

Flow analysis by Capillary Electrophoresis

Subjects: [Chemistry](#), [Analytical](#)

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Flow analysis is the science of performing quantitative analytical chemistry in flowing streams. Capillary electrophoresis (CE) is an analytical method that separates ions in a narrow channel. Separation is based on ions electrophoretic mobility with the use of an applied voltage. Because of its efficiency and speed of analysis, capillary electrophoresis (CE) is a prospective method for the monitoring of a flow composition withdrawn from various processes (e.g., occurring in bioreactors, fermentation, enzymatic assays, and microdialysis samples).

flow analysis monitoring capillary electrophoresis open-source hardware

1. Introduction

Flow analysis is the science of performing quantitative analytical chemistry in flowing streams ^[1]. It can be any liquid material stream withdrawn from a chemical or bioreactor, from a chemical process, or an environmental source (such as a river or similar source). Although information about the composition of a particular flow can be obtained by using a set of chemical sensors, those sensors' lack of the required selectivity and sample processing and separation is an essential part of the flow analysis.

Capillary electrophoresis (CE) is an analytical method that separates ions in a narrow channel. Separation based on ions electrophoretic mobility with the use of an applied voltage. The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius. The migration speed is directly proportional to the applied voltage - the greater the voltage, the faster the mobility. Neutral species are not affected, only ions move with the electric field. CE is used most predominately because it gives faster results (compared to other separation methods such as gas chromatography (GC) or liquid chromatography (LC)) and provides high-resolution separation (compared to other separation methods). It is a useful technique because there is a large range of different CE modes available such as capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MEKC), capillary electrochromatography (CEC), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP) ^[2].

This entry describes the possibilities of the capillary electrophoresis for flow analysis (known also as flow injection analysis (FIA)) and demonstrates that CE has advantages over other, more popular separation methods such as LC–mass spectrometry and that the online interfacing of CE is simple in principle but involves complications due to the need for supporting instrumentation. Analysis of some possible examples of published FIA–CE applications demonstrates that the need for supporting instrumentation results in FIA–CE interfacing, which is clumsy and still unsuitable for miniaturization. This explains why FIA–CE applications are not widespread yet and many attractive applications are overlooked.

2. Coupling FIA to CE Online

Separation methods involve the necessary time for separating the sample into components, and flow analysis must consider this time. If the flow composition varies with time (and this variation is usually of interest), then there is a certain time interval during which the analysis must be completed to obtain a realistic estimate of the variation course occurring in the flow. We call this interval the time (temporal) resolution of the flow analysis.

When the changes in the flow composition are slow compared to the time needed for sample collection, processing, and analysis, the monitoring can be performed offline with any available commercial or homemade instrument based either on the LC or CE method. Online monitoring is needed when the process is analyzed with a short temporal interval, and this requires rapid CE.

FIA–CE coupling was first demonstrated by Kaljurand et al. ^[3] and shortly afterward by Kuban et al. ^[4]. Horstkotte and Cerdà reviewed operational principles of coupling of FIA with CE ^[5]. The interface is a quite simple piece of inert plastic (material depends on the chemical composition of the flow) that contains drilled channels for the flow, capillary, and electrode. The interface is usually kept at ground potential to avoid the difficulties of high voltage isolation. An interface schematic is represented in **Figure 1**.

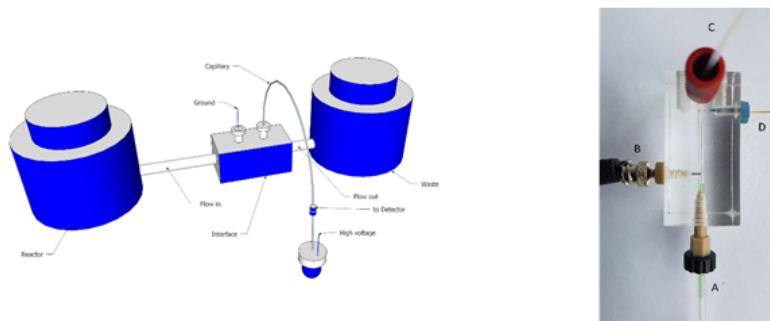


Figure 1. FIA-CE interfacing by direct insertion of a capillary into the flow channel. Left: experimental setup, right: photo of an interface. A - flow in, B - electrode, C - flow out, D - capillary.

While the interface itself is simple, the CE analysis process requires supporting equipment for interchanging the sample and the background electrolyte (BGE), for providing liquid flows for rinsing capillaries (such as NaOH or H₂O), as well as equipment for sample cleaning and processing, since the direct sampling of flow is rarely possible. An example of a set-up that consists of all listed supporting equipment is the publication by Kuban and Karlberg [6]. The equipment was applied to the analysis of real samples such as soft drinks, vinegar, and wine. Advantages of FIA-CE coupling for monitoring of various bioprocesses were demonstrated by Breadmore's group [7][8][9] by Turkia et al [10][11][12][13] by Hausers group [14] and by Kaljurand et al [15][16][17][18][19].

3. Open Access CE Instrumentation for Monitoring

The use of CE for the online analysis of flows requires usually specific instrumentation, and to complete this in a laboratory is frequently beyond the capabilities of a typical analytical chemist. Here, a helpful new trend in various fields in measurement science (including analytical chemistry) is becoming steadily popular. This trend, known as "open-source hardware", could be of great help to those individuals who are willing to prepare their own FIA-CE instrumentation. The Open Source Hardware Association defines it as such: "Open source hardware is hardware whose design is made publicly available so that anyone can study, modify, distribute, make, and sell the design or hardware based on that design." Ideally, open-source hardware uses readily available components and materials, standard processes, open infrastructure, unrestricted content, and open-source design tools to maximize the ability of individuals to make and use hardware [20]. This new paradigm is becoming widely accepted in scientific communities, and open-source hardware is finding its steady place in chemistry research. There are several publications of general interest, which describe how to implement open-source hardware in chemical research [21][22]. For CE, particularly, Kuban et al. provided the most up-to-date information on open-source hardware and software resources, enabling the construction and utilization of an open-source CE instrument. They demonstrated that the unique flexibility, low cost, and high efficiency of CE makes it particularly suitable for open source instrumental development [23]. They provided an overview of hardware and software sources, with emphasis on the availability of open-source information on the web and in the scientific literature. Hauser's group reported several successful open source set-ups composed of commercially available parts without the requirement of mechanical and electronic workshop facilities [24][25]. To make the fabrication of CE instruments especially easy for interested persons, the latter publication even includes a "shopping list" of the needed parts, together with vendors.

As an example of an open-source application, the simplest possible FIA-CE instrumentation is shown in **Figure 2** [26]. It consists of two micro-peristaltic pumps, which provide a sample and BGE solutions to the flow gating cross [27] (can be an Upchurch PEEK cross). A high-voltage power supply provides the needed separation voltage. A contactless conductivity detector can be built in house by the open-source description provided by do Lago [28]. The set-up was used for successful monitoring of the contents of the cations in tap water using acetic acid BGE [26]. However, the flow gating sampler has a disadvantage: it is not robust enough. Its optimal performance depends critically on the width of the gap between the sample and separation capillaries. In addition, the need for the continuous supply of the BGE is in contrast with one of the main advantages of CE: low consumption of chemicals. For example, Takasago miniature peristaltic pump discharge rate is 0.2 mL/min [29]. This means that during a one-hour run the sampler consumes about 12 mL BGE.

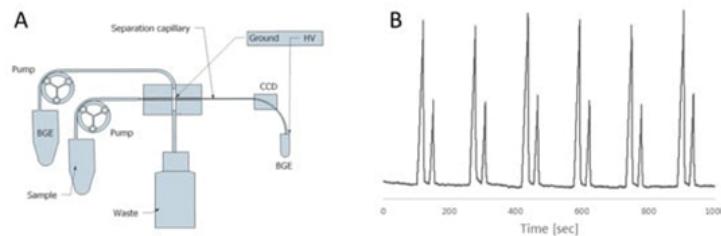


Figure 2. An example of a simple FIA-CE interface. (A)—experimental set-up. (B)—set of electropherograms of anions of Tallinn (Estonia) tap water (recorded by switching of the peristaltic pumps). Peaks: first, Cl^- and second, SO_4^{2-} . Electrophoretic conditions: BGE- 1M acetic acid, $\text{HV} = 10$ kV, capillary: 20 cm length and 75 μm internal diameter.

It is obvious that some experience in CE technology will help to complete even such a trivial setup. The cost of the parts is low, except for the contactless conductivity detector. The detector seems to be the main obstacle in achieving the goals of building open source FIA-CE instrumentation. Although one has a very detailed description and instructions on how to build an open-source contactless conductivity detector, it is still difficult to imagine that a person not experienced in electronics could complete this task. For using optical detectors, we have a similar problem: Detailed open-source descriptions are available ((see, e.g. [30]) but here, competence in optics would be useful. Therefore, the development of a cheap and simple detector for CE is urgently required for achieving the goals of open source hardware. For example, a similar problem seems to have found a solution in gas chromatography. Metal oxide semiconductor (MOS) gas sensor detectors appear to be cheap detectors for gas analysis. The appearance of gas sensors has made possible the building of an extremely simple and robust yet still powerful gas chromatograph, which can be built by a layman and which would be useful for citizen science [31].

4. Conclusions

The FIA-CE combination for the monitoring of flow has some advantages, which justifies further research to overcome the present obstacles. CE is the only analytical separation method that is amenable for miniaturization. If the supporting equipment can be reduced correspondingly, the appearance of separation-based sensors can be expected. These sensors could then be used online for many attractive applications, with bioreactor monitoring *in situ* being one of the obvious applications. This includes designing miniaturized bioreactors and cell culture systems *in vitro* and monitoring nutrients and metabolic products. Moreover, neurochemistry or drug metabolism and behaviors in awake, freely roaming animals could be monitored. Even direct telemetric control of such animals could be considered: observing the natural behavior of an animal and correlating it with biochemical events in the brain.

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