

LOW-COST CAPILLARY ELECTROPHORESIS INSTRUMENTATION FOR ASSESSMENT OF RAIN WATER IONIC COMPOSITION

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Abstract: The objective of this Short Term Scientific Mission was to test the suitability of capillary electrophoresis for determination of rain water chemical composition. The fully functional, open-hardware, low-cost instrument was assembled and tested. An original protocol for determination of the chemical content of rain water was established and validated in the pilot test. Capillary electrophoresis proved its highest suitability for the detection and quantification of ions in different samples, indicating substantial differences between clean and dirty snow waters. The hydrostatic sample injection method has been found very efficient and straightforward in manual and laboratory routines. An important deliverable of the Short Term Scientific Mission was a set of multimedia materials for the promotion of CE technique and especially low-cost CE instrumentation.

Keywords: capillary electrophoresis, rain water, ionic composition, COST Action CA16215

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1. INTRODUCTION

Capillary electrophoresis (CE) is an analytical chemistry method with a high potential for implementation in different real-life applications. It is capable of separation, quantification and characterization of polar and charged molecules in complex mixtures. The application field of CE includes both scientific research and industry (Seiman et al. 2009; Kobrin et al. 2014; Renzi et al. 2005; Lim et al. 2014; Gregu et al. 2016; Koenka et al. 2016). In general, the technology is well developed, including several commercial instruments available on the market, for example 7100 CE System from Agilent (2019) and Capel-205 from Lumex (2019). However, the constraint is the rather high cost of such instruments and the very limited possibility for these in-field applications, especially when compact size and portability are essential.

CE has been identified by COST Action CA16215 as one of the “model” techniques for promotion of the open hardware concept. For that reason, the Action supported a Short-Term Scientific Mission where feasibility of a CE prototype instrument was tested toward its implementation as part of the multisensory system for monitoring the environment in cities. The objective of the mission was to learn theoretical and practical knowledge necessary for properly implementing capillary electrophoresis in diverse application fields, with a special emphasis on the determination of rain water chemical composition. The paper presents some of the experiences gained, summarizing key technical aspects of the sensor and basic methodology for the proper CE implementation. It is therefore dedicated for those who are not familiar with CE, to demonstrate the advantages of the method and to convince them the usefulness of it.

2. MATERIAL AND METHODS

2.1. Low-cost and open-hardware capillary electrophoresis instrument

A prototype CE instrument used in this research was originally developed by the analytical chemistry research group at Tallinn University of Technology as the result of a multi-annual research programme. The instrument (**Figure 1**) is custom-made and contains low-cost components easily accessible on the market. Part of the sensor is custom 3D printed to minimize costs but also to allow easy adaptation of the system for specific requirements. The general schematic of the CE instrument and the configuration used in this research are presented in **Figure 1**. Dimensions of this prototype are 120 mm x 70 mm x 130 mm (width x length x height, respectively) and its mass (without batteries) is 550 g.

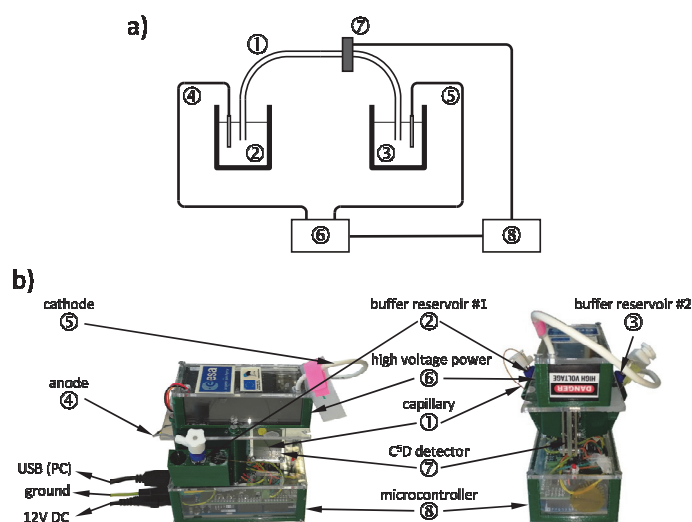


Figure 1. (a) Schematic of capillary electrophoresis instrument and (b) hardware configuration of the prototype used for measurement of the rain water chemical composition

Both ends of capillary ① are immersed during measurement in background electrolyte (BGE) reservoirs ② and ③ connected with electrodes to the high voltage power supply with negative polarity ⑥ (cathode) and ground ④ (anode). Separation by CE is based on two electro-kinetic phenomena: electrophoresis and electroosmosis. The electrically charged ions of the rain water migrate in the BGE under the presence of an electric field. Under steady-state conditions, the two opposite forces (electrical force and frictional force on a spherical ion) balance each other and a final electrophoretic velocity (v_{ep}) is reached, which remains constant at a constant electric field strength (**Equation 1**). As the velocity of different ions vary in various buffers and in function of the electrical current, it is possible to separate these ions from complex mixtures (Landers 2007).

$$v_{ep} = \frac{q * E}{6\pi r * \eta} = \mu_{ep} * E \quad (1)$$

where q is the charge, E is electric field strength, calculated as the voltage applied divided by the total length of the capillary, $6\pi r$ corresponds to the Stokes' radius of the particle, η is the viscosity of the surrounding medium. By simplifying the equation, μ_{ep} becomes the electrophoretic mobility of the charged solute in the separation medium considered.

The presence of separated ions can be detected with a Capacitively Coupled Contactless Conductivity Compensated Detector (C^5D) that measures capacitance changes of the liquid passing through the capillary. It is an advanced variant of C^4D detector invented by Da Silva & Do Lago (1998) and Zeeman et al. (1998). The whole system can be controlled with a real-time low-cost Arduino microcontroller ⑧, equipped with a custom electronic board interfacing all system components (Jaanus et al. 2016). The operation software controls the CE electrical voltage and C^5D frequency (300 kHz – 2 MHz), acquires signals from the detector and detects current within the capillary. The result of measurement is an electropherogram corresponding to the time-resolved capacitance changes correlating to the quantity of ions separated from the mixture and passing by the detector.

2.2. System adjustment

Analyte standard solutions have been prepared from the previously dried (6 hours at 100°C) salts of NaCl (Honeywell), $MgSO_4$ (Fisher Chemical), $Ba(OH)_2 \cdot 8H_2O$ (LachNer), $HCO_2Li \cdot H_2O$, $CuCl_2 \cdot H_2O$, $C_2H_8N_2O_4 \cdot H_2O$, $CaCl_2$ (Sigma-Aldrich). All solutions were prepared with ultra-pure water for ion chromatography (Sigma-Aldrich). Background electrolytes were prepared from the following chemicals: 2-(N-Morpholino)ethanesulfonic acid hydrate (MES), L-Histidine (His) (Fisher Chemical) and acetic acid (AA) (Honeywell). 1M NaOH solution and acetonitrile (ACN) (Sigma-Aldrich) were used for the capillary conditioning.

Polymicro (Optronics) fused silica capillary with 50 μm inner and 350 μm outer diameter, and total 40cm and effective 20 cm lengths, was prepared for the CE analysis. The capillary was cleaned before the first run following the procedure as described in the following chapter. Capillary conditioning was also performed between runs by flushing capillary with BGE.

The reference 100 μM salt mixture was used for optimization of the BGE choice. In this case, a clear separation of all peaks (without overlapping and with baseline resolution) as well as no saturated C^5D detector readings were desired.

As a result of the CE instrument adjustments, the optimal configuration most suitable for assessment of rain water was:

- CE conditions: 8.6 kV, -16.0 μ A
- C⁵D detector: excitation frequency of 2.0 MHz
- Buffer: 6 M acetic acid
- Sample introduction: hydrodynamic, gravimetric, 10cm of diss-level and 15 s

2.3. Sample collection and preparation

Samples of the rain water were collected from the city of Tallinn (Estonia) on February 8, 2019. The fresh snow was gathered from the snowdrift interior (5 cm below the outer surface) and then melted in the laboratory at room conditions (20 °C). Similarly, a dirty sample of the snow deposited along the transportation road was collected as a not-pure water trial. Finally, the tap water was sampled directly from the waterpipe as a drinking water example. All the samples were stored in closed containers as shown in **Figure 2**.



Figure 2. Water samples used for determination of chemical composition

All the water samples were filtered before measurement with a mechanical filter (type SF-NYL25-45) in order to remove any solid-state contaminations and particles. An ordinary syringe-based solution was identified as the most efficient filtering technique, performing well with water samples.

2.4. Measurement protocol

Such pre-processed (filtered) samples were ready for a direct measurement on the CE instrument. The sequence of actions related to the system preparation, together with installation of a brand-new capillary, include:

1. refresh instrument by dusting with ethanol or isopropanol
2. inspect electrodes (electrical connections, tightness, broken wires)
3. clean electrodes with ethanol or isopropanol
4. inspect detector cell (plug, hole for pass of capillary, cleanse)
5. prepare and/or inspect capillary:
 - a. cut to the length or cut-out broken edges
 - b. search for microcracks
 - c. inspect capillary edges
 - d. measure capillary total length
 - e. mark position of the detector (effective length)
6. clean capillary outside surface with ethanol or isopropanol
7. install capillary in the detector cell
8. clean and regenerate inside capillary by forcing flow of following liquids in a specific order and duration:
 - a. 1M sodium hydroxide (NaOH) for 10 minutes
 - b. 0.1M sodium hydroxide (NaOH) for 5 minutes
 - c. distillate water (H₂O) for 2 minutes
 - d. acetonitrile (ACN) for 5 minutes
 - e. distillate water (H₂O) for 2 minutes
 - f. (BGE), same as used in subsequent analysis, for 5 minutes
9. install capillary in the buffer reservoirs #1 and #2 and connect anode and cathode electrodes
10. apply high voltage and check the current (low value indicates improper capillary preparation, air bubble in the capillary, problem with electrical wiring or power supply)

The use of standard plastic syringes is a recommended solution to implement low-cost cleaning of capillary. It allows both application of vacuum or over-pressure on one side of the capillary, while perceiving the microflow of liquid that confirm openness of the tube. The air tightness can be assured by using a laboratory gum gasket and syringe needles as capillary guides when setting up the cleaning procedure.

The proper CE measurement requires a precise injection of the sample to capillary. There are several techniques usable, even if the simplest is a hydrostatic “syphon effect” approach. The sequence of actions to properly implement sample injection is presented in **Figure 3**. The sample injection initiates when a single end of the cleaned capillary filled with the buffer (a) is immersed in the investigated sample solution (b). It is important to assure that the initial level of the sample liquid is below the reference level of both buffer reservoirs. The proper sample injection is occurring when the sample liquid container rises over the reference level at an appropriately selected and consistent (over the series of measurements) height. Both the height and time of lifting affect the quantity if the sample liquid injected. It was found that for the needs of the rain water ionic composition diss-level of 10 cm and lifting time of 15 seconds were optimal conditions. The injection process ends when the sample container with capillary immersed is moved back below the reference level (d) and the capillary containing trace sample liquid is finally re-installed in the buffer reservoir (e) lifted to the reference level.

It has to be mentioned that no electrical field should be applied to the system when implementing hydrostatic samples injection in contrast to the electro-kinetic approach. In the second case, the sample liquid solution is mixed with the buffer in a separate container (f). The capillary is gently moved to that container, together with the electrode, assuring minimal diss-levelling and avoiding air bubble creation (g). The electrical current is applied between temporary and #2 reservoirs, promoting the certain quantity of the tested liquid injection (h). The procedure is finalized when capillary with the sample injected is re-installed in the buffer reservoir #1 (i) lifted to the reference level. The proper measurement and liquid separation starts afterward, and the measurement procedure is identical for both sample injection methodologies.

The quantity of the sample injected with the electro-kinetic solution depends primarily on analytes charge and electroosmotic flow, secondly on the sample concentration, capillary diameter, electrical voltage applied and time of injection. Those conditions illustrate the biggest limitation of the electro-kinetic injection resulting in “electrokinetic bias”. It can be used effectively and reliably with diverse background electrolytes that produce electroosmotic flow (EOF) with the same or higher than analytes velocity. Electrokinetic injection cannot be used reliably in the case of 6 M acetic acid, as the EOF velocity is lower than of the analyte. Although, electro-kinetic injection of rain water can be implemented with His and MES background electrolytes, where pH of the separation liquid varied from 4 to 8.

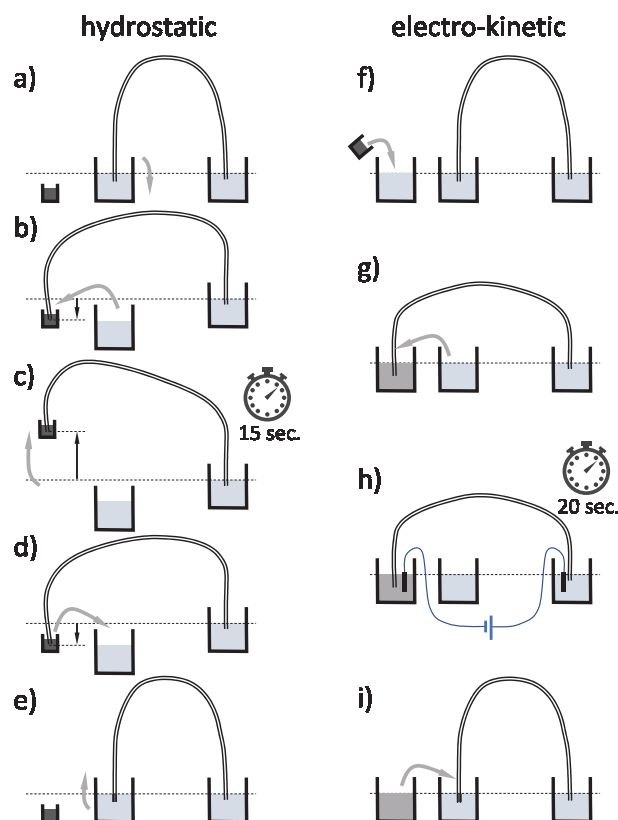


Figure 3. Procedure of the tested liquid injection to the capillary by means of hydrostatic “syphon effect” (left) and electro-kinetic (right) methods.

As for more, this first approach study shows good potential for the implementation of hydrostatic injection in the automatic system by changing from siphoning effect to pressurised or vacuum injection modes (Pham et al. 2014; Xu et al. 2009).

The liquid separation and acquisition of electropherograms starts immediately after sample injection, when the high voltage electricity passes from anode, through capillary, to cathode. At the same time, conductivity changes as measured by a C^5D sensor are recorded and stored for further post-processing. The duration of experiment depends on many factors, such as BGE type, electrical voltage, ions mobility, temperature, capillary length and diameter, among others.

2.5. Analysis of electropherograms

The electropherogram contains time resolved changes of the conductivity as recorded by C^5D when the electrical current flows from BGE reservoir #1 to #2. The deviation of conductivity values from the baseline is correlated with the kind and amount of separated ions. The simple approach for analysis of electropherograms is visual identification of peaks and manual marking of the baseline. The area and shape of the peak, along with the identified retention time, are then used for qualitative and quantitative analysis. Even if manual evaluation of experimental results is acceptable as a laboratory routine, it is not a suitable approach for automatic analysis of data. For that reason, an original software was developed in LabView 2018 (National Instruments Corp., USA) and implemented for detection and quantification of peaks. Wavelets were used here as an algorithm for the baseline detection, subtracted from the original electropherogram (Perrin et al. 2001). Peak detection was implemented as a derivative computation, and area under the peak was an integration of C^5D signal.

3. RESULTS

Typical electropherograms of the rain water acquired during the mission are presented in **Figure 4**. The reference liquid data are also provided to simplify data interpretation. A great advantage of using the reference solution is a possibility to introduce chemical markers, simplifying the peak identification and, especially, determination of the time when first separated ions reach the detector location. It is noticeable that a low-cost, open-hardware CE instrument was capable to accurately detect several ions, both in the reference liquid as well as in the characterized water samples.

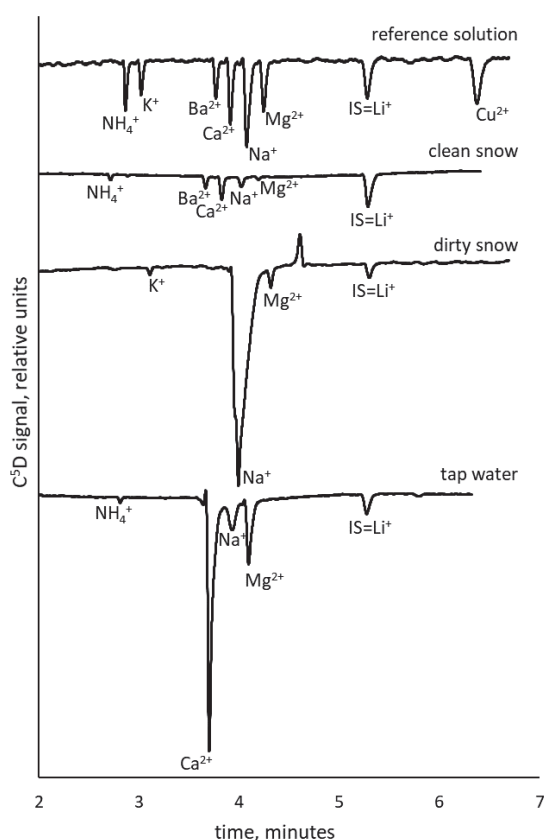


Figure 4. Capillary electropherograms of the investigated water samples: reference solution of model ions $100 \mu\text{M}$ (a), clean snow (b), dirty snow (c) and tap water (d). CE conditions: 6 M acetic acid, 2 MHz , 8.5 V . Capillary: $50/350 \mu\text{m}$, $20/40 \text{ cm}$, BGE changed after each experiment. Injection is hydrodynamic: lifting above diss-level 10 cm and 15 seconds .

Table 1. Inorganic cations composition detected in the water samples.

ANALYTE	PURE SNOW (μM)	DIRTY SNOW (μM)	TAP WATER (μM)
NH_4^+	12	<i>n.d.</i>	13
K^+	<i>n.d.</i>	49	<i>n.d.</i>
Ba^{2+}	29	<i>n.d.</i>	<i>n.d.</i>
Ca^{2+}	36	<i>n.d.</i>	1031
Na^+	13	2546	128
Mg^{2+}	2	708	357
Cu^{2+}	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>

Relative standard deviation of the presented results did not exceed 20% of the mean value, *n.d.* – not detected

Results of quantitative analysis of electropherograms are summarized in **Table 1**, separately for clear, dirty snow and tap waters. Ammonium, sodium and magnesium cations were quantified in all water samples. On the contrary, copper was found only in the reference solution (**Figure 4**). The presence of barium ions in the clean samples and potassium in the dirty snow samples can be explained by residual contamination remaining as an effect of the student's fireworks festival taking place around the sample collection place (Steinhauser et al. 2008). The relatively high amount of sodium identified in the dirty snow was interpreted as an effect of street maintenance with sea salt used in winter to dissolve the snow from the roads of Tallinn. Similarly, a fairly high concentration of magnesium present in the dirty snow water sample was explained by seasonal road care activities.

It was found that the tap water composition differs from that declared by the Tallinna vesi - Tallinn's tap water supplier (Veepuhastusjaam 2017). Mean difference was noticed in ammonium ion, exceeding 20 times nominal concentration. The presence of a high amount of calcium was also observed. No potassium, barium or copper ion traces were detected. Sodium content correlated well with the data provided by Tallinna vesi. Even so, the sodium ion concentration in Tallinn's tap water was 67 times lower than the level recommended by the World Health Organization. It has to be stated however, that discrepancy between Tallinna Vesi and results obtained in this pilot test might be due to some unidentified problems in instrument calibration, as related to a very short time of the STSM visit.

4. CONCLUSION

Capillary electrophoresis proofed its highest suitability for detection and quantification of ions in different samples of water including that collected as snow. The fully functional, open-hardware, low-cost CE instrument was assembled and tested. An original protocol for determination of the chemical content of rain water was established and validated in the pilot test. Hydrostatic sample injection method has been found very efficient and straightforward in manual and laboratory routines.

The pilot research results indicated substantial differences between clean and dirty snow water. While detected ions concentration in the clean snow water varied from 2 μM (Mg^{2+}) to 36 μM (Ca^{2+}), the dirty snow contained higher ions concentration ranging from 49 μM (K^+) to 2.54 mM (Na^+). Tallinna tap water sodium ions concentration was 67 times lower than WHO limitations, with no barium nor copper ions identified.

An important deliverable of the Short-Term Scientific Mission was a set of multimedia materials for promotion of CE technique and, especially, low-cost CE instrumentation. It includes series of movies presenting:

- procedure of water samples collecting
- presentation of capillaries and preparation (inspection, cutting, measurement)
- cleaning of capillaries sequence, including handling and adopting of standard syringes for optimal and minimal cost operation
- installation of the capillary in the instrument and filling with the background electrolytes
- presentation of the CE instrument, its components and functionalities
- common trouble corrections (hints and tips)
- routine operation (measuring water samples) with CE
- sample injection procedure
- basic operation of the software

All movies are available on the COST Action CA16215 website (<http://portasap.eu/>).

5. ACKNOWLEDGMENTS

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