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Improving precision of manual hydrodynamic injection in capillary electrophoresis with contactless conductivity detection

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ABSTRACT

Reproducible injection in capillary electrophoresis has been difficult to achieve with manual injection techniques using simple injection devices, such as gravity injection (siphoning) or hydrodynamic sample splitting. We demonstrate that the injection reproducibility can be improved using very simple means. With hydrodynamic sample splitter, a passive micro-metering valve can be inserted in-line to regulate the sample flow rate through the splitter interface. A significant improvement of both reproducibility and repeatability was achieved. The reproducibility of RSD of the peak areas improved from 25.4% to 4.4%, while the repeatability was below 4.1% when micro-metering valve was used. Additional simple correction that can be used to further improve the variability of injected sample volumes in any hydrodynamic injection mode in CE with conductivity detection was proposed and verified. The measured EOF peak can serve as a simple indicator of the injected volume and can be effectively used for additional correction. By a linear function between the injection volume and the peak area of the EOF, the RSD values of peak areas for both manual gravity injection and hydrodynamic sample splitter were further improved below 2% RSD. The linearity of the calibration curve was also significantly improved. The proposed correction works even with slight differences in matrix composition, as demonstrated on the analysis aqueous soil extract of model mixture of five nerve agent degradation products.

1. Introduction

Capillary electrophoresis has enjoyed relatively high popularity in last decades due to its superior performance in regard to efficiency, separation times, selectivity, and minimal sample and electrolyte consumption. It has however not, to a large extent, replaced the liquid chromatographic techniques used for routine analysis in analytical laboratories, owing to several shortcomings that currently limit CE, especially in quantitative analysis. For instance, only gel electrophoretic methods are listed in the Official Methods of Analysis handbook's latest edition [1]. Among the most cited drawbacks of CE appear low sensitivity, matrix dependence and low reproducibility. Perhaps the low reproducibility is one of the major problems that CE is being faced with and has been subject to several recent reviews [2,3]. The factors that can have an effect on the reproducibility of CE separation are numerous and include for instance poor sampling precision, changes in EOF induced by temperature and viscosity changes, adsorption of sample compounds onto the capillaries, various inherent sampling and detection biases, improper buffering of the electrolytes, decomposition due to electrolysis etc. Consequently there is no simple and/or generally applicable solution to improve the poor reproducibility of CE. Several approaches were however suggested to improve the reproducibility of the peak areas of the analytes in CE that are obtained through integration of the measured peaks in the electropherograms. The peak area is a representation of the analyte concentration, and its accurate assessment is important for quantitative analysis. Peak area in CE, however, is prone to change significantly between runs. For instance if EOF changes due to the sample component adsorption, the peak areas will change as well, as they depend greatly on the analyte migration velocity through the detector. Dividing the peak areas by their respective migration times [4] is commonly applied and can to some extent eliminate the peak area variation. This correction however accounts only for the changes due to the differences in migration velocities. More often, internal standardization is practiced [5–7], as this can also account for the errors in injection volumes. However it may not be universally applicable in all cases, as it may be difficult to find a suitable IS, especially for complex sample matrices.

In a well-controlled CE method, e.g. when repeatable migration times are obtained, poor peak area precision most often relates to the poor repeatability of the sample injection. This applies especially for the manual injection techniques. Manual injection is

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susceptible to the variations induced by the operator, because it is more difficult to control all the procedures exactly in the same way manually as can do the automated instruments. The repeatability issues have been most pronounced in the home-built field portable CE instruments that are otherwise one of the strongest CE assets. The development of portable CE instruments has seen a remarkable surge in the last decade, as more than ten articles on newly developed portable CE instruments appeared [8–17] including a recent comprehensive review [18]. A quick overview of the published work reveals that the sampling precision is not very good, typically in the range of 10–15% RSD of peak areas. The sampling devices used in existing portable CE instruments make use of either manual electrokinetic [8–13,16] or hydrodynamic injections [12,13,15–17]. Electrokinetic injection, has been shown to suffer from multiple biases [19,20] and is thus difficult to apply for quantitative analysis. Manual hydrodynamic injection, such as siphoning injection, though suitable for quantitation, is not robust enough, nor sufficiently reproducible to allow operation under difficult conditions. Some of the portable CE instruments include a simple sampling carousel, similar to those present in the commercial instruments that is operated manually, however the general performance of these instruments still calls for improvement. Automated injection devices have been difficult to implement in the home-built portable instruments because they add to an increased complexity. Thus, the development of a simple injection method with high precision for portable CE instruments is probably one of the most important tasks to be resolved. The injection should preferably be done without any capillary movement and ideally the two capillary ends should be in fixed position during the sample injection and analysis. The attempts to simplify the design of manual samplers in portable CE instrument were shown by Kaljurand's group [17,21]. The tedious manual hydrodynamic or EK injection was replaced by a flow splitting device(s) of various geometries. However even with this simplified injection, based on flow splitting, the reproducibility of peak areas was no better than 9% RSD.

Surprisingly, not much effort has been devoted to improvement of the data evaluation techniques with respect to precision of sample injection in CE. In a sole report from 2006, Erny and Cifuentes [22] have shown that the hydrodynamically injected sample plug length can be estimated by measuring the electrophoresis current. It was shown that the current increases suddenly when the injected water plug exits the separation capillary. The measured dip can be used for correcting the injection imprecision. This approach is however only suitable for relatively large injected volume of samples having sufficiently different conductivity from that of the BGE. If this condition is not fulfilled the current monitoring approach may not work.

In here, we propose several ways to improve the performance of the manual hydrodynamic injection techniques. We show that with hydrodynamic sample splitter, an inclusion of a simple micrometering valve helps regulate the injection reproducibility to an acceptable level. We also show that with conductivity detection, the measured EOF peak correlates well with the injected volume and can be used for correcting the injection imprecision with simple manual HD injection. By a linear function between the injection volume and the peak area of the EOF (EOF peak area can be simply integrated from the registered electropherogram) we show that further improvement in injection precision can be achieved. The RSD values of peak areas for both manual gravity injection and hydrodynamic splitter device can be improved to below 2% RSD. The calibration linearity also improves in the same way. We demonstrate the applicability of this approach on determination of a model solution of five degradation products of chemical warfare agents, that are commonly analysed by portable CE instruments.

2. Experimental

2.1. Materials and methods

2.1.1. Electrophoretic system

A purpose-built CE instrument with either manual siphoning injection or hydrodynamic sample splitter was employed for all electrophoretic runs. The separation voltage was provided by a high voltage power supply unit (Spellman CZE2000R Start Spellman, Pulborough, UK) that was operated at a potential of −18 kV applied at the detection side of the separation capillary. The separation capillaries used were fused-silica (FS) capillaries (75 μm I.D., 375 μm O.D., 45 cm total length, 35 cm effective length, Microquartz, GmbH, Munich, Germany). The separation capillaries were preconditioned with 0.1 M NaOH for 30 min, deionized water for 10 min and with respective background electrolyte (BGE) solution for 10 min. Between two successive injections, the capillary was flushed with BGE solution for 1 min. All CE experiments were performed at ambient temperature.

2.1.2. Injection

Injection of standard solutions and real samples was carried out either hydrodynamically or using an in-house built sample splitter injector. In a hydrodynamic (HD) injection mode one capillary end with the sample vial was elevated to a fixed height of 15 cm for a specific time interval (typically 10 s, manually timed) and injection was carried out by siphoning effect. The hydrodynamic sample splitter consisted of a splitter interface machined in a piece of polyimide block. The schematic of the splitter injector is shown in Fig. 1. The splitter includes a 3 cm long horizontal flow through channel of 1 mm I.D. to which two vertical channels of the same diameter are connected. A separation capillary and a grounding Pt electrode were tight fitted into a PTFE tubing (350 μm I.D., 1/16″ O.D.), inserted in the two vertical channels and secured with 1/16″ flangeless fittings (Upchurch Scientific, Oak Harbor, WA, USA). One side of the horizontal channel of the splitter interface was connected with the injection syringe via a micro-metering valve (P446, Upchurch Scientific), while the other side included a 10 cm long, 250 μm I.D. PTFE tubing directed to waste. The splitting ratio was adjusted by choosing the length and I.D. of this waste tubing. A fixed volume of sample (500 μL) was delivered by a 1 mL disposable plastic syringe and injected manually by forcing the sample to flow by the splitting point in the splitter interface. The sample injection was followed by the BGE injection to clean the interface from the remaining sample before the application of high voltage.

2.1.3. Detection system

A high voltage capacitively coupled contactless conductivity detector (C4D) was used. It was described in a recent publication [23]. It consists of a detector cell, an external ac voltage source for excitation and an external detector circuitry for processing the cell current. The excitation voltage was provided by a circuitry based on a MAX038 oscillator (Maxim Integrated Products, Sunnyvale, CA, USA). The oscillator operated at various frequencies between 100 and 400 kHz and a voltage booster using a high voltage operational amplifier (PA91, Apex Microtechnology, Tucson, AZ, USA) produced an output of maximum 360 V_{pp} (peak-to-peak). The detector was operated at 200 kHz and 300 V_{pp} in all experiments. Data were collected using in-house written software and a 20 bit sigma-delta data acquisition card (Lawson Labs Inc., Malvern, PA, USA).

2.2. Chemicals

2.2.1. Reagents, standards, electrolytes

All chemicals were of reagent grade and deionized (DI) water (MilliQ Water System, Millipore, Molsheim, France) was

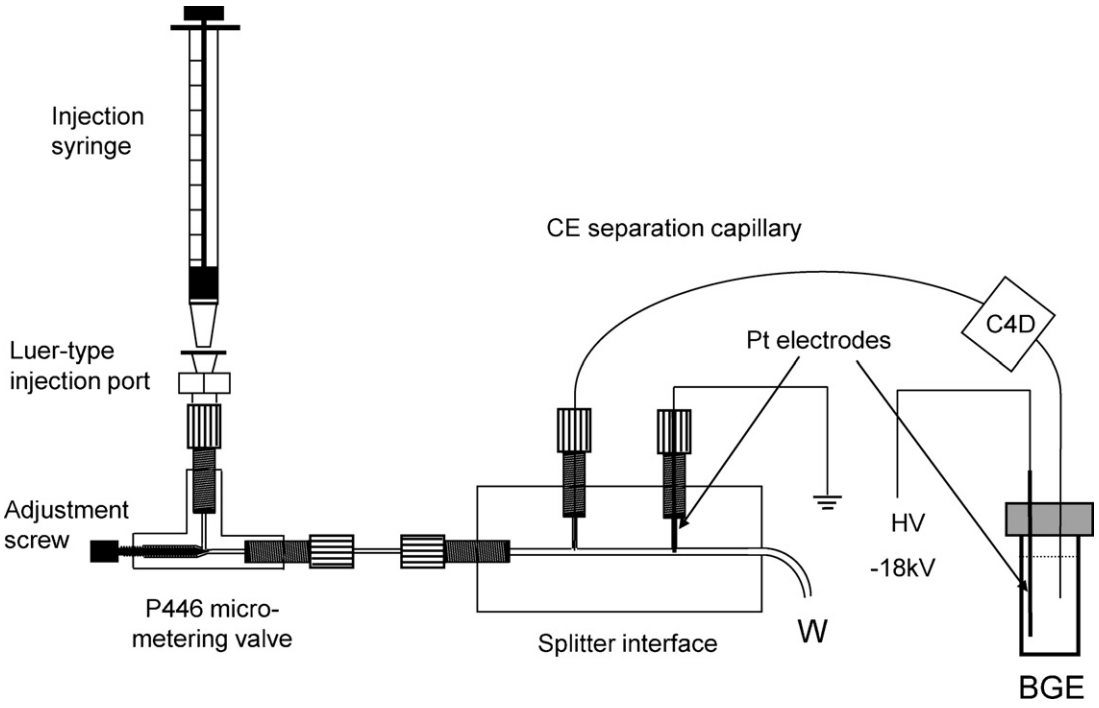

Fig. 1. A schematic of the hydrodynamic sample splitter.

used throughout. Stock solutions of phosphonic acids (10 mM) were prepared from pure acids (butylphosphonic acid, BPA, Alfa Aesar; propylphosphonic acid, PPA, Alfa Aesar; methylphosphonic acid, MPA, Sigma–Aldrich) or their sodium salts (pinacolylmethylphophonate, PMPA; ethylmethylphosphonate, EMPA; 2-aminoethyldihydrogenphosphonate, AEDHPA, all Sigma–Aldrich). All multi-ion standard solutions were prepared daily from these stock solutions and were diluted with DI water.

Background electrolyte (BGE) solutions for CE measurements were prepared daily from 100 mM stock solutions of 2-(N-Morpholino)ethanesulfonic acid (MES, Sigma–Aldrich) and L-histidine (HIS, Sigma–Aldrich).

Preparation of samples containing nerve agent degradation products. Laboratory floor sample: 1 mL of 1 mM mixture of five phosphonic acids was applied to the laboratory floor, let dry for 30 min and sampled with DI water pre-moistened Ghost wipe (Environmental Express, Mt. Pleasant, SC, USA) tissue. The tissue was transferred to a plastic sample vial with 10 mL of DI water and the phosphonic acids from the tissue were extracted into water. The extract was filtered through a 0.45 μm filter (Filtropur S, Sarstedt, Numbrecht, Germany) and internal standard (AEDHPA) at 400 μM was added to the sample, followed by direct injection into a CE system.

Soil sample: 1 mL of 1 mM phosphonic acid mixture was applied to 5 g of soil sample, let stand for 30 min and transferred to plastic sample vial with 10 mL of DI water. The phosphonic acids in soil sample were extracted by vigorous shaking for 1 min, followed by filtration through a 0.45 μm filter, addition of internal standard and direct injection into the CE system.

3. Results and discussion

3.1. Optimization of the separation of the nerve agent degradation products

A model mixture of five phosphonic acids (degradation products of nerve agents) was selected for the proposed study. All the selected phosphonic acids have relatively low pKas and a BGE with low pH can be used for their separation. The conductivity of the acids, on the other hand, is rather significant, and C4D can be advantageously used for their sensitive detection. A simple MES/HIS background electrolyte presents a good choice for an efficient separation. MES/His based electrolytes were previously used for separation of CWA degradation products in co-electroosmotic mode [24–26]. In this mode the fast inorganic anions migrate before the phosphonic acids and the monitoring of the EOF may require a significant time delay after the analytes have been separated. In the current work the separation mode was counter-electroosmotic, e.g. the phosphonic acids migrate against the EOF, but are eventually swept to the detector by the EOF. In this case the EOF peak migrates before the analytes and its detection and evaluation is convenient without introducing any delays. Initially, the concentration of MES and HIS was varied between 5 and 20 mM, with best performance achieved at low, 7.5 mM concentration of each. Separation of all selected phosphonic acids including internal standard was achieved in less than 7 min in a 7.5 mM MES/HIS BGE with pH 6 at −18 kV. For qualitative and quantitative analysis, the inclusion of internal standard is sometimes useful and we have chosen to include 2-aminoethyldihydrophosphonic acid (AEDHPA). It was observed that this internal standard actually produces 2 peaks in the electropherograms, that effectively embrace the separated phosphonic acids and can be used both for qualitative identification of the peaks and quantitative analysis.

An example of the separation of 5 phosphonic acids including AEDHPA as an internal standard is shown in Fig. 2. Note the different *y*-scale intensities on the electropherograms. The electropherogram of a standard mixture of phosphonic acids includes several points of interest that were identified and were applied to correct the performance of the injection devices used. First, at approximately 1–1.5 min, there is a cluster of small cationic analytes, that may include some common inorganic cations such as K^+, Na^+, Ca^{2+}, Mg^{2+} present either in the standard solutions or as background contaminants in the real samples. The cationic analytes are followed by a large negative EOF peak at about 2 min. EOF peak appears typically when C4D detection is used, as the conductivity of all migrating zones is monitored. We assume that the EOF zone contains only

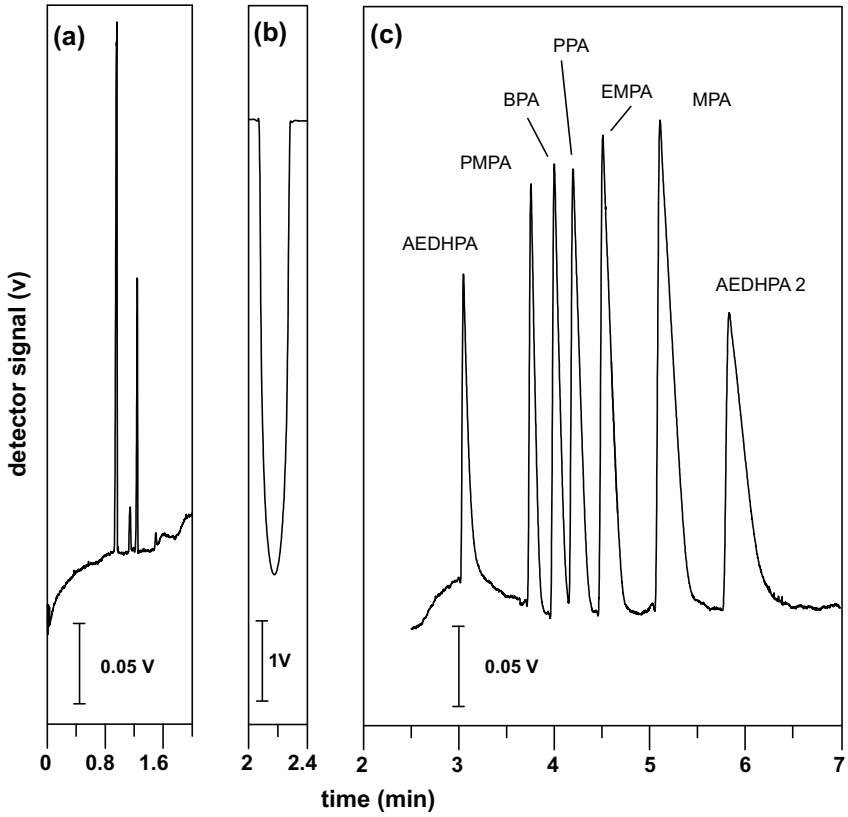

Fig. 2. Electropherogram of standard mixture. (a) Cationic analytes, (b) EOF peak, (c) analytes and IS. Sample: 100 μM each phosphonic acids, IS-AEDHPA 400 μM, separation conditions: −18 kV. C4D detection. BGE: 7.5 mM MES/HIS, pH 6.

water and possibly neutral compounds that may be present in real samples. The EOF peak is of particular interest, as we have noticed that there is a linear relationship between the integrated EOF peak area and the injection volume. It can be thus used for correcting injection imprecision typically encountered with simple manual HD injection procedures. Third, after the EOF peak, the analytes of interest and internal standard migrate between 3 and 7 min, as they are eventually swept by the EOF to the detector.

3.2. Performance of simple injection devices and their improvements

In the initial experiments conducted with hydrodynamic siphoning injection or using a hydrodynamic sample splitter, we have encountered rather large variations of peak areas and consequently also variations of migration times. After careful examination of the electropherograms, we have concluded that the irregularities are not due to the changed performance of the capillary and EOF (which may be very often the case), but due to the poor performance of the injection devices. While the repeatability of the peak areas with the HD siphoning injection could be maintained at approximately 5% when extreme care was taken to reproducibly perform all the injection sequences, the repeatability of the peak areas with the hydrodynamic sample splitter was typically no better than 30% RSD. These values were in agreement with the previously achieved data [21] using a similar instrumental setup and the hydrodynamic sample splitter. It was extremely difficult to achieve exactly the same injected amounts using the HD sample splitter, as it is rather difficult to control the flow rate of the sample by simply pushing the syringe piston by hand. While acceptable results could be obtained when used by a single, experienced, person, the failure to reproduce the exact injection amount has been even more evident when different people operated the same instrument.

To asses the performance of the splitter injection device, four volunteers were instructed to carefully inject 500 μL amount of the same sample using the same pressure in the splitter injector. Duplicate injection was performed by each person. The final injected amounts differed as much as 100% between the operators (9.2 nL vs. 18 nL, calculated from Eq. (1), see Section 3.3) as is shown in a bar graph in Fig. 3A. The reproducibility for the splitter injector without the micro-metering valve was 25.4% ($n = 8$). The injection volume from duplicate injections by the same person differed also significantly, the calculated repeatability ($n = 2$) values ranged from 2% to 15.7%. The injected amount not only influences the peak areas but also migration times of the peaks, as with the larger injection amounts, excessive peak tailing was observed due to the capillary overloading. Fig. 4 shows an example, where the hydrodynamic sample splitter was used without the micro-metering valve and the applied pressure for sample injection was high (A) and low (B). The insert in Fig. 4 shows the recording of the EOF peak for both injections. It is quite apparent, that the EOF peak area differs significantly between the injections and correlates well with the injection amount and the peak areas of the analytes. Due to the significant differences of the signal between the analytes and the EOF, the EOF peak in the electropherograms showing the separation of the analytes was clipped.

In general, splitter injectors are prone to the applied flow rate/pressure differences and to achieve a stable performance, a precise pumping mechanism is required. Tsukagoshi et al. [27] have achieved RSD values of peak areas lower than 3% using a precisely controlled syringe pump that delivered the sample and BGE portions with an exactly defined flow rates for a defined time interval. A syringe pump is however difficult to apply in a portable instrument as it adds additional level of complexity and power consumption.

It is also not compatible with the simple sampling schematic that has been developed with the current portable CE instrument.

In order to improve the current performance of the HD sample splitter, we have included an in-line micro-metering valve between the injection port and the splitting point of the interface (see Fig. 1). The function of the micro-metering valve is to restrict the manually applicable pressure and thus achieve uniform sample flow rates through the splitter interface. A similar principle was recently used by Hauser et al. [28] to achieve reproducible flow in an SIA-CE injection system. By setting the thumbwheel of the micro-metering valve into a predefined position, the maximum manually applicable pressure from the injection syringe and consequently the speed of pressure rise is limited leading to improved reproducibility of the injection. The same operators were asked to perform duplicate injection of the same solutions. Fig. 3B shows that although there was still some difference in the injected amounts, the low to high injection volume now varied by maximum of 17%, which is a significant improvement compared to the previously presented data. The

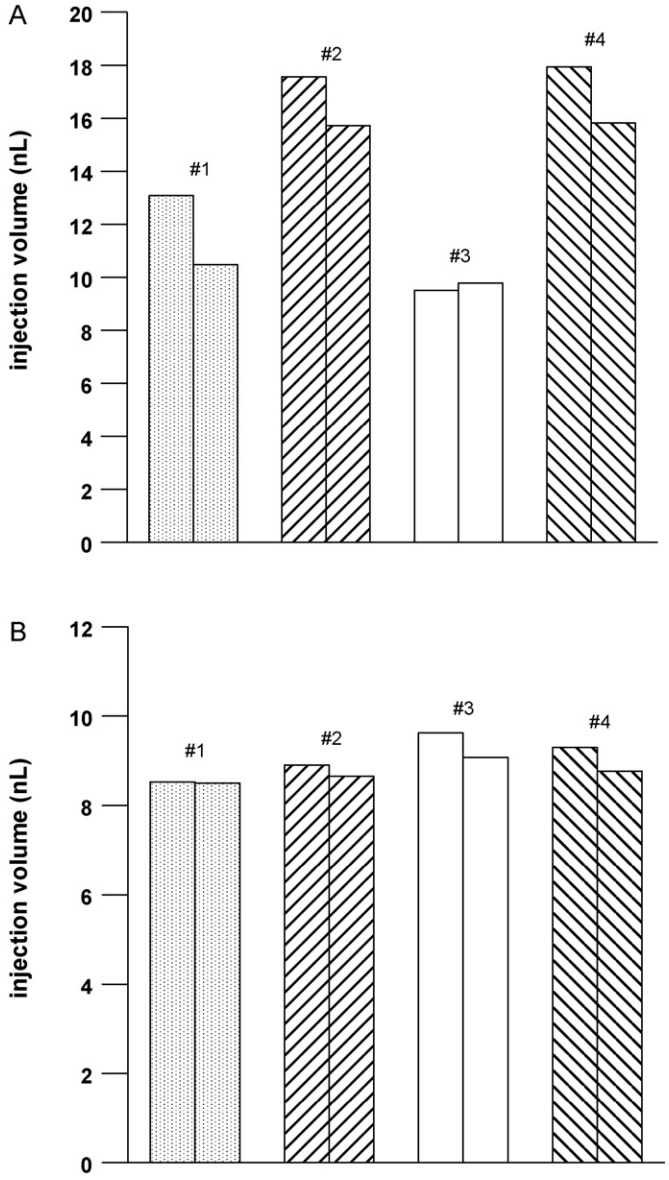

Fig. 3. Difference in injection volumes injected by four different operators (#1–#4) without (A) and with (B) the micro-metering valve. The injected volumes were obtained from the linear equation between EOF peak area and the theoretically calculated injected volumes from Eq. (1).

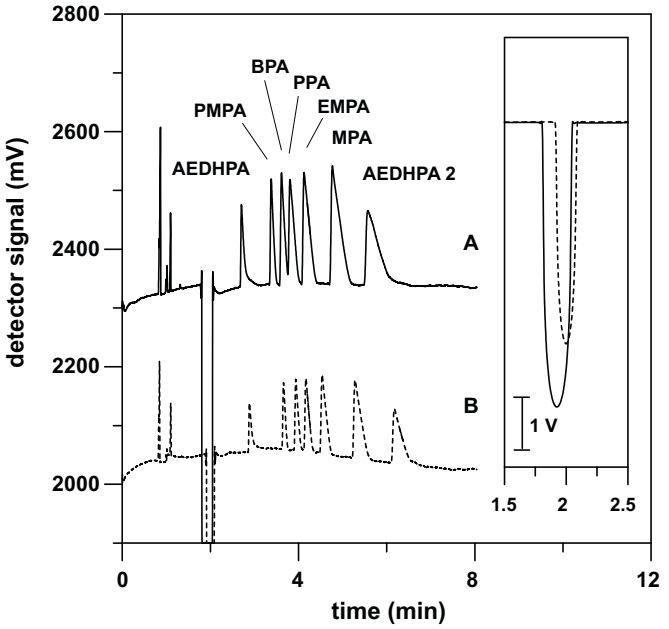

Fig. 4. Comparison of different injected amounts using a splitter injection device without the micro-metering valve. (A) High pressure applied, (B) low pressure applied. 500 μL sample injected in both cases. Electrophoretic conditions the same as in Fig. 2. The insert shows the EOF peak registered in case A (solid line) and case B (dotted line).

reproducibility for the splitter injector with the micro-metering valve was improved to 4.4% ($n = 8$). The calculated repeatability ($n = 2$) values also improved and ranged from 0.2% to 4.1%.

3.3. Improving performance of the HD injection using the EOF peak area

The achieved reproducibility of the manual injection may still be unacceptable in some cases and the goal of the present investigation was to improve the precision beyond the achieved values using a simple correction based on the EOF peak measurement.

The counter EOF separation of the phosphonic acids offers and excellent opportunity for this correction, as the EOF peak migrates ahead of the separated analytes and its measurement does not require additional analysis time or any extra device to be used (see Fig. 2). Obviously this approach is not applicable to an analysis with very slow EOF, for instance in the protein analysis. However, the separation of small anions or cations is typically preceded or immediately followed by an EOF peak and it is thus not very complicated to include this peak in the measured/evaluated data. To study the dependence of the EOF peak properties on the injected amount, we have used a hydrodynamic siphoning injection, as it can be relatively precisely controlled and the injected sample volume can be calculated based on the Hagen–Poiseuille equation:

$$F = \frac{\Delta p \pi r^4}{8 \eta L} \quad (1)$$

where F is the volumetric flow rate, Δp is the pressure difference, r is the capillary radius, η is the solution viscosity and L is the capillary length. The injection volume can be calculated as the product of the volumetric flow rate, F and the injection time, t_{inj} for which the capillary end is elevated. The achievable pressure difference, Δp can be easily calculated from the weight of the liquid column inside the capillary:

$$\Delta p = h \rho g \quad (2)$$

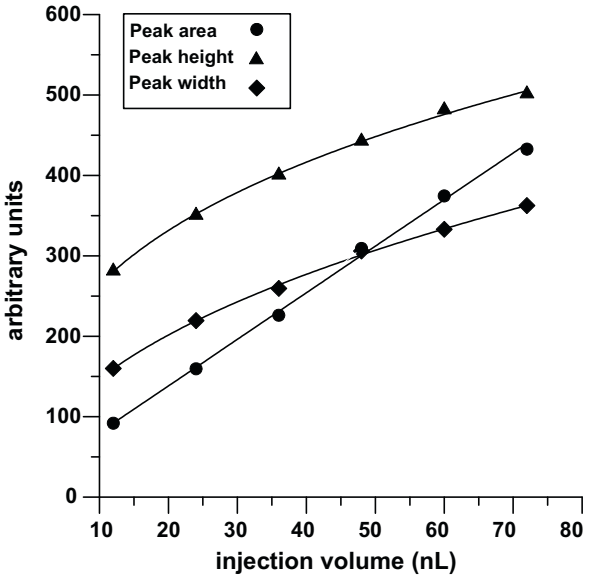

Fig. 5. The dependence of the EOF peak height, width and area on the injection volume. Note that the integration parameters were scaled to fit into a single graph.

where h is the height difference, ρ is the liquid density and g is the acceleration due to the gravity. By knowing the elevation height, injection duration and other capillary and solution parameters, the exact volume injected can be calculated. By substituting the values used in real experiment, $L = 0.5$ m, $\eta = 0.00089$ kg s^{-1} m^{-1}, $r = 37.5 \times 10^{-6}$ m, $h = 0.15$ m, $\rho = 1000$ kg m^{-3}, $g = 9.8$ m s^{-2}, the injection volumes for t_{inj} equal to 5–30 s injections varies between 12.8 and 77 nL. Various parameters of the EOF peak can be measured. Those include for instance EOF peak width at the baseline, EOF peak height and EOF peak area.

Fig. 5 shows the relation between the injection volumes and the selected parameters of the EOF peak. It is evident that both peak width and peak height give a non-linear dependence that can be best fitted by a power function (coefficient of variance, r^2, equals to 0.997 and 0.998, respectively), while the EOF peak area gives a strictly linear relationship ($y = 27.79x + 45$; $r^2 = 0.998$). Thus, the area of the EOF provides a good estimate of the total hydrodynamically injected sample plug. It could be argued that the peak width of the EOF peak is the simplest parameter to be measured, as the EOF peak area may sometimes be difficult to assess because the EOF peak signal will be out of range of the detector. However as the peak width is influenced by the dispersion in the system and during the injection/separation, it does not produce a linear relationship and it is rather difficult to implement for this type of correction.

Two sets of experiments were performed to verify the effect of the EOF peak area correction and its applicability to improve the CE performance. In the first set, an error of the injected amount of sample introduced into the separation capillary by HD siphoning injection was simulated by varying the injection time between 8 and 12 s. This may correspond to a worst-case scenario that may be encountered when different operators inject in the same CE instrument. Ten randomized injections were performed keeping the injection height the same, but varying the injection times in the selected range. The peak area of each analyte was integrated, and the RSD was calculated. Then the integrated EOF peak area was used for simple linear correction-by dividing each analyte peak area by the EOF peak area. Internal standard (IS) method was also used, i.e. dividing each analyte peak area by the peak area of an internal standard (AEDHPA) that was added at 400 μM concentration to the mixture of analytes. The results are shown in the first part of Table 1. In the second experiment, the injection conditions for the consecutive injections were kept exactly the same. Ten injections of the same sample for 10 s were performed. The results are shown in the second part of Table 1

Table 1
Repeatability of HD injection, expressed as RSD of peak area% ($n = 10$).

	No correction	EOF PA correction	IS method
Variable injection time 8–12 s			
PMPA	14.4	1.5	2.3
BPA	14.7	1.5	2.5
PPA	14.3	0.9	1.5
EPA	14.3	1.9	2.7
MPA	15.1	1.9	2.4
The same injection time, 10 s			
PMPA	4.3	1.5	2.3
BPA	4.2	1.9	2.6
PPA	3.9	1.3	2.4
EPA	4.4	1.5	1.4
MPA	5.3	1.6	2.2

Obviously, with the worst-case scenario (first set of experiments), the RSDs of peak areas were as high as 15% RSD. However a simple correction by the EOF peak area improved the results significantly, the RSD being below 2%. Slightly higher values (1.5–2.7%, RSD) could be achieved using the IS method. A very similar trend was observed also for the second set of experiments; here the improvement is not that radical due to the initially relatively good RSD values, however, again it was improved from 5% to below 2%.

Table 2 shows the results of EOF correction for the splitter injection device. Similarly to the HD siphoning injection, two sets of experiments were designed. In the first case, the volume of the injected sample was varied between 400 and 600 μL, while in the second case it was exactly 500 μL. 10 repeated injections were performed. Again, the correction by EOF peak area resulted in dramatic improvement of the RSD of peak areas in both experiments, being below 2% in most cases.

It is thus evident that the proposed correction efficiently works in any type of hydrodynamic sample injector and significant improvement in the analytical performance of the instruments even with simple, manually operated, injection devices can be achieved. This may have a significant consequence for the acceptance of CE, especially in field portable experiments, where precision is often of concern.

3.4. The effect of EOF peak area correction on the calibration data

The calibration curves were constructed from 5 data points along the calibration range of 0–100 μM. Triplicate injections of each calibration standard were carried out. Internal standard (AEDHPA) was added to all calibration solutions at 400 μM concentration. When raw peak areas of the phosphonic acids were used for plotting the calibration data, the linear fit produced the coef-

Table 2
Repeatability of the hydrodynamic sample splitter, expressed as RSD of peak area% ($n = 10$).

	No correction	EOF PA correction	IS method
Variable injection volume, 400–600 μL			
PMPA	23.4	1.7	2.8
BPA	18.4	2.1	3.0
PPA	17.7	2.0	2.8
EPA	22.8	1.7	2.4
MPA	24.8	1.3	2.5
The same injection volume, 500 μL			
PMPA	6.9	2.0	1.6
BPA	8.5	2.0	2.1
PPA	6.8	2.0	3.1
EPA	10.5	1.3	2.3
MPA	12.0	1.6	2.1

Table 3
Figures of merit for the analytical method.

	r^2 (a)/r^2 (b)/r^2 (c)	Linearity	LOD (μM)
PMPA	0.9948/0.9998/0.9994	0–100 μM	5.0
BPA	0.9921/0.9998/0.9999	0–100 μM	4.7
PPA	0.9841/0.9998/0.9995	0–100 μM	4.7
EPA	0.9919/0.9995/0.9999	0–100 μM	4.2
MPA	0.9926/0.9997/0.9992	0–100 μM	4.1

(a) No correction; (b) EOF peak area correction; (c) IS method.

ficient of variation, r^2, between 0.9919 and 0.9948. The r^2 values are rather low, most probably due to the variations in the injection volumes, as described earlier. However, when the suggested correction with EOF peak area was applied to the calibration data, the r^2 values improved significantly and were in the range 0.9995 and 0.9998. Similar results were also obtained when IS correction was used (r^2 between 0.9992 and 0.9999). The results are summarized in Table 3, together with the LOD data for 5 phosphonic acids that were determined from the calibration curve, based on the 3 S/N criteria.

The proposed correction using the EOF peak area improved the linearity of the calibration curve significantly and results comparable to IS method were achieved.

3.5. The effect of sample matrix

The improved linearity achieved with the proposed correction suggests that the EOF peak area correction is applicable even if the total concentration of the ions in the sample differs, i.e. slight differences in the matrix composition may be tolerable. We have investigated the influence of matrix and its effect on the proposed EOF correction using two types of samples. Five phosphonic acids were applied to soil sample and a laboratory floor and extracted after 30 min as described in Section 2. In both cases the extraction solvent was DI water. The condition of a fairly similar matrix composition is fulfilled, though some ions may be co-extracted from the samples, which indeed was the case of the soil sample. In Fig. 6 an electropherogram of a standard solution containing 100 μM of the phosphonic acids and the soil extract are shown. In the left insert of Fig. 6 the separation of inorganic cations from the standard solution and soil extract is shown. The amount of cations in soil sample is significantly higher than in the standard solution. The laboratory floor wiped with DI water did not show any increase of cations, as it was relatively clean (results not shown). The right insert of Fig. 6 shows the electropherogram section with the EOF peak. It is clear that the EOF peak areas for the standard and the soil extract are the same, e.g. slight difference in sample matrix does not influence significantly the EOF peak area and hence the proposed correction. A detailed study would however be required to investigate the influence of different solvents and/or samples containing very large concentrations of ions, for instance sea water or biological samples. Because the selected model analytes are soluble in water and aqueous extraction is suitable, the investigation of different solvents was outside the scope of this work.

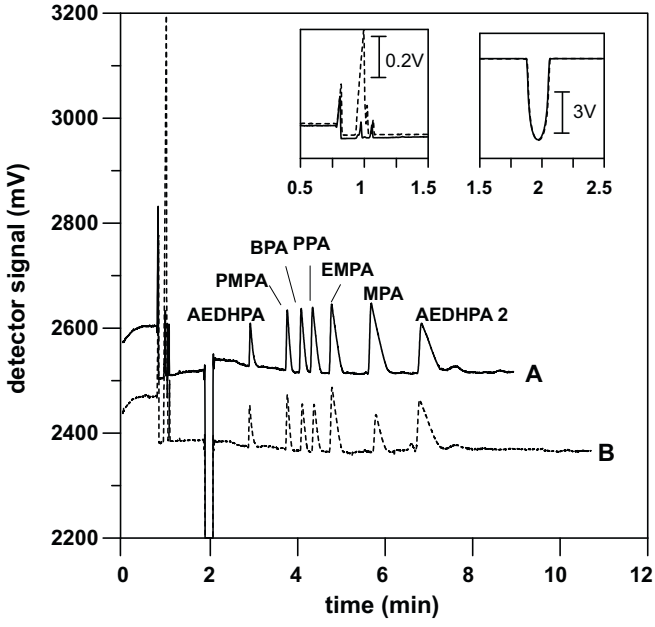

Fig. 6. Electropherogram of a standard and soil extraction sample. (A) Standard: 100 μM each phosphonic acids, IS–AEDHPA 400 μM, (B) soil extract-added IS: AEDHPA 400 μM. Other conditions the same as in Fig. 2. The left insert shows the separation of cations in a standard solution (solid line) and soil extract (dotted line). The right insert shows two nearly identical EOF peaks recorded for standard solution and soil extract.

4. Conclusions

In manual hydrodynamic injection devices in CE, improved reproducibility can be achieved in different ways. When using hydrodynamic sample splitter, a passive micro-metering valve can be inserted in-line to regulate the sample flow rate through the splitting interface. A significant improvement of both reproducibility and repeatability can be achieved. Additional simple correction that can be used to further improve the variability of injected sample volumes in any hydrodynamic injection mode in CE with conductivity detection has been proposed and verified. This correction consists of monitoring the EOF peak together with the peaks of analytes. The integrated EOF peak area is used to correct the injection error introduced by various injection modes and devices, for instance by HD siphoning and HD sample splitter. The proposed correction improves the RSD of peak areas to less than 2%. The same correction can be applied even for extreme cases in which the injected volume differences vary by 50%. It can be also used to significantly improve the linearity of calibration curves and provides results comparable to the IS method. As the proposed correction can be simply applied to any electropherogram without a need for additional device or monitoring of additional separation parameters, it can have a significant impact on the acceptance of CE technique for quantitative analysis.

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