FIA Coupled to Capillary Electrophoresis – A Tool for Process Monitoring?

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Abstract

The potential of the hyphenated technique flow injection-capillary electrophoresis (FIA-CE) for process monitoring is reviewed. The combination of these techniques is advantageous because FIA enables automated and rapid sample pre-treatment, while CE allows multianalyte determinations. It is also easily adapted to the on-line arrangements preferred for process monitoring. Samples can be introduced into FIA systems by either classical valve injection or hydrodynamic, valveless injection techniques. Similarly, samples can be introduced into the CE system by electroosmotic flow traction, electrokinetic injection, hydrodynamic injection, or a combination of electrokinetic and hydrodynamic methods. Successful application of the FIA-CE approach has been described for drug analysis (dissolution rate analysis), paper and pulp liquors (anions) and food analysis (beverages). Depending on the application, relative standard deviation values in the range of 0.6-6% have been reported.

Keywords On-line process monitoring, flow injection analysis, capillary electrophoresis, FIA-CE

1. Introduction

Process monitoring requires techniques that are selective and sensitive, while providing rapid feedback. Several types of electrode and optical probe can offer a rapid response but are limited by relatively poor selectivity, sensitivity and versatility. For most separation techniques such as gas chromatography (GC), high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) the reverse is true. These techniques are tedious but extremely versatile since multianalyte determinations are possible. Wet chemistry methods, on the other hand, are both selective and sensitive and can yield a relatively rapid response. These methods have been automated for several decades now, using segmented flow analysis (SFA), flow injection (FIA) or sequential injection (SIA), and utilised to monitor a variety of processes [1-4]. However, most SFA, FIA or SIA techniques are capable of determining only one analyte at a time. Nevertheless, in spite of this shortcoming, there are obvious advantages of performing wet chemistry in a flow system, especially the possibility of automating sample pre-treatment procedures such as dialysis, filtration, gas diffusion, analyte enrichment and dilution. Consequently, the ideal match would be to combine a rapid separation technique with FIA. This paper reviews some of the reasons for the interest in hyphenating CE and FIA techniques to develop a new tool for process monitoring.

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2. FIA-CE interfacing

Several technical solutions for interfacing FIA and CE have been reported, both for on-line [5,6] and off-line [7] operations. Conceptually, on-line interfacing is to be preferred for process monitoring, mainly for the greater rapidity it allows. The interface should have a low dead volume to minimise sample dispersion. Fang and co-workers [6] have designed an interface that connects the FIA and CE parts of the system via a funnel-shaped reservoir into which the high voltage electrode is immersed, see Fig. 1 (A). The interface described in our original report on FIA-CE [5] was housed in a piece of plexiglas, also depicted in Fig. 1 (B). Rimmer and Dorsey have constructed an interface comprising a commercial cross-fitting of the type commonly used in FIA and chromatography for merging streams [8]. The inlets designed for adding reagents were used to house the capillary and the platinum electrode providing the high voltage.

3. FIA injection techniques

Automated and reliable injections in FIA could be provided by either a programmable rotary valve or a hydrodynamic system [2-4]. The latter requires fully programmable pumps and its applicability for

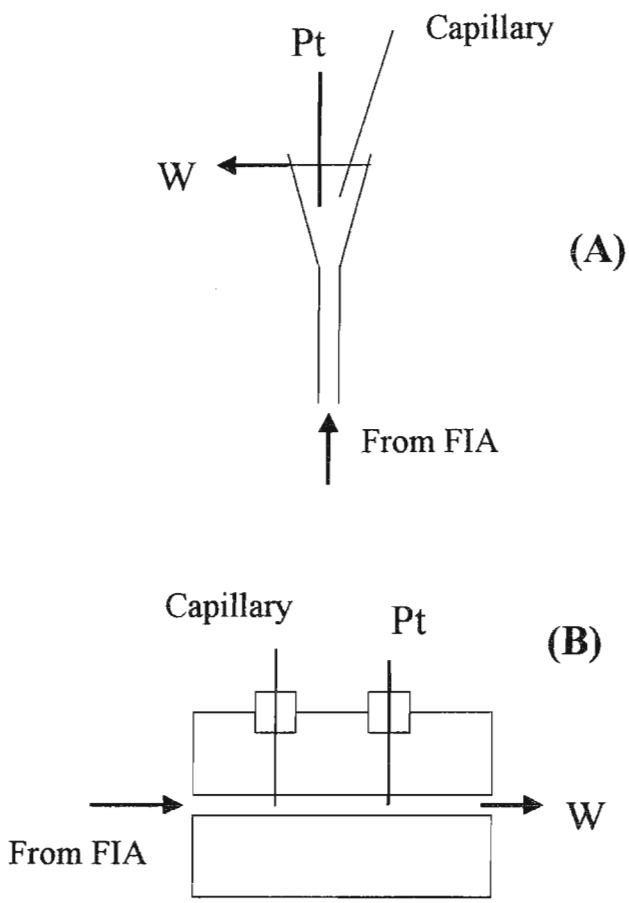

Fig. 1 FIA-CE interfaces designed by (A) Fang et al. [6] and (B) Kuban et al. [5]. Pt = platinum electrode; W = waste.

process monitoring has already been demonstrated [9]. When hydrodynamic injection is performed in an FIA system the two pumps operate intermittently. Essentially, the manifold configuration comprises two flow systems connected via a common duct. One pump is devoted to sample acquisition, usually in a closed loop configuration, loading the sample into the duct while the second pump is idle. When the duct has been filled with sample the first pump is stopped and the second is started, taking the contents of the duct to the detector, via reagent merging points and reaction coil(s) as required.

4. CE injection techniques

The two most common techniques for introducing samples in capillary electrophoresis (CE) are hydrodynamic and electrokinetic injection. Both techniques are relatively simple to perform, and they permit automation - provided that the injected samples are free from particles and colloids. Thus, the word "hydrodynamic" has two meanings depending on whether it is used in FIA or CE. This can be confusing, particularly when FIA and CE are combined. Hydrodynamic injection in CE is accomplished through forcing the sample into the capillary by pressure (or vacuum), or by siphoning. The high voltage is switched off during this process. Usually, an internal standard is added to the sample to compensate for the irreproducibility of the injected sample portions.

In contrast, when electrokinetic injection is performed in CE the high voltage is on during the whole process. Internal standards are also added to the samples in electrokinetic injection systems, to compensate for bias caused by conductivity variations from sample to sample.

Electrokinetic injection offers various attractive features including instrumental simplicity, pre-concentration potential through sample stacking, and injection selectivity. Hydrodynamic injection allows repeated injections of constant amounts of analyte, regardless of variations in conductivity, while still maintaining the option of automating the sample pre-treatment procedures. It is even possible to introduce samples through techniques involving a combination of these two injection principles.

5. CE detection techniques

UV-visible detectors are commonly utilised in CE [CE monographs]. However, such detectors are restricted by the short light path length across the capillary, which reduces their sensitivity. The indirect mode is often used for detecting inorganic ions or cations, since most of these ions do not absorb in the UV-visible range. Other methods of detecting analytes in CE include conductometric, fluorimetric, amperometric and mass spectrometric techniques.

6. Operational principles of FIA-CE systems

FIA-CE systems can be designed for both electrokinetic and hydrodynamic injection modes. Fig. 2 shows a typical FIA-CE system based on valve injection (FIA) and electrokinetic injection (CE). The sample (S) can be pumped continuously from a process stream and passed through the FIA valve furnished with by-pass coils for both the sample and the electrolyte (E) streams. When the FIA valve is activated the electrolyte stream carries the sample plug into the FIA-CE interface. Preferably, this FIA system should have a dispersion coefficient of unity, meaning that the edges of the

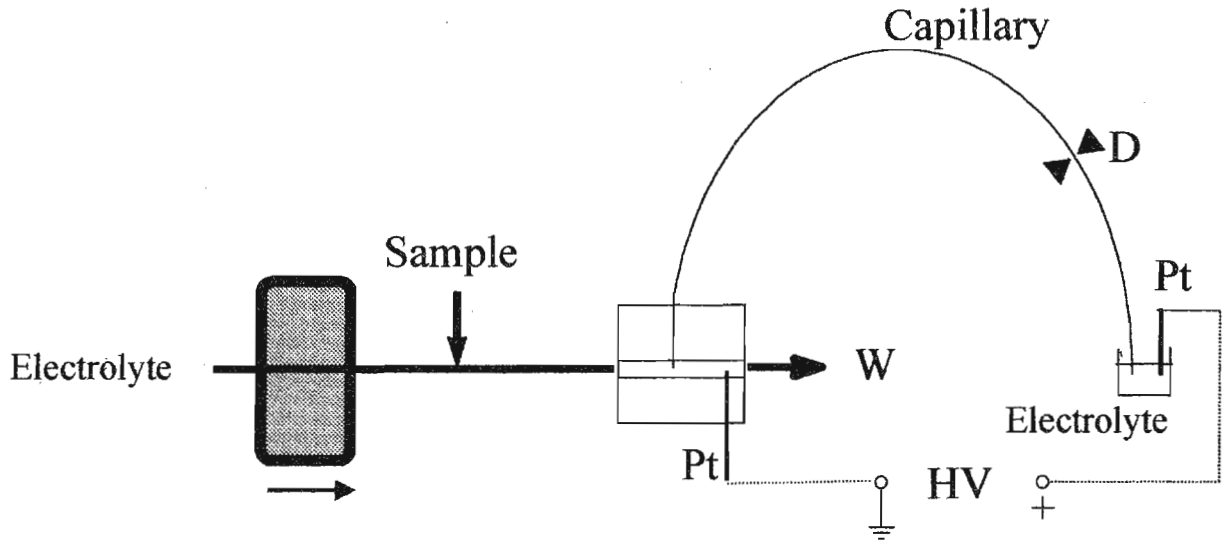

Fig. 2 An FIA-CE system based on electrokinetic injection. Pt = platinum electrodes; HV = high voltage; D = detector; W = waste.

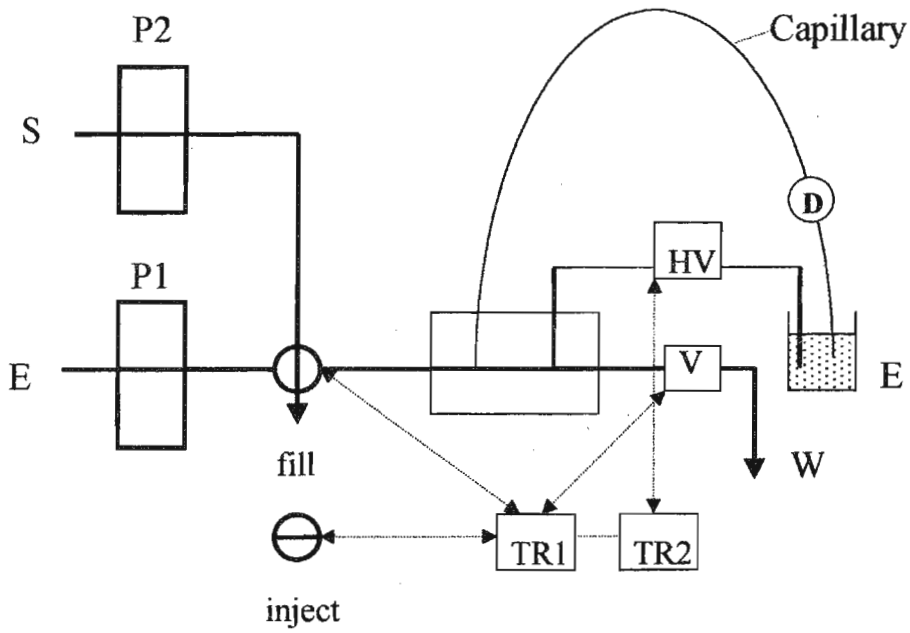

Fig. 3 An FIA-CE system based on hydrodynamic injection (in the CE part). S = sample; E = electrolyte solution; HV = high voltage; TR1, TR2 = time relays; V = pinch valve; W = waste; D = detector; P1, P2 = pumps.

sample plug are slightly mixed with electrolyte leaving the centre portion intact. When the sample passes the aperture of the capillary (which is mounted in the interface), a small portion migrates into it, driven by the applied electrical field since the high voltage is switched on during the entire analytical cycle.

FIA-CE systems can be used to determine a wide range of analytes, ranging from small, inorganic anions and cations to large organic, charged species in aqueous samples, food and process liquids with complex matrices.

Fig. 3 shows apparatus for FIA-CE based on hydrodynamic injection [17]. The two peristaltic pumps, P1 and P2, continuously deliver electrolyte (E) and sample (S) solutions, respectively. On changing the FIA-valve from the "fill" to the "inject" position, a certain amount of sample, typically 200 µl, is injected into the electrolyte stream and carried towards the capillary positioned

in the FIA-CE interface. Synchronously with the injection, the high voltage (HV) is interrupted and a counting sequence for TR1 is initiated. The exact duration of the HV interruption is set by TR2. When the sample plug reaches the capillary opening in the flow channel of the FIA-CE interface, the electromagnetic valve (V) closes the outflow channel for a precisely defined period of time, usually 0.1-1 s. Thus, a small sample fraction is hydrodynamically injected into the capillary, since closing the outflow of the interface channel causes a temporary overpressure. When the injection step has been completed, HV is applied across the two platinum electrodes, and separation of the ionic constituents present in the sample is initiated.

Pu and Fang have designed a "bias-free" sample introduction system for FIA-CE [18], based on sample traction through an induced electroosmotic flow. In this system, a Nafion joint is applied to a fracture in the capillary, close to the capillary inlet. The end of the capillary is inserted through a liquid reservoir, which is clamped around the fractured region of the capillary. One platinum electrode is positioned in this reservoir and the other in the funnel-shaped reservoir where the end of the capillary is immersed. The two platinum electrodes are intermittently connected to the high voltage supply. When the sample is injected into the FIA system and subsequently provided to the funnel reservoir, the platinum electrode in the crack reservoir is connected, thereby creating an electroosmotic flow at the capillary outlet so that sample can be aspirated into its aperture in a bias-free manner. Once aspirated, the platinum electrode in the funnel reservoir is connected so that separation of the analytes in the inserted sample can commence.

7. Approaches for process monitoring using FIA-CE

The FIA-CE technique could be adapted for process monitoring in several different ways. Off-line arrangements can always be used, but such operations are time-consuming and labour intensive, so they will not be discussed further here. On-line configurations are definitely preferable. The versatile FIA system can then be used for sample clean-up. An interesting concept for non-invasive systems that can be used to follow batch processes has been proposed by Kuldvee and Kaljurand [19], in which the container housing the process stream to be monitored is provided with a dialysis membrane, and an external acceptor stream collects the analyte ions. The acceptor stream and an electrolyte stream are then pumped intermittently to a common tube into which the capillary is immersed. The injection principle is a combination of hydrodynamic and electrokinetic mechanisms.

Another FIA-CE arrangement has been successfully used by Fang's group for drug resolution analysis (DRA) [20]. In their apparatus, the sample was aspirated from the dissolution vessel, filtered and injected into the flow injection system (25µl). The obvious advantage of this arrangement is, of course, its multicomponent monitoring ability. Sample throughputs as high as 60 h^{-1} were achieved, with relative standard deviations in the range 0.8-1.6%. Thus, FIA-CE seems to be a godsend for future DRA applications.

Finally, Kuban and Karlberg have described a valveless FIA-CE system suitable for process monitoring [21]. Its analytical capability was demonstrated for on-line monitoring of the major anions in kraft pulping liquors. This system allowed the oxidation occurring in a black liquor sample to be monitored in terms of changes in the concentrations of sulphite, sulphide and thiosulphate ions. Typical standard deviation values in the calibration tests were in the range 2-6% for the studied anions.

8. Conclusions

The FIA-CE technique opens new possibilities for process monitoring. It is a simple concept that combines all the advantages of two mature techniques: the automated, versatile sample pre-treatment offered by FIA, together with the high resolution, multicomponent options provided by CE. Sample pre-treatment techniques such as dialysis, filtration, gas diffusion, derivatisation and ion exchange have already been applied successfully. It is assumed that this list will be rapidly expanded in years to come, and that FIA-CE methods for monitoring many more processes will be developed.

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(Received May 19, 2000)

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