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A Micromachined Injection Device for CZE: Application to Correlation CZE

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A micromachined injection device is described, which can be connected to a fused-silica capillary. This allows injection without interrupting the current, which is an advantage in CZE and essential in correlation CZE. A typical property is the excellent connection, with low dead volume, between the capillary and the microchip. This results in very good repeatability of the peak parameters as is demonstrated by conventional electrokinetic injections. Correlation CZE experiments showed a considerable improvement in S/N ratio at high as well as at low concentrations. An 8-fold improvement in detection limit is achieved. The repeatability of peak height and peak area in correlation CZE was even better than obtained with conventional CZE.

Although UV analysis has one of the highest detection limits of all detection techniques in CZE, it is still the most popular because of its simplicity and versatility. The detection limits in the order of 10^{-6} mol/L can be improved by increasing the optical path length. By using rectangular capillaries instead of conventional capillaries, better detection limits have been achieved.[1,2] A bubble-shaped cell to extend the optical path length has been introduced, resulting in a 3-fold increase in signal.[3,4] A 6-fold improvement in signal-to-noise (S/N) ratio by using z-shaped cells[5] has been described. The bent section of the capillary reduced the light transmission through the capillary. However, a microchannel flow-through cell without any bent parts did not lead to a considerable improvement.[6] The favorable effect of a focusing lens in front of the capillary was demonstrated,[7] and in combination with a z-shaped cell, a more than 10-fold improvement was obtained.[8] Joining capillaries to increase the path length at the detection point resulted in a 3−5-fold improvement.[9,10] A multireflection cell can increase the sensitivity 40 times[11] but is practical only with a laser as the light source.

Summarizing, a large variety of advanced UV detection modes have been introduced, practically leading to a 3−10-fold improvement of the S/N ratio.

Changes in the sampling conditions can also be used to improve the detection limit. However, injecting a larger volume results in a loss of resolution. Preconcentration, e.g., by stacking techniques, can sometimes be a solution.

As an alternative, correlation CZE has been developed.[12] Injection according to a specified pattern results in a detector signal with an increased amplitude compared with the detector signal of a conventional injection. By means of a cross-correlation procedure, a so-called correlogram is calculated. A correlogram is almost similar to the electropherogram obtained after a conventional injection. The main advantage of this procedure is an improved S/N ratio. As a preliminary result, a 5-fold improvement has been reported.[12]

As already mentioned, the injection procedure is different in comparison with conventional CZE. While in conventional CZE one waits after the injection until the entire electropherogram has been observed, in correlation CZE injections are made semicontinuously during the migration of sample injected previously. To prevent the influence of the subsequent injections on each other, the current flow may not be interrupted during the injection procedure. The consequence is the need for a special injection device for correlation CZE.

Another point of importance is the difference between the actual and the assumed size and shape of the injection.[13] This difference consists of a systematic error, which is correlated with the injection pattern, and a random error, which is not. The systematic error is not problematic in conventional CZE because of calibration procedures. However, in correlation CZE, these disturbances lead to so-called ghost peaks in the correlogram[14] due to the correlation with the injection pattern. Ghost peaks are images of the main peak. Their properties have been studied thoroughly, and their relative positions can be predicted theoretically.[15]

On the other hand, random errors will have less influence in correlation CZE, as compared to conventional CZE, because they are not correlated with the injection pattern and thus are diminished by averaging over all injections. Since these disturbances influence the results in conventional CZE, several injection

(1) Tsuda, T.; Sweedler, J. V.; Zare, R. N. *Anal. Chem.* **1990**, *62*, 2149.
(2) Cifuentes, A.; Poppe, H. *Chromatographia* **1994**, *39*, 391.
(3) Heiger, D. N. *High Performance Capillary Electrophoresis: An Introduction*; Hewlett Packard: Waldbronn, Germany, pp 100−101.
(4) Xue, Y.; Yeung, E. S. *Anal. Chem.* **1994**, *66*, 3575.
(5) Chervet, J. P.; van Soest, R. E. J.; Ursem, M. *J. Chromatogr.* **1991**, *543*, 439.
(6) Kim, S.; Kim, W.; Hahn J. H. *J. Chromatogr., A* **1994**, *680*, 109.
(7) Bruin, G. J. M.; Stegeman, G.; van Asten, A. C.; Xu, X.; Kraak, J. C.; Poppe H. *J. Chromatogr.* **1991**, *559*, 163.
(8) Moring, S. E.; Reel, R. T.; van Soest, R. E. J. *Anal. Chem.* **1993**, *65*, 3454.
(9) Liu, S.; Dasgupta, P. K. *Anal. Chim. Acta* **1993**, *283*, 747.
(10) Hows, M. E. P.; Perrett, D. *Chromatographia* **1996**, *43*, 200.
(11) Wang, T.; Aiken, J. H.; Huie, C. W.; Hartwick, R. A. *Anal. Chem.* **1991**, *63*, 1372.
(12) Van der Moolen, J. N.; Louwerse, D. J.; Poppe, H.; Smit, H. C. *Chromatographia* **1995**, *40*, 368.
(13) Van der Moolen, J. N.; Boelens, H. F. M.; Poppe, H.; Smit, H. C. *J. Chromatogr., A* **1996**, *744*, 103.
(14) Mars, C.; Smit, H. C. *Anal. Chim. Acta* **1990**, *228*, 193.
(15) Mulder, R.; Hoogerbrugge, P. J.; Smit, H. C. *Chemom. Intell. Lab. Syst.* **1987**, *1*, 243.

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systems have been designed in order to improve the reproducibility of the sample introduction. Reproducibility is an important parameter in assessing the random injection error. Deml et al.[16] introduced in 1985 an electric sample splitter, as in capillary GC. Others developed rotary-type injectors, as in HPLC,[17,18] injection with valves,[19] and split flow injection.[20]

Automated CZE systems became available in 1987, which proved to be more reproducible.[21−24] Despite the automation, electrokinetic sample injection was not as reproducible as the injection reproducibility in HPLC. Lukacs and Jorgenson[25] remarked in 1985 that reproducing the duration of the injection is the major difficulty. The problem is in the delay in reaching a higher or lower voltage or pressure level, which is not reproducible. Lee and Yeung[26] reported a RSD of 0.8% for the time course of the current during injection. It has been pointed out before[13,27] that the (electric) first-order time constant of the CZE system is an important parameter when the size and shape of the injection plug is being quantified. To improve the reproducibility of the injection, a trapezium-shaped injection has been proposed.[28] Some modern commercial (automated) CZE systems offer the possibility of this voltage ramping.

Nevertheless, these injection methods still require interruption of the applied voltage during injection and therefore cannot be used as an injection device for correlation CZE. Tsuda and Zare[29] developed a split injector that maintains the applied voltage during (electrokinetic) sample injection. The sampling device introduced by Kaljurand et al.[30] offers this possibility too. The latter authors claimed a peak height repeatability of 1%. Of interest is the fluid joint, described by Amankwa and Kuhr.[31] A small precapillary is used as a microreactor for on-line peptide mapping, but it can also be used as an injection device. As will be outlined, these kinds of injection devices offer the advantage of maintaining the applied voltage during injection, resulting in an improved injection reproducibility. Besides, these devices can also be used as coupling devices in comprehensive two-dimensional applications.

In the last seven years, there has been a growing interest in the use of micromachined separation devices. Although the first reports were in the field of GC[32] and LC,[33] microchips are currently most intensively studied in CZE. The first report of a successful separation by CZE on a microchip was by Manz et al. in 1992.[34] The use of computer-controlled high-voltage relays to switch the high voltage to control the direction of the fluid flow offers the advantage that no moving parts, like integrated microvalves and micropumps as in μ-LC, are required.[35,36]

The valveless control, however, is subject to leakage between channels at the intersection point due to diffusive effects, but mainly due to hydrodynamic effects.[35,37] This fluid leakage can be expected to limit the effectiveness of valveless switching.[38] A reproducibility of 2.7% has been reported[39] for a sample injection method with the buffer reservoir floating. Active control of the potential of each reservoir at all times is required to reduce unwanted leakage effects.[40] Jacobsen et al.[39] introduced the expression "pinched sample loading" for this control of the potentials and reported a reproducibility of 1.7% RSD. It is to be expected that further improvement can be obtained by placing integrated electrodes not only at the reservoirs but also at the injection cross, to allow independent control of the potentials in the reservoirs and the separation column.[36] Effenhauser et al.[41] used a kind of loop injection and reported a peak area reproducibility of about 2%.

In this article, it is demonstrated that microchips are very suitable as injection devices for correlation CZE. Microchip technology has been chosen for the development of a new device, because it allows accurate mass fabrication, and thus identical systems, and the possibility to join channels at an intersection with low dead volume.[42,43]

EXPERIMENTAL SECTION

Reagents. A 0.03 mol/L phosphate buffer at pH 6.01 was used as background electrolyte (BGE). Sodium phosphate was obtained from Merck (Darmstadt, Germany). Benzyltrimethylammonium chloride (BTMA, Aldrich, Steinheim, Germany), benzyltriethylammonium chloride (BTEA, Merck), and benzyltributylammonium chloride (BTBA, Merck), dissolved in buffer, were used as sample. The buffer and the sample solutions were filtered through a 0.45 μm filter.

Equipment. The main part of the home-made system used for correlation CZE (Figure 1) was described previously.[12] It is based on a CZE system (Table 1), consisting of a fused-silica capillary, a high-voltage power supply, platinum electrodes in vials for sample and buffer at both sides of the capillary, and a UV detector. A personal computer was used to control the injection procedure by means of a high-voltage relay and to acquire the data obtained from the UV detector. All results were obtained using a home-made high-voltage relay. It consist of two normal relays, physically separated by two Perspex rods. This is done

(16) Deml, M.; Foret, F.; Bocek, P. J. *Chromatogr.* **1985**, *320*, 159.
(17) Tsuda, T.; Mizuno, T.; Akiyama, J. *Anal. Chem.* **1987**, *59*, 799.
(18) Debets, A. J. J.; Mazereeuw, M.; Voogt, W. H.; van Iperen, D. J.; Lingeman, H.; Hupe, K.-P.; Brinkman, U. A. Th. *J. Chromatogr.* **1992**, *608*, 151.
(19) Verheggen, Th.P. E. M.; Beckers, J. L.; Everaerts, F. M. *J. Chromatogr.* **1988**, *452*, 615.
(20) Tehrani, J.; Macomber, R.; Day, L. *J. High Resolut. Chromatogr.* **1991**, *14*, 10.
(21) Honda, S.; Iwase, S.; Fujiwara, S. *J. Chromatogr.* **1987**, *404*, 313.
(22) Rose, D. J.; Jorgenson, J. W. *Anal. Chem.* **1988**, *60*, 642.
(23) Schwartz, H. E.; Melera, M.; Brownlee, R. G. *J. Chromatogr.* **1989**, *480*, 129.
(24) Moring, S. E.; Colburn, J. C.; Grossman, P. D.; Lauer, H. H. *LC-GC* **1990**, *3*, 46.
(25) Lukacs, K. D.; Jorgenson, J. W. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1985**, *8*, 407.
(26) Lee, T. T.; Yeung, E. S. *Anal. Chem.* **1992**, *64*, 1226.
(27) Huang, X.; Coleman, W. F.; Zare, R. N *J. Chromatogr.* **1989**, *480*, 95.
(28) Warburton, J. *Int. Labmate* **1993**, *18*, 27.
(29) Tsuda, T.; Zare, R. N. *J. Chromatogr.* **1991**, *559*, 103.
(30) Kaljurand, M.; Ebber, A.; Somer, T. *J. High Resolut. Chromatogr.* **1995**, *18*, 263.
(31) Amankwa, L. N.; Kuhr, W. G. *Anal. Chem.* **1993**, *65*, 2693.
(32) Terry, S. C.; Jerman, J. H.; Angell, J. B. *IEEE Trans. Electr. Dev.* **1979**, *26*, 1860.
(33) Manz, A.; Miyahara, Y.; Miura, J.; Watanabe, Y.; Miyagi, H.; Sato, K. *Sens. Actuators* **1990**, *B1*, 249.
(34) Manz, A.; Harrison, D. J.; Verpoorte, E. M. J.; Fettinger, J. C.; Paulus, A.; Lüdi, H.; Widmer, H. M. *J. Chromatogr.* **1992**, 593, 253.
(35) Fan, Z.; Harrison, D. J. *Anal. Chem.* **1994**, *66*, 177.
(36) Jacobsen, S. C.; Hergenröder, R.; Moore, A. W.; Ramsey, J. M. *Anal. Chem.* **1994**, *66*, 4127.
(37) Harrison, D. J.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C. S.; Manz, A. *Science* **1993**, *261*, 895.
(38) Harrison, D. J.; Manz, A.; Fan, Z.; Lüdi, H.; Widmer, H. M. *Anal. Chem.* **1992**, *64*, 1926.
(39) Jacobsen, S. C.; Hergenröder, R.; Koutney, L. B.; Warmack, R. J.; Ramsey, J. M. *Anal. Chem.* **1994**, *66*, 1107.
(40) Seller, K.; Fan, Z.; Fluri, K.; Harrison, D. J. *Anal. Chem.* **1994**, *66*, 3485.
(41) Effenhauser, C. S.; Manz, A.; Widmer, H. M. *Anal. Chem.* **1993**, *65*, 2637.
(42) Harrison, D. J.; Fan, Z.; Seiler, K.; Manz, A.; Widmer, H. M. *Anal. Chim. Acta* **1993**, *283*, 361.
(43) Jacobsen, S. C.; Hergenröder, R.; Koutney, L. B.; Ramsey, J. M. *Anal. Chem.* **1994**, *66*, 1114.

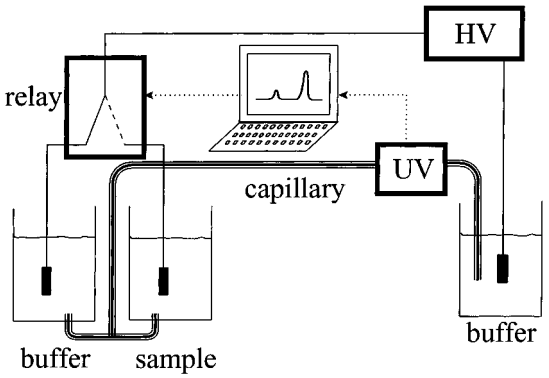


Figure 1. General setup of a correlation CZE system.

Table 1. List Of Equipment

apparatus/ condition	type and supplier
HV power supply	HCN 35-35000 (0−35 KV), FuG, Rosenheim, Germany
capillary	75 μm i.d., 280 μm o.d., fused silica, LC Packings, Amsterdam, The Netherlands
capillary length	76 cm
capillary length to detector	59 cm
UV detector	Linear UVIS 200, Linear Inst. Fremont, CA
UV wavelength	210 nm
data amplifier	Model 113 preamplifier, Princeton Applied Research Corp., Princeton, NJ
A/D card	DAS-1601, Keitley MetraByte, Gorinchem, The Netherlands
computer	386, 16Mb RAM
software	custom-written, based on Matlab v4.2

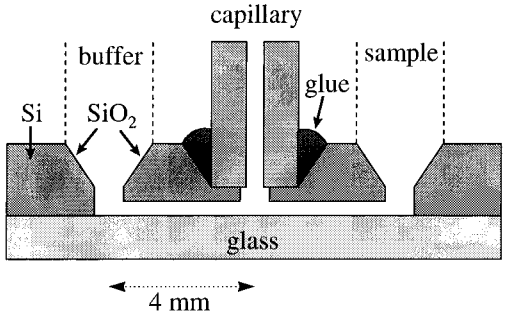


Figure 2. Design of the microchip injection device.

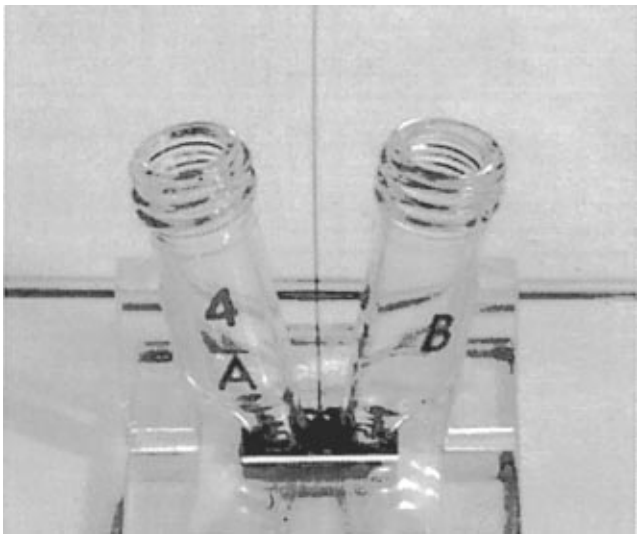


Figure 3. Photograph of the microchip injection device.

in order to keep the high voltage separated from the computer control. Some experiments were done using a commercial high-voltage relay (type TMR-10, Torr Laboratories, Inc., Los Angeles, CA) instead of the home-made relay. No differences between experiments with the commercial HV relay and the home-made HV relay were noticed. The capillary, the electrodes, the vials, and the CZE cell of the detector were placed in a thermostated (25 ± 1 °C) safety box.

Micromachining of the Injection Device. Devices were manufactured under contract by Twente MicroProducts (Enschede, The Netherlands) using silicon micromachining technology. The channel and the connections were realized by a combination of dry and wet etching steps in a silicon substrate. The silicon was then anodically bonded to a glass sheet to cover the channel. The channel was electrically isolated with a thin layer of silicon oxide in order to sustain electric fields up to 250 V/cm across the channel and pressures up to 120 bar. The channel dimensions were matched to the inner diameter of the capillary (75 μm) to minimize the dead volume at the connection of capillary and microchip (Figure 2). The channel from the reservoirs to the T-piece, 75 μm wide and 75 μm deep, had a length of 4 mm. A cylindrically shaped connection between the T-piece and the capillary was made with length 75 μm and diameter 85 μm.

After the capillary was polished with a 0.5 μm diamond lapping film, it was glued on the microchip using an epoxy resin (Araldite No. 2014). The capillary was positioned by a micromanipulator and a microscope. Finally, glass 1 mL reservoirs were glued onto the microchip (Figure 3).

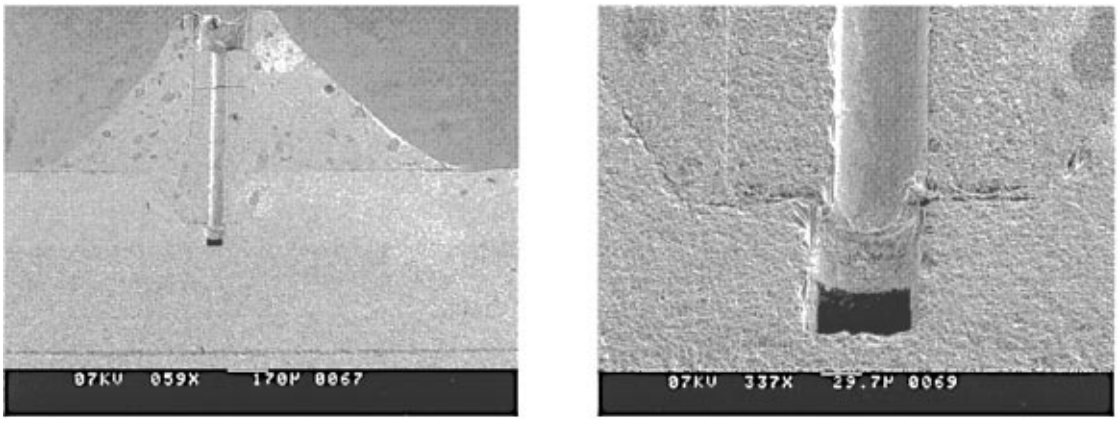
In order to inspect the connection between the capillary and the microchip, one of the devices was cut in two equal pieces. Scanning electron microscope (SEM) photographs of the cross section were made by Twente MicroProducts (Figure 4). In the left photograph, the contours of the capillary, resin, and microchip are visible. The black rectangle in the middle of the right photograph is the cross section of the channel to the vial. It can be seen that the connection between the microchip and the capillary is excellent.

Procedures. Every morning, buffer was degassed with helium and the capillary was rinsed with 0.1 mol/L sodium hydroxide, water, and buffer for several minutes.

Electrokinetic single injections were made by switching the high voltage (10 kV) for 5 s to the sample vial. The injection pattern used in correlation CZE is a pseudorandom binary sequence (PRBS).[12,44] A random pattern can be used also, but optimal results are obtained with a PRBS.[45] In order to make a true comparison with the conventional injections, the length of the clock period (CP) of the PRBS was chosen equal to the conventional injection time (5 s). The PRBS length in time has to be equal to or larger than the electropherogram length. So, because of the electropherogram length of about 12 min, the minimal PRBS length was 255 CP. Finally, at least two sequences were necessary, because the first sequence cannot be used for the correlation procedure. In all experiments, the applied voltage was 10 kV.

The amplified detector signal was digitized (2 Hz) by a 12-bit analog-to-digital (A/D) converter. The data processing included

(44) Engelsma, M.; Boelens, H. F. M.; Louwerse, D. J.; Kok, W. Th.; Smit, H. C. *Anal. Chim. Acta* **1993**, *272*, 309.
(45) Kaljurand, M.; Valentin, J. R.; Shao, M. *Anal. Chim. Acta* **1996**, *324*, 29.



a b

Figure 4. SEM photographs of the microchip cross section: (a) 59% enlargement; (b) 337% enlargement.

correction for linear drift of the (digitized) detector signal and, in the case of correlation experiments, cross-correlation between the detector signal and a pattern close related to the injection pattern.[44]

To determine the peak parameters, a peak-fitting procedure was applied to the correlogram or the electropherogram, using a nonlinear regression software package with a Fraser−Suzuki peak model[46] and a second-order polynomial describing the baseline. Noise levels, expressed as standard deviations, were calculated from the main baseline part of the correlogram or electropherogram. All software used in the data processing was custom written for Matlab (The MathWorks, Inc., Natick, MA). The calculated S/N ratio was expressed as the ratio between peak height and the standard deviation of the noise.

RESULTS AND DISCUSSION

Stability of the system during an experiment is one of the most important demands in the performance of correlation techniques.[47] Important aspects concerning stability in CZE are variations in migration rate, e.g., caused by variations in temperature or pH, and detector disturbances, such as drift of the detector signal. In order to prevent variations in temperature, the system was thermostated on 25 ± 1 °C. Variations in pH can be diminished by not using small vials for sample and buffer. Variations in the migration rate can be determined by measuring the repeatability of the migration time. Figure 5 demonstrates the repeatability of five consecutive single injections. The relative standard deviation (RSD) of the migration time for these injections was better than 0.4%, while it was better than 0.8% ($n = 20$) for a series measured over two days. These results make clear that the variations in the migration rate are sufficiently low to perform correlation CZE.

Detector disturbances are an important cause of lack of stability in CZE. This can be demonstrated by the correlogram of a linearly drifting detector signal (Figure 6a). A significant baseline disturbance appeared in the correlogram (Figure 6b). In a simulation, the correlogram of a noise-free signal (Figure 6c) with a comparable linear drift as the detector signal in Figure 6a has been calculated. The correlogram (Figure 6d) shows the same disturbance as in Figure 6b. As a result of a simple correction for the linear drift in the detector signal, a correlogram with a flat baseline has been obtained (Figure 6e). Although this specific

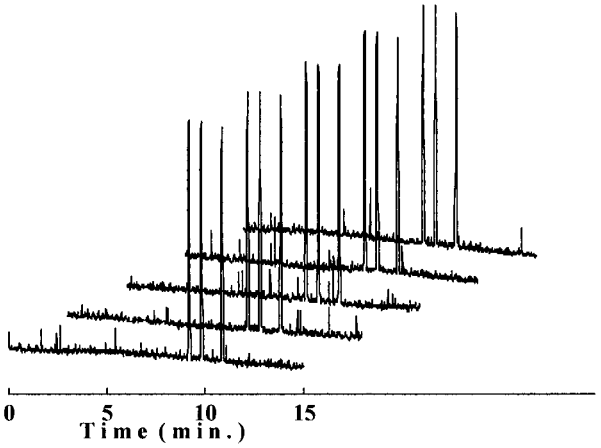


Figure 5. Electropherograms of five consecutive single injections of 10^{-4} mol/L sample. Injection time 5 s; voltage 10 kV; sample time 0.5 s; gain preamplifier 2000.

disturbance can be corrected for easily, other disturbances, e.g., $1/f$ noise, in this case not present in this detector signal, are more difficult to correct for.

As was noted in the introduction, reproducibility is an important parameter in assessing the random injection error. For several reasons, an improved reproducibility with the microchip device compared to conventional electrokinetic injections is to be expected. First, in conventional injections the injected amount is influenced by "ubiquitous injection". Grushka and McCormick[48] were the first to demonstrate that the actual insertion of the capillary into or withdrawal of the capillary from the sample solution results in an ubiquitous injection of sample into the capillary. According to Fishman et al.,[49,50] this extraneous injection, described by the authors as spontaneous fluid displacement, is caused by an interfacial pressure difference across a droplet at the inlet of the capillary as it is removed from a sample solution. It is clear that this phenomenon cannot occur with the microchip injection device.

Second, as a result of applying a voltage, the temperature in the capillary will rise. Huang et al.[27] observed a temperature rise

(46) Scheeren, P. J. H.; Barna, P.; Smit, H. C. *Anal. Chim. Acta* **1985**, *167*, 65.
(47) Lub, T. T.; Smit, H. C. *Anal. Chim. Acta* **1979**, *112*, 341.
(48) Grushka, E.; McCormick, R. M. *J. Chromatogr.* **1989**, *471*, 421.
(49) Fishman, H. A.; Amundi, N. M.; Lee, T. T.; Scheller, R. H.; Zare, R. N. *Anal. Chem.* **1994**, *66*, 2318.
(50) Fishman, H. A.; Scheller, R. H.; Zare, R. N. *J. Chromatogr., A* **1994**, *680*, 99.

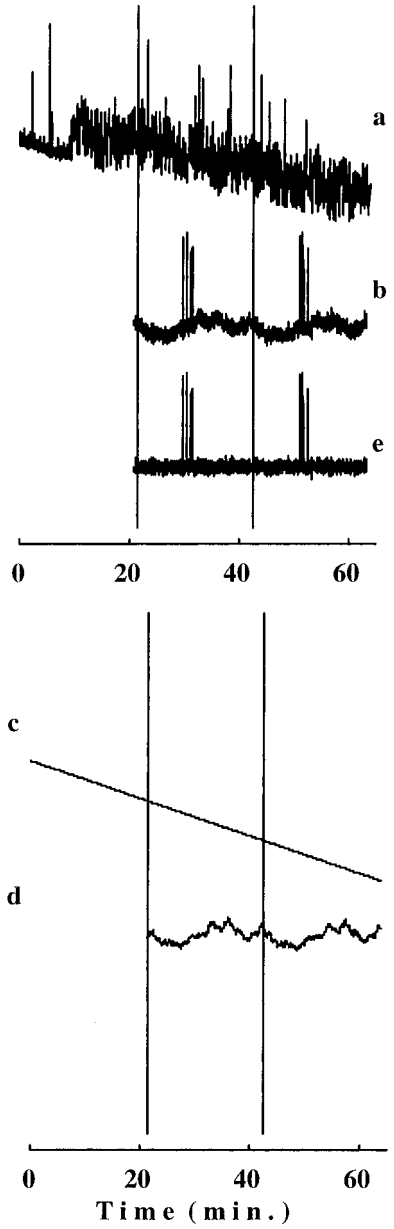


Figure 6. Influence of linear drift on the correlation procedure: (a) detector signal; (b) correlogram of detector signal a; (c) simulated linearly drifting baseline; (d) simulated correlogram of signal c; (e) correlogram of detector signal a after correction for the linear drift. Sample 4×10^{-6} mol/L; PRBS = 255 CP, CP = 5 s; three sequences; voltage 10 kV; sample time 0.5 s; gain preamplifier 2000.

of 12 K over a period of less than 10 s for an applied voltage of 20 kV. This temperature leap will have a large influence on the electrophoretic conditions (e.g., viscosity). Using the microchip injection device, fast switching of the applied voltage results in a stable mean temperature in the capillary and in the injection device.

Finally, the delay in reaching a higher or lower voltage or pressure level is often not reproducible,[26] as was noted in the introduction. The rise time to maximum voltage is determined by the time constant of the power supply and can be neglected with modern supplies. The fall time depends mainly on the discharge time constant of the CZE system. This fall time can be minimized by adding a resistance across the capillary.[27,51] With the present system, voltage is maintained during sample injection. The time needed for the relay to switch (in the order of 10 ms) is small compared to the fall time of discharging the capillary system (in the order of seconds[27]). So, only a small voltage drop will occur.

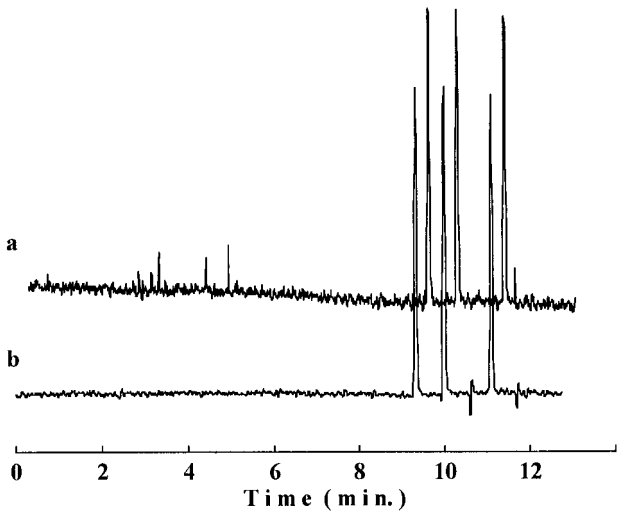


Figure 7. Electropherogram (a) vs correlogram (b) of 10^{-4} mol/L sample. PRBS = 255 CP, CP = 3 s; injection time single injection 3 s; voltage 10 kV; sample time 0.5 s; gain preamplifier 2000.

The RSD of peak height and peak area of the five consecutive single injections (Figure 5) was found to be better than 1.1%, while it was better than 1.3% for the series of 20 injections measured over two days. These results are even better than those obtained with hydrodynamic injection on most commercial instruments.[52,53] Only experiments using internal standards showed better results.

To demonstrate the potential of correlation CZE, a comparison has been made between an electropherogram and a correlogram. All experiments have been done under the same conditions. Figure 7 shows the difference between an electropherogram and a correlogram of a concentration of a 10^{-4} mol/L sample. About a 4-fold improvement in S/N ratio in the correlogram compared to the electropherogram is demonstrated. Apart from the large peaks of the components, some other peaks can be observed to the right of each component peak. Their positions relative to the main peaks are constant, which indicates that these extra peaks are noncomplementary[15,54] ghost peaks. Although the observed ghost peaks are relatively small, they suggest a systematic injection error, as was noted in the introduction. Noncomplementary ghost peaks do not influence the magnitude of the main peak, and it is possible to correct for their presence[15,44] by performing one extra experiment. The cause of the injection error is not clear and needs more investigation.

The RSD of the migration time between five correlograms was better than 0.1%. The repeatability of peak height and peak area is better than 1.0% RSD. These results are a little bit better than the repeatability results of the five consecutive single injections.

Correlation CZE has been developed in the first place to decrease the detection limit in CZE. Therefore, results obtained with a low sample concentration have been compared (Figure 8).

(51) Kuhr, W. G.; Yeung, E. S. *Anal. Chem.* **1988**, *60*, 2642.

(52) Wätzig, H.; Dette, C. *Fresenius J. Anal. Chem.* **1993**, *345*, 403.

(53) Kunkel, A.; Degenhardt, M.; Wätzig, H. Symposium on High Performance Capillary Electrophoresis, Orlando, FL, January 1996; Poster 531.

(54) Engelsma, M.; Louwerse, D. J.; Boelens, H. F. M.; Kok, W. Th.; Smit, H. C. *Anal. Chim. Acta* **1990**, *228*, 209.

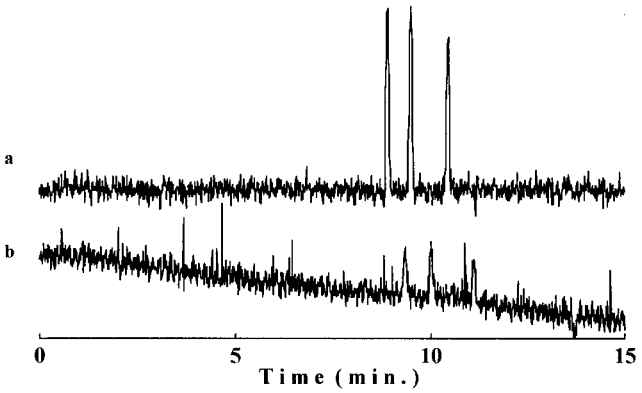


Figure 8. Correlogram (a) vs electropherogram (b) of 4×10^{-6} mol/L sample. PRBS = 255 CP, CP = 5 s; injection time single injection 5 s; voltage 10 kV; sample time 0.5 s; gain preamplifier 5000.

The correlogram resulted in a S/N ratio of 33, vs a S/N ratio of 4 for the electropherogram. So, an 8-fold improvement is proved with these experiments. It is clear that the accuracy of the peak parameters is also much better. This improvement is obtained with only a moderate cost in terms of time; twice the time of an electropherogram is needed, because two sequences, with the length of an electropherogram each, have to be injected. Spikes, occurring quit often in electropherograms, can disturb the electrophoretic peaks, especially at low concentrations. As can be seen in Figure 8, no spikes are present in the correlogram. The cross-correlation procedure spreads the spike over the entire correlogram. So, large spikes will result in some extra noise in the correlogram, but they will not disturb the peaks.

CONCLUSIONS

Microchip technology is very well suited for accurate fabrication of complex structures. It is demonstrated that connecting a microchip injection device to a fused-silica capillary can be done quite easily. SEM photographs proved that the connection is excellent, with low dead volume. The repeatability of peak height and peak area of the electrokinetic injection was very good, with RSD values better than 1.1%.

Although it was sufficient to allow correlation CZE experiments, the stability is to be improved. Detector disturbances especially should be controlled better.

An improved repeatability with the microchip injection device compared to conventional injections has been demonstrated. With correlation CZE, the injection repeatability was even better than the repeatability with conventional CZE with the microchip injection device.

Correlation experiments showed a considerable improvement in S/N ratio at high as well as low concentration. An 8-fold improvement in detection limit has been demonstrated. Probably, a higher improvement will be obtained if detector disturbances can be prevented. A possible cause of these disturbances may be the intensity fluctuation of the D_2 lamp in the detector. Measurement against a reference will improve the results then. Combining correlation CZE and advanced detection techniques, as for instance the z-shaped cell with a focusing lens in front of the capillary, is also desirable.

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