

**A MICRO-INJECTOR FOR CAPILLARY ELECTROPHORESIS**

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Complete List of Authors:	Sáiz, Jorge Koenka, Joel García-Ruiz, Carmen; University of Alcalá, Department of Analytical Chemistry Müller, Beat; EAWAG, Chwalek, Thomas; EAWAG, Hauser, Peter; The University of Basel, Department of Chemistry
Keywords:	micro-injector, capillary electrophoresis, contactless conductivity detection

Short Communication**A MICRO-INJECTOR FOR CAPILLARY ELECTROPHORESIS**

Jorge Sáiz^{1,2,4}, Israel Joel Koenka⁴, Carmen García-Ruiz^{1,2}, Beat Müller³, Thomas Chwalek³, Peter C. Hauser^{4*}

¹ Department of Analytical Chemistry, Physical Chemistry and Chemical Engineering, University of Alcalá, Ctra. Madrid-Barcelona Km 33.6, 28871 Alcalá de Henares (Madrid), Spain.

² University Institute of Research in Police Sciences (IUICP), University of Alcalá, Ctra. Madrid-Barcelona Km 33.6, 28871 Alcalá de Henares (Madrid), Spain.

³ EAWAG, Swiss Federal Institute of Aquatic Science and Technology, 6047 Kastanienbaum, Switzerland.

⁴ University of Basel, Department of Chemistry, Spitalstrasse 51, 4056 Basel, Switzerland.

*Corresponding author.

E-mail address: Peter.Hauser@unibas.ch (P. C. Hauser)

Phone: ++41 61 267 10 03; Fax: ++41 61 267 10 13

Keywords: micro-injector, microfluidics, capillary electrophoresis, oral fluid.

Abbreviations: BGE, background electrolyte; His, histidine; HV, high voltage; MES, 2-(N-morpholino)ethanesulfonic acid; PMMA, poly(methyl methacrylate); C⁴D, capacitively coupled contactless conductivity detection, CBI, capillary batch injection; GUI, graphical user interface.

Abstract

A novel micro-injector for capillary electrophoresis for the handling of samples with volumes down to as little as 300 nL was designed and built in our laboratory for analyses in which the available volume is a limitation. The sample is placed into a small cavity located directly in front of the separation capillary, and the injection is then carried out automatically by controlled pressurization of the chamber with compressed air. The system also allows automated flushing of the injection chamber as well as of the capillary. In a trial with a capillary electrophoresis system with contactless conductivity detector, employing a capillary of 25 μm diameter, the results showed good stability of migration times and peak areas. To illustrate the technique, the fast separation of 5 inorganic cations (Na^+ , K^+ , NH_4^+ , Ca^{2+} and Mg^{2+}) was set up. This could be achieved in less than 3 min, with good limits of detection (10 μM) and linear ranges (between about 10 μM and 1000 μM). The system was demonstrated for the determination of the inorganic cations in porewater samples of a lake sediment core.

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Capillary electrophoresis (CE) is well known for being a separation technique which requires only very small samples volumes. The internal diameters of the capillaries employed are in the range from 75 μm (for detection by optical absorption measurement) down to 10 μm (for detection by contactless conductivity measurement or mass-spectrometry). Typically a sample plug of about 1 cm length is injected, therefore the sample volumes consumed for a single separation run are between about 1 nL and 50 nL in dependence on the capillary diameter. However, in practice the volumes of samples needed are significantly higher. The commercial benchtop instruments employ automated injectors based on sampling trays fitted with vials into which the capillary ends are temporarily dipped and injection is carried out by pressurization of the container or applying a vacuum at the other end of the capillary. The minimum sample volume that can be handled with these instruments is perhaps about 50 μL . For purpose built CE instruments built in laboratories around the world (see [1-22]) different approaches have been employed. The most simple instruments are based on manual hydrodynamic injection by syphoning in which the end of the capillary is placed into a vial containing the sample and lifting this to a defined height for a defined period of time. This allows working with sample volumes down to about 10 μL [23, 24]. However, it is difficult to carry this out with high precision, and it is tedious in particular when working with field portable instruments out of doors. Purpose built CE-instruments which have employed automated injection have usually been based on either passing a sample plug to an injection cell with a sequential-injection manifold [9, 16, 18, 25] or on a flow-through arrangement employing a sample loop [13, 15, 19, 20, 26]. However, the sample volumes to be inserted into the capillary are too small to be manipulated directly with standard fluid handling apparatus based on rotary injection valves or standard stepper motor driven syringe pumps. Therefore these systems work with larger volumes and employ split injection

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3 methods as introduced by Kubáň *et al.* [26]. Such automated split injector systems have
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5 also been incorporated into field portable instruments [11, 13, 14, 16, 19], but require
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7 relatively large sample volumes of at least 500 μL .
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12 On the other hand, for certain applications only very limited sample volumes are available.
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14 An example encountered in our laboratories is the analysis of porewater in lake sediments.
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16 The extraction from the sediment core brought to the surface only provides a few tens of
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18 microliters of sample [23, 24] and the amount of liquid that can be withdrawn from the
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20 sediment decreases strongly with depth. If the volume that needs to be extracted for
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22 analysis from the core can be reduced, it is also possible to obtain a higher resolution in
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24 the depth profile, thus the ability to work with the smallest possible volumes is highly
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26 desirable. A further study in progress based on highly limited sample volumes is the
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28 determination of leachable nutrients on rock surfaces. While these projects would not have
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30 been possible without CE, manual siphoning injection has been mandatory in order to
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32 handle the small sample volumes. Also clinical analysis, such as the CE-C⁴D methods
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34 developed in our laboratory for the determination of the only weakly UV-absorbing
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36 creatine [27], valproic acid [28, 29], carnitines [30], lactic acid [31] or uric acid [32], can
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38 benefit from a possible reduction in sample size. In recognition that the capability of CE
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40 for the analysis of very low sample volumes has not been fully realised, recently
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42 Grundmann and Matysik developed a new sampler based on the technique of capillary
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44 batch injection (CBI) [33]. Their injection system employed a separate capillary connected
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46 to a ultra-high precision syringe pump to pick up the sample. In the second step, the end of
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48 this sampling capillary was then moved with computer controlled micromanipulators and
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50 butted directly to the end of the separation capillary. The sample previously picked up was
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3 then dispensed into the latter for injection proper. This allowed very precise injections into
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5 the separation capillary with efficiencies of up to 100 % [33].
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10 The injector presented here is based on a small cell into which the sample is placed
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12 manually, but the actual injection into the capillary and flushing operations are then
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14 carried out automatically under computer control. Mechanical movements of any parts are
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16 not necessary. A schematic drawing of the injection cell can be seen in Figure 1A. It
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18 consists of a small block of PMMA with dimensions of a 30 x 25 x 20 mm (H x W x D).
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20 The vertical channel has a diameter of 5 mm on top but narrows to a width of 0.66 mm at
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22 the bottom. The separation capillary meets this narrow section in a 90° angle. Additional
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24 ports serve for connection of the electrophoretic ground electrode, as entry points for air
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26 and background electrolyte (BGE), and as a fluid drain. A removable lid fitted to the top
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28 with an o-ring provides an air-tight seal. The fluidic connections were made with 1/16"
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30 o.d. and 0.01" i.d. tubings using standard 1/4"-28 flangeless fittings and the electrode was
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32 mounted likewise.
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39 The complete assembly is shown in Fig. 1B. Compressed air is employed for propulsion of
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41 fluids and the pressure is regulated with miniature electronic pressure controllers (OEM-
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43 EP, Parker Hannifin Corporation, Cleveland, OH) with pressure ranges from 0 to 15 psi.
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45 Three switching valves (AV201-T116, LabSmith Inc, Livermore CA, USA), labelled V1,
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47 V2 and V3, were used in combination with the pressure regulators to control all the fluidic
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49 steps. The electrophoretic separation was carried out with a high voltage (HV) power
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51 supply from Spellman (CZE2000, Pulborough, UK). A purpose made detector with high
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53 voltage excitation and a measuring cell based on a previous design [34, 35] was used in
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55 combination with an e-corder 401 and the software Chart (eDAQ, Denistone East NSW,
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3 Australia) for data acquisition. Silica capillaries (Polymicro, Phoenix AZ, USA) of 25 μm
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5 i.d. and 375 μm o.d. were used. For safety, the HV electrode and the BGE vial were
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7 placed inside a cage made of PMMA. The pressure controllers and the high voltage
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9 module were controlled with a personal computer via an Arduino microcontroller board
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11 (www.arduino.cc) and the valves via a proprietary interface from LabSmith. The
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13 sequencing of steps was programmed with the graphical user interface (GUI)
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15 *Instrumentino* [36]. This is a versatile software package, based on the programming
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17 language *Python*, which was recently developed in our laboratory and simplifies the
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19 control of experimental assemblies of different hardware components. It was primarily
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21 designed for parts interfaced via an Arduino board, but also allows the integration of other
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23 components which are connected directly to the personal computer. This software is
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25 available for download under an open source licence for general use
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27 (http://www.chemie.unibas.ch/~hauser/open-source-lab/instrumentino/index.html).
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35 The sequence of operations is illustrated in Fig. 2. First, BGE is delivered to the micro-
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37 injector by opening valve V1 (Step 1) while the lid remains closed. Then, the injector is
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39 pressurized to flush the capillary with BGE by opening V2 (Step 2). Next, the BGE is
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41 removed from the nano-injector by gravity flow following the opening of V3 to waste and
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43 V2 to air (Step 3). The lid of the micro-injector is then manually opened and a drop of
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45 sample placed in the sample receptacle with a micro-syringe (Step 4). The lid is closed
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47 again and part of the sample plug is injected into the capillary by applying air pressure
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49 (Step 5). The amount of sample injected is optimized for the task at hand by setting the
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51 pressure and the duration of its application. Following this, the injector chamber is flushed
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53 with BGE in order to remove excess sample and to fill the injector with BGE (Step 6). The
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55 high voltage is then turned on and the separation is carried out.
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6 Due to the design of the micro-injector, in which the bottom of the sample receptacle is
7 immediately adjacent to the capillary inlet and consists of a narrow channel, it was
8 possible to employ sample volumes down to as little as 300 nL. But note that it is possible
9 to work with larger volumes as well (up to 100 μL) if so desired, as the channel above the
10 intersection with the capillary widens. CE is a versatile technique, which can be optimized
11 in different ways in order to meet different objectives, *i.e.* high resolution, low detection
12 limits, or fast analysis times. Relatively high resolutions and wide linear ranges can be
13 obtained in short separations when the parameters are well optimized. In Fig. 3A the
14 separation of 5 inorganic cations within less than 3 min is shown. A fused silica capillary
15 of 25 μm i.d. was used to achieve good peak resolution [16]. A BGE consisting of 30 mM
16 2-(N-morpholino)ethanesulfonic acid and histidine (MES/His) at pH 6.0 with 2 mM 18-
17 crown-6 was used because it allowed the separation on NH_4^+ and K^+ while Ca^{2+} and Na^+
18 were still baseline-resolved. The capillary had a total length of 60 cm and an effective
19 length of 49 cm. Injection was performed at 2 psi for 2 s and the separation was carried
20 out at -30 kV. The sample volume placed in the micro-injector was 300 nL. The LODs for
21 the cations were 10 μM . The stability of migration times was studied over 10 separations,
22 obtaining good RSD values of around 0.3%. The peak areas could be reproduced with
23 RSD values between 7 and 10 %. The linear ranges for the cations extended from 10 μM
24 to 1500 μM in the case of NH_4^+ , K^+ and Ca^{2+} and from 10 μM to 1000 μM for Na^+ and
25 Mg^{2+} . These results show a good stability of the system and the electrophoretic procedure,
26 which is comparable to that of other automated systems produced in our laboratories, even
27 though the sample volume is much reduced.
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4 The system was then used with the same electrophoretic conditions for the analysis of
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6 porewater samples withdrawn from a sediment core of Lake Baldegg in Switzerland [24].
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8 The electropherogram obtained for one of the samples is shown in Fig. 3B. In Fig. 3C a
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10 depth profile for the cations is shown. Notable is the absence of ammonium until a certain
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12 depth, which presumably is due to more pronounced reducing conditions at lower levels.
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17 In conclusion, it has been found possible to create an automated CE-injector suitable for
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19 sample volumes down to 300 nL with acceptable reproducibilities. It is expected, that,
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21 besides the demonstrated porewater analysis, the method will be useful for other
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23 applications in environmental studies. The overall system is fairly compact and the
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25 techniques employed are not more complex than those previously implemented in
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27 automated portable CE-instruments reported by our group. Therefore the system is
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29 suitable for ready implementation in such instruments as well and expected to be
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31 sufficiently robust for field applications. It may also prove useful for clinical applications
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33 such as the analysis of biopsy samples or blood samples from small infants. Note, that it
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35 may be possible to refine the injection approach to further reduce the required sample
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37 volume, perhaps by constructing an injector using microlithographic techniques. However,
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39 it has to be borne in mind that ultimately the sample needs to be transferred from
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41 somewhere, even if directly from the sampling site into the instrument, and that the
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43 handling becomes increasingly more difficult as the volume is reduced.
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48 49 **Acknowledgements**

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Figure Caption:

Fig. 1 Schematic drawings of the injector cell (A) and the complete CE-C⁴D system with automated microinjector (B). The system was supplied with compressed air, which was regulated by two pressure controllers. This, in combination with three valves (V1, V2 and V3), allowed to carry out all the steps for the analysis of samples.

Fig. 2 Steps for the analysis of a single sample (drawings not to scale). 1) Buffer delivery to the micro-injector; 2) Capillary flushing; 3) Draining of the injector; 4) Sample placement; 5) Sample injection; 6) Flushing of the injector and buffer delivery prior to the electrophoretic separation.

Fig. 3 Determination of 5 inorganic cations. A) Electropherogram for an aqueous solution of standards: 500 μM NH_4^+ , K^+ , Ca^{2+} , Na^+ and Mg^{2+} . B) Determination of NH_4^+ , K^+ , Ca^{2+} , Na^+ and Mg^{2+} in a porewater sample of a lake sediment. C) Depth profiles for the 5 cations. Details on the sampling procedure can be found in a previous publication [24]. Conditions for the CE-C⁴D analysis are given in the text.

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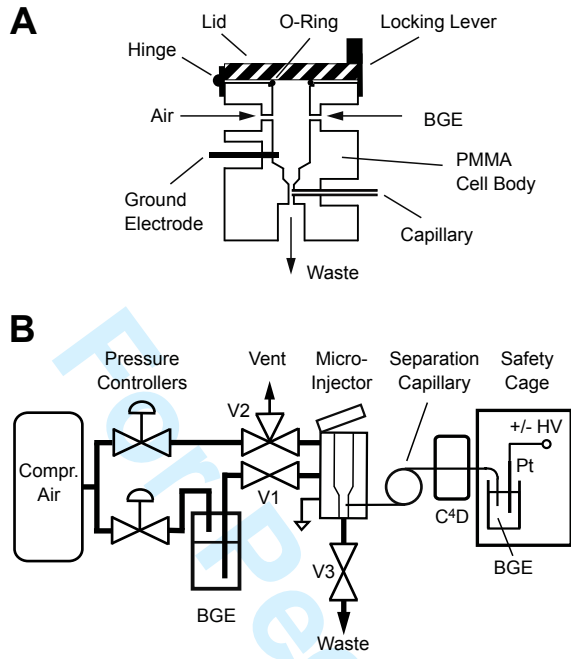


Fig. 1

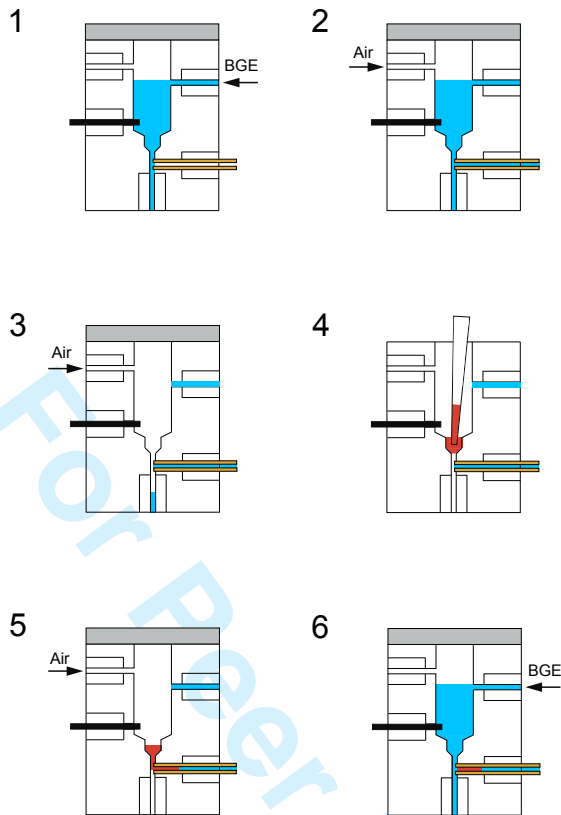


Fig. 2

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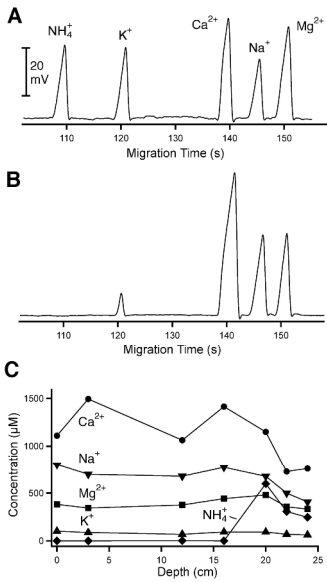


Fig. 3

276x386mm (300 x 300 DPI)