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Cross-correlation capillary electrophoresis in unmodified commercial equipment

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Abstract

The present contribution deals with the use of unmodified commercial equipment for cross-correlation capillary electrophoresis (CE). Complex injection sequences have been injected by switching sample and background electrolyte vials according to a pre-programmed order. Because of the nature of the auto-sampler used, no sequence could be injected in commercial instrument without high voltage (HV) interruptions. Apparently, this is the main reason why obtained signal-to-noise (S/N) ratios are lower than theoretical. Specific ways of programming the injection sequence and use of modified injection sequence in the correlation process have been used to overcome the deleterious effect caused by this sample injection. An electro-osmotic flow (EOF) marker was used as a sample to find optimal conditions for cross-correlation experiments. Best results demonstrate S/N ratio improvements around 2.8 compared to 4.0 theoretical maximum.

Keywords: cross-correlation CE, deconvolution, multiple input, multiplex measurement

1. Introduction

Low detection limits (LOD) and good signal-to-noise (S/N) ratios have always been desired in analytical separation techniques. Unfortunately in capillary electrophoresis (CE) the most widely used UV detection faces relatively low concentration sensitivity due to a very short optical path length. In combination with small sample volumes this results in low mass sensitivity. Improving sensitivity of CE has been an important research field during past few decades while CE has enjoyed its growing popularity. On the instrumental side two different approaches have been used to achieve lower LODs in CE. Either the optical path length of detector could be increased with the use of Z- [1] and bubble-shaped [2] detector shells or rectangular shaped capillaries [3]. The second approach is exchanging UV detection for more sensitive detection method like laser induced fluorescence (LIF) or others. Investments in instrumentation are quite likely needed for these kinds of modifications. Therefore, techniques for increasing sensitivity that do not need any changes in instrumentation are preferred. One option could be compressing a long sample plug into a very narrow highly concentrated sample zone: stacking [4-6]. Stacking and several similar sample pre-concentration techniques can provide better sensitivity compared to regular UV detection.

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Only limitation is the fact that the sample matrix should have very low conductivity compared to the background electrolyte (BGE).

The apparently the most simple way of improving S/N ratio and therefore increasing sensitivity of CE is the averaging of signals obtained from multiple analyses of the same sample [7-9]. With this technique there is no need for modifications in the instrumentation. The idea behind signal averaging is the fact that accumulated peak size is proportional to the number of signals *n* while the accumulated noise amplitude is proportional to $\sqrt{n}$. This means a S/N ratio improvement factor is $\sqrt{n}$. Signal averaging is widely used in spectroscopy, like NMR, mass spectrometry, etc. Downside of this averaging technique is its extreme time consumption – to achieve 10 fold S/N ratio improvement theoretically 100 analyses must be performed and in practice even more. Such time consumption could be tolerated in spectroscopy, where the length of analysis is in milliseconds, but not in separation sciences where the length of a single analysis is in the order of minutes.

One possible solution to reduce the time consumption of averaging techniques could be measuring of overlapping analyses. This technique to achieve better sensitivity is known as cross-correlation CE. It is based on the injection of multiple discrete sample plugs into the separation capillary according to a well defined injection sequence and treating the whole detector signal as a sum of single injections. This means that after the experiment has been performed it is possible to (cross-)correlate the detector signal of multiple overlapping peaks with the injection sequence in order to obtain an electropherogram that corresponds to the single injection. This correlated electropherogram has an improved S/N ratio as it is the average of all overlapping injections.

Most often injection sequences are created using pseudo random binary sequence (PRBS) generators. This specific generator can construct injection sequences with a length of 2^n-1, where *n* is an integer number that corresponds to "flip-flops" in PRBS generator. If *n* is 1, then experiment could be considered as regular single injection. Theoretically, the S/N improvement factor can be calculated as:

$$S/N = \frac{2^{n-1}}{\sqrt{2^n}} = \frac{\sqrt{2^n}}{2}$$

Cross-correlation CE has successfully been performed using UV [10-13], LIF [14-16] or even amperometric detection [17]. It has been used in conventional CE [18, 19] and in microchip CE [14, 17, 21, 22]. Hata et al. have even reported using cross-correlation CE technique for MEKC analyses [23]. All cross-correlation CE experiments reported before have been done using some sort specific sample injection device that is not available in commercial equipment. Some examples are: pneumatic auto-samplers [12, 24], a microchip with a high-voltage switch [10, 11], a capillary with an additional inlet in the middle [18], photolytic optical gating [15], etc. What is common for all these injection techniques is that the sample sequence can be injected without any high voltage (HV) interruptions. In this way it is easier to achieve S/N ratio improvements close to the theoretical value as good results could be optained only with rapid high precision sample injection. Reduced precision however, soon causes a number of deleterious effects such as low frequency correlation noise and artificial peaks carrying no chemical information (ghost peaks).

A study of literature reveals that possible use of commercial intruments for cross-correlation experiments has not been investigated thoroughly. In this contribution we would like to report the results of cross-correlation CE experiments performed in an unmodified commercial instrument.

2. Experimental

2.1. Instrumental

Experiments were performed in a conventional Beckmann P/ACE 5500 capillary electrophoresis system equipped with UV-detection and auto-sampler. The CE system consisted of a 55.4 cm long (49.4 cm to detector), 75 μm I.D. × O.D. 360 μm fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA). The UV detector was set to 214 nm and the signal was registered at the frequency of 10 Hz. Injection and separation voltage were always 10 kV. The Auto-sampler of the P/ACE system was programmed normally or according to a complex pseudo random binary sequences of ones and zeroes.

The correlation process of multiple injection electropherograms was performed by solving a system of linear equations with the aid of fast Fourier transform. Software for the correlation process was written and run in MatLab (The Math Works, Inc., Natick, MA, USA). Before the correlation electropherograms were processed in Matlab to remove spikes and baseline drift.

2.2. Analytes

All analyses were performed with 15 mM tris(hydroxymethyl)aminomethane (TRIS) borate as a background electrolyte solution (BGE) at pH 8.46. All solutions were prepared by dissolving chemicals of analytical grade in deionized water (Milli-Q). The analytes - dimethyl sulfoxide (DMSO), 2,5-dimethyl benzene sulfonic acid, cinnamic acid, m-hydroxy benzoic acid, and p-nitro phenol were obtained from from Merck (Darmstadt, Germany). TRIS and boric acid were purchased from Sigma-Aldrich (Steinheim, Germany).

2.3. Procedures

At the beginning of each day the capillary was rinsed with 1 M NaOH (5 min) and water (3 min) and then thoroughly flushed with the BGE for 5 minutes. In between the separate runs the capillary was additionally flushed with BGE for 2 minutes.

3. Results and discussion

3.1. Sample injection modes

Using the P/ACE system it is possible to use pressure or HV mode for the sample injection. It is obvious that hydrodynamic or electro-kinetic injections would affect the peak-broadening and reproducibility of the migration times differently. To find out which of the sampling modes would suite for cross-correlation CE better, the following experiment was carried out. 10 injections were made in a row at regular intervals. In between those injections BGE was introduced into the capillary. The whole sequence of sample and BGE plugs was inserted into capillary before registration of the electropherogram begun. In total, four different combinations of sampling were tested, as both the sample and BGE can be either injected in electro-kinetic or hydrodynamic mode. As sample the mixture of EOF marker – dimethylsulfoxide (DMSO) – and anion – phenol – was used. The injection parameters were chosen in such a way that both the hydrodynamic injection and the electro-kinetic injection would introduce the same amount of sample i.e. the sample plug with the same length. The length of pressurized injection was 1 second and the corresponding length of the electro-kinetic injection with the same amount sample injected was 2.1 seconds. This relation between electro-kinetic and hydrodynamic injection was calculated using EOF value, Poisseuille's law and the fact that hydrodynamic injection is made at 0.5 psi. The lengths of the injection of BGE zones were 27.9 and 57.9 second for hydrodynamic and electro-kinetic injections respectively. The lengths of BGE injections were chosen so that the distance between the peaks would be exactly 1 minute in electropherograms.

Results are depicted in figure 1. Hydrodynamic injection seems to be main cause of peak broadening, as the best results have been achieved when both sample and BGE were injected electro-kinetically. The total time during which the pressure is applied is crucial. When the sample is introduced electro-kinetically and the BGE hydrodynamically the peak-broadening is unacceptable, but the peak-broadening is much smaller when the sample is injected by pressure and BGE electro-kinetically. Pressurized injection causes peak broadening because of its non-laminar flow profile. Best results are obtained when both the sample and the BGE are injected electro-kinetically, therefore this combination is used in all further experiments.

3.2. Programming injection sequence

The Beckman's P/ACE 5500 system is using an auto-sampler system that switches vials at inlet or outlet of the capillary. Obviously it is not capable of switching vials while the separation process is running and HV is on. This

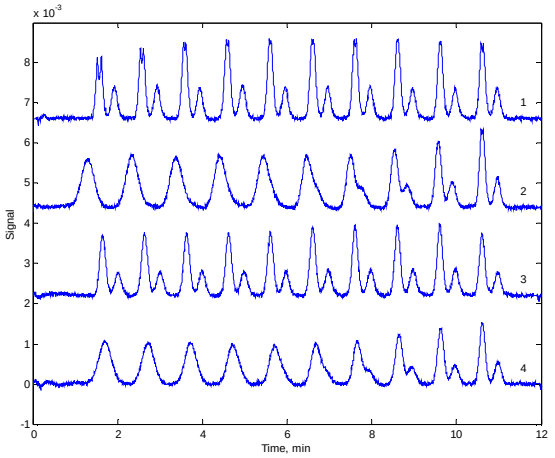

Figure 1. Different combinations of hydrodynamic and electro-kinetic injections. 1 – sample and BGE introduced by migration, 2 – sample introduced by migration, BGE by pressure, 3 – sample introduced by pressure, BGE by migration, 4 – sample and BGE introduced by pressure.

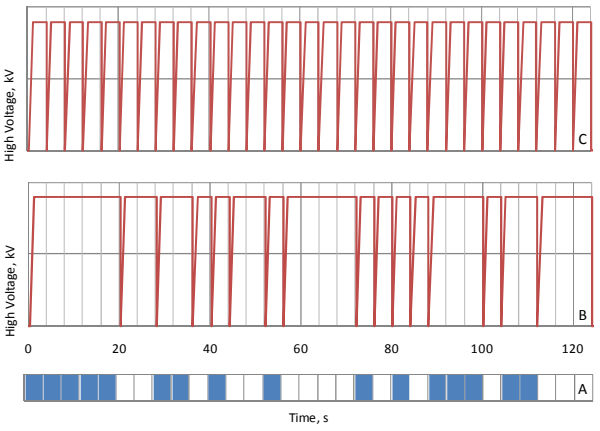

Figure 2. Two options to program injection sequence in P/ACE system. A – injection sequence, B – HV profile, if voltage is interrupted only for changing vials, C – HV profile, if voltage is interrupted after every injection.

means that injection of the randomized sequence of sample and BGE injections cannot be done without HV interruptions.

There are two options how to program randomized injection sequences. Firstly, it is possible to inject all consecutive sample or BGE injections in a row. In this case HV is interrupted only for replacing sample with BGE vials and vice versa (Figure 2B). This kind of sequence seems attractive as it uses minimal number of HV interruptions. Therefore, it could save some analysis time. There are some negative aspects if there is a systematic error in the injection system of P/ACE system due to the fact that it takes some time for the HV to reach its value after it has been switched on (so-called ramp) and later it takes also some time for the HV to reach value 0 kV after the power supply has been switched off. This sort of systematic error should be no problem in a regular single injection analysis as it has the same impact on all analyses. In case of cross-correlation CE the impact of this kind of systematic error could be detrimental as these errors are accumulating. Moreover, while using an injection program with a minimum number of HV interruptions, these errors are spread across the injection sequence unevenly, which makes it difficult to compensate its effect. Therefore, another option to program a randomized injection sequence would be to make every single injection separately (Figure 2C). This means that the number of HV interruptions is much higher than in case the HV is interrupted only for vial change. On the other hand, these interruptions are equally distributed through the whole injection sequence. Thus, it could be possible to calculate a corrected injection sequence and use this in the correlation process.

The P/ACE system has a correction mechanism for the injection length. It takes into account the time it takes for HV to reaches its value at the start of the injection and to reach value 0 kV at the end of the injection. In practice, at the start of the injection the system waits till the HV reaches half of its value before it starts counting the time. At the end of the injection, it switches off the HV exactly one second before the end of the injection. This means the excessive amount of sample that is injected at the start of the injection is compensated with a moderate amount injected at the end of the injection.

To determine if the correction mechanism of the injection system is good enough for cross-correlation CE, the following experiment was done. Two experiments consisting of two sample injections in a row were carried out. In the first experiment in between two sample injections a 3 minute long BGE injection was made. For comparison, in the second experiment instead of a long BGE plug there were made 45 short plugs of 4 seconds. The sample mixture consisted of EOF marker (DMSO) and an anionic component (m-hydroxybenzoic acid). After the experiment two electropherograms were aligned according to the second EOF marker peak. Apparently the distance between two marker peaks where 45 HV interruptions were made was smaller than in case of no interruptions. This is evidence of the systematic error of the injection system. By the distance between two EOF marker peaks it can be calculated that

the systematic error of one injection (or HV interruption) is about 0.13 s. This means that for every injection the systematic error is larger than one data point (0.10 s at 10 Hz) and this sort of error could not be considered negligible. All the multiple input experiments described further were programmed so that every single injection was made separately and HV was interrupted after every injection.

3.3. Procedures to modify the injection sequence for use in correlation process

Systematic errors of HV interruptions are spread equally across the whole injection sequence – there is one HV interruption for every injection in the sequence. Because of this systematic error all injections are actually a little bit shorter than they should be and the whole sequence in the detector output is also shorter if compared to the injected sequence. Part of the detector signal used for correlation must have the same length as the injection sequence. Now because they are not, additional data is used in the correlation process that does not correspond to any injection in the injection sequence and therefore converts into low frequency noise in the correlated electropherogram.

To avoid this kind of additional noise in the correlated electropherograms both the injection sequence and the corresponding detector signal should have the same length. Now because it is difficult to manipulate the detector signal without distorting it, a modification of the injection sequence was carried out. In the modification process the injection sequence was shortened till it had exactly the same number of points as the detector signal corresponding to one injection sequence. Actual procedures for modifying the injection sequence and then correlating the detector signal were the following. First, the interval of the detector signal which corresponds exactly to one injection sequence was found. It was done manually by finding similar looking points in the detector signal assuring that the distance between them should correspond to one sequence. It is easy when the sample mixture consists only of one component as the detector signal looks very similar to the injection sequence. After the detector signal of n points was determined, a modified injection sequence corresponding to the detector signal had to be generated. For that, the detector signal was elongated by a factor p that was equal to the length of the original sequence. Now a new sequence with $p \times n$ points was constructed according to the ideas reported here [14]. Basically in the original sequence every sample injection was replaced by the value *1* and BGE injection with the value *-1*. Between every value corresponding to sample or BGE injection $n-1$ values of *0* were added. Correlation was done using the modified injection sequence. After that the correlated electropherogram was shortened to the original length of the detector signal by averaging every p points.

3.4. Randomizing injection sequence

There are different options that should be considered when generating randomized injection sequences for correlation CE. Firstly, a sample injection of randomized size could be made at regular intervals. Secondly, sample injections with the same length could be injected at randomized intervals. And thirdly, both the length of injections and the intervals between them could be randomized. Experiments were carried out to find the most suitable injection sequence type for correlation CE experiments with conventional equipment. All randomized sequences were generated with a pseudo random binary sequence (PRBS) generator and were based on the same PRBS sequence with the length of $2^5-1=31$ elements. Therefore the total amount of a sample injected into capillary during one sequence was the same for all three types of sequences, though all sequences had a different length. The theoretical maximum signal-to-noise (S/N) ratio improvement for all sequences was equal to 2.83. Relatively short PRBS sequences were chosen to keep the total length of CE experiments as small as possible. Besides, the duty cycle of the sequence of regular injections and randomized intervals is very low compared to the other options and therefore it would be difficult fitting a longer injection sequence into the effective length of the capillary.

Results of these experiments are presented in figure 3. Comparing S/N ratio improvements to theoretical maximum, best results are obtained with the sequence of regular injections at randomized intervals. Worst S/N ration improvement was obtained using the sequence of injections with randomized length at regular intervals. S/N ratio improvement of a sequence where both size and interval of injections was randomized is slightly worse than in case of regular injections and randomized intervals. This sequence corresponds to an exact output of the PRBS generator, therefore its duty cycle is highest and therefore maximum practical S/N ratio improvement should be highest as more injection could be fitted into the effective length of the separation capillary. All experiments below

are performed using sequences where the both length of the injections and the intervals between them were randomized.

3.5. Optimal sequence length

In the following experiment three different lengths of the injection sequence were used. PRBS series with 31, 63 and 127 elements should give theoretical S/N ratio improvements of 2.8, 4.0 and 5.6 respectively. Results of these three experiments with their correlated electropherograms and single injection for comparison are depicted in figure 4. All three correlated electropherograms have improved S/N ratio compared to the single injection, but to different extent. Improvement factors calculated according to standard deviations and compared to the single injections for 31, 63 and 127 element long PRBS sequences are 2.4, 2.8 and 2.1 respectively. This means best S/N ratio is obtained with the medium length sequence, while highest S/N gained compared to theoretical is achieved with the shortest sequence. There are several simple explanations why experiments with the longest sequence have worst results. During a longer sequence more injections are made, therefore the number of HV interruptions and accumulated systematic errors is also higher with resulting detrimental effect. Besides, during the longer experiment it is more likely that EOF drift could occur and have its own negative effect on the results. Moreover, with shorter sequences it is possible to choose the part of detector signal used for the correlation process, as the signal needed for correlation is small compared to the whole signal. In case of PRBS length 127, the signal needed for correlation is almost as long as the whole registered signal. Therefore, possibilities to choose the best part of a signal for correlation are very limited. While planning multiple input CE experiments it is important to find a good compromise between the sequence length and the expected S/N improvement, because rules-of-thumb that longer sequences always guarantee better S/N ratio do not work in practice.

3.6. Signal post-processing

For a successful correlation process to obtain a nice electropherogram with a higher S/N ratio, the original detector signal must be clean of any spikes and without significant baseline drift. This sort of disturbance will

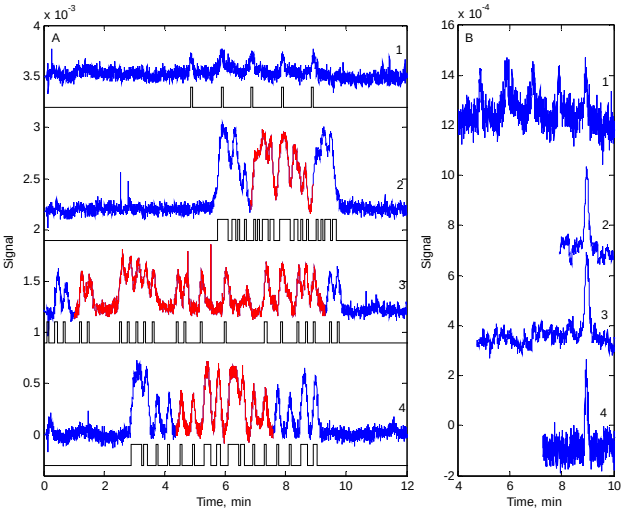

Figure 3. Different options for randomizing injection sequence. A – detector signal, B – correlated electropherograms, 1 – 5 consecutive single injections, 2 – size of injections and interval between them has been randomized, 3 – only interval between injections has been randomized, 4 – only size of injections has been randomized. Detector signal marked with red is part of a whole signal that was used for correlation. Experimental conditions: length of one PRBS element – 4 s, BGE – 40 mM TRIS Borate, sample – 200 μM DMSO

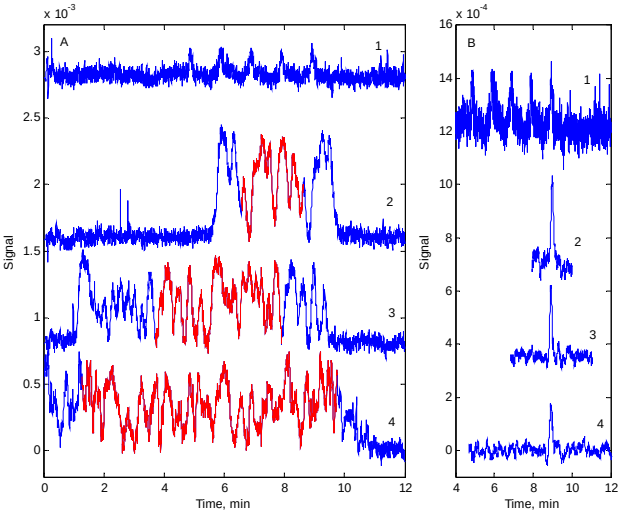

Figure 4. Multiple input CE experiments using randomized injection sequences of different length. A – detector signal, B – correlated electropherograms, 1 – 5 consecutive single injections, 2 – PRBS 31, 3 – PRBS 63, 4 – PRBS 127 elements. Detector signal marked with red is part of a whole signal that was used for correlation. Experimental conditions: length of one PRBS element – 4 s, BGE – 40 mM TRIS Borate, sample – 200 μM DMSO

transform into additional low frequency baseline noise in the correlated electropherogram. Figure 5 demonstrates how this sort of baseline disturbances affects the results of the correlation process. Spike or spikes will transfer into a higher number spikes with lower intensity (Figure 5.1) that are located in the correlated electropherogram according to the injection sequence [25]. Baseline drift transfers into low frequency noise and drift (Figure 5.2). To get a maximum result out of our experiments, all signals were manually processed first for removing spikes and then deleting baseline drift before correlation.

3.7. Sample mixtures with several components

Experiments so far have been performed with only one component in the sample solution. This way it was easier to investigate options and limitations of multiple input techniques in commercial CE equipment. These experiments lead the way for implementing cross-correlation CE for analyses of sample mixtures. Before analyzing more realistic samples, mixtures of three and four components were used. Experiments with sample mixtures (figure 6) reveal several drawbacks that make the use of cross-correlation CE for analyses not very practical at the moment. First of all, it was found out that every additional component in the sample mixture makes it more difficult to achieve S/N ratio improvement. At this early stage it was possible to achieve small S/N ratio gain with mixture of three components (figure 6A), but in case of four components the single injection electropherogram had better S/N ratio than correlated electropherogram of multiple input technique (figure 6B). To be more specific, high frequency detector noise in the actual detector signal (figure 6.2) is replaced by low frequency noise in the correlated electropherograms (figure 6.1). According to [26], such kind of low frequency noise is caused by the reproducibility problems of sample injections and non-stationarities of the system like EOF drift. To be correct, the single injection mode P/ACE system is very reproducible as has been tested over and over again, but in multiple input mode there is lack of reproducibility. Reason for this is probably the fact that the first and the last injections in a sequence have been treated differently, as first injections have been migrated/separated by short HV pulses while last injections have been treated by constant HV. These reproducibility problems cause some portion of signal to transpose in correlated electropherograms into artificial low frequency noise instead of analyte peaks. In case of complex samples this effect is multiplied by the number of components and from a certain number of components it is not possible to gain any S/N ratio improvement. Moreover, in case one component has a higher peak than others, low

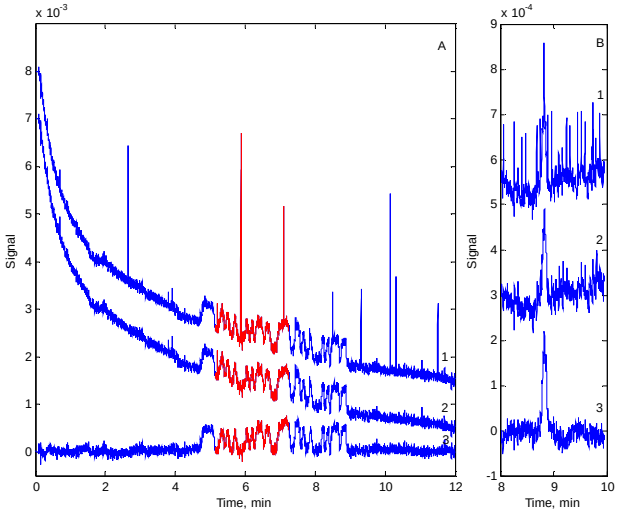

Figure 5. Detrimental effect of spikes and baseline drift. Here are present detector signal (A) as it was recorded (1), and after removal of spikes (2) and baseline drift (3) and corresponding correlated electropherograms (B). Experimental conditions: injection sequence – 31 element PRBS, length of one element – 4 s, BGE – 40 mM TRIS Borate, sample – 200 μM.

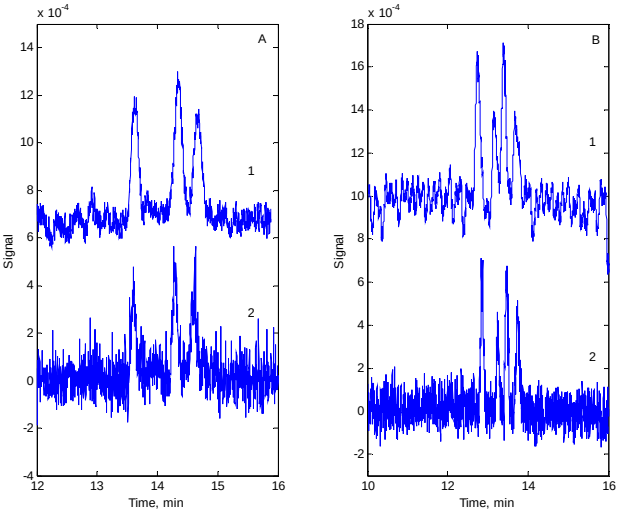

Figure 6. Multiple input CE results with sample mixture of several components. 1 – correlated electropherogram, 2 – single injection for comparison, A – sample mixture of three components, B – sample mixture of four components.

frequency noise of this component hides smaller peaks of other components, limiting dynamic concentration range within a single sample mixture.

4. Conclusions

In commercial instruments there are limitations concerning cross-correlation CE experiments. The whole injection sequence must fit into the effective length of the separation capillary. This sets maximum theoretical S/N gain that could be achieved in P/ACE system. In practice the S/N gain is limited by the fact, that the auto-sampler of P/ACE system is not capable of working without HV interruptions that are needed for changing sample and BGE vials. A high number of HV interruptions lowers the precision of the injection system and causes low frequency noise in correlated electropherograms, limiting S/N ratio gain. This unwanted noise is proportional to the size of a peak and to the number of components in the sample mixture. Therefore, satisfactory S/N ratio improvement at the moment could be achieved only with very simple sample mixtures. Though, a specific way of programming the injection sequence and modification of the sequence used in the correlation process can at least partly reduce the deleterious effects caused by low precision of injection system.

References

1. J. P. Chervet, R. E. J. Van Soest, M. Urzem, J. Chromatogr. 541 (1991) 439.
2. Y. Xue, E. S. Yeung, Anal. Chem. 66 (1994) 3575.
3. T. Tsuda, J. V. Sweedler, R. N. Zare, Anal. Chem. 62 (1990) 2149.
4. D.S. Burgi, R.L. Chien, Anal. Chem. 63 (1991) 2042.
5. J. L. Beckers, P. Boček, Electrophoresis 21 (2000) 2747.
6. M. Urbanek, L. Křivankova, P. Boček, Electrophoresis 24 (2003) 466.
7. J. A. McReynolds, S. A. Shippy, Anal. Chem. 76 (2004) 3214.
8. C. T. Culbertson, J. W. Jorgenson, J.Microcolumn Sep. 11 (1999) 652.
9. M. F. Regehr, S. K. Paliwal, F. E. Regnier, J. Chromatogr. A. 659 (1994) 247.
10. H. van der Moolen, D. J. Louwerse, H. Poppe, H. C. Smit, Chromatographia 40 (1995) 368.
11. H. van der Moolen, H. Poppe, H. C. Smit, Anal. Chem. 69 (1997) 4220.
12. R. Kuldvee, M. Kaljurand, H. C. Smit, J. High Res. Chromatogr. 21 (1998) 169.
13. J. A. McReynolds, L. Gao, J. Barber-Singh, S. A. Shippy, J. Sep. Sci. 28 (2005) 128.
14. J. C. Fister III, S. C. Jacobson, J. M. Ramsey, Anal. Chem. 71 (1999) 4460.
15. K. L. Braun, S. Hapuarachchi, F. M. Fernandez, C. A. Aspinwall, Electrophoresis 28 (2007) 3115.
16. T. Kaneta, K. Kosai, T. Imasaka, Anal. Sciences 19 (2003) 1659.
17. R. Guchardi, M. A. Schwarz, Electrophoresis 26 (2005) 3151.
18. K. Hata, T. Kaneta, T. Imasaka, Anal. Chem. 76 (2004) 4421.
19. K. L. Braun, S. Hapuarachchi, F. M. Fernandez, C. A. Aspinwall, Anal. Chem. 78 (2006) 1628.
20. L. Gao, E. E. Patterson II, S. A. Shippy, Analyst. 131 (2006) 222.
21. K. Hata, Y. Kichise, T. Kaneta, T. Imasaka, Anal. Chem. 75 (2003) 1765.
22. K. Hata, T. Kaneta, T. Imasaka, J. App. Phys. 105 (2009) 102018.
23. K. Hata, T. Kaneta, T. Imasaka, Electrophoresis 28 (2007) 328.
24. A. Seiman, M. Kaljurand, A. Ebber.,Anal. Chim. Acta. 589 (2007) 71.
25. M. Kaljurand, E. Kullik. Computerized Multiple Input Chromatography. Chichester : Ellis Norwood. 1989, 225.
26. J. N. van der Moolen, D. J. Louwerse, H. Poppe, H. C. Smit, Chromatographia 368 (1995) 7.