Background conductivity independent counter flow pre-concentration method for capillary electrophoresis

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Short Communication

Background conductivity independent counter flow pre-concentration method for

capillary electrophoresis

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Abstract

A pre-concentration method for anions is presented, which relies on a trap created by applying an electric field against a hydrodynamic flow of the sample. The trapping zone is created in front of a cation exchange membrane which allows the isolation of the electrode and thus prevents any interference by electrolysis products. Pre-concentration factors of up to 20 were demonstrated for nitrate and formate as model analyte ions and were linearly related to the sample volume passed through the trap. A discrimination between the ions was found possible by adjustment of the hydrodynamic flow velocity. The method was also found to be suitable for the preconcentration of an anion (nitrate at 100 µM) in presence of a second anion at a very high concentration (50 mM formate). The detection limits for the four anions chloride, nitrate, perchlorate and formate could be lowered from 4, 4.3, 4.2 and 7.2 µM obtained without trapping respectively to 127, 142, 139 and 451 nM with trapping.

In comparison to chromatography, capillary electrophoresis has the advantages of relative simplicity and a low demand for consumables. This allows its implementation as low cost and portable systems (see for example [1, 2]). A hindrance to wider acceptance are the relatively high limits of detection, which for optical detectors are due to the short available pathlengths. For contactless conductivity detection (C^4D) these also seldom are lower than 1 µM, due to the presence of a background conductivity. For this reason there has been a strong interest in the development of analyte pre-concentration methods. An overview can be found in a recent review [3] and in a systematic guide [4] by Breadmore and coworkers. For capillary electrophoresis so-called stacking methods have been developed. These rely on the local creation of an electric field gradient in the sample zone, which leads to a faster movement of the ions causing them to pre-concentrate, *i.e.* to stack, at the zone boundary. However, with the possible exception of transient isotachophoresis, this is only possible for samples of low conductivity [4]. An alternative method, which is subjected less to this limitation, is counter-flow gradient electrofocusing. This makes use of a hydrodynamic flow in the direction opposite to the electrokinetic movement of the ions. By creating a gradient, or a step, in the electric field, a trapping zone is established as the ions experience forces driving them to the same area from either side. The topic has been reviewed by Shackman and Ross in 2007 [5] and the more recent developments have been summarized by Breadmore *et al.* [3] in 2015.

The field strength can be modified by changing the diameter of the channel, but this also alters the hydrodynamic flow velocity proportionally. Thus it is necessary to create a discontinuity by modifying the field with the help of an electrode which is in direct or indirect contact with the trapping zone. As described in the reviews, a variety of in-channel arrangements have been proposed. The challenge lies in the avoidance of

interference through electrolysis products created at the electrodes (including the possible

bubble creation due to oxygen or hydrogen evolution) and in achieving a robust

arrangement on a scale that does not introduce bandbroadening. Good implementations are

not easily achieved. Alternatively, as in Pressure Assisted Electrokinetic Injection

(PAEKI) [6-8], the trapping is carried out at the inlet of the separation channel. However,

this can be expected to be still affected by variations in the background conductivity (see

e.g. the extensive discussions in [9, 10]).

The device reported herein was designed with the intention of avoiding problems with

electrolysis products and bubble formation, effects of varying background conductivities

of samples and to obain a sharp sample plug inside the capillary for good resolution in the

separation step. A sketch of the trapping cell, made from polymethyl methacrylate

(PMMA), is given in Fig. 1A. It has the shape of an isosceles trapezoid and contains a

flow through channel of 20 mm length with a diameter of 0.4 mm which has two 90°-

turns. At these corners the channel is contacted by two cation exchange membranes

(Nafion 117, Sigma-Aldrich, St. Louis, MO, USA). The chambers behind these

membranes are filled with a buffer solution consisting of 0.5 M 3-(N-

morpholino)propanesulfonic acid (MOPS) + 0.5 M sodium MOPS and contain electrodes

made from stainless steel. A separation capillary (fused silica, 365 μm OD, 10 μm ID, 50

cm length, Polymicro Technologies, Phoenix, AZ, USA) is mounted perpendicularly at the

halfway point of the channel in the trapping device. As illustrated in Fig. 1B, fluid

handling of the system is achieved with a pair of 20 μL syringe pumps (SPS01-020-C360,

LabSmith, Livermore, CA, USA), 3 two way valves (AV201-T116, LabSmith) and a 4

port selector valve (AV202-C360, LabSmith) which connects either ports A-C and B-D or

A-D and B-C. The system is completed with a high voltage DC module capable of

delivering up to 500 V (HMD-0.5R10-24-5-ER, Hivolt, Hamburg, Germany) and a second

module with a maximum voltage of 30 kV (Spellman CZE2000, Pulborough, UK). A

purpose made C^4D cell, similar to the one reported by Tanyanyiwa *et al.* [11], placed 20

cm from the inlet, was used for detection and its signal was captured with an e-corder

(eDAQ, Denistone East, NSW, Australia) and processed with the Chart software package

(eDAQ). All parts of the system were automatically operated using a purpose made

graphical user interface software based on Instrumentino [12, 13].

Trapping of anions is achieved by pumping sample through the channel against a voltage

applied through the two cation exchange membranes as illustrated in Fig. 1A. This leads to

an accumulation of anions in front of the membrane on the left hand side if their

electrokinetic velocity is higher than the hydrodynamic flow velocity. The use of the

membranes prevents the creation of bubbles in the trapping channel. The current through

the membranes is maintained by the transport of cations. It is thought that the charge of

the anions pre-concentrated in front of the membrane is balanced by cations passed

through the membrane. The fact that the trapping voltage is applied through the

membranes directly across the sample solution also leads to the creation of consistent field

strengths regardless of the conductivity of the sample.

The process is started by filling the channel with sample solution, followed by a period in

which both the hydrodynamic flow and the electric field are applied. The flow through

arrangement allows a flexible choice of sample volume. Subsequent to the trapping step

the volume of the trapping zone is moved with the help of a syringe pump to the inlet of

the separation capillary, where part of it is then injected hydrodynamically by

pressurization, followed by flushing of the channel with the electrophoretic background

electrolyte. The separation of the trapping zone from the capillary inlet assures a high

integrity of the sample plug which enters the capillary. Analysis by zone electrophoresis is

then carried out by application of the high voltage across two separate electrodes.

In Fig. 2 results obtained for formate and nitrate are reported, having mobilities of

$56.6 \cdot 10^{-9}$ and $74.1 \cdot 10^{-9}$ $m^2 \cdot s^{-1} \cdot V^{-1}$ respectively (as calculated from the ionic conductivities

tabulated in [14]). In Fig. 2A the enhancement factors for the two ions are plotted against

different applied hydrodynamic flow rates while keeping the applied voltage constant. It

can be seen that the enhancement factors are found to drop for flow rates higher than

approximately 400 and 560 $\mu m \cdot s^{-1}$ for formate and nitrate, respectively. The values match

very well the expected limits in the flow rates for which trapping should be possible.

These were calculated from the mobilities of the ions and the applied field strength of 75

$V \cdot cm^{-1}$ as 424 and 555 $\mu m \cdot s^{-1}$ for the two ions. This clearly demonstrates that the

implementation of the concept works. The fact that some enhancement is observed for

both ions also for higher flow rates than these maxima must be due to imperfections

caused by the laminarity of the hydrodynamic flow and distortion of the electric field in

the angled arrangement of the trapping zone in front of the membrane. Sets of experiments

were also carried out for field strengths of 35, 150 and 250 $V \cdot cm^{-1}$ and the highest

possible flow rates for maximal trapping were also found to correlate well with the

expected values.

In Fig. 2B it is shown that the enhancement factor scales linearly with the volume of the

sample passed through the cell. The experiment was carried out with a flow velocity of

318 $\mu m \cdot s^{-1}$ and again an applied field strength of 75 $V \cdot cm^{-1}$. Under these conditions both,

formate and nitrate are efficiently trapped. It is, however, also possible to selectively trap

the ion with the higher mobility, namely nitrate. The mixture of formate and nitrate was

passed through the cell at the higher flow velocity of 477 μm·s^{-1}, which according to

prediction and the results of Fig. 2A is too high for efficient trapping of formate. As can

be seen in Fig. 2C, nitrate is trapped as efficiently as for the lower flow rate, but as

expected, formate is only trapped to a small extent. Why the enhancement factor for

formate even drops slightly with increasing volume is not clear.

The effect of the composition of the solution was then tested. Pre-concentration

experiments were carried out for solutions containing 100 μM potassium nitrate and up to

50 mM sodium formate. In all cases, nitrate was successfully pre-concentrated, despite the

high background conductivity of the solutions due to the formate salt. An

electropherogram for a pre-concentrated standard solution of 100 μM potassium nitrate

and 50 mM sodium formate can be seen in Fig. 3A. An expanded view of the nitrate peak

is shown in the inset together with the peak for nitrate for the solution not subjected to the

trapping experiment demonstrating the increase in concentration achieved for the high

ionic background.

Finally, it was examined if the system can be employed for lowering the LODs for CE-

C^{4}D by examining the behaviour for the four anions chloride, nitrate, perchlorate and

formate. Without carrying out the trapping step LODs of 4, 4.3, 4.2 and 7.2 μM were

determined for the 4 anions respectively under the CE-C^{4}D conditions employed.

Trapping from a volume of 150 μL with an applied field strength of 150 V·cm^{-1} and a

flow velocity of 663 μm·s^{-1} resulted in LODs of 127, 142, 139 and 451 nM. The

separation of the ion mixture following pre-concentration from 500 nM, which is well

below the LOD without pre-concentration, is shown in Fig. 3B. Note, that the flow

velocity of 663 $\mu m \cdot s^{-1}$ corresponds to a volumetric flow rate of 5 $\mu L \cdot min^{-1}$ for the set-up

employed which means that the trapping experiment for the 150 μL of standard solution

had a duration of 30 min.

In conclusion, a fully automated pre-concentration system based on balancing

hydrodynamic and electrokinetic flows was built and integrated into a miniaturized

capillary electrophoresis instrument. In contrast to the electrokinetic stacking methods

employed in capillary electrophoresis, the method has the advantage of working even for

high conductivity samples and a discrimination between slow and fast ions could be

achieved by adjustment of the flow rate. The results are preliminary and there is room for

improvement of the pre-concentration factors and the trapping times. Although only

anions were tested thus far, the same principle should be applicable for cations as well,

using anion-exchange membranes and reverse voltages.

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Figure Captions:

Fig. 1   A) Pre-concentration cell. B) Microfluidic system. HV = high voltage, BGE = background electrolyte.

Fig. 2   A) Enhancement factors for nitrate and formate as a function of the applied flow velocity for an applied field strength of 75 V·cm^{-1} and a volume of 15 µL. Standard solution: 50 µM potassium nitrate, 100 µM sodium formate. The two vertical lines represent the theoretical maximal flow rates at which trapping is possible. Injection: 20 s at 100 kPa. Separation voltage: 25 kV. Background electrolyte: 30 mM histidine, 30 mM acetic acid.

B) Enhancement factors for nitrate and formate as a function of the volume of sample passed through the trapping cell for an applied flow velocity of 318 µm·s^{-1} and an applied field strength of 75 V·cm^{-1}. Other conditions as for Fig. 2A.

C) Enhancement factors for nitrate and formate as a function of the volume of sample passed through the trapping cell for an applied flow velocity of 477 µm·s^{-1} and an applied field strength of 75 V·cm^{-1}. Other conditions as for Fig. 2A.

Fig. 3   A) Separation of a pre-concentrated high conductivity standard solution. Hydrodynamic flow velocity: 265 µm·s^{-1}. Field strength: 35 V·cm^{-1}. Standard solution: 15 µL of 100 µM potassium nitrate, 50 mM sodium formate. BGE: 300 mM histidine, 300 mM acetic acid. Other conditions as for Fig. 2A. A magnification of the pre-concentrated nitrate peak (full line) is overlaid on the non-concentrated nitrate peak (dotted line) in the inset.

B) Separation of 500 nM chloride, nitrate, perchlorate and formate following pre-

concentration. Hydrodynamic flow velocity: 663 μm·s^{-1}. Field strength: 150

V·cm^{-1}. Volume of standard solution: 150 μL. Other conditions as for Fig. 2A.

Figure 1

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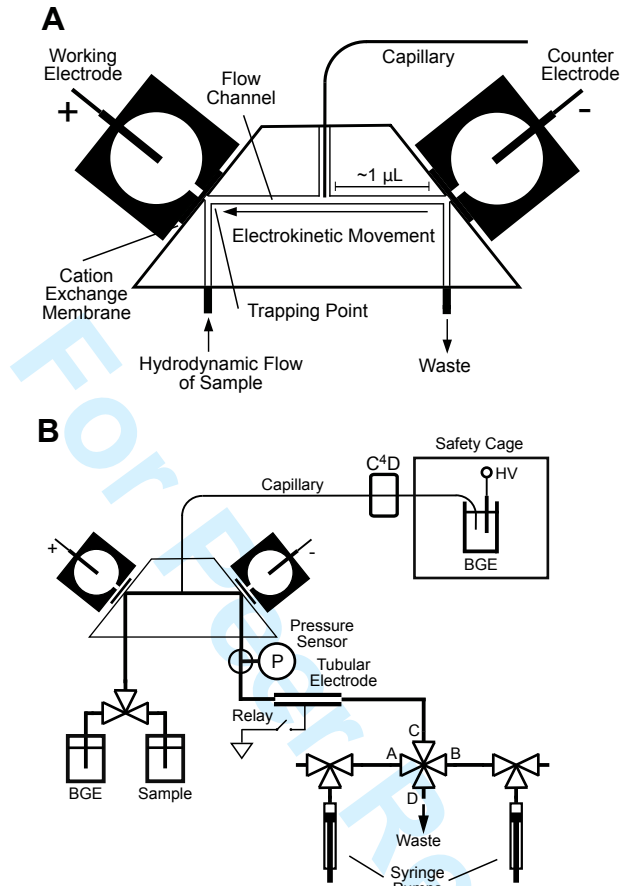43
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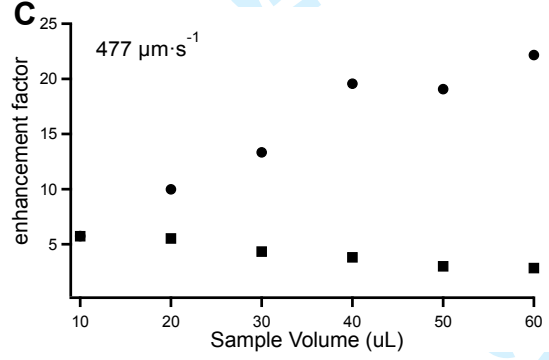48
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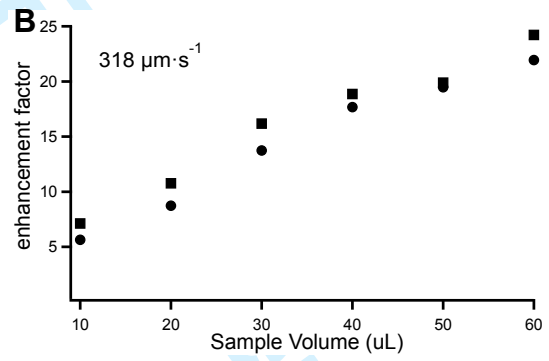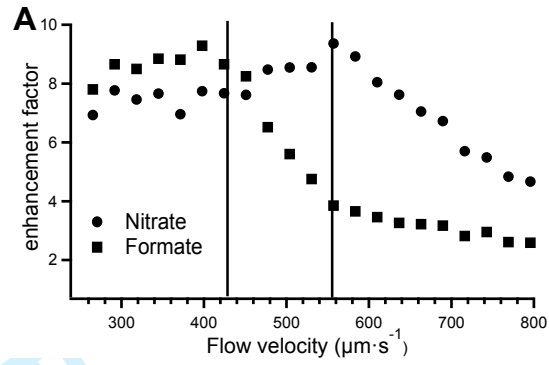52
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Figure 2

Figure 3