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| Complete List of Authors: | Koenka, Israel; THE UNIVERSITY OF BASEL, DEPARTMENT OF CHEMISTRY<br>Sáiz, Jorge; Universidad de Alcala de Henares, Department of Chemistry<br>Rempel, Paul; THE UNIVERSITY OF BASEL, DEPARTMENT OF CHEMISTRY<br>Hauser, Peter; THE UNIVERSITY OF BASEL, DEPARTMENT OF CHEMISTRY |
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# A microfluidic breadboard approach to capillary electrophoresis

Israel Joel Koenka[1], Jorge Sáiz[1,2,3], Paul Rempel[1], Peter C. Hauser[1]*

[1] Department of Chemistry, University of Basel, Spitalstrasse 51, 4056 Basel, Switzerland.

[2] Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering - University of Alcalá, Ctra. Madrid-Barcelona km 33.6, Alcalá de Henares 28871, Madrid, Spain.

[3] University Institute of Research in Police Sciences (IUICP), University of Alcalá, Ctra. Madrid-Barcelona Km 33.6, 28871 Alcalá de Henares (Madrid), Spain.

Corresponding author,

* Peter C. Hauser, E-mail: Peter.Hauser@unibas.ch

Tel: +41 61-267-1003 Fax: +41 61-267-1013

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**ABSTRACT**

A breadboard approach for electrophoretic separations with contactless conductivity detection is presented. This is based on miniature off-the-shelf components such as syringe pumps, valves, and pressure controllers which could be set up in a very compact overall arrangement. It has a high flexibility for different tasks at hand and the common operations of hydrodynamic injection and capillary flushing are automated. For demonstration of the versatility of the proposition, several very diverse configurations and modes of electrophoresis were successfully implemented, namely standard capillary zone electrophoresis, pressure assisted zone electrophoresis, the simultaneous separation of cations and anions by dual-capillary zone electrophoresis, the separation of cationic amino acids by isotachophoresis as well as the separation of small carboxylic acids by gradient elution moving boundary electrophoresis. The system also allows fast separations, as demonstrated by the analysis of 6 inorganic cations within 35 seconds. The approach addresses respective limitations of either, conventional capillary electrophoresis instruments as well as electrophoretic lab-on-chip devices, while maintaining a performance in terms of detection limits and reproducibility comparable to standard instrumentation.

## INTRODUCTION

Capillary electrophoresis (CE) is a very flexible method. Not only is it suitable for the determination of essentially any ionic species but also different modes of separation, such as isotachophoresis (ITP), are possible besides the most commonly employed capillary zone electrophoresis (CZE). Generally, compromise operating conditions have to be established with regard to the analytical goals. Low limits of detection are achieved by injecting relatively large amounts of sample, which then require long separation times. Large dynamic ranges require modest injected amounts. Fast separations are only possible with small sample plugs in short separation channels and fast injection procedures, but this will impart a loss of sensitivity. It is important to realise that optimization with regard to these parameters therefore requires full flexibility in the amount injected, the applied voltage, and crucially, in the length of the separation channel. For an illustration of this seldom dicussed aspect see for example Mai *et al.*[1]

Besides the length of the separation channel, also its diameter has a strong bearing on the separation efficiency as the Joule heat produced on application of the separation voltage creates a temperature gradient which leads to differences in migration velocity. It is therefore highly desirable to employ the narrowest possible capillaries. On the other hand, the standard UV-absorbance detection is not readily feasible with internal diameters of less than 50 μm because of the shortening of the optical pathlength and the required reduction in the size of the aperture. An alternative detection method is contactless conductivity detection, which is universal and simpler in configuration than optical detection. As had been demonstrated by Mayrhofer *et al.* in 1999, capacitively coupled contactless conductivity detection ($C^4D$) can be used with narrower separation channels of 25 or 10 μm diameter.[2] This is possible with negligible effect on the limit of detection.[3] Besides improved resolution, a further important benefit of the use of such narrow capillaries is the

possibility of employing hydrodynamic pumping, which in capillaries of larger diameters incurs significant bandbroadening[3] as the laminarity of the flow is limited in narrow channels. When $C^4D$ is employed in CZE concurrent pumping may therefore be used for optimization of the residence time of the analytes in the electric field in order to obtain best analysis times or best resolution,[3] or for the compensation of the electroosmotic flow (EOF) in the determination of anions.[4] Pumping also allows the placement of one or more samples plugs at desired positions along the capillary length prior to optimized separations.[5] Han *et al.* have also employed pressure driven CE to overcome variations in the electroosmotic flow.[6] In comparison to UV-detection C4D therefore is not only simpler and less expensive, but also enables to two benefits of narrower capillaries, *i.e.* improved resolution and the ability to employ hydrodynamic pumping for further optimization.

A common goal in CE is the determination of cations and anions in the same sample (for a review see Sáiz *et al.*[7]), which not only requires different applied polarities, but often different buffers are needed, as well as a surface modification of the capillary in order to reverse the electroosmotic flow. The concurrent use of two separation channels is the best approach as the operating conditions can be optimized independently.

In practice, capillary electrophoresis is usually carried out in conventional capillaries with commercial instruments available from several manufacturers, which were either designed for zone electrophoresis or for isotachophoresis. These instruments feature a high degree of automation, but on the other hand are not well suited for full optimization with regard to capillary diameter, capillary length, fast injections, the use of hydrodynamic manipulations or the employment of multiple capillaries. $C^4D$, while becoming a regular option for some newer commercial instruments, can only be retrofitted as a 3rd party module to instruments of the industry leaders which feature fixed built-in UV-detectors. Alternatively,

electrophoretic separations may also be implemented on lab-on-chip devices which contain a separation channel embedded in a planar substrate made of glass or a polymer. A large number of publications has appeared on the evaluation of such systems (for a recent review see for example Castro and Manz[8]). However, this arrangement also has certain practical problems, such as the presence of siphoning effects if the chips are not placed perfectly horizontal, difficulties in flushing of the manifold, geometric constraints for setting up efficient detection, and a lack of flexibility in particular with regard to the length of the separation channel. Several workers have thus proposed the use of interlocking microfluidic building blocks for the flexible construction of devices, but electrophoretic separations have not been demonstrated.[9, 10][Bhargava, 2014 #55] In a different approach to alleviate design and construction constraints, Garcia and coworkers proposed the use of standard capillaries and off-the-shelf connectors to emulate the standard elongated cross configuration of electrophoretic lab-on-chip devices[11] and demonstrated the separation of inorganic cations. It also should be borne in mind, that for efficient use electrophoresis in conventional capillaries and on lab-on-chip devices has to be automated and also the latter require a periphery with pumps and valves for flushing and sample introduction. A further discussion of some of the aspects of capillary electrophoresis on either platform and a comparison with liquid chromatography can be found in a review by Breadmore.[12]

The miniature microfluidic breadboard approach to electrophoretic separations and the adoption of contactless conductivity detection proposed here has evolved from our work with both, conventional capillaries and lab-on-chip devices, conducted over a number of years, in which we sought to develop cost effective instruments from components of standard size and methods for such applications as field analysis, automated monitoring, and the concurrent determination of cations and anions (see for example[1, 5, 7, 13-18]). The term *breadboard* is borrowed from the electronics laboratory where electronic circuits are

arranged by plugging components into a support with a grid of holes. It is demonstrated that it is possible to set up very flexible CE systems, which can be rearranged for different needs as they may arise, from standard capillaries, miniature fluidic interconnects, miniature syringe pumps, and miniature pressure controllers as components which are all reusable in different configurations. The approach alleviates the respective limitations of conventional CE instrumentation as well as of lab-on-chip devices with regard to full flexibility of separation channel length and internal cross-section, fast and precise injections, best detection geometry, the use of hydrodynamic pumping, the use of multiple separation channels *etc.* without incurring compromises in performance compared to those platforms.

## EXPERIMENTAL

### Instrumentation

The miniature syringe pump (SPS01), valves (AV201), air tight liquid reservoirs (T116-BBRES), interconnects (T116-203, T116-204), and connecting tubing were obtained from LabSmith (Livermore, CA). The fused silica capillaries were sourced from Polymicro (Phoenix, AZ) except for the coated low-EOF capillary (Guarant from Alcor Bioseparations, Palo Alto, CA). Separation voltages were applied by using ±30 kV high voltage modules (CZE2000) from Spellman (Pulborough, UK) and pressurization was achieved with pneumatic pressure controllers (OEM-EP) from Parker Hannifin (Etoy, Switzerland). The LabSmith parts were connected to a LabSmith electrical interface board. The pressure regulator and high voltage units were controlled with a purpose built electronic interface based on an Arduino microcontroller board (www.arduino.cc) and attached solid state relays for power switching and digital-to-analog convertors for setting voltages. Each system was connected to a personal computer via a separate universal serial bus (USB) connection and controlled with a single program running on the computer,

which is based on the open source and *Python* based *Instrumentino* package.[19, 20] The program interacts with the LabSmith components by making use of an application programming interface (API) from the device manufacturer installed on the personal computer and with the Arduino attached components via a purpose written software package running on the microcontroller board. Detection was carried out either with an in-house built conductivity detector ($C^4D$)[21, 22] or a commercially available version from eDAQ (Denistone East, NSW, Australia). An e-corder 401 and the Chart software (both eDAQ) were used for data acquisition.

**Reagents and samples**

All chemicals used were of analytical grade. Sodium hydroxide, L-histidine (His), 2-(N-morpholino)ethanesulfonic acid (MES), 18-crown-6, sodium nitrite, propionic acid, lithium chloride, DL-lactic acid (Lac) and L-lysine (Lys) were obtained from Fluka (Buchs, Switzerland). Potassium perchlorate, calcium nitrate, ammonium fluoride, potassium acetate and magnesium sulfate were purchased from Merck (Darmstadt, Germany). Formic acid, acetic acid and butyric acid were obtained from Sigma-Aldrich (Buchs, Switzerland), arginine (Arg) was obtained from Acros (Geel, Belgium). The sample of Himalayan rock salt was bought at a local supermarket. All solutions were prepared using ultrapure water, purified using a Milli-Q system (Millipore, Bedford, MA).

## RESULTS AND DISCUSSION

**Components**

The essential components required in setting up a capillary electrophoresis system are depicted in schematic form in Fig. 1. Pumps are required for fluid handling if the system is to be automated. Several hydrodynamic operations are required, *i.e.* flushing of capillaries and manifold as well as sample uptake and hydrodynamic sample injection into the

capillary. Different pumping options are possible. Small peristaltic or membrane pumps are available. These are not suited for precise operations and only produce limited pressures, but may still be useful for such tasks as aspiration of a sample into a manifold. Stepper motor driven syringe pumps allow the precise movement of solutions, including withdrawal, and are suitable for high pressures. However, the volume resolution of syringe pumps is generally not adequate for directly metering the small volumes in the low nanoliter to picoliter range to be injected into the separation capillaries. Stepper motor driven pumps are also not ideal when a smooth, pulse free hydrodynamic flow is required concurrent with a separation run. For these tasks it is preferable to employ controlled pressurization, which alleviates the aforementioned shortcomings and was therefore adopted for our work. A hydrodynamic flow can be achieved by using a solution container pressurized with a compressed gas in the headspace and a valve for flow control. Electronic pressure controllers are available which allow the electronic setting of the desired gas pressure. This approach is also useful if pumping from the high voltage side is desired. Conventional pumps and valves are not designed to be exposed to a liquid at a high voltage, but pressurization with a compressed gas provides inherent electrical isolation. Small valves are available from different suppliers; important parameters are low internal volumes and a low displacement volume of solutions on actuation. It is also desirable that the valve is of the latching type, *i.e.* it consumes power only on switching, which is critical if the set-up is to be battery operated.

The capillaries usually employed for separation are made of fused silica, have an outer diameter of 365 µm and are available with a wide range of internal diameters. For optical detection 75 or 50 µm IDs have to employed, but as discussed in the introduction, narrower capillaries of 25 or even 10 µm ID in combination with $C^4D$ are often preferable as better separation efficiencies are obtained and hydrodynamic pumping is possible. The

available channel cross-sections of a standard capillary are therefore comparable to those of separation channels on the chip platform. The use of $C^4D$ also has the advantage of allowing easy adjustment of the effective separation length to the detector as it is not necessary to remove the protective polyimide coating in order to create a UV transparent window. Commercially available low dead volume interconnects (T-pieces and crosses) for these standard capillaries may be used for making up a manifold.

If the standard UV-absorption detection is desired, commercial detectors may be integrated, but these are expensive and bulky. It is, however, also feasible to construct relatively compact UV-detectors based on newly available deep-UV light-emitting diodes.[23-27] Conctactless conductivity detection has the advantages discussed in the introduction. Furthermore also the detection cells can be very small enabling precise positioning on short capillaries. These detectors are commercially available, but with some attention to detail well performing units can also be built in-house.[15, 21, 28, 29]

A large range of high voltage modules with different maximum voltage levels and current as well as a range of secondary features are available from different suppliers. For precise control of the voltage the simplest models should be avoided in favor of devices which include a feedback mechanism for regulating the output. Many modules also provide monitor signals which allow to keep track of the actual output voltage and sometimes also of the current, which are both very useful features. A reversible polarity is highly desirable, as this allows to change between cation and anion separation via electronic control, but this is a rare feature. Discussions of small high voltage modules can be found in Lewis *et al.*[30] and Blanes *et al.*[31] Attention has to be paid to the potential hazard of the electrophoretic separation voltage. This is best addressed by enclosing the high voltage bearing parts in an insulated case which is fitted with a microswitch to interrupt the high voltage on opening.

To avoid the exposure of a fluid handling manifold based on pumps and valves at the inlet end of a capillary to the separation voltage it always has to be applied at the detection end of the capillary, which is the reverse of the usual arrangement. This is not a problem with optical detectors and $C^4D$, but would not allow the use of end-capillary detection schemes.

**Standard Zone Electrophoresis**

The set-up for a standard zone electrophoretic separation with hydrodynamic injection is shown in Fig. 2A and consists of a sequential injection analysis (SIA)-CE arrangement. Such a set-up was first described by Ruzicka and coworkers[32] and has previously been employed successfully on a conventional scale in our group.[18, 29, 33-36] The manifold consists of a miniature syringe pump and 3-way valves instead of the selection valve more commonly employed in SIA and an electronically controlled pressurization section. The syringe pump serves for delivery of the background electrolyte, sample uptake and sample transport to the injection point. Standard T-pieces were employed for connection of the capillary and the ground electrode to the fluidic manifold. Injection of sample into the capillary and capillary flushing is achieved by pressurization using the electronic pressure controller. Setting the time and pressure allows precise control of the desired length of the sample plug injected into the capillary. The entire manifold can fit into a space of approximately 20 cm by 20 cm (not including the power supplies and cylinder of pressurized gas).

The separation of a standard solution of 6 common inorganic cations ($NH_4^+$, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Li^+$) is shown in Fig. 2B. A BGE optimized for best separation of these ions consisting of 30 mM lactic acid and histidine (Lac/His) (pH 4.9) and 4 mM 18-crown-6 was employed. The crown ether allows the separation of ammonium from potassium, while lactate modifies the mobilities of $Ca^{2+}$ and $Mg^{2+}$ through complexation. The

performance in terms of linear ranges, correlation coefficients, LODs and reproducibilities is illustrated by the data of Table 1. These are generally comparable with the parameters obtained for conventional CE-$C^4D$, but it is noteworthy that the Lac/His buffer allows to work with larger linear ranges for these cations than the more commonly employed MES/His buffer.[13] This BGE is therefore suitable for the analysis of samples in which the analytes are present at very different concentrations. For such samples, dilution is not an option as that will lower the concentrations of certain analytes below their LODs. An example for such an application is the analysis of Himalayan rock salt, usually sold in health stores. Unlike normal table salt, rock salts are taken directly from the mines and therefore contain impurities. The electropherogram for a solution of 0.175 mg/mL of a rock salt sample is shown in Fig. 2C. Using the Lac/His buffer it was possible to determine $K^+$ at 17.5 µM, $Ca^{2+}$ at 18.5 µM and and $Mg^{2+}$ at 14.3 µM alongside $Na^+$ at a concentration 1950 µM.

**Fast Separation**

Impressively fast separations have been reported for electrophoretic separations on lab-on-chip devices, and this is usually considered to be one of the main selling points if not a unique feature of the approach. It appears that it is commonly perceived that this is largely due to the special injection mode based on the cross formed between injection and separation channels. However, it has repeatedly been demonstrated over the years that very fast separations are also possible in short conventional capillaries when used in arrangements that allow fast and precise injection operations.[37-45]

The analysis time for the separation presented in the previous section with 2 minutes is relatively short for capillary electrophoresis, but an even faster separation is possible using the same set-up of Fig. 2A simply by reducing the effective capillary length to 10 cm and

the injection time to 0.5 s in order to place only a short sample plug into the capillary and thus maintain the resolution. The separation of the 6 cations within 35 s is illustrated in Fig. 3. However, as discussed in the introduction, this is achieved at the cost of a loss of sensitivity and under these conditions the LODs for $NH_4^+$, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $Li^+$ were determined as 9.6, 11, 14, 11, 12 and 27 µM respectively, which are about 4 times higher than those for the separation reported in the previous section.

**Pressure Assisted Capillary Electrophoresis (PACE)**

As discussed in the introduction, when using narrow capillaries of 25 or 10 µm ID it is possible to employ hydrodynamic pumping in CE without penalty in laminar flow induced bandbroadening. One possible application of PACE, *i.e.* for speeding up the analysis, as implemented with the manifold shown in Fig. 2A is illustrated in Fig. 4. As can be seen in Fig. 4A, under conditions necessary for the separation of a group of anions, phosphate will arrive only much later at the detector. Note, that the negative going peak for phosphate is nothing unusual for $C^4D$. The time saving that can be achieved by introducing a hydrodynamic flow is illustrated in Fig. 4B. Pressurization was turned on following the passage of the last peak of the early analytes. The introduction of pressure was found not to have an adverse effect on the repeatability of the migration time for the phosphate peak as the relative standard deviation was found to be even better for pressurization than without (0.6% *vs.* 0.9% RSD for migration time respectively, n = 9). Similarly, also the reproducibility for the area of the phosphate peak was found to be improved (12% *vs.* 6.5% RSD with and without hydrodynamic flow respectively). It was also found that the migration times and peak areas for phosphate obtained with pressurization were also not adversely affected when the system was disassembled and then reassembled between measurements (0.4% and 6.5% RSD for migration time and peak area for 9 measurements in 3 sets with disassembly between the sets).

**Dual Capillary Zone Electrophoresis**

The use of two separation channels is not readily possible with conventional commercial CE instrumentation, but in principle not a significant complication as the required components are significantly less costly than for other quantitative analytical methods, such as HPLC. Dual channel electrophoresis as previously reported for conventional scale fluidic set-ups[13, 15] could therefore relatively easily be implemented in the here proposed microfluidic breadboard approach as shown in Fig. 5A. It is possible to achieve this using a single syringe pump and pressurization section. Using this configuration, inorganic cations ($NH_4^+$, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Li^+$) and anions ($Cl^-$, $NO_2^-$, $NO_3^-$, $SO_4^{2-}$, $ClO_4^-$) were simultaneously analyzed, as can be seen in Fig. 5B. The same BGE was used for both types of ions and the effective lengths of the capillaries and separation voltages were adjusted so both cations and anions were baseline separated and appear within the same time frame. If it is desired to use different BGEs for the cations and anions, as well as inject individually optimized amounts, this will also be possible with an extended system.

**Isotachophoresis (ITP)**

In ITP, a sample plug is introduced between a leading electrolyte (LE), which has a higher mobility than the analyte ions, and a terminating electrolyte, with a lower mobility. The only change in the instrumentation necessary for performing ITP is the ability to manipulate three solution (LE, TE, sample) rather than only two (BGE, sample) as in CZE. This could be implemented by employing an additional 3-way valve as shown in Fig. 6A. In order to demonstrate its use, an ITP separation of cationic amino acids[46] was performed, as can be seen in Fig. 6B.

**Gradient Elution Moving Boundary Electrophoresis (GEMBE)**

GEMBE is an electrophoretic separation method relying on the simultaneous application of a hydrodynamic flow and an electric field which was introduced by Ross and coworkers in 2007.[47-51] This generates two opposing forces on each ion, and at first a relatively high counter-pressure is applied to prevent any analyte ion from entering the capillary. The pressure is then linearly decreased (while keeping the voltage constant) which reduces the opposing force, allowing the different analytes to enter the capillary in turn, from fastest to slowest. This creates a set of boundaries moving against the applied pressure, towards the detector. Each new segment includes all of the ions from the earlier segments, so the measured conductivity is increased stepwise. The implementation of this less commonly employed mode of electrophoresis on the microfluidic platform is illustrated in Fig. 7A. The length of the capillary from the injection point to the detector was as short as 3.5 cm and a controlled counter-pressure was applied again by pressurization with compressed nitrogen using the electronic pressure controller. Pumping from the high voltage end of the capillary does not present a problem when using this approach as the only contact with the solution at the elevated voltage is via the compressed nitrogen. The analysis of a mixture of four carboxylic acids in 160 s is illustrated in Fig. 7B. The counter pressure was slowly reduced from 100 to 50 psi in steps of 0.5 psi, demonstrating the fine level of pressure control available in this system. The rate of the reduction of the pressure affects the analysis time but also the separation. It is also possible to evaluate the GEMBE data in peak mode, simply by forming the first derivative of the electropherogram, as illustrated in Fig. 7C.

## CONCLUSION

It was found possible to assemble very compact electrophoresis instrumentation from off-the-shelf miniature components. This alleviates the limitations of commercial

electrophoresis instruments in terms of implementing different electrophoretic modes. The standard building blocks needed for hydrodynamic injection, capillary flushing and detection can quickly be rearranged for different purposes and the *Instrumentino* package allows easy adaptation of the software control. The contactless conductivity detector can be placed anywhere on the capillary without the need to produce a detection window which allows to easily adjust compromise conditions between the degree of separation and analysis time. The performance of the microfluidic breadboard approach was found to be similar to commercial CE systems. Furthermore, fast separations are possible, but the approach has none of the limitations of lab-on-chip electrophoretic devices with regard to the inflexibility of the separation length and the geometrical constraints for detection. It also should be borne in mind, that lab-on-chip electrophoresis devices require a periphery with pumps and valves for flushing and sample introduction, and the chip is only part of a larger fluidic manifold. The breadboard approach thus provides high flexibility in a small space at moderate cost without penalty in performance.

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Table 1

Validation data for the determination of cations.

| Ion | Linear range (μM) | Correlation coefficient $r^2$ | Limit of Detection[a] (μM) | Reproducibility of migration time (%RSD)[b] | Reproducibility of peak area (%RSD)[b] |
|---|---|---|---|---|---|
| $NH_4^+$ | 5-4000 | 0.998 | 2 | 0.7 | 3.4 |
| $K^+$ | 5-4000 | 0.998 | 3 | 0.7 | 4.0 |
| $Na^+$ | 5-3000 | 0.999 | 3.5 | 0.7 | 3.7 |
| $Ca^{2+}$ | 5-750 | 0.996 | 2.5 | 0.8 | 4.0 |
| $Mg^{2+}$ | 5-750 | 0.996 | 2.5 | 0.8 | 4.6 |
| $Li^+$ | 10-4000 | 0.997 | 7 | 1.0 | 4.0 |

[a] Peak heights corresponding to 3 times the baseline noise.

[b] Determined for 200 μM $NH_4^+$, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Li^+$. n = 9.

**Figure Captions:**

Fig. 1 Components for building microfluidic CE systems.

Fig. 2 A) Schematic drawing of a microfluidic system for zone electrophoresis with automated hydrodynamic injection and capillary flushing employing the SIA approach.

B) Separation of cations at 200 µM. BGE: 30 mM Lac/His pH 4.9 and 4 mM 18-crown-6. Capillary: 25 µm ID, 365 µm OD, 35 cm effective length, 50 cm total length. Injection: 22 s at 1.6 psi, occupying 10 mm of the capillary length as calculated by the Hagen-Poiseuille equation. Separation voltage at detection end: -30 kV.

C) Separation of a sample of Himalayan rock salt in water at 0.175 mg/mL. Note, that the large peak for $Na^+$ is measurable but truncated in the figure.

Fig. 3 Fast separation of 200 µM $NH_4^+$, $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $Li^+$ in water. Capillary: 25 µm ID, 365 µm OD, 10 cm effective length, 50 cm total length. Injection: 0.5 s at 1.6 psi, occupying 0.2 mm of the capillary length as calculated by the Hagen-Poiseuille equation. Other conditions as for Figure 2.

Fig. 4 Separation of anions with pressure assistance.

A) Normal zone electrophoretic separation of anions. BGE: 30 mM Lac/His pH 4.9. Capillary: 10 µm ID, 365 µm OD, 20 cm effective length, 50 cm total length. Injection: 20 psi, 10 s. Separation voltage at detection end: +30 kV. $Cl^-$ 300 µM, $NO_3^-$ 300 µM, $ClO_4^-$ 300 µM, $H_2PO_4^-$ 600 µM.

B) Separation of the anions with superimposed hydrodynamic flow to speed up the migration of the phosphate. A pressure of 50 psi was continuously applied from t = 100 s.

Fig. 5 Concurrent electrophoretic separation of cations and anions.

A) Extended manifold for two separation capillaries.

B) Cations. BGE: 30 mM Lac/His pH 4.9. Capillary: 25 µm ID, 365 µm OD, 35 cm effective length, 60 cm total length. Injection: 6 psi, 7 s. Separation voltage at detection end: -30 kV. $NH_4^+$ 200 µM, $K^+$ 400 µM, $Na^+$ 400 µM, $Ca^{2+}$ 200 µM, $Mg^{2+}$ 200 µM, $Li^+$ 200 µM.

C) Anions. BGE: 30 mM Lac/His pH 4.9. Capillary: 25 µm ID, 365 µm OD, 50 cm effective length, 60 cm total length. Injection: 6 psi, 7 s. Separation voltage at detection end: -30 kV. $Cl^-$ 200 µM, $NO_3^-$ 400 µM, $ClO_4^-$ 200 µM, $SO_4^2$ 200 µM, $MnO_4^-$ 400 µM.

Fig. 6 Isotachophoretic separation of amino acids.

A) Manifold for the injection of two separate electrolytes.

B) Isotachopherogram for a standard solution of 2 mM His, Lys and Arg in water. LE: 10 mM potassium acetate and 52.3 mM acetic acid (pH 4.0). TE: 10 mM Ala. Low EOF-Capillary: 25 µm ID, 365 µM OD, 60 cm effective length, 75 cm total length. Injection for 10 s at 6 psi. Separation voltage at detection end: - 30 kV.

Fig. 7 GEMBE separation.

A) Manifold for pressure application from the detection end of the capillary.

B) GEMBE-electropherogram of 200 µM formic, acetic, propionic and butyric acids (all 200 µM). Capillary: 10 µm ID, 365 µM OD, 3.5 cm effective length, 30

cm total length. Separation voltage at detection end: - 30 kV. Pressure gradient: 100 psi to 50 psi over 400 steps of 0.5 psi.

C) Derivative of the raw electropherogram given in part B.

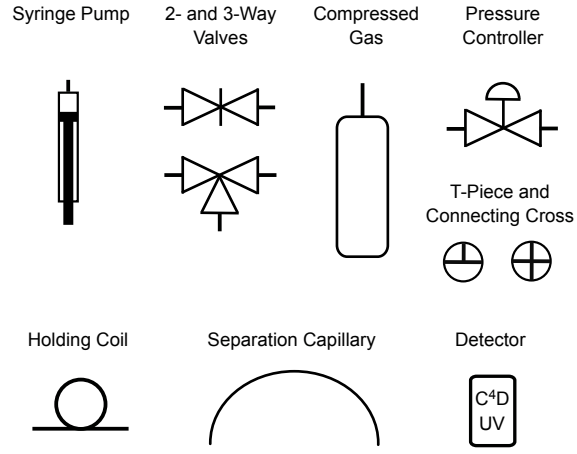

Syringe Pump
2- and 3-Way Valves
Compressed Gas
Pressure Controller
T-Piece and Connecting Cross
Holding Coil
Separation Capillary
Detector
C4D
UV

Fig. 1

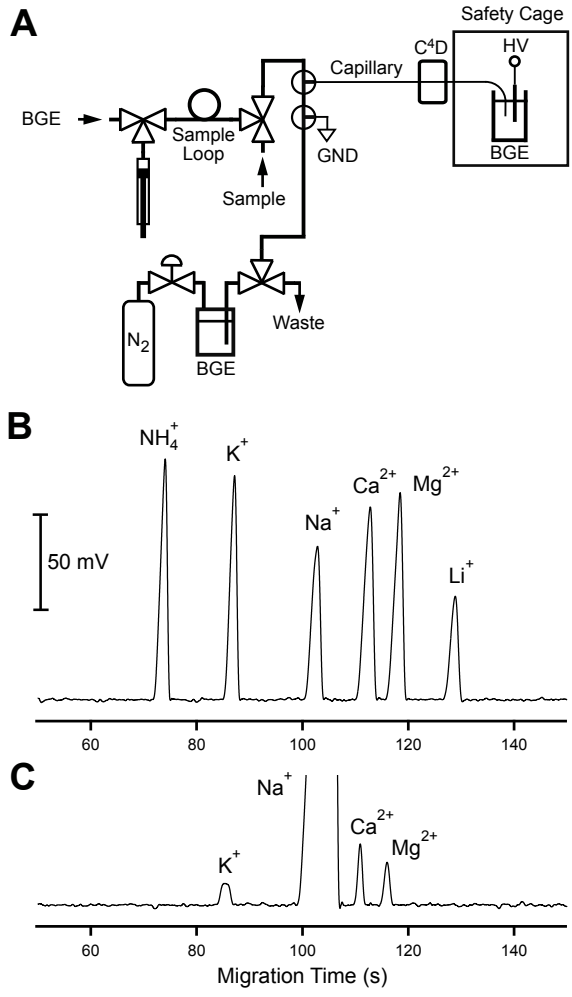

A
Safety Cage
C4D
HV
Capillary
BGE
Sample Loop
GND
Sample
BGE
N2
Waste
BGE
B
NH4+
K+
Na+
Ca2+
Mg2+
Li+
50 mV
60
80
100
120
140
C
Na+
Ca2+
Mg2+
K+
60
80
100
120
140
Migration Time (s)

Fig. 2

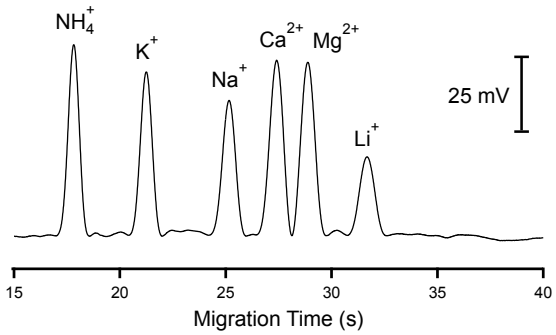



Fig. 3

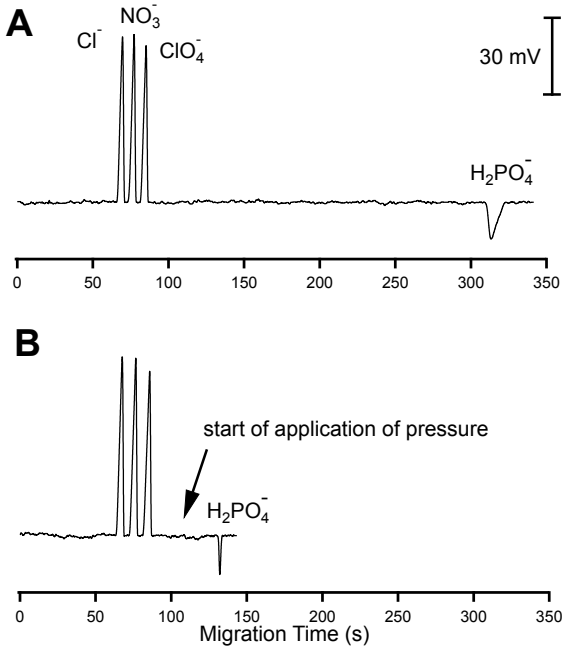

A
Cl-
NO3-
ClO4-
30 mV
H2PO4-
0
50
100
150
200
250
300
350
B
start of application of pressure
H2PO4-
0
50
100
150
200
250
300
350
Migration Time (s)

Figure 4

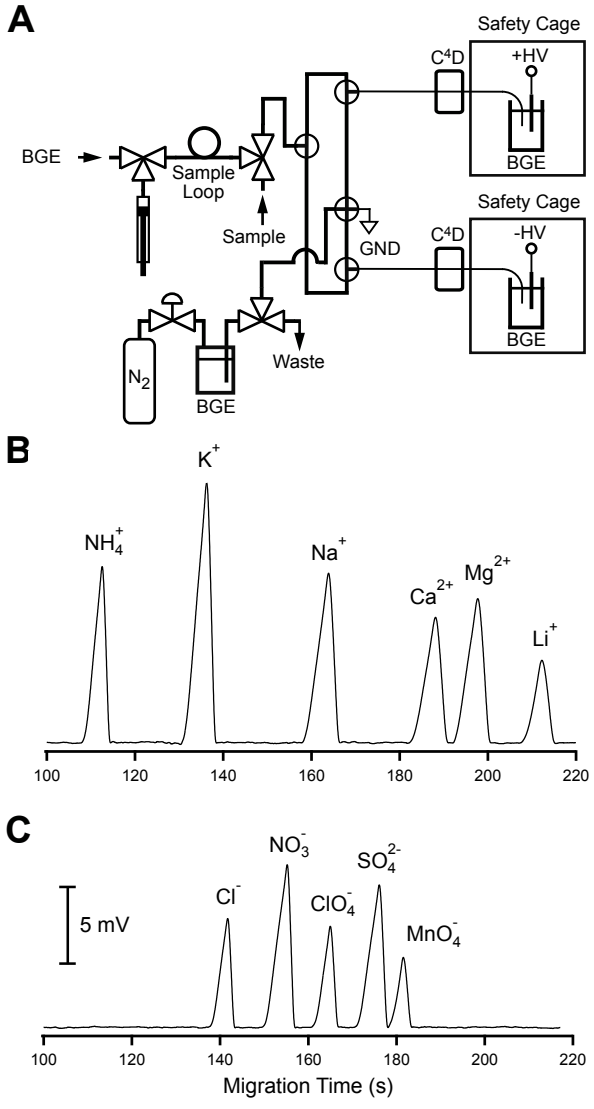
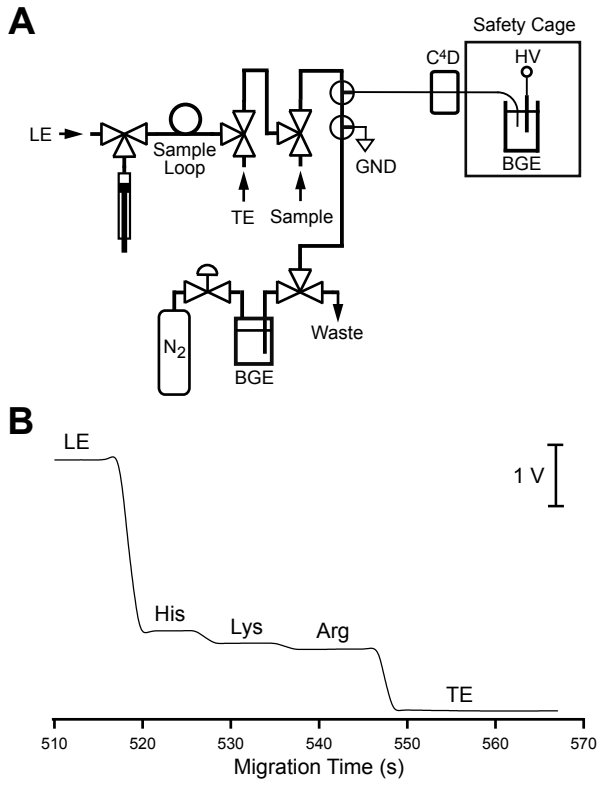

A
Safety Cage
C4D
+HV
BGE
BGE
Sample Loop
Sample
GND
Safety Cage
C4D
-HV
BGE
N2
BGE
Waste
B
K+
NH4+
Na+
Ca2+
Mg2+
Li+
100
120
140
160
180
200
220
C
NO3-
SO4 2-
Cl-
ClO4-
MnO4-
5 mV
100
120
140
160
180
200
220
Migration Time (s)

Fig. 5


A
Safety Cage
C4D
HV
LE
Sample Loop
GND
BGE
TE
Sample
N2
BGE
Waste
B
LE
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His
Lys
Arg
TE
510
520
530
540
550
560
570
Migration Time (s)

Fig. 6

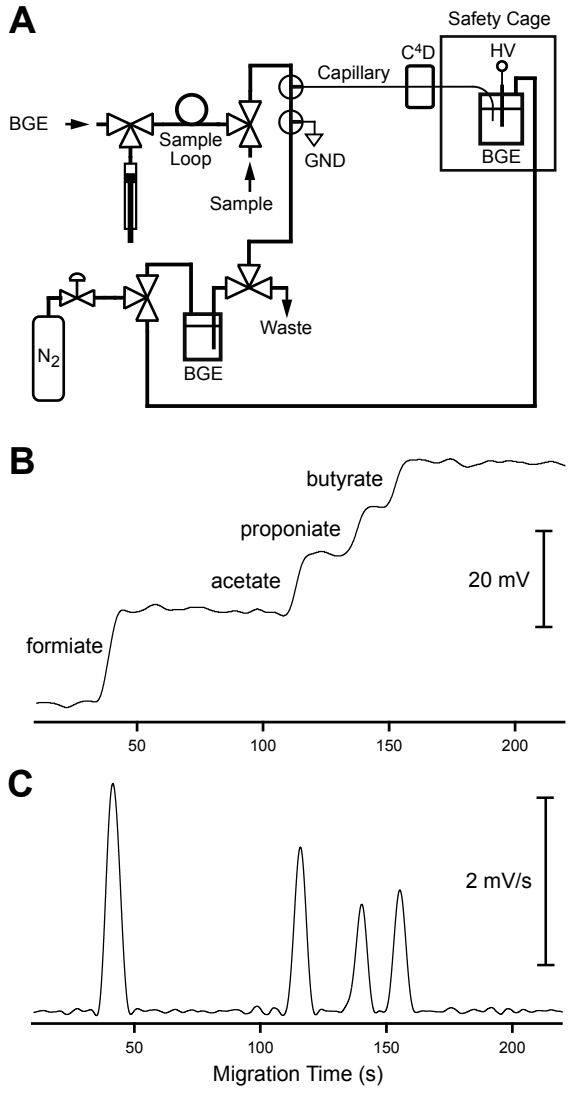
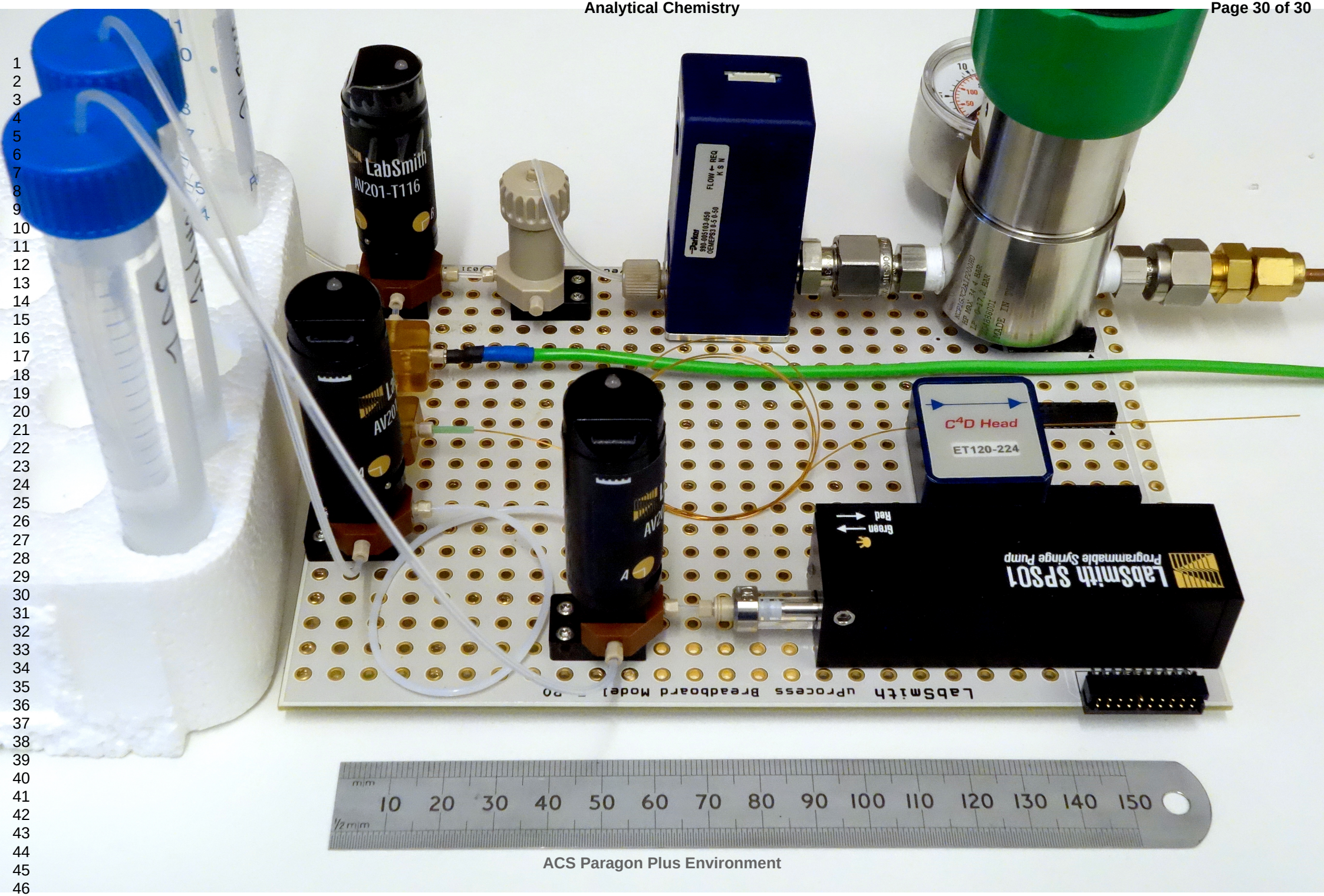

A
Safety Cage
C4D
HV
Capillary
BGE
Sample Loop
GND
Sample
BGE
Waste
N2
BGE
B
butyrate
proponiate
acetate
formiate
20 mV
50
100
150
200
C
2 mV/s
50
100
150
200
Migration Time (s)

Fig. 7

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