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Large-volume stacking for quantitative analysis of anions in capillary electrophoresis
II. Large-volume stacking without polarity switching

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Abstract

Several methods have been reported for performing 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 from the capillary.

In this work, this removal is performed using the redissolution of an electroosmotic flow modifier that will act as a pump, without the need for polarity switching. The performance of this method is evaluated and optimized for the separation of arsenious acids. Then, the possibility of the quantitative aspect of this stacking procedure is discussed.

Keywords: Large-volume stacking; Stacking; Anions; Tetradecyltrimethylammonium bromide; Arsenic acid; Monophenylarsonic acid; Monomethylarsonic acid

1. Introduction

It has been shown in a previous paper [1] that large-volume stacking with polarity switching allows a ten- to twenty-fold sensitivity enhancement to be attained in capillary electrophoresis of slow-moving anions that are separated in a fused-silica capillary under a positive voltage as regards their resistance to the electroosmotic flow.

However, polarity switching is an experimental constraint that can lead to non-reproducible results if the current is not monitored properly during the backout step. Therefore, the aim of this study is to suppress the polarity switching step by performing an automatic stacking process.

Both slow- and fast-moving anions can be separated under a negative voltage using a fused-silica capillary that has been dynamically coated by the addition of an electroosmotic flow modifier to the running buffer, either to suppress or to reverse the electroosmotic flow. In this separation configuration, Burgi [2] reported a technique of large-volume stacking using the redissolution of the electroosmotic flow modifier in the sample plug to restore negative charges on the capillary wall and to use the regenerated electroosmotic flow to pump the large sample plug out of the capillary without polarity switching. For this purpose, diethylenetriamine (DETA) is added to the separation buffer to suppress the

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electroosmotic flow by reducing the ζ potential of the capillary wall [3].

In a first step, we studied the performances of this stacking method using DETA with organic and inorganic arsenious compounds as the anions of interest [4].

In a second step, we used another cationic surfactant (TTAB: tetradecyltrimethylammonium bromide) to optimize the performance of the stacking process by controlling the electroosmotic flow, by preventing the slow-moving anions from being pushed out of the capillary and by maximizing the efficiency of the stacking procedure.

Finally, the quantitative analysis of low concentrated samples of anions with large-volume stacking using TTAB under precise sampling conditions was discussed.

2. Experimental

Experiments were carried out on a SpectraPhoresis 1000 (Thermo Separation Products) using PC 1000 software. The capillaries were untreated fused-silica capillaries (44 cm×75 μm I.D.) with a detection window located 8 cm from the capillary extremity. UV detection was at 195 nm. The run temperature was 40°C. The anion standard consisted of monophenylarsonic acid (BzAs), monomethylarsonic acid (MMA) and arsenic acid (AsV) [1].

The running buffer consisted of 20 m*M* disodium hydrogenphosphate (Na_2HPO_4) and the required amount of electroosmotic flow modifier (DETA or TTAB), adjusted to pH 6.0 with orthophosphoric acid. Prior to any change in the concentration of the modifier, the capillary was rinsed for 2 min with deionised water, then for 2 min with the running buffer without modifier and finally for 2 min with the buffer containing the new modifier concentration. At the end of the day, the capillary was rinsed for 2 min with 1 *M* NaOH and for 5 min with deionised water.

2.1. Standard separation

The standard sample consisted of 20 ppm MMA and AsV, and 5 ppm BzAs in 0.2m*M* Na_2HPO_4, pH 9.0. Injection was performed under a low calibrated vacuum over 2 s, giving rise to an injected volume corresponding to 1.0% of the whole capillary volume. By applying a negative voltage (−20 kV), the separation occured with 78 μA average current.

2.2. Stacking process

The standard sample is diluted to the required concentration using a 0.2 m*M* Na_2HPO_4 solution. The sample is injected under low vacuum for 165 s, which corresponds to an injected volume representing 80.0% of the capillary volume. A negative voltage (−20 kV) is then applied and the stacking and separation processes occur successively, without the need for polarity switching. The total migration time of the analyte includes the successive backout and separation times.

3. Results and discussion

All types of anions migrate in the direction of the detection end under a negative voltage, if the electroosmotic flow is suppressed or reversed (neutral or positively charged capillary wall). This can be achieved either with a static or a dynamic capillary wall coating. This study is based on the use of a dynamic coating (with electroosmotic flow modifier present in the running buffer) and supposes a reversible adsorption of the electroosmotic flow modifier on the capillary wall. These conditions can lead to a stacking effect of a large volume of anions in a low conductivity matrix. A large volume of low conductivity sample containing no modifier is injected hydrodynamically into the capillary (which has been pre-equilibrated with a buffer containing the modifier). A negative voltage is then applied to the injection end. The modifier adsorbed on the capillary wall in contact with the sample region dissolves rapidly, restoring negative charges to the capillary surface and, consequently, a local electroosmotic flow towards the injection end. The value of this local electroosmotic flow may not be as high as that of the original bare silica capillary, since dynamic coating is always accompanied by a memory effect resulting from residual surfactant molecules adsorbed on the capillary wall. Nevertheless, the sample plug is electroosmotically pushed out while the negative

species migrate rapidly towards the boundary between the sample zone and the buffer under the enhanced electric field. As the sample plug is pushed out, the modifier contained in the running buffer progressively coats the capillary wall again, slowing down and finally reversing the electroosmotic flow; separation of stacked species starts to occur without the need for polarity switching.

The main problem with this method is the electroosmotic flow mismatch caused by the adsorption and the desorption of the electroosmotic flow modifier. As this phenomenon is not instantaneous, a transitory state exists before the system is equilibrated, after the sample plug has been pushed out of the capillary. As a result, migration times using stacking conditions are different from migration times using standard conditions of injection and the apparent velocity of the analytes passing through the detection window can not be evaluated very accurately. Therefore, peak-corrected areas and the stacking factor, F, as defined in a previous work [1] can not be calculated properly and the stacking method is evaluated only in terms of sensitivity enhancement (peak-height enhancement).

In this study, we compared the 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 80% of the capillary volume).

3.1. Large-volume sample stacking using DETA

DETA was used as an electroosmotic flow modifier [3] in order to suppress the electroosmotic flow by reducing the ζ potential on the capillary wall. Under our working conditions (pH 6.0), an increase in the concentration of DETA, up to 2mM, significantly decreased electroosmotic mobility to a quite constant value (Table 1). Any further increase in the concentration of DETA drastically affected the peak efficiency because of the increase in the electric current and in Joule heating. Consequently, by using experimental conditions where the concentration of DETA is 2mM, the reduced electroosmotic flow made it possible to separate arsenious compounds under a negative voltage, their electrophoretic mobilities being higher than the electroosmotic mobility.

Table 2
Sensitivity enhancement and efficiency after large-volume sample stacking

Compound	SE	$N_{stacking}$	$N_{standard}/N_{stacking}$
AsV	15.2	3800	27.4
MMA	22.4	5500	16.5
BzAs	20.2	4250	15.8

Conditions: 2 mM DETA, pH 6. The sample was prepared in 0.2 mM Na_2HPO_4.

Results listed in Table 2 illustrate a 15–22-fold sensitivity enhancement, corresponding to peak-height enhancement. Then, peak efficiency values need to be calculated using the effective solute migration times represented by the difference between the global experiment time and the backout time (time needed to pump the low conductivity plug out of the capillary). This backout time cannot be measured with precision, but it can be evaluated by measuring the time needed for the electric current to reach 95% of the value obtained under classical conditions of injection. Consequently, peak efficiencies are estimated, but will not be used as precise values.

Estimated peak efficiency values obtained using this large-volume stacking method are largely lower than those obtained under standard conditions of injection, despite an optimization of the field enhancement factor γ ($\gamma=100$): The order of magnitude of peak broadening given by the square root of $N_{standard}/N_{stacking}$ ranges from 4.0 to 5.2.

Table 1
Electroosmotic mobility measured with different concentrations of DETA in the running buffer (pH 6.0)

[DETA] (mM)	0	1	2	3	4	5
m_{eo}[a] (10^5 cm^2 V^{-1} s^{-1})	+63	+9.8	+6.2	+6.1	+6.0	+6.0

[a] A positive value of m_{eo} indicates that the electroosmotic velocity is in the same direction as the electric field. A negative value indicates that m_{eo} is in the opposite direction to the electric field.

Since a Gaussian peak area is given by:

$$S = \sqrt{2\pi}\,\sigma h \quad (1)$$

S being the area, σ the standard deviation and h the height of the Gaussian peak, and assuming that the number of theoretical plates is inversely proportional to σ^2, we can deduce the following expression:

$$F = SE\sqrt{\frac{N_{\text{standard}}}{N_{\text{stacking}}}} \quad (2)$$

Consequently, the dispersion introduced by the stacking method can explain the deviation of the experimental sensitivity enhancement (*SE*) values compared with the expected *SE* value equal to 80. Nevertheless, imprecision in peak efficiency values prevent us from using F values that could be calculated from Eq. (2).

The dispersion introduced by the stacking process can result from three main causes. First, the increase in the global migration time due to the backout process leads to an increase in peak dispersion caused by longitudinal diffusion ($\sigma^2 = 2Dt$). Nevertheless, this phenomenon cannot account for such a loss of efficiency. Second, due to the kinetics of DETA adsorption–desorption, which are not instantaneous, the separation of solutes starts to occur before the system has been completely equilibrated and involves solute dispersion. Lastly, the global electroosmotic flow is the result of two opposite contributions, thus creating an electroosmotic flow mismatch at the boundary between the sample zone and the buffer zone, resulting in additional solute dispersion.

In conclusion, large-volume sample stacking using DETA enables good sensitivity enhancement (15–22-fold increase in peak height without an increase in noise) to be attained, however, the dispersion introduced by the method leads to an important loss in efficiency that seems to be caused by the transitory state that is due to the DETA adsorption–desorption process on the capillary wall. Consequently, we studied another electroosmotic flow modifier that made it possible to control more efficiently the electroosmotic velocity of the running buffer.

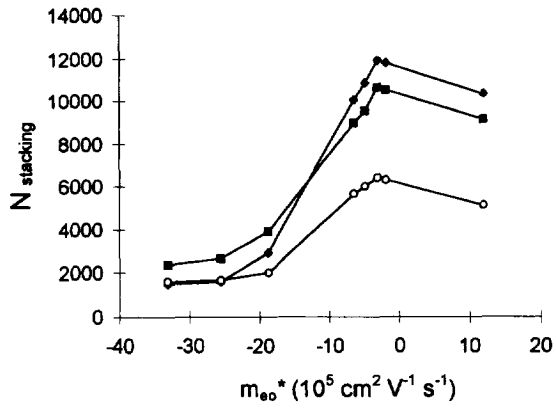


Fig. 1. Efficiency as a function of electroosmotic mobility in the running buffer using large-volume stacking in the presence of TTAB. Solutes: (♦) AsV; (■) MMA; (○) BzAs. *, Same as [a] in Table 1.

3.2. Large-volume sample stacking using TTAB

We studied TTAB as a cationic flow modifier. The effect of the concentration of TTAB in the running buffer on the electroosmotic flow is illustrated in Table 3, showing that the electroosmotic velocity can be reduced, suppressed or reversed, depending on the concentration of TTAB.

The performance of large-volume sample stacking, using the redissolution of TTAB as a pump, has been tested in terms of estimated peak efficiency (Fig. 1) and sensitivity enhancement (Fig. 2) for various TTAB concentrations in the running buffer, i.e. for different electroosmotic mobility values. As the electroosmotic flow is reversed, the efficiencies of

Table 3
Influence of TTAB concentration on electroosmotic mobility

[TTAB] (m*M*)	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45
m_{eo}[a] (10^5 cm^2 V^{-1} s^{-1})	+12.0	−2.0	−3.2	−5.0	−6.5	−18.8	−25.6	−33.1

[a] Same as in Table 1.

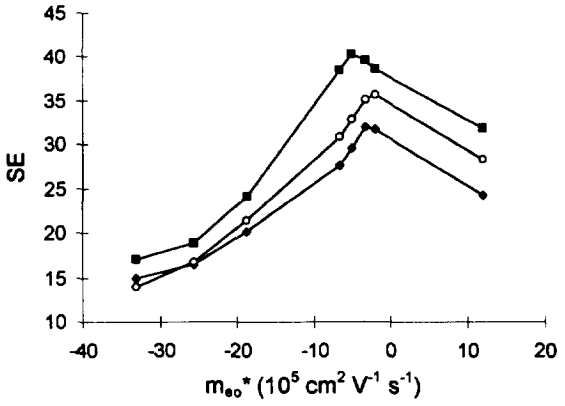


Fig. 2. Sensitivity enhancement as a function of electroosmotic mobility in the running buffer using large-volume stacking in the presence of TTAB. Solutes: (♦) AsV; (■) MMA; (○) BzAs. *, Same as [a] in Table 1.

electrophoretic peaks first increase, reach maximum values for m_{eo} values close to $-3\cdot10^{-5}$ cm^2 V^{-1} s^{-1}, and then dramatically decrease when the electroosmotic flow is too strongly reversed. The optimum peak efficiency values are still lower than efficiencies observed under classical injection conditions, but are twice as high as the efficiencies reached using DETA operating conditions. Moreover, curves representing sensitivity enhancement SE versus electroosmotic mobilities (Fig. 2) reveal maximum SE values ranging from 30 to 40 for the three solutes when the electroosmotic mobility is close to $-3\cdot10^5$ cm^2 V^{-1} s^{-1}, leading to sensitivity enhancement that is almost twice as high as the sensitivity enhancement obtained using DETA operating conditions.

Thus, comparison of Figs. 1 and 2 shows that both efficiency and sensitivity enhancement pass through a maximum corresponding to the same operating conditions. Electropherograms obtained under these optimum conditions are shown in Fig. 3. As a result, the use of TTAB at its optimum concentration, instead of DETA, allows maximum SE values close to 30–40 (15–20 for DETA), with a two-fold higher efficiency and shorter analysis times to be reached.

In conclusion, the performance obtained with TTAB justify its use instead of DETA for maximizing the sensitivity enhancement while limiting the loss of resolution introduced by the stacking process.

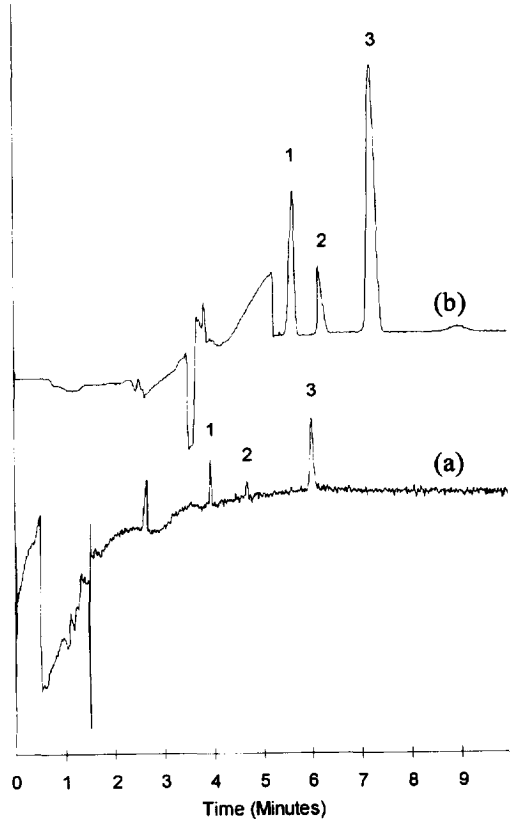


Fig. 3. Electrophoregrams obtained under optimal TTAB concentration conditions ($m_{eo}=-3\cdot10^{-5}$ cm^2 V^{-1} s^{-1}). (a) Classical injection (1% of the capillary volume) of a solution containing 20 ppm AsV and MMA and 5ppm BzAs (pH 9.0) (×10 vertical magnification). (b) Large-volume injection (80% of the capillary volume) of a solution containing 20 ppm AsV and MMA and 5 ppm BzAs (pH 9.0). Solutes: (1) AsV; (2) MMA and (3) BzAs.

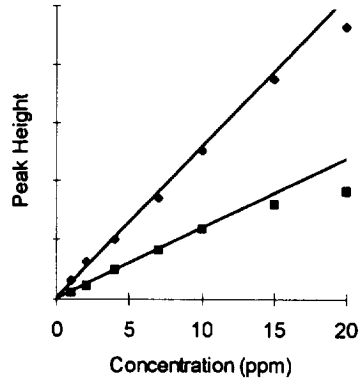
3.3. Quantitative analysis in large-volume stacking using the redissolution of TTAB as a pump

The quantitative aspect of the stacking procedure was studied using the TTAB concentration corresponding to the optimum sensitivity enhancement previously determined ($m_{eo}=-3\cdot10^{-5}$ cm^2 V^{-1} s^{-1}). Samples were obtained by dissolving weighed amounts of solutes in a mixture of running buffer–water (1:99, v/v), which lead to sample solutions with the same conductivity. As a result, the backout time and the electroosmotic mobility in the sample region were identical during successive injection steps. However, effective migration times of solutes

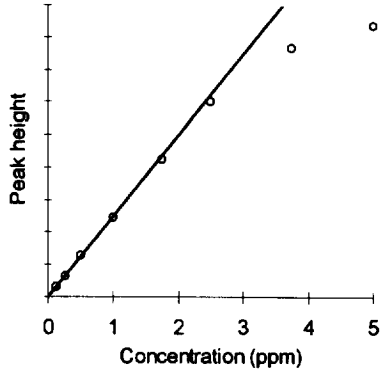
deduced from solute migration times and backout time differed from solute migration times using classical injection conditions, preventing us from calculating reliable peak-corrected areas. Nevertheless, according to Wätzig [5], calibration functions can also be reliable using peak heights. Fig. 4a,b illustrate calibration curves for the three solutes. Linear curves were obtained up to concentrations close to 10 ppm for AsV ($r^2=0.9973$) and MMA ($r^2=0.9996$), and close to 3 ppm for BzAs ($r^2=0.9998$). For higher concentrations, deviations from the linear curve were obtained, due to reduced peak heights resulting from loss of peak efficiency.

Moreover, good repetability was achieved, since R.S.D.s ($n=5$) ranged from 0.6 to 1.1% for migration times and from 1.0 to 2.7% for SE values.

In conclusion, this study demonstrates the possibility of carrying out quantitative analysis using a large-volume stacking process without polarity switching.



(a)



(b)

Fig. 4. Peak height versus concentration for (a) ♦= AsV and ■=MMA and (b) ○=BzAs, using the large-volume stacking process with TTAB for hydrodynamically injected sample volumes corresponding to 80% of the capillary volume (samples prepared in 0.2 m*M* Na_2HPO_4, pH 9.0).

4. Conclusions

Large-volume stacking without polarity switching has been optimized by using TTAB as the electroosmotic flow modifier. This method enhances the sensitivity by 30–40 fold, while minimizing the loss of efficiency and allowing quantitative analysis for the determination of diluted samples in low conductivity matrices. Further investigations are being carried out to test other electroosmotic flow modifiers to further reduce the loss of efficiency resulting from the backout process.

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