for a rectangular function is defined as $(1/\sqrt{12})\tau$.

Fig. 1a(1) shows a situation when the diffusion variance is comparable to the injection variance. The standard deviation for the Gaussian longitudinal

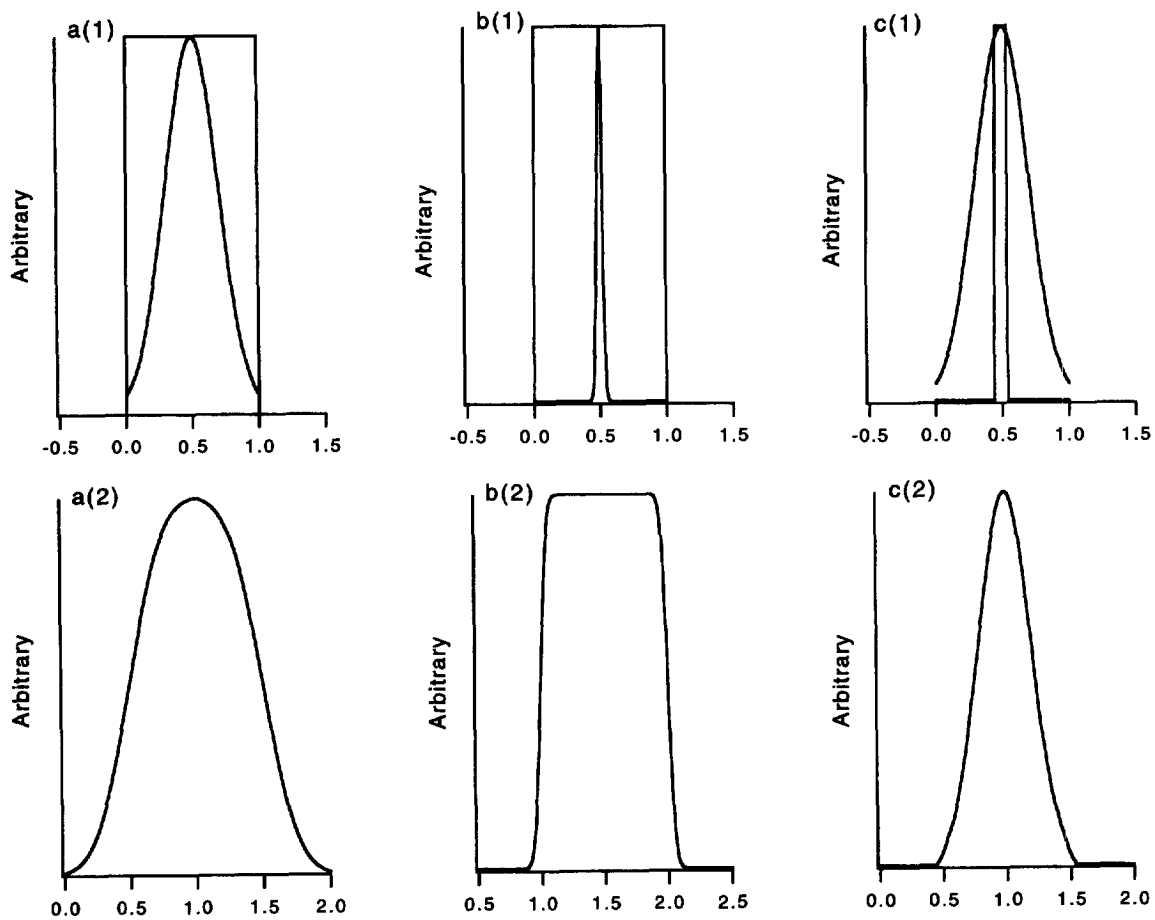

Fig. 1. The convolution of two functions. In this figure, a(1), b(1) and c(1) are arbitrary functions before being convoluted; a(2), b(2) and c(2) are the convoluted functions.

diffusion factor is 0.2, and the length of injection, τ, is equal to 1. The convoluted peak shown in Fig. 1a(2) has a variance that can be calculated from:

$$\sigma^2_{Tot} = \sigma^2_{LD} + \sigma^2_{Inj} = 0.2^2 + (1/12) \times 1 = 0.123$$

The peak width of the convoluted peak ($4\sigma_{Tot}$) is about 1.4, as shown in the figure.

Fig. 1b(1) shows a situation when the injection contributes much more than diffusion to the total variance. The τ for injection is still equal to 1, but the σ for diffusion is 0.02, 10 times smaller than that in Fig. 1a. The total variance of the convoluted peak in this case is 0.0837, and the peak width is 1.15, as shown in Fig. 1b(2). Fig. 1c(1) shows a situation when the injection τ is 0.093, and the σ_{LD} is 0.2. The total variance and the peak width of the convoluted peak are 0.0486 and 0.88, respectively.

3. Experimental

3.1. Apparatus

A Beckman P/ACE 5500 CE system was used for the experiments. The UV absorption was monitored at 254 nm. System Gold software from Beckman was used for data collection, analysis and system control through a 486 PC computer. A 57 cm long (50 cm to detector)×75 μm I.D., fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used for the separations.

3.2. Chemicals

Adenosine (A), guanosine (G), uridine (U) and thymidine (T) are free bases. Adenosine 5′-mono-, di- and tri phosphate (AMP, ADP, ATP); uridine 5′-mono, di- and tri phosphate (UMP, UDP, UTP); cytidine 5′- mono-, di- and tri phosphate (CMP, CDP, CTP); guanosine 5′ mono-, di- and tri phosphate (GMP, GDP, GTP); and thymidine 5′ mono-, di- and tri phosphate (TMP, TDP, TTP) are sodium salts (Sigma, St. Louis, MO, USA). Penciclovir (PCV, a nucleoside analog), 9-(4-hydroxy-3-hydroxymethyl-but-1-yl)guanine, was provided by SmithKline Beecham Pharmaceuticals (Brentford, UK). Hydroxypropyl-β-cyclodextrin (HP-β-CD) was purchased from Aldrich (Milwaukee, WI, USA).

The nucleosides and nucleotides were dissolved in distilled water to prepare stock solutions with concentrations ranging from $5.0 \cdot 10^{-2}$ to $1.0 \cdot 10^{-3}$ *M*. A solution of $5.53 \cdot 10^{-3}$ *M* PCV was prepared by dissolving the solid in water and was stored at −20°C. The frozen samples were thawed and a mixture of the above nucleosides and nucleotides was prepared by mixing 20 μl of each component from its stock solution; the resulting concentrations were $2 \cdot 10^{-4}$ to $2.5 \cdot 10^{-4}$ *M*. This mixture was further diluted by adding 20 μl of the mixture into 200 μl of electrophoresis buffer ($1.8 \cdot 10^{-5}$ to $2.3 \cdot 10^{-5}$ for each analyte) before being injected onto the capillary.

3.3. CE methods

The electrophoresis buffer was 160 m*M* borate (pH 9.1) with 60 m*M* HP-β-CD. The buffer was prepared from distilled, deionized water filtered through a 0.2 μm filter prior to use. Before the capillary was used the first time, the capillary was treated with 0.1 *M* NaOH for 15 min, followed by a 5 min rinse with deionized water and a 5 min rinse with electrophoresis buffer. Between runs, the capillary was cleaned and equilibrated with a 5 min rinse with the running buffer. Sample injection was carried out by applying a 0.5 p.s.i. (3.447 kPa) pressure to the sample vial for a specified period of time. The injection times are specified in the text. A voltage of 25 kV was used for the separation, and a temperature of 20°C was maintained throughout the experiment.

4. Results and discussion

4.1. The injection plug

The average linear velocity of a liquid in a capillary tube under pressure is described by Poiseuille's equation. However, the pressurized flow gives a parabolic flow profile at the front of the injection plug. It should be noted that Poiseuille's law only describes the average velocity of a sample plug as illustrated in Fig. 2.

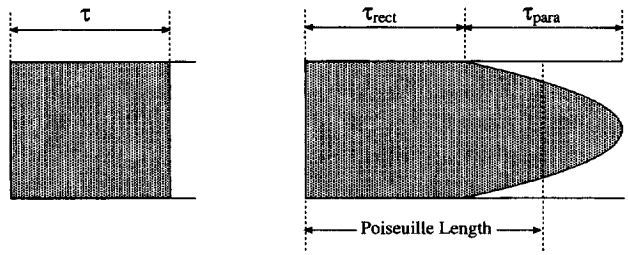

Fig. 2. The ideal and non-ideal injection plugs. (a) The ideal plug can be described by a rectangular function. (b) The non-ideal plug consists of a rectangular portion and a parabolic portion. The length calculated by Poiseuille's equation using the average flow-rate underestimates the injection length. The parabolic portion has a negative-sigmoidal concentration profile.

A perfect plug injection is demonstrated in Fig. 2(a), and the signal profile (or the profile of the amount of analyte along the capillary) is represented by a rectangular function. The plug length, as defined earlier, is τ. Fig. 2(b) demonstrates a non-ideal situation where the injected sample plug has two components: τ_{rect} and τ_{para}. The value of τ_{rect} is shorter than the length of a perfect plug calculated by Poiseuille's equation. The value of τ_{para}, however, is often overlooked. The real sample length should be longer than the one calculated by Poiseuille's equation, as shown in Fig. 2(b). If the effective length of the sample is used (including the parabolic front), the variance would be larger than that calculated from the ideal plug injection.

To obtain the real injection length, a narrow plug of thymidine was injected onto the capillary, and pushed through the capillary by a pressure of 20 p.s.i. (137.9 kPa). The absorption was monitored at 254 nm, and the result is shown in Fig. 3.

The values of τ_{rect} and τ_{para} can be obtained by analyzing Fig. 3. From the center of gravity of the absorption signal, the average linear velocity of the sample is obtained; from the width of the peak, the width of the parabolic profile can be estimated. τ_{para} is obtained from the band broadening observed in the chromatogram at the lower part of Fig. 3, and τ_{rect} is calculated by subtracting τ_{para} from the product of the maximum linear velocity of the analyte and the injection time.

The viscosity of the liquid is calculated by Poiseuille's equation:

$$\eta = \frac{\Delta P r^2}{8UL_c} \tag{16}$$

where U is the average linear velocity measured from Fig. 3 (2.87 cm/s), ΔP is the pressure (20 p.s.i.), r is the radius of the capillary ($37.5 \cdot 10^{-4}$ cm) and L_c is the total capillary length (57 cm). When p.s.i. is replaced by $6.893 \cdot 10^4$ g/(cm s), η, the apparent viscosity, is calculated to be $1.69 \cdot 10^{-2}$ g/(cm s), or 1.69 cP, for 160 mM borate buffer with 60 mM HP-β-CD.

The average linear velocity during the injection, under a pressure of 0.5 p.s.i., can be obtained by rearranging Eq. (16). The average flow-rate is 0.63 mm/s.

The Gaussian shaped peak shown in Fig. 3 is the result of the band (peak) broadening caused by the pressurized flow, and the peak width is equal to τ_{para}. The retention time for the peak pushed through by a pressure of 20 p.s.i. is 17.4 s, and the peak width is 8.3 s. Note that without molecular diffusion, the thin layer of injected sample would have flown through the capillary at a rate of two times the average flow-rate, and with a uniform concentration profile throughout the capillary. The parabolic front is the result of molecular diffusion in a small diameter capillary [12]. Although the length of the parabolic portion is proportional to the square root of the injection time, the distance traversed by the injected fluid is proportional to the injection time.

The maximum flow-rate, or the flow-rate of the parabolic front was obtained by using the effective

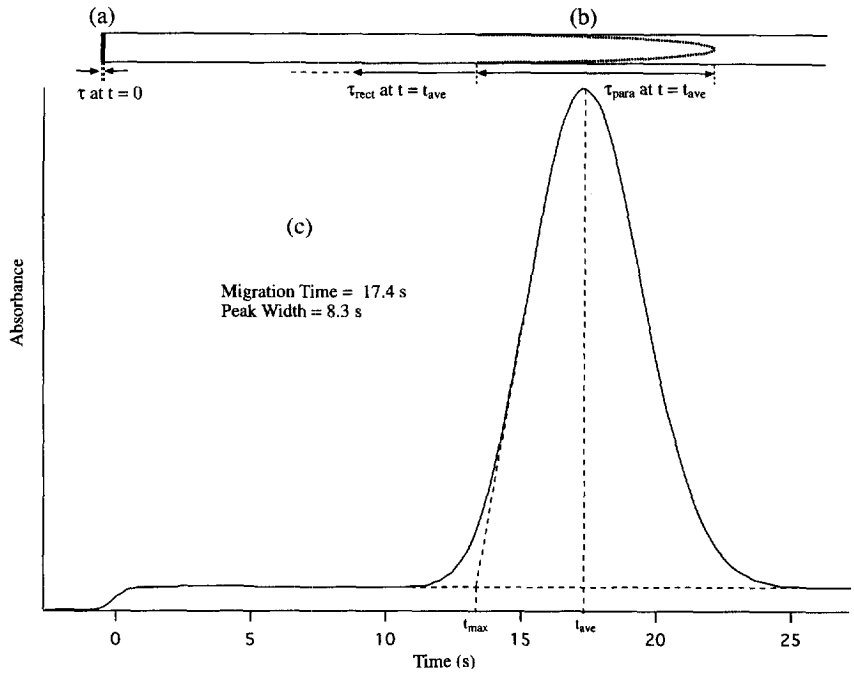

Fig. 3. The absorption signal from pushing a thin plug of thymidine through a 57 cm capillary (50 cm from inlet to the detection window)×75 μm I.D., with a pressure of 20 p.s.i.. (a) A thin layer sample plug injected onto the capillary. (b) The rectangular and parabolic components caused by a 20 p.s.i. pressure for 17.4 s. (c) The absorption signal. Note that (b) and (c) are not drawn to scale because (b) is the band on the column, and (c) is the time domain signal. The value of t_{ave} is used to calculate the viscosity, and t_{max} is used to calculate the total injection length.

capillary length divided by the time at the starting point of the peak [which is $17.4-(0.5\cdot 8.3)=13.2$ s, or t_{max} in Fig. 3]. The peak width in Fig. 3 is much longer than the injection length (τ at $t=0$), and the band broadening is solely contributed by the pressure induced parabolic flow. It should be noted that the Gaussian shaped peak in Fig. 3 results from a thin layer of sample which outlines the front of the parabolic flow profile. In a real injection, the plug is filled with sample as shown in Fig. 2(b), and the concentration profile for τ_{para} has a negative sigmoidal shape. Therefore, the injected sample as a whole can be considered close to a rectangular plug. The length of the sample plug can be calculated by:

$$\tau = \tau_{rect} + \tau_{para} = U_{max}\Delta t \qquad (17)$$

Where Δt is the injection time and U_{max} is the maximum flow-rate obtained from dividing the effective capillary length by t_{max}. In this case, because the pressure used for injection was 0.5 p.s.i., the maximum velocity of the sample during the injection is obtained by:

$$\frac{500\text{ mm}}{13.2\text{ s}} \times \frac{0.5\text{ p.s.i.}}{20\text{ p.s.i.}} = 0.95\text{ mm/s}$$

4.2. The injection variance

Fig. 4 shows a set of electropherograms with different injection lengths. It can be observed that with a 2 s (s for seconds, not the "s" in Eq. (4)) injection, the total peak variance is mainly contributed by diffusion. The peak widths are significantly larger for peaks with longer migration times. On the other hand, it can be observed that when the injection time is increased, the peak width increases significantly for all peaks.

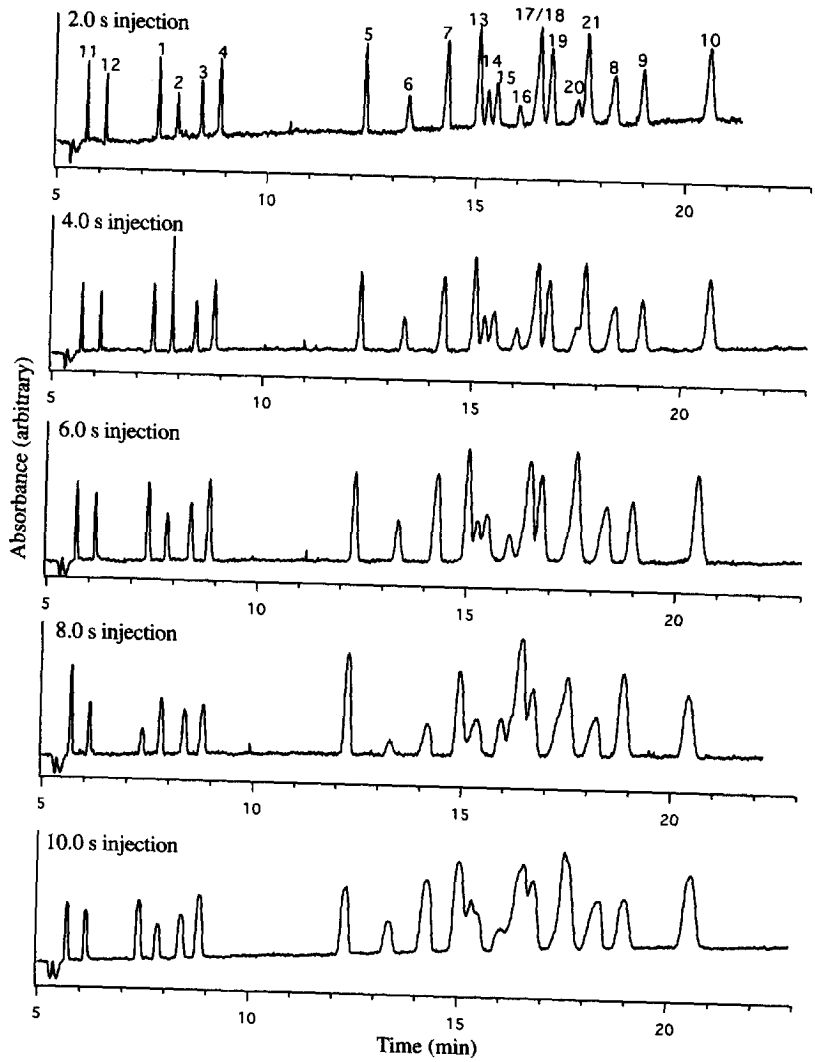

Fig. 4. The electropherograms of a group of nucleosides and their phosphates with different injection times. The peaks are: 1. A; 2. C; 3. G; 4. U; 5. TMP; 6. AMP; 7. ATP; 8. UTP; 9. CDP; 10. UDT; 11. T; 12. PCV; 13. GMP; 14. ADP; 15. GTP; 16. CMP; 17/18. TTP/TDP; 19. GDP; 20. CTP; 21. UMP.

Because of the additivity of the variance, when a variance, σ^2_{Inj}, is introduced from the injection, this variance will contribute directly to the final peak width, and can be described by the second moment of the injection function $[(1/12)\tau^2_{\mathrm{Inj}}$ for a rectangular profiled injection, $(1/16)\tau^2_{Inj}$ for a Gaussian shaped injection).

The standard deviation of the peak contributed by a rectangular profiled injection plug can be calculated by:

$$\sigma_{Inj} = \frac{\tau_{\mathrm{Inj}} t_{\mathrm{R}}^{\mathrm{A}}}{\sqrt{12} L_{\mathrm{D}}} \tag{18}$$

where τ_{Inj} is the plug length, $t_{\mathrm{R}}^{\mathrm{A}}$ is the migration time of the analyte A, and L_{D} is the effective length of the

capillary (from the injection end to the detector). L_D/t_R^A is the velocity of the analyte, $\tau_{Inj}t_A^R/L_D$ is the time required for the plug to go through the detector, and $1/\sqrt{12}$ of this time is defined as the standard deviation, σ_{Inj}. For a Gaussian profiled injection, the $1/\sqrt{12}$ in Eq. (18) is replaced by 1/4.

The same plug length contributes very differently to the total variance of each analyte peak. The variance is smaller for faster migrating analytes, and bigger for slower migrating analytes. This is a unique phenomenon that only happens when pressure injection is used. Eq. (18) has to be used to calculate the standard deviation for each analyte. The dependence of the variance on the injection time for each analyte studied is demonstrated in Fig. 5. It is obvious that the injection contribution to the total variance is much larger for slower migrating peaks because the time required for the same plug length to migrate through the detection window is much longer.

4.3. Total variance and injection variance

The relationships between the total variance of selected analytes and the variance introduced by injection are shown in Fig. 6. It is important to note that the variance of each contributing factor has to be converted to temporal variance because the peak width is usually expressed in time (s). Except for the 8 s and 10 s injections, the total variance is obtained by fitting each peak with a Gaussian function:

$$P_G = K0 + K1 \cdot \exp\left[-\left[\frac{(x - K2)}{K3} \right]^2 \right] \quad (19)$$

where $K2$ is the migration time (or the center of gravity of the peak) in min, and $60 \cdot (K3)/\sqrt{2}$ is the σ_{Tot} in s. Because the peaks in the 8 s and 10 s injection runs are no longer Gaussian, the peak width at the baseline was measured and used as $4\sigma_{Tot}$. The width of peak number 2 in the 4 s injection experiment was also used as $4\sigma_{Tot}$ because the peak height was marred by a bubble. The total variance is σ_{Tot}^2 in s^2. The injection variances of both the rectangular and Gaussian profiles are calculated and shown in Fig. 6. The plug length is calculated by Eq. (17).

It is observed that the variance calculated based on Gaussian profiled plugs underestimates the variance contributed by injection, especially when the plug

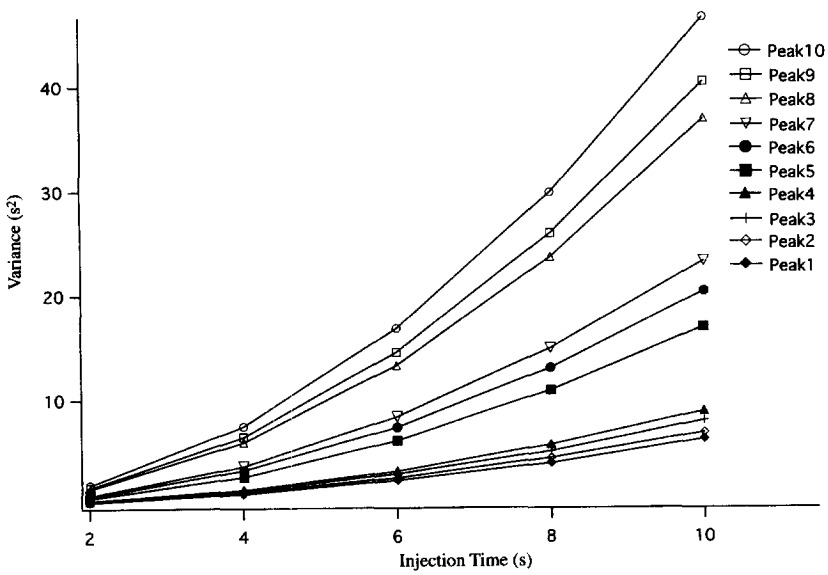

Fig. 5. The variance contributed from the same injection length to the peaks of analytes with different migration rates. The longer injection length contributes much more to the slower moving analytes than to the faster moving analytes. The peak identification is the same as the peaks numbered in Fig. 4.

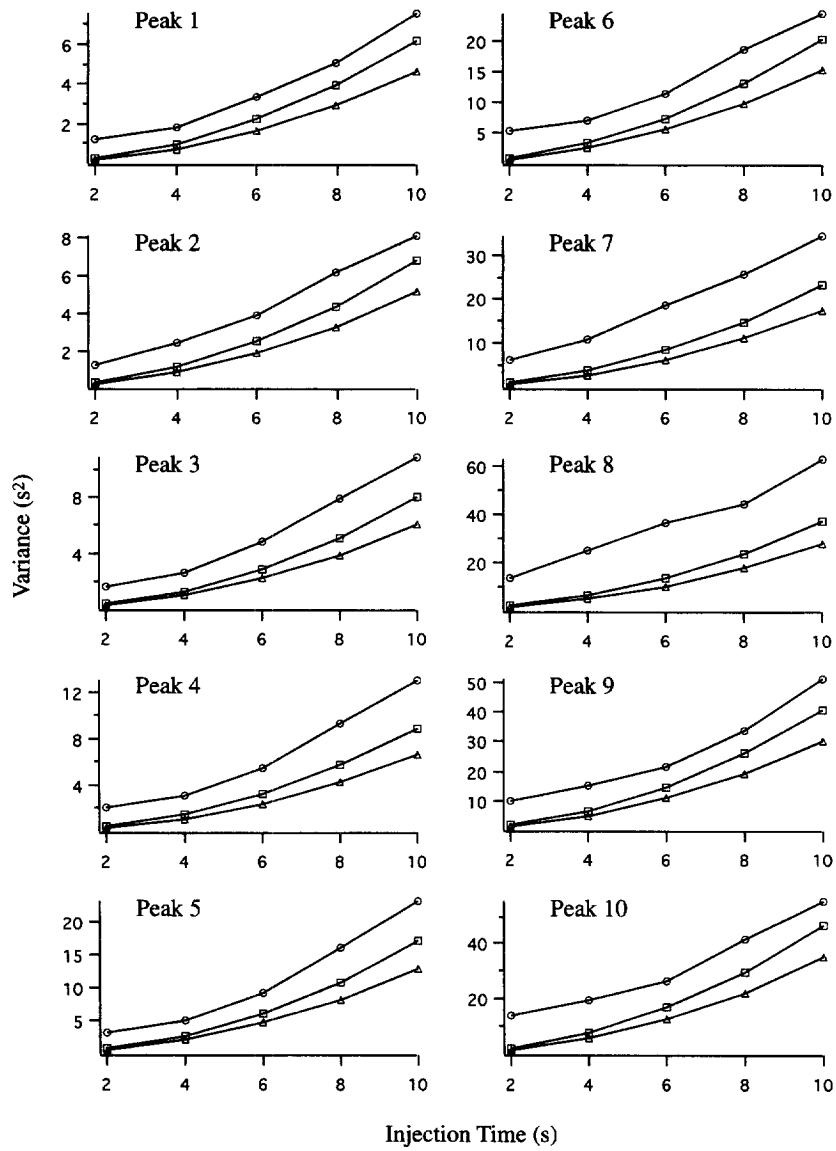

Fig. 6. The variances contributed from the injection and the final peak variances for selected peaks are plotted against the injection time. The circles are the measured total peak variance, the triangles are the variances calculated by assuming the injection plugs have Gaussian concentration profiles and the squares are the variances calculated assuming the injection plugs have rectangular profiles. The lengths of the sample plugs are calculated from Eq. (17). Peaks 1 to 10 in Fig. 4 are analyzed.

length is large. The difference is not as big between a rectangular profile and a Gaussian profile when the injection plug is small. The rectangular sample plug may have become close to a Gaussian profile during the time between injection and the start of separation because of longitudinal diffusion. For a longer injection length, the profile more closely resembles a rectangular profile. The difference between σ^2_{Tot} and σ^2_{Inj} is the variance caused by longitudinal diffusion (σ^2_{LD}) and other factors ($\sigma^2_{\mathrm{Other}}$). The σ^2_{LD} is larger for slower migrating peaks because it is determined by the Einstein equation ($\sigma^2_{\mathrm{LD}} = 2Dt(t_{\mathrm{R}}/L_{\mathrm{D}})^2$), where

D is the diffusion coefficient, t is the time allowed for the sample to diffuse which is mainly the migration time and $(t_R/L_D)^2$) converts the distance domain variance to the time domain variance). The total variance of each peak increases linearly with the injection variance with a slope valued between 12 and 16, and an intercept equal to the variance caused by diffusion and other factors.

By examining Fig. 4 in more detail, one can see that the peaks in the electropherogram with the 2 s injection resemble the situation demonstrated in Fig. 1c, peaks 1 to 4 in the 10 s injection run resemble Fig. 1b, and the peaks in the 4 s or 6 s runs resemble Fig. 1a. This can be explained by the convolution of the injection function (close to rectangular) and the diffusion function (Gaussian).

Moore and Jorgenson [7] have demonstrated that with an optical gated injection method, the total variance correlates very well with the sum of the injection variance and the longitudinal diffusion variance. The optical gated injection has the advantage that the sample plug is almost a perfect rectangular plug for each analyte, but the disadvantage that the length of each sample plug injected is different because of the different migration velocities of the analytes during the injection. However, this "disadvantage" determines that the variance introduced by the injection for each analyte is the same. This can be applied to electrokinetic injection as well. Electrokinetic injection has the disadvantage that less of the slower migrating component in a sample is injected with a smaller volume than the faster moving ones. The advantage is, however, that the slower migrating component has a narrower plug width than the faster moving components, and this improves the resolution in cases where the limit of detection is not a critical issue.

Although the average velocity of a sample pushed by a constant pressure can be calculated by Poiseuille's equation, the parabolic flow profile adds more variance to the injected plug. Optical gated injection and electrokinetic injection, on the other hand, have nearly perfect rectangular concentration profiles, and the length of the plug can be easily calculated. However, Moore and Jorgenson's results [7] did suggest that for slower migrating analytes, a Gaussian profiled injection will fit their data better with a variance of $(1/16)\tau^2$. This is reasonable because if the plug length is very narrow, the rectangular plug will diffuse to a Gaussian concentration profile rather quickly. When the injection variance is between $(1/12)\tau^2$ and $(1/16)\tau^2$, it is likely that the concentration profile of the injection plug is in between a rectangular and a Gaussian, resulting from the convolution of an injection function (rectangular) and a diffusion function (Gaussian).

It should be noted that only the length, not the volume, of an injected sample plug contributes directly to peak broadening.

5. Conclusion

The variance contribution of a cylindrical injection to the final peak is similar for all analytes, moving fast or slowly in the capillary, for optical gated injection, or for electrokinetic injection. This is because faster moving analytes are injected with a longer plug, and slower moving analytes are injected with a shorter plug. In pressure induced injection, however, the plug length is identical for all analytes. It is important to distinguish between band broadening and peak broadening because the same sample length requires a different time to migrate through the detection window for analytes moving at different velocities. The injection variance is smaller for faster moving analytes, and larger for slower moving analytes.

Because the variance from injection is directly transferred to the analyte peak, as a rule of thumb, the injection length should be kept low as long as the detection limit is not a problem. If the separation of the slower moving analytes in a CE separation process is the main challenge, one should consider using electrokinetic injection.

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