ELSEVIER

Journal of Chromatography A, 744 (1996) 103–113

JOURNAL OF CHROMATOGRAPHY A

Origin and correction of bias caused by sample injection and detection in capillary zone electrophoresis

J.N. van der Moolen, H.F.M. Boelens, H. Poppe, H.C. Smit*

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands

Abstract

It is well known that electrokinetic sample introduction leads to a bias in CZE due to differences in the electrophoretic mobility of the substances. Therefore, a correction by multiplying the peak area with the migration time is often deemed necessary. With on-column detection, the area of a peak in the electropherogram depends on the velocity of the zone. In CZE every component has a different velocity, leading to a bias in detection. For that reason another correction by dividing the peak area by the migration time can be used. It will be shown that the injection and detection bias are inversely proportional to each other. This means that no correction of peak area is necessary for these biases in the case of electrokinetic injection being used in combination with UV detection, in contrast with hydrodynamic injection.

Keywords: Detection, electrophoresis; Injection methods

1. Introduction

The use of correlation techniques to improve the signal-to-noise ratio in chromatography has been known since 1970 [1]. Recently, the application of a correlation technique in capillary zone electrophoresis (CZE), resulting in correlation capillary zone electrophoresis (CCZE) [2], showed a considerably improved detection limit in comparison to conventional CZE. The major drawback of the use of CCZE is the high demand on the injection system. Reproducible and fast injections over a long time period are essential. Non-ideal injections lead to so-called ghost peaks and correlation noise in general [3]. Because of the strong relation between the injection and detection in CCZE, a study of the quality of the injection in CZE in relation to the detection has been made.

The two most commonly applied sample introduction methods in CZE are hydrodynamic (HD) and electrokinetic (EK) injection. The equipment for EK injection is simpler than the equipment for HD injection. Because of the parabolic flow profile, HD injection results in a more broadened zone than EK injection. However, HD injection does not change the composition of the injected sample, while EK injection does.

Various articles have been published during the last 15 years describing a bias caused by EK sample introduction. Jorgenson and Lukacs [4] were the first to point out that as the electrophoretic mobilities of the substances differ, EK injection discriminates between species. This discrimination also depends on the composition (pH, conductivity) of the sample solution. In this report we have assumed that the sample solution has been matched carefully to the buffer with respect to pH and conductivity. This has been considered to be good practice in CZE, and a pre-requisite for reliable results when using EK injection. Discrimination between components in

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00440-2

itself should not be a problem, because calibration has to be performed in quantitative analyses. Nevertheless, Huang et al. [5] proposed to correct for this kind of bias by multiplying peak area ratios by the migration time ratios of the components. According to the authors, but for reasons unclear to us, this correction would be valid only if the applied voltage during the EK injection is nearly the same as during the CZE run. Despite the feasibility of this correction, the injection bias was a reason for Huang et al. [6,7] to use only HD injections. Other authors referred to the bias in a comparison with HD injection [8–12] or injection by spontaneous fluid displacement [13]. A few [14,15] authors did use the correction together with EK injection. The quantitative precision can be improved by the use of internal standards [16]. Lee and Yeung [17] showed that the precision can be improved also by proper correction of the bias. Theoretically, this correction would eliminate fluctuations due to changes in electroosmotic flow.

Another kind of bias in CZE occurs as a result of the detector selected. With an on-column concentration detector, like a UV or fluorescence detector, as is usually applied, the area of a peak in the electropherogram depends on the velocity of the zone. In chromatographic techniques, as for example HPLC, all components move through the detector cell with the same velocity, the velocity of the mobile phase. However, in CZE every component has a different velocity. Thus a slowly migrating analyte will contribute more to the peak area than a faster migrating analyte.

As an alternative, peak height can be used as the quantitative parameter instead of peak area, because peak height is less influenced by velocity differences. However, in contrast to the peak area, the peak height will be influenced by zone broadening caused by, e.g., diffusion or thermal influences. Nevertheless, if peak area has been chosen as quantitative parameter, one will have to deal with bias caused by velocity differences during detection. Again, calibration has to be performed in quantitative analyses, so this bias should not be a problem also. A means of dealing with the bias was pointed out by Hjerten et al. [18], who were the first to pay attention to this bias. The authors corrected for it by dividing the peak width or peak area by the migration time of the component. In their article no justification for this correction procedure was supplied. Zhu et al. [19] confirmed this aspect experimentally and other authors used the division by the migration time of the components to normalise peak areas [20,21]. Moring et al. [22] and Goodall et al. [23] point to another reason for using this normalisation; in quantitative analyses, it will increase the reproducibility as it will also correct for possible variations in zone velocities between experiments. Altria [24] showed the importance of peak area normalisation in the quantitative determination of drug related impurities. Huang et al. [25] stated that it would be more correct to multiply the width by the zone velocity instead of dividing it by the migration time, which may be of importance if results obtained on different systems have to be compared. However, as the instantaneous zone velocity is not measured, the estimation from the migration time is generally the only alternative.

In this publication it will be shown that this normalisation procedure will not only correct for differences in velocity between components or variations in migration times, but it will also offer the possibility to compare the results of experiments, performed under different circumstances like voltages, capillary lengths or detection cells, more correctly. Furthermore, some attention will be paid to the correction procedure if injection circumstances vary.

In the case of EK sample introduction being used in combination with UV detection, both the described injection and detection bias are present. If the correction procedures for both biases are compared, it can be noticed that they are inversely proportional to each other. This means that in this specific case no correction of peak area is necessary, provided that the sample solution has the same pH and conductivity as the buffer. The theoretical explanation will be supported by experimental results.

2. Theory

2.1. Injection

As has been discussed previously [26], the quantity Q of species k introduced into the capillary by sample injection is given by

$$Q_k = \pi r^2 \cdot c_k \cdot l_k \quad (1)$$

where r is the capillary inner radius, c_k is the concentration of species k in the sample solution and l_k is the sample zone length. However, this equation is only valid if an ideal, block shaped injection pattern is assumed. In case the actual injection pattern is not ideal, the extra zone broadening caused by injection leads to more complicated equations. Some problems concerning non-ideal injection patterns will be discussed in detail later.

In the case of an ideal EK injection the length of the sample zone of species k is determined by

$$l_k = t_{inj} \cdot u_{tot,k,inj} \quad (2)$$

where t_{inj} is the injection time and $u_{tot,k,inj}$ is the total velocity of species k during injection. In general, the total velocity $u_{tot,k}$ depends on the voltage V, the capillary length L and the total mobility of k, $\mu_{tot,k}$:

$$u_{tot,k} = \frac{V}{L} \cdot \mu_{tot,k} \quad (3)$$

Since the total mobility is the sum of the electrophoretic mobility μ_k and the electroosmotic mobility μ_{osm},

$$\mu_{tot,k} = \mu_k + \mu_{osm} \quad (4)$$

Eq. 2 becomes

$$l_k = t_{inj} \cdot \frac{V_{inj}}{L} \cdot (\mu_k + \mu_{osm}) \quad (5)$$

Where V_{inj} is the injection voltage. Combining Eqs. 1 and 5 leads to

$$Q_k = \pi r^2 \cdot c_k \cdot t_{inj} \cdot \frac{V_{inj}}{L} \cdot (\mu_k + \mu_{osm}) \quad (6)$$

This equation shows clearly the dependence of the injected quantity on the electrophoretic mobility of components.

Assuming a constant velocity during a run, the electrophoretic mobility of a component can be determined by measuring its migration time t_k:

$$t_k = \frac{l}{u_{tot,k}} = \frac{l \cdot L}{V \cdot (\mu_k + \mu_{osm})} \quad (7)$$

where l is the capillary length from the point of injection to the point of detection.

Multiplication of Q_k and t_k results in

$$Q_k \cdot t_k = l \cdot \pi r^2 \cdot c_k \cdot t_{inj} \cdot \frac{V_{inj}}{V} \quad (8)$$

which does not contain component dependent parameters apart from the concentration of species k.

As mentioned previously, Huang et al. [5] stated that it will only be possible to correct for the bias of the EK injection, if the applied voltage during the EK injection is nearly the same as during the CZE run. Nevertheless, Eq. 8 indicates that the correction is always possible. Furthermore it is possible to compare the results of CZE experiments, in the case of different experimental conditions being employed. Since the area of electrophoretic peaks is proportional to the injected quantity Q_k, it is concluded that in theory the bias in peak area caused by injection can be removed by multiplying the peak area with the migration time of the component.

2.2. Detection

A component moving through the detector cell of a UV detector will result in a peak-shaped detector signal having a certain height, width and area. For quantitative analyses, both peak height and peak area can be used. When it is first assumed that the optical detection window is infinitely small, the instantaneous detector signal of component k, $S_k(t)$, will be given by

$$S_k(t) = \epsilon_k \cdot b \cdot c_k \quad (9)$$

where ϵ_k is the molar absorptivity of species k, b is the pathlength of the light, the inner diameter of the capillary, and c_k is the concentration of species k. In contrast to the peak height, the peak area is not affected by zone broadening, caused by, e.g., diffusion. For that reason the area of the peak is often considered to be the preferable intensity parameter. The value of the peak area is then determined by the integral of the detector signal with respect to time:

$$A_k = \int S_k(t)\mathrm{d}t = \epsilon_k \cdot b \cdot \int c_k \mathrm{d}t \quad (10)$$

From Eq. 7 follows the dependency of the time on the length and the velocity:

$$dt = \frac{dz}{u_{tot,k}} \tag{11}$$

and considering that the concentration integrated over z should be equal to the amount per area cross section, Eq. 10 becomes

$$A_k = \epsilon_k \cdot b \cdot \int c_k \frac{dz}{u_{tot,k}} = \frac{\epsilon_k \cdot b}{u_{tot,k}} \cdot \int c_k dz$$

$$= \frac{\epsilon_k \cdot b}{u_{tot,k}} \cdot \frac{Q_k}{\pi r^2} \tag{12}$$

where Q_k is the injected quantity.

When the detection window is finite the total signal integral will be the average of Eq. 12 over the window length l_{det}. As a result this will only differ from Eq. 12 by a constant factor, to be combined with πr^2:

$$A_k = \alpha \cdot \frac{\epsilon_k \cdot b}{u_{tot,k}} \cdot Q_k \tag{13}$$

This equation describes clearly the dependence of the peak area on the velocity of the component. It follows also that the peak area can be corrected by multiplying it with the velocity. Assuming a constant velocity during a run, the velocity can be replaced by an expression using the migration time:

$$A_k^* = \frac{A_k}{t_k} \tag{14}$$

where A_k^* is the corrected, velocity independent, peak area.

Therefore, analogous to the description of the correction of the injection bias described before (Eq. 8), the migration time can be used to get rid of the differences in peak area. To correct for the injection bias the peak area has to be multiplied by the migration time. The detection bias, however, can be removed by dividing the peak area by the migration time (Eq. 14). One should be aware of the fact that both corrections can not compensate for velocity differences that occur during an experiment, e.g., as a result of changing mobilities, electroosmotic flow or voltage.

Since the peak area depends on the migration time (velocity), the reproducibility of the peak area will also depend on the reproducibility of the migration time. Korman et al. [21] studied the reproducibility of migration time, peak area and corrected peak area. The experiments with HD injection showed that in case of low reproducibility (1–5%) of the migration times the reproducibility of the area is also low (1–5%), but that the reproducibility of the corrected area remained good (less than 1%). Quantitative analyses of drug related impurities, and the chiral separation of a racemic pharmaceutical by Altria [24] confirmed the necessity of the peak area normalisation.

2.3. Electrokinetic injection combined with UV detection

Eq. 13 shows not only the dependence of the peak area on the velocity, but also on the injected amount. In case of EK injection the injected quantity has been described by Eq. 6. Combining both equations leads to

$$A_k = \alpha \cdot \frac{\epsilon_k \cdot b}{u_{tot,k}} \cdot \pi r^2 \cdot c_k \cdot t_{inj} \cdot \frac{V_{inj}}{L} \cdot (\mu_{tot,k})$$

$$= \alpha^* \cdot \epsilon_k \cdot b \cdot c_k \cdot t_{inj} \cdot \frac{V_{inj}}{V} \tag{15}$$

Although both the injected quantity with EK injection and the peak area with UV detection depend on the velocity, this equation proves that the peak area with UV detection after EK injection does not depend on the velocity. The only component specific parameters in the equation are the molar absorptivity and the concentration. The importance of this is that no correction of the peak area will be necessary, if both EK injection and UV detection are used. Since on-column UV detection is widely used in CZE [24,27], it is important to note that for peak area determination the so-called discrimination disadvantage of EK injection is not relevant.

3. Experimental

The CZE system used (Table 1) consists of a Prince programmable injection system for capillary electrophoresis, an UV detector, a 50 μm I.D. fused-silica capillary, and a recorder.

A 0.03 mol/l phosphate buffer at pH 6.01 was used as background electrolyte. Sodium phosphate

Table 1
List of equipment and conditions

Apparatus/Condition	Type
CZE system	Prince, Lauer Labs, Emmen, The Netherlands.
UV detector	Linear UVIS 200, Linear Inst. Fremont, CA, USA.
UV wavelength	210 nm.
Capillary	50 μm I.D., 375 μm O.D., Fused-Silica, Polymicro Technologies, Phoenix, AZ, USA.
Recorder	Kipp and Zonen, Delft, The Netherlands.
Capillary length	73 cm
Capillary length to detector	58 cm
Injection time	3 s
Injection pressure (HD)	100 mbar
Injection voltage (EK)	5, 10 or 15 kV
Separation voltage	5, 10 or 15 kV
Concentration sample	10E−4 mol/l
UV–Vis Spectrophotometer	Philips PU 8700 Series, Philips, Eindhoven, The Netherlands.
Cell spectrophotometer	Quartz glass Suprasil, 1.001 cm

was obtained from Merck (Darmstadt, Germany). Benzyltrimethylammonium chloride (BTMA, obtained from Aldrich, Steinheim, Germany), benzyltriethylammonium chloride (BTEA) and benzyltributylammonium chloride (BTBA, both obtained from Merck), dissolved in buffer, were used as sample. The buffer as well as the sample solution were filtered through a 0.45 μm filter. The capillary was rinsed each day with sodium hydroxide, water and buffer.

The peak height h, the peak width at $0.25h$, $w_{0.25}$, and the migration time t of the recorded electrophoretic peaks have been manually determined. The peak area was calculated from the measured peak height and peak width, according to [28]:

$$A = 0.753 \cdot h \cdot w_{0.25} \tag{16}$$

After A/D conversion, the time course of the injection voltage was integrated on a pc.

The UV-spectrum of the components was recorded with phosphate buffer as the reference.

4. Results and discussion

According to Eq. 15 the measured peak area depends on only two component-specific parameters, the concentration and the molar absorptivity. Only solutions with a known concentration have been used in the experiments. The measured UV spectra of the components show comparable pictures. The molar absorptivities of the components have been determined at a wavelength of 210 nm. From the results (Table 2) it can be concluded that only small differences exist between the molar absorptivities of the components. Peak heights and peak areas have been corrected for the small differences in molar absorptivity and concentration, so any difference in signals and peak areas has to be attributed to different electrophoretic behaviour.

4.1. Detection

In the study of bias between components caused by on-column UV detection, HD injections were

Table 2
Molar absorptivities of the components

Component	Concentration (10^{-4} mol/l)	λ_{max} (nm)	ϵ_{max} ($l \cdot mol^{-1} \cdot cm^{-1}$)	ϵ_{210} ($l \cdot mol^{-1} \cdot cm^{-1}$)
BTMA	1.040	209.6	0.842	0.833
BTEA	0.9803	209.6	0.890	0.890
BTBA	1.001	209.7	0.918	0.918

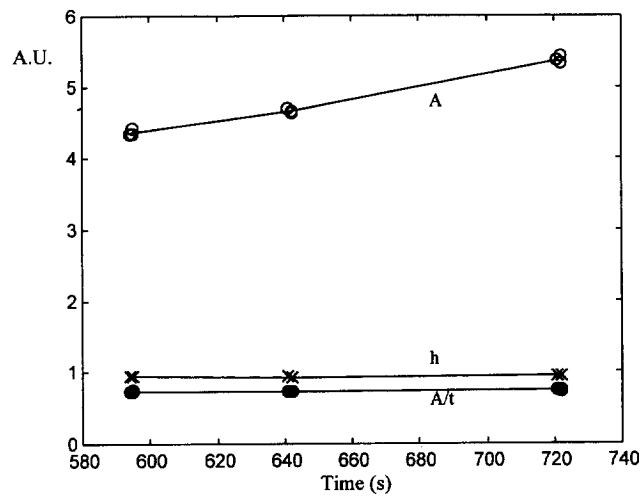

Fig. 1. Relation between peak parameters and migration time of three HD injected components. h=peak height; A=peak area; A/t=corrected peak area.

performed in order to ensure that equal amounts of the components were injected. As can be seen in Fig. 1 the peak area increases from the component with a low migration time (BTMA) to the component with a higher migration time (BTBA), but the peak heights are the same for all components. The corrected peak areas are approximately equivalent. The reproducibility of the peak parameters is better than, or equal to 1% (n=3). It is concluded that dividing the peak area by the migration time can be used to correct the detection bias between components.

The fact that peak area at a fixed injection quantity is inversely proportional to the total velocity (Eq. 13) indicates that experiments can be compared only if the same voltage is applied (Eq. 3). However, the correction described in Eq. 14 can be employed even if this is not the case. This has been studied by performing several experiments at which the separation voltage has been varied. If the experimental results gathered in Fig. 2 are examined, some remarks can be made:

- The changes in peak area are more or less in agreement with the change in migration time.
- The correction of peak area according to Eq. 14 gives satisfactory results.

The above confirms that it will be possible to correct adequately for the bias in peak area, if peaks recorded under different experimental conditions have to be compared. The reproducibility of the parameters is better than 1.5% (n=5).

Fluctuations of the voltage or the total mobility between experiments will decrease the reproducibility of the migration time and the peak area via the detection bias (Eq. 13). Yet, the reproducibility of the migration time is good in the present set of experiments (R.S.D.<0.5%, n=5). As a result the correction applied does not improve the reproducibility of the peak area, as has been shown previously in literature [21].

4.2. Injection

The injection bias between experiments has also been studied. Several experiments with different injection voltages were performed. An increase in the injection voltage, should lead to an proportional increase in the introduced amount of sample (Eq. 6). As a result the peak area should increase with a factor equal to the ratio between the voltages.

As can be seen in Table 3, the measurements do not completely confirm this expectation. At higher voltage the peak area is relatively smaller. This can possibly be caused by the shape of the injection. It was pointed out before [25] that the RC time constant (where R=resistance of electrolyte inside the capillary, and C=capacitance of circuit) of the

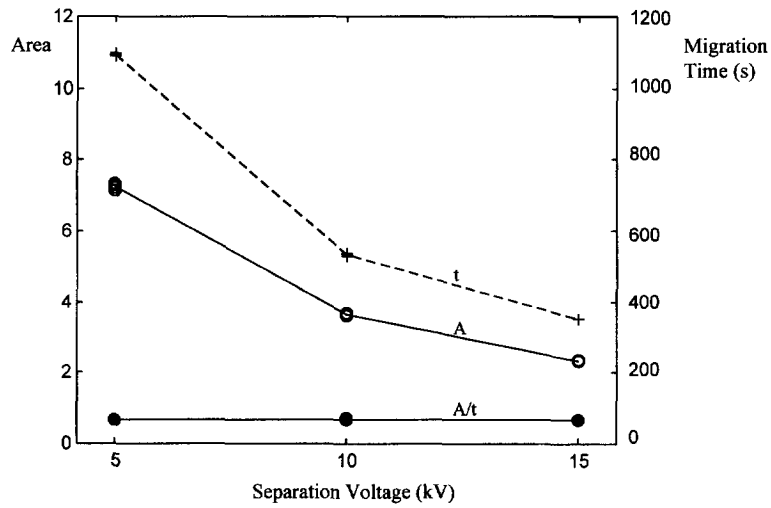

Fig. 2. Correction for detection bias between experiments with different separation voltage. $V_{inj.} = 10$ kV; component = BTMA; A = peak area; A/t = corrected peak area; t = migration time;

system is an important parameter when quantifying the size and shape of the injection plug. Another problem can be the injection of sample as a result of the withdrawal of the capillary from a sample solution, as was recently noted by Fishman et al. [13]. Because of the importance of the injection in the study of correlation CZE, and in order to quantify the influence of an abnormal shape, the actual injection pattern at 5, 10 and 15 kV has been studied.

The injection bias between components has not been studied, because experiments to demonstrate this bias would demand another detector. Moreover, the effect of this specific bias has already been discussed in the literature [5,14,15].

4.3. Shape of the injection

In an ideal case, the shape of the injection should be a block, which means that the time to reach a

Table 3
Experiments to study the injection bias, with corresponding R.S.D. values ($n = 5$)

V (kV)	V_{inj} (kV)	Electrophoretic Peak Area (AU·s)						Injection Area (AU·s)	
		BTMA		BTEA		BTBA			
		10^{-3}	RSD[a] (%)	10^{-3}	RSD[a] (%)	10^{-3}	RSD[a] (%)	10^{-3}	RSD (%)
5	5	3.84	0.5	3.68	0.6	3.76	1.1	0.70	1.07
	10	7.26	1.2	7.18	1.4	7.28	1.1		
10	10	3.64	1.0	3.58	0.5	3.58	0.9	1.37	0.43
	15	5.09	0.9	5.04	0.9	5.09	0.8		
15	10	2.33	0.7	2.30	1.8	2.33	0.6	1.98	0.33
	15	3.32	1.3	3.25	1.5	3.28	0.7		
5	$R_{10,5}$	1.888	0.012	1.954	0.017	1.936	0.015	1.960	0.008
10	$R_{15,10}$	1.399	0.008	1.408	0.007	1.415	0.008	1.444	0.003
15	$R_{15,10}$	1.423	0.010	1.412	0.015	1.410	0.007		

[a] For the ratios the standard deviation is given instead of the R.S.D. value.

higher or lower voltage or pressure level is infinitely small. However, in practise there is a short but finite time taken. This time is not reproducible due to the differing electrical capacity of the system from run to run. To improve the reproducibility of the injection [29], a trapezium shaped injection can be performed (Fig. 3).

In theory, the area, i.e., the voltage-time integral, of a 15 kV injection should be three times as large as the area of a 5 kV injection, and the area of a 10 kV injection twice as large. Table 3 compares the area of the measured injection voltage at 5, 10 and 15 kV with the electrophoretic peak area. As can be seen, the area ratios of the injection voltages are also smaller than the theoretical values. This agrees in part with the results presented in the former section on electrophoretic peak areas, so the main differences in electrophoretic peak area are probably caused by differences in the injected amount of the components.

To explain these differences, the shape of the injection has been studied in more detail. Fig. 4 clearly shows the difference between the theoretical shape (trapezium) and the actual shape of the injections. The difference is possibly caused by the time constant of the first order system. This time constant results in an extra injected amount, which is constant for all EK injections. For that reason, the influence is relatively smaller for larger injection areas. It has also been noticed that the injection time applied by the system at 15 kV was larger than the injection time at 5 kV and 10 kV, although the programmed injection time was the same. As a consequence the initial width of the injected zone will already be larger.

From these results it can be concluded that it is very important to have knowledge of the size and shape of the injection, because it might have a large influence on the results. Therefore, it is recommended to record the time course of the injection voltage.

4.4. Electrokinetic injection combined with UV detection

As has been explained in the theoretical part of this report, the detection bias will be cancelled out if EK-injection is used. Eq. (15) also shows that peak areas will be equal, as long as experiments are done with equal ratio between the injection voltage and the separation voltage. Table 4 roughly confirms this suggestion; the small decrease in peak area at higher voltages is mainly attributed to the injection imperfections. Thus, it is concluded that the use of UV detection compensates for the bias in peak area caused by EK sample injection.

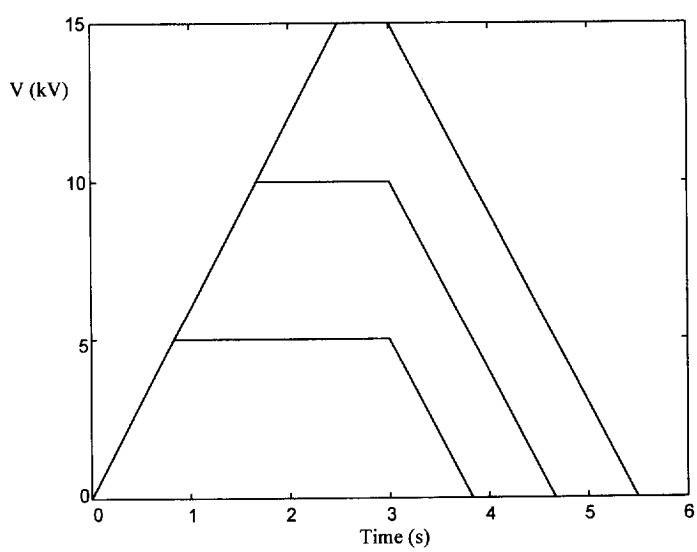

Fig. 3. Trapezium shaped injection pattern. Voltage ramping = 6 kV/s.

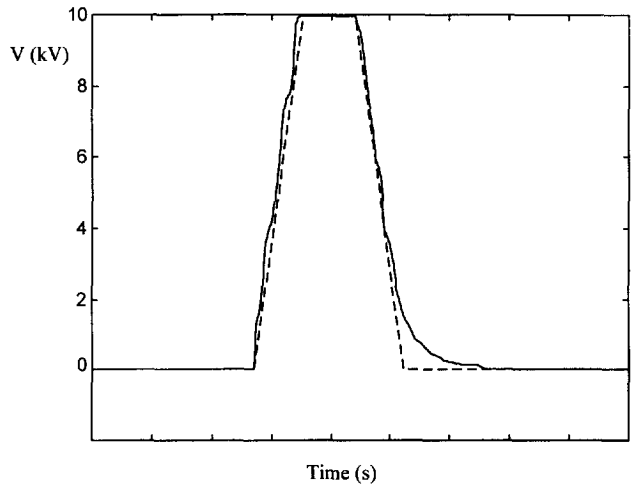

Fig. 4. Shape of the injection.

If, however, HD-injection is used, the detection bias will not be cancelled out and then peak areas have to be corrected for. This phenomenon can be clearly illustrated, comparing the results of experiments with EK injection and the results of experiments with HD injection. Fig. 5 illustrates the similarity between the uncorrected peak areas of typical experiments with EK injection and the corrected peak areas of the experiments with HD injection. All other experiments with EK injection (not shown) give comparable pictures, even if the injection voltage differs from the separation voltage.

These results demonstrate that the peak area ratio between peaks will not be affected by differing velocities, if UV detection is used in combination with EK sample injection. It was found that the injection voltage does not have to be equal to the separation voltage, as was assumed by Huang et al. [5]. However, if the reproducibility of the migration time is low (1–5%), a correction of the peak area might be still necessary.

5. Conclusion

It has been shown that the peak area ratio between peaks will not be affected by differing velocities, if UV detection is used in combination with EK sample injection. It was found that the injection voltage does not have to be equal to the separation voltage. Provided that the sample solution has the same pH and conductivity as the buffer, the peak area ratio of peaks in one electropherogram will not change for these experiments. If HD injection is used, the detection bias will not be cancelled out and peak areas have to be corrected for.

It has been shown also that size and shape of the injection have a large influence on quantification. For

Table 4
Comparison between experiments with both injection and detection bias ($V=V_{inj}$), with corresponding R.S.D. values ($n=5$)

V(kV)	5		10		15		$R^{5,10}$		$R^{10,15}$	
V_{inj}(kV)	5		10		15			S_R		S_R
	$A(10^{-3}$ AU·s)	R.S.D. (%)	$A(10^{-3}$ AU·s)	R.S.D. (%)	$A(10^{-3}$ AU·s)	R.S.D. (%)				
BTMA	3.84	0.5	3.64	1.0	3.32	1.3	1.06	0.005	1.10	0.008
BTEA	3.68	0.6	3.58	0.5	3.25	1.5	1.03	0.004	1.10	0.008
BTBA	3.76	1.1	3.58	0.9	3.28	0.7	1.05	0.007	1.10	0.006

For the ratios the standard deviation is given instead of the R.S.D. value.

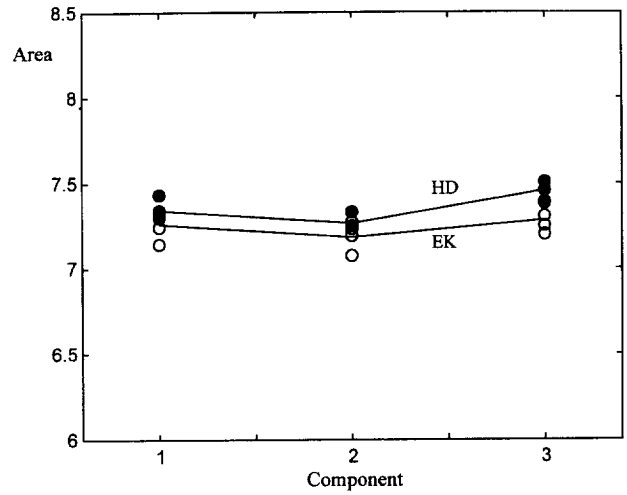

Fig. 5. Comparison for three components between corrected peak area after HD injection, and uncorrected peak area after EK injection.

this reason, knowledge about the size and shape of the injection is desirable. It is recommended to record the time course of injection voltage as well as electropherograms, because this will enable one to correct for variations.

Acknowledgments

The investigations were supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organisation for Scientific Research (NWO) under grant 700-344-003.

References

[1] H.C. Smit, Chromatographia, 3 (1970) 515.
[2] J.N. van der Moolen, D.J. Louwerse, H. Poppe and H.C. Smit, Chromatographia, 40 (1995) 368.
[3] R. Mulder, P.J. Hoogerbrugge and H.C. Smit, Chemometr. Intell. Lab. Syst., 1 (1987) 243.
[4] J.W. Jorgenson and K.D. Lukacs, Anal. Chem., 53 (1981) 1298.
[5] X. Huang, M.J. Gordon and R.N. Zare, Anal. Chem., 60 (1988) 375.
[6] X. Huang, J.A. Luckey, M.J. Gordon and R.N. Zare, Anal. Chem., 61 (1989) 766.
[7] X. Huang, M.J. Gordon and R.N. Zare, J. Chromatogr., 480 (1989) 285.
[8] Th.P.E.M. Verheggen, J.L. Beckers and F.M. Everaerts, J. Chromatogr., 452 (1988) 615.
[9] D.J. Rose and J.W. Jorgenson, Anal. Chem., 60 (1988) 642.
[10] M.J. Gordon, X. Huang, S.L. Pentoney and R.N. Zare, Science, 242 (1988) 224.
[11] A. Weston, P.R. Brown, P. Jandik, A.L. Heckenberg and W.R. Jones, J. Chromatogr., 608 (1992) 395.
[12] P. Coufal, H.A. Claessens and C.A. Cramers, J. Liq. Chromatogr., 16 (1993) 3623.
[13] H.A. Fishman, R.H. Scheller and R.N. Zare, J. Chromatogr. A, 680 (1994) 99.
[14] Y.-F. Cheng and N.J. Dovichi, Science, 242 (1989) 562.
[15] R.D. Smith, H.R. Udseth, J.A. Loo, B.W. Wright and G.A. Ross, Talanta, 36 (1989) 161.
[16] E.V. Dose and G.A. Guiochon, Anal. Chem., 63 (1991) 1154.
[17] T.T. Lee and E.S. Yeung, Anal. Chem., 64 (1992) 1226.
[18] S. Hjerten, K. Elenbring, F. Kilar, J.-L. Liao, A.J.C. Chen, C.J. Siebert and M.-D. Zhu, J. Chromatogr., 403 (1987) 47.
[19] M. Zhu, D.L. Hansen, S. Burd and F. Gannon, J. Chromatogr., 480 (1989) 311.
[20] M.W.F. Nielen, J. Chromatogr., 588 (1991) 321.
[21] M. Korman, J. Vindevogel and P. Sandra, J. Microcol. Sep., 5 (1993) 525.
[22] S.E. Moring, J.C. Colburn, P.D. Grossman and H.H. Lauer, LC·GC Int., 3 (1990) 46.
[23] D.M. Goodall, S.J. Williams and D.K. Lloyd, Trends Anal. Chem., 10 (1991) 272.
[24] K.D. Altria, Chromatographia, 35 (1993) 177.
[25] X. Huang, W.F. Coleman and R.N. Zare, J. Chromatogr., 480 (1989) 95.

[26] K.D. Lukacs and J.W. Jorgenson, J. High Resolut. Chromatogr. Chromatogr. Commun., 8 (1985) 407.
[27] G.J.M. Bruin, G. Stegeman, A.C. van Asten, X. Xu, J.C. Kraak and H. Poppe, J. Chromatogr., 559 (1991) 163.
[28] N. Dyson, Chromatographic Integration Methods, RSC Chromatography Monographs, Cambridge, UK, 1990, pp. 9–29.
[29] J. Warburton, Int. Labmate, 18 (1993) 27.