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Large-volume stacking for quantitative analysis of anions in capillary electrophoresis
I. Large-volume stacking with polarity switching

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Abstract

Several methods have been reported describing on-column concentration for sensitivity enhancement in high-performance capillary electrophoresis. One technique of interest consists of a large-volume hydrodynamic injection, followed by the removal of the large plug of low conductivity sample matrix out of the capillary using polarity switching. In this work the performance of this method is evaluated for the separation of arsenious acids and a comparison is made between the increase in peak area and peak height to evaluate the real sensitivity enhancement. Finally, the use of this stacking procedure for quantitative analysis is discussed.

Keywords: Large-volume stacking; Stacking; Anions; Dimethylarsinic acid; Monomethylarsonic acid; Arsenic acid

1. Introduction

High-performance capillary electrophoresis (HPCE) has been shown to be an efficient technique for the separation of charged species [1,2]. This high efficiency and the small injected volumes enable HPCE to reach very good mass sensitivity, but the short optical path length associated with on column UV detection considerably limits the concentration sensitivity. Therefore, several methods have been reported [3,4] which enhance the sensitivity of HPCE, such as sample concentration using on-line isotachophoresis [5,6] or sample stacking [7–10].

Slow moving anions (anions having an electrophoretic mobility inferior to the bulk electroosmotic flow) can be separated using a positive voltage in an untreated fused-silica capillary, with regard to their resistance to the electroosmotic flow. Chien and Burgi developed a method called large-volume stacking to stack anions in such separation conditions [10]: a large plug of low conductivity sample is introduced hydrodynamically into the capillary and a negative voltage is applied at the injection extremity. The large solvent plug is then electroosmotically pushed out of the capillary while the negative species stack-up at the boundary between the sample zone and the background electrolyte. Once the main part of the low conductivity zone has been pushed out of the capillary, the positive voltage is applied for separation to occur. This method eliminates the

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negative effects of the low conductivity plug [11] on the separation performances.

In this work, the effects of large-volume stacking on the separation of organic and non organic arsenious compounds [12] are investigated, both in terms of sensitivity enhancement and quantitative analysis.

2. Experimental

Experiments were carried out with a SpectraPhoresis 1000 (Thermo Separation Products) using PC 1000 software. The capillaries were untreated fused-silica capillaries (70 cm×75 μm I.D.) with detection window located 8 cm from the capillary extremity. The detection was UV detection at 195 nm. The run temperature was 40°C. The anions standard (Table 1) consisted of dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and arsenic acid (AsV).

The running buffer consisted of 20 m*M* disodiumhydrogenphosphate (Na_2HPO_4) adjusted to pH 6.0 with orthophosphoric acid. The capillary was washed with the running buffer for 2 min prior to each experiment. At the end of the day, the capillary was rinsed for 2 min with 1 *M* NaOH and for 5 min with deionised water.

Table 1
Arsenious compounds of interest

Name	Formula	pK_a
Dimethylarsinic acid (DMA)	CH_3 | O=As–OH | CH_3	9.3
Monomethylarsonic acid (MMA)	CH_3 |	3.6
	O=As–OH | OH	8.2
Arsenic acid (AsV)	OH |	2.3
	O=As–OH |	6.9
	OH	11.4

2.1. Standard separation

The standard sample consisted of DMA, MMA and AsV, at a 10 ppm concentration in 0.2 m*M* Na_2HPO_4 (pH 6.0). The sample was hydrodynamically injected at a calibrated low vacuum during 5 s, with a corresponding injected volume representing 1.0% of the whole capillary volume. Then, a positive voltage (25 kV) was applied leading to an average current i_0=82 μA.

2.2. Stacking process

The standard sample was diluted to the required concentration using a 0.2 m*M* Na_2HPO_4 solution. Hydrodynamic injections were performed under high vacuum for 12 s which corresponds to an injected volume representing 50.0% of the capillary volume. In a first step, a negative voltage (−25 kV) was applied and the large plug of sample was electroosmotically pumped out of the injection extremity of the capillary. The electric current through the capillary was initially largely lower than its value reached during an identical classical capillary electrophoresis separation (i.e., i_0). As the low conductivity injected plug was pumped out of the capillary, the current continuously increased up to its normal value: this meant that the main part of the low conductivity injected plug had been pushed out of the capillary and the stacking process could be considered complete. Consequently, the high voltage was switched from the negative to the positive value. The experimental current was monitored very carefully and polarity switching was operated as the current had reached 78 μA, i.e., 95% of its standard value i_0. This corresponds to a backout time $t_{backout}$ equal to 0.8 min. The time needed for the high voltage supply to change from −25 kV to +25 kV (t_{+-}) was equal to 0.1 min.

3. Results and discussion

3.1. Performance of large-volume stacking with polarity switching

Stacking anions separated in an untreated fused-silica capillary according to their resistance to the

electroosmotic flow first consists of the hydrodynamic injection into the capillary of a large plug of low conductivity sample. Then, a negative voltage is applied at the injection end of the capillary, and the electroosmotic flow causes the large plug to move in the direction of the injection end, hence pushing this plug out of the capillary. Meanwhile, the negatively charged species contained in this low conductivity plug are subjected to a strong local electric field strength and consequently move with a very high electrophoretic velocity towards the boundary between the sample plug and the buffer zone. Once anions reach this boundary, their velocity is slowed down in the lower field strength of the higher conductivity buffer, resulting in the stacking of the analytes at the boundary. When the main part of the large sample plug has been removed from the capillary, most of the negative species are concentrated at the boundary and the sample plug is now a thin zone of stacked species. At this moment, polarity is switched (a positive voltage is applied to the injection end) and separation starts to occur under classical conditions.

To evaluate the performances of this method, we studied the peak areas and peak heights obtained either with a standard hydrodynamic injection process (injected volume representing 1% of the capillary volume), or with a large sample plug stacking process (injected volume representing 50% of the capillary volume), using the same solute sample at a concentration of 10 ppm.

As in capillary electrophoresis with UV detection, peak areas depend on the apparent velocity of the migrating analytes, they were corrected by peak migration times according to:

$$S_c = \frac{S}{t_M} \tag{1}$$

where S_c is the corrected peak area, S the measured peak area and t_M the migration time of the analyte of interest.

The total peak migration time (T) measured on the electrophoregram corresponds to the time needed for the stacking process to occur ($t_{backout}$), to the polarity switching time (t_{+-}) and to the peak migration time (t_M) according to:

$$T = t_M + t_{backout} + t_{+-} \tag{2}$$

Effective peak migration time t_M, deduced from Eq. (2), is identical to effective migration time ($t_{M(standard)}$) of the same analyte with classical conditions of hydrodynamic sample injection.

Consequently, the corrected peak areas with a stacking process can be calculated according to Eq. (3):

$$S_c = \frac{S}{T - t_{backout} - t_{+-}} = \frac{S}{t_M} = \frac{S}{t_{M(standard)}} \tag{3}$$

Corrected peak area enhancement due to the stacking process is evaluated by the stacking factor F defined as follows:

$$F = \frac{S_c}{S_{c(standard)}} \times \frac{C_{inj(standard)}}{C_{inj}} \tag{4}$$

where C_{inj} is the injected solute concentration, (standard) corresponding to experiments carried out with classical conditions of hydrodynamic injection. The F value characterizes the stacking process yield, representing the amount of injected sample that has really been stacked. In our conditions, an F value equal to 50 will mean that the whole amount of injected sample has been stacked without any loss of sample during the stacking process.

Another parameter of interest that can be used in order to characterize the performance of the method is the sensitivity enhancement (SE), similar to F but related to peak heights:

$$SE = \frac{h}{h_{(standard)}} \times \frac{C_{inj(standard)}}{C_{inj}} \tag{5}$$

h being the peak height.

Comparison of F and SE is useful to check the loss of efficiency due to the stacking effect. For example, if $F = SE$, no peak broadening has occurred due to large injection or dispersion during the stacking process, and consequently peak area and peak height vary in the same way. Burgi and Chien [13] have shown an optimum value for the conductivity ratio between the sample and the running buffer, corresponding to a maximum efficiency. They defined the field enhancement factor γ as follows:

$$\gamma = \frac{E_1}{E_2} = \frac{\rho_1}{\rho_2} \tag{6}$$

where ρ_1 and ρ_2 are the sample region and the

running buffer zone resistivities respectively, E_1 and E_2 the local electric field strengths in corresponding regions. Because of a balance between peak-narrowing due to stacking and peak-broadening generated by the electroosmotic flow mismatch at the boundary between the sample and the run buffer [14], there exists an optimum γ value, corresponding to a minimum band width after stacking, and thus a maximum efficiency. In our study, this optimum γ value has been found to be around 100: the optimum stacking conditions are obtained using a sample conductivity 100 times lower than the running buffer conductivity. Under such conditions, corresponding F and SE values are listed in Table 2.

Those results clearly demonstrate that first, F values lower than 50 mean that a part of the injected species of interest are lost during the backout step, and secondly, SE values lower than F values mean that the stacking process introduces dispersion, leading to lower efficiency than that obtained under standard conditions of injection.

Even if this loss of efficiency does not affect the solute separation because of the high selectivity of the system, it limits the main purpose of the stacking process, that is, a sensitivity enhancement of the method.

Such experiments illustrate that during the backout step of the stacking process, the global electroosmotic velocity across the whole capillary is higher than the electrophoretic velocity of analytes in the sample plug, and consequently part of them are flown out of the capillary. As this global electroosmotic velocity is still decreasing during the backout step, an optimum sample plug length corresponding to the maximum length may exist, that can be injected without loss of the analyte, depending on the electrophoretic velocity of analyte in the sample zone and the bulk electroosmotic flow velocity itself varying according to the sample plug length.

To calculate this optimum plug length, one has to express the apparent velocity of an analyte in the sample plug as a function of the plug length during the backout step.

At the beginning of this step, the capillary can be divided into two distinct zones: the sample zone (zone 1) with resistivity ρ_1 and the buffer zone (zone 2) with resistivity ρ_2. (Fig. 1a). Nevertheless, the stacking of the analytes at the boundary between these two zones will result in a modification of both

Table 2
Comparison of calculated x_0 and measured F and SE for sample stacking of 1 ppm samples (injected volume representing 50% of the capillary volume), $t_{backout}=0.8$ min, $t_{+-}=0.1$ min

pH		x_0 (%)	F^a	R.S.D.%[a] (F)	SE^a	R.S.D.%[a] (SE)
6.0	DMA	9.2	11.6	2.9	11.1	1.3
6.0	MMA	27.5	34.8	3.1	14.3	1.5
6.0	AsV	34.1	40.6	2.1	16.0	0.8
7.8	DMA	29.2	34.3	2.5	15.2	1.0
7.8	MMA	33.3	42.1	3.5	16.4	1.8
7.8	AsV	50.1	49.3	1.3	18.5	0.3

Sample dissolved in Na_2HPO_4 0.2 mM (pH 6.0); run buffer pH, 6.0 and 7.8.
[a] Calculated from 5 experiments.

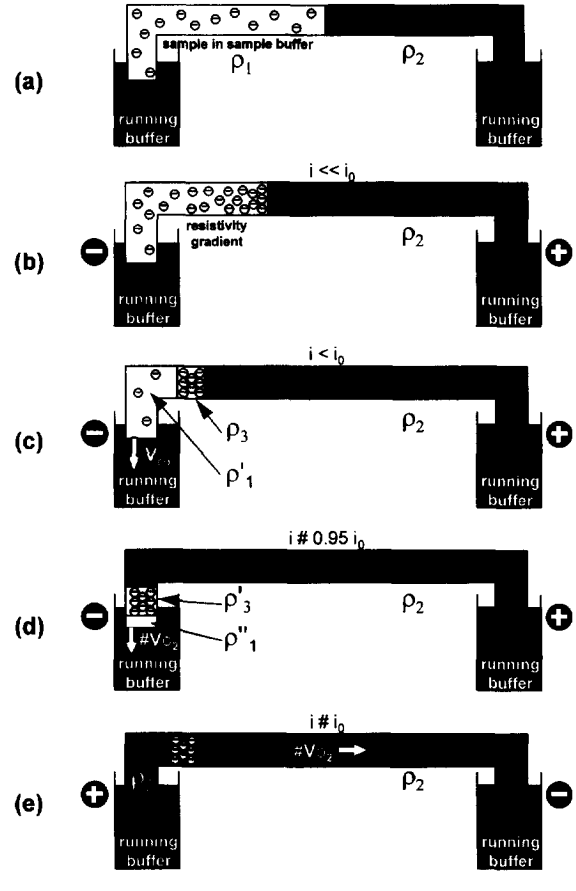

Fig. 1. Schematic representation of the stacking process: (a) hydrodynamic injection of a large sample volume, (b), (c) and (d) backout step, (e) classical separation process after polarity switching.

the composition and the conductivity of the sample zone according to Kohlrausch regulating function [15,16], because of a displacement of buffer ions in the sample zone when analytes migrate towards the boundary. Consequently, after a transitory state (Fig. 1b), the stacking process leads to the creation of a third zone with resistivity ρ_3 in which analytes are stacked, and a modification of the sample buffer plug resistivity ρ'_1 (Fig. 1c).

However, further calculations are performed using the same simplified model as that used by Chien and Burgi [7], assuming that this model corresponds to the initial state and neglects the influence of the sample ions on the resistivity of the sample zone. Hence, it is only an approximated model for the rest of the backout step. Consequently, the local electric field strength in the sample plug is calculated according to relationship [7], knowing that this model is true at the beginning of the stacking process, but is approximated as long as the stacking process is taking place.

$$E_1 = \frac{\rho_1 E_0}{\rho_1 x + \rho_2 (1 - x)} \tag{7}$$

E_0 is the overall electric field strength and x the fraction of capillary filled with the sample plug. Eq. (7) can be modified using Eq. (6):

$$E_1 = \frac{\gamma E_0}{1 + (\gamma - 1)x} \tag{8}$$

In turn, the local electrophoretic velocity in the sample plug can be expressed as:

$$v_{ep} = m_{ep_1} \frac{\gamma E_0}{1 + (\gamma - 1)x} \tag{9}$$

where m_{ep1} is the electrophoretic mobility of the analyte of interest in the sample zone. This mobility can be influenced either by the viscosity of the buffer or by its conductivity, in so far as the thickness of the electric double layer around a charged analyte depends on the ionic strength of the background electrolyte. Nevertheless, the electrophoretic mobility of the two analytes of interest is shown not to vary significantly between the sample zone and the buffer zone at constant pH value. According to Fig. 2, the values of m_{ep} for AsV and MMA are not significantly influenced by γ, hence by the buffer conductivity.

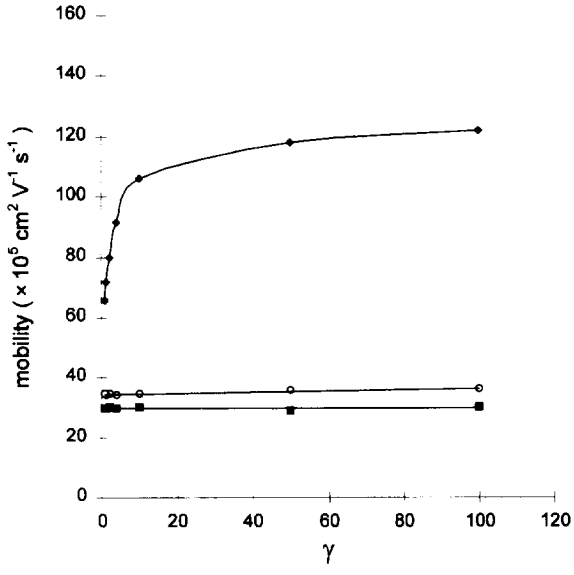

Fig. 2. Mobilities in a capillary filled with the sample buffer as a function of $\gamma = C_2/C_1$ ($C_2 = 20$ mM, pH 6.0). (♦) Electroosmotic mobility, (○) electrophoretic mobility of AsV and (■) electrophoretic mobility of MMA.

Thus, m_{ep1} will be treated as a constant at given pH for the rest of the study and will be noted m_{ep}.

In a second step, to calculate the apparent velocity of analytes, one needs to calculate the overall electroosmotic velocity during the backout process. Chien and Burgi [13] expressed this velocity as:

$$v_{eo} = x v_{eo_1} + (1 - x) v_{eo_2} \tag{10}$$

where v_{eo1} and v_{eo2} are the local electroosmotic velocities in the sample plug and in the running buffer, respectively, proportional to the respective local electric field strengths:

$$v_{eo_1} = v_{o_1} \frac{E_1}{E_0} = v_{o_1} \frac{\gamma}{1 + (\gamma - 1)x} \tag{11}$$

and

$$v_{eo_2} = v_{o_2} \frac{1}{1 + (\gamma - 1)x} \tag{12}$$

where v_{o1} and v_{o2} are the electroosmotic velocities generated under E_0 in a capillary filled with sample and filled with the running buffer respectively. Those velocities can be expressed in both cases according to:

$$V_{o_i} = m_{o_i} E_0 \quad (13)$$

where m_{oi} are the corresponding electroosmotic mobilities.

The electroosmotic mobility m_{o2} is a constant for a given running buffer, whereas the electroosmotic mobility in the sample region (m_{o1}) is a function of the sample conductivity. Thus m_{o1} can be expressed as:

$$m_{o_1} = m_{o_2} r(\gamma) \quad (14)$$

where $r(\gamma)$ can be defined as the reduced electroosmotic mobility in the sample zone, and is a function that can not be approximated by a simple model (see Fig. 2). In our working conditions, experimental values of $r(\gamma)$ vary between 1.0 and 1.9. Combining Eqs. (10,11) Eqs. (12–14) leads to:

$$v_{eo} = \frac{E_0}{1 + (\gamma - 1)x}[(m_{o_2}\gamma r(\gamma) - m_{o_2})x + m_{o_2}] \quad (15)$$

The apparent velocity of an analyte is the result of its electrophoretic velocity and the overall electroosmotic velocity:

$$v_{app} = \frac{E_0}{1 + (\gamma - 1)x}[\gamma m_{ep} + m_{o_2} + x(m_{o_2}\gamma r(\gamma) - m_{o_2})] \quad (16)$$

Solving the equation $v_{app} = 0$ leads to x_0 which is the sample plug length corresponding to a reversal of the sign of the apparent velocity:

$$x_0 = -\frac{\gamma m_{ep} + m_{o_2}}{m_{o_2}\gamma r(\gamma) - m_{o_2}} \quad (17)$$

As γ value is very large, Eq. (17) can be approximated to:

$$x_0 = -\frac{\gamma m_{ep} + m_{o_2}}{m_{o_2}\gamma r(\gamma)} = -\left(\frac{m_{ep}}{m_{o_2} r(\gamma)} + \frac{1}{\gamma r(\gamma)}\right) \quad (18)$$

Moreover, assuming that $100 \leq \gamma r(\gamma) \leq 190$, Eq. (18) becomes:

$$x_0 = -\frac{m_{ep}}{m_{o_2} r(\gamma)} \quad (19)$$

Hence, for a given analyte, if the injected plug length x_{inj} is higher than x_0, at the beginning of the backout (negative voltage applied), the apparent velocity of the analyte is in direction of the injection end (in the same direction as the electroosmotic flow): the analyte migrates in this direction and consequently, it is not stacked. For $x = x_0$, the sign of the apparent velocity changes and when $x < x_0$, the apparent velocity is in the direction of the detection end (opposite to the electroosmotic flow) resulting in the stacking of the analyte at the boundary (Fig. 1, steps b and c).

At the end of the backout step (Fig. 1, step d), the x value becomes very small, close to the relative length of a sample plug injected under conventional hydrodynamic injection. Assuming that $x \ll 1$ and that the value of γ has decreased because of the stacking process (creation of a zone with resistivity ρ'_3), such that $1 < \gamma \ll 100$, Eqs. (9,10) become:

$$v_{ep} \# m_{ep_1} \gamma E_0 \quad (20)$$

$$v_{eo} \# v_{eo_2} \quad (21)$$

Hence, the sign of the apparent velocity of the analyte at the end of the backout (step d) depends on its electrophoretic mobility and on the new value of γ, i.e., on resistivity ρ'_3.

As a consequence, two different situations can occur for a given analyte: first, if the value of γ is large enough, the apparent velocity is still in the direction opposite to the electroosmotic flow and no species are lost during this final stacking step. Second, if the value of γ is lower (close to 1), the apparent velocity of the analyte changes to the same direction as the electroosmotic flow. In this case, the stacked species contained in the plug of resistivity ρ'_3 begin to migrate towards the zone with resistivity ρ''_1. Nevertheless, when entering this zone, as $\rho''_1 \gg \rho'_3$, the species experience a much larger electric field (larger value of γ), and are automatically driven back to the plug of resistivity ρ'_3.

Consequently, there are two possibilities for the sign of the apparent velocity of a stacked analyte at the end of the backout. Nevertheless, both cases allow the species of interest to stay in the capillary, and the stacked analytes will not be flown out of the capillary during this step.

When the main part of the sample buffer plug has been pushed out of the capillary, polarity is switched

and a positive voltage is applied to the injection extremity. This leads to a situation identical to that obtained by hydrodynamically injecting a small sample plug of low conductivity. Due to an electroosmotic flow mobility close to m_{o2}, running buffer is pumped into the capillary through the injection end, and the sample plug is between two high conductivity running buffer zones, as it is in classical operating conditions (Fig. 1, step e). At this moment, as the current across the capillary is close to its standard value i_0, the sample plug has a minor influence on the electric field and the separation can take place as it occurs during a classical run.

As a consequence of Eq. (19), if the relative injected plug length is higher than x_0, the total injected sample will not be stacked and part of it will be lost. Secondly, for a given buffer (given m_{o2}), and for a given sample (given γ), x_o only depends on the electrophoretic mobility of the analyte of interest. Thus, according to Eq. (19), two different analytes with two different electrophoretic mobilities will not be stacked in the same way, leading to different F values for each analyte. This phenomenon leads to a bias similar to that observed under conventional electrokinetic injection, where the injected amount directly depends on the electrophoretic mobility of the analyte.

Under the present experimental conditions, calculated values of x_0 for $\gamma = 100$ are listed in Table 2. It appears that F values increase with increasing x_0 values. Consequently, the performances of the stacking process increase with increasing x_0, hence with increasing m_{ep}. This is clearly illustrated by comparing F values at two different pH values (Table 2). An increase of the buffer pH value has three major effects: first, an increase of bulk electroosmotic mobility, second an increase of electrophoretic mobility of arsenious compounds, which become more and more ionized with pH (see Table 1), and third, a change of the phosphate buffer conductivity. It can be observed that the stacking factor and the sensitivity enhancement increase with increasing pH and consequently the predominant parameter influencing the performances of the stacking process seems to be the increase of the electrophoretic mobility of the analyte.

However, it also appears that for calculated x_0 values inferior to x_{inj} (50%), F is larger than x_0 probably because of approximations introduced by the simplified model used for the local electric field strength in the sample zone (Eq. (7)). Nevertheless, the x_0 value is useful both to evaluate the maximum injectable amount and to predict the variations of the stacking factor F. Moreover, the example of AsV at pH 7.8 shows that for an injected plug relative length $x_{inj} = 50\%$ close to the calculated value of x_0 (50.1%), the value obtained for the stacking factor is close to 50, confirming the fact that the analytes are not flown out at the end of the backout step.

To minimize the loss of analytes and consequently improve the performances of large-volume stacking with polarity switching, one has to increase the value of x_0. According to Eq. (19), this can be achieved either by an increase of the electrophoretic mobilities of the analytes, or by reducing the value of m_{o1}. On the one hand, the electrophoretic mobilities of the anions of interest will increase by increasing pH, leading to consecutive higher migration times. On the other hand, according to Fig. 1, a decrease of 10% of the m_{o1} value can be achieved by reducing γ from 100 to 10. Nevertheless, such a low γ value giving rise to improved stacking factor has tremendous consequences on efficiency. For example same experiments as in Table 2, carried out at pH 6.0 for a γ value equal to 50, lead to a 10% increase of the stacking factor but with a two fold loss in efficiency compared to that obtained for $\gamma = 100$. Thus, if peak area values are higher, peak height values might be lower, limiting the method interest in term of sensitivity enhancement.

In conclusion, the present study demonstrates that the sensitivity enhancement through large-volume stacking is limited by two phenomena: first, loss of analytes for injected sample plug relative length larger than x_0 and second, loss of efficiency when x_0 values are improved by decreasing γ values. Nevertheless, a 10 to 20 fold improvement in the sensitivity of the technique can be obtained using this stacking procedure.

3.2. Quantitative analysis using large-volume stacking with polarity switching

To perform quantitative analysis, migration times have to be identical for different sample concentrations. But here, $t_{backout}$ depends on the electroosmotic mobility in the sample zone (m_{o1}), therefore on the sample conductivity. As a consequence, if

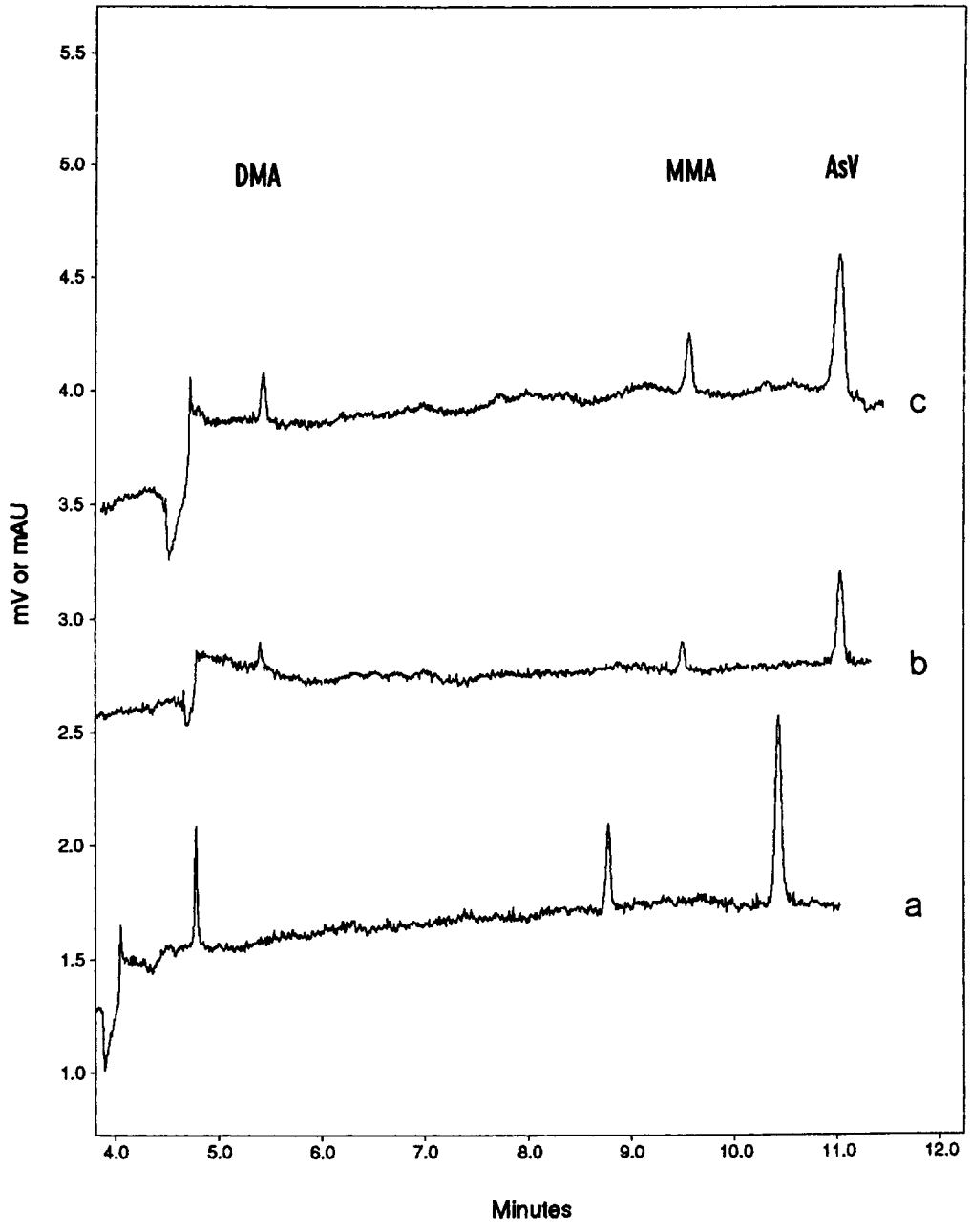

Fig. 3. Comparison of electrophoregrams using classical hydrodynamic injection and using large volume stacking with polarity switching. (a) Classical hydrodynamic injection of 10 ppm samples (1% of the capillary volume), (b) stacking of an injected volume representing 50% of the capillary volume of 250 ppb samples, (c) idem (b) with 500 ppb samples. $t_{backout}=0.8$ min, $t_{+-}=0.1$ min. Sample dissolved in Na_2HPO_4 0.2 m*M* (pH 6.0), run buffer pH 6.0.

samples are prepared in pure water, $t_{backout}$ and m_{ol} will be dependent on the analyte concentration and both the global migration time and the stacking factor will be different for each sample concentration, thus limiting the quantitative aspect of the analysis. Consequently samples were dissolved in a solution possessing a conductivity largely higher than the conductivity of the same sample dissolved in pure water, but low enough for the efficient stacking process to occur. As a consequence, samples were prepared in a hundred times diluted running buffer corresponding to optimal stacking conditions ($\gamma = 100$ is the optimal value for efficiency). Identical global migration times and stacking factors can be obtained in such operating conditions (Fig. 3).

The study of corrected peak areas using large-volume stacking as a function of injected concentration leads to linear calibration curves (Fig. 4) with good correlation factors for injected volume representing 50% of the capillary length and sample concentrations ranging from 125 ppb to 1 ppm.

This study shows that quantitative analysis can be carried out by using large-volume sample stacking provided that all samples had the same conductivity. Moreover, good repeatability can be achieved, as R.S.D. values obtained with 5 experiments vary between 1.8 and 2.7% for migration times and 1.3 and 2.8% for corrected peak areas for injected concentrations ranging from 125 ppb to 1 ppm.

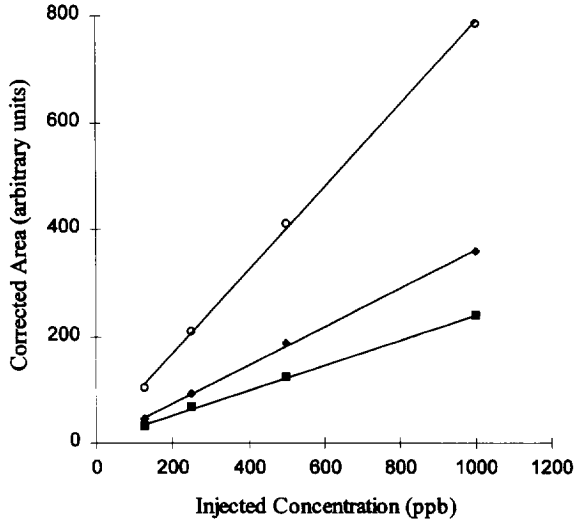

Fig. 4. Calibration curves obtained for MMA (■), DMA (♦) and AsV (○): stacking process for hydrodynamically injected sample volumes corresponding to 50% of the capillary volume. The correlation factors are r^2(DMA) = 0.9992, r^2(MMA) = 0.9992 and r^2(AsV) = 0.9995. Experimental conditions similar to those in Fig. 3.

4. Conclusions

Large-volume stacking with polarity switching has been proved to be a powerful tool for sensitivity enhancement in capillary electrophoresis, even though its use is limited for slow moving anion analysis. Nevertheless, a 10 to 20 fold sensitivity enhancement can be reached for the quantitative analysis of highly diluted samples in low conductivity matrices.

However, polarity switching is an experimental constraint that can lead to non reproducible results if the current is not monitored properly. Therefore, further investigations will be carried out to develop and optimize an automatic stacking procedure that does not require any polarity switching during the analysis.

References

[1] F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, J. Chromatogr., 169 (1979) 11.
[2] J.W. Jorgenson and K.D. Lukacs, Anal. Chem., 53 (1981) 1298.
[3] R. Weinberger, Practical Capillary Electrophoresis, Academic Press, San Diego, CA, 1993.
[4] M. Albin, P.D. Grossmann and S.E. Moring, Anal. Chem., 65 (1993) 489A.
[5] D.S. Stegehuis, H. Irth, U.R. Tjaden and J. van der Greef, J. Chromatogr., 538 (1991) 393.
[6] C. Schwer and F. Lottspeich, J. Chromatogr., 623 (1992) 345.
[7] R.L. Chien and D.S. Burgi, J. Chromatogr., 559 (1991) 141.
[8] R.L. Chien and D.S. Burgi, Anal. Chem., 64 (1992) 1046.
[9] R.L. Chien and D.S. Burgi, J. Chromatogr., 559 (1991) 153.
[10] D.S. Burgi and R.L. Chien, Anal. Biochem., 202 (1992) 306.
[11] J.L. Beckers and M.T. Ackermans, J. Chromatogr., 629 (1993) 371.
[12] M. Albert, C. Demesmay, M. Porthault and J.L. Rocca, Analusis, 20 (1992) 383.
[13] D.S. Burgi and R.L. Chien, Anal. Chem., 63 (1991) 2042.
[14] R.L. Chien and J.C. Helmer, Anal. Chem., 63 (1991) 1354.
[15] F. Kohlrausch, Ann. Phys. Chem, 62 (1897) 209.
[16] F.E.P. Mikkers, F.M. Everaerts and Th.P.E.M. Verheggen, J. Chromatogr., 169 (1979) 1.