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**Interfacing commercially available capillary electrophoresis to sample preparation and/or detection systems to solve analytical problems**

**Abstract:** This review presents the state-of-the-art of interfacing sample preparation and detector systems with commercially available capillary electrophoresis (CE) equipment. The analytical potential of combined sample preparation units into commercially available CE equipment under integrated or coupling approaches, allowing at-line, on-line, and in-line arrangements to automate sample preparation is discussed. Some examples of the analytical applications are reported when using different sample treatment procedures, such as cleanup and preconcentration, extraction/filtering, gas extraction/diffusion, extraction with membrane and hollow fiber, and dialysis and extraction with supported liquid membrane. CE interfaced detectors are also discussed by distinguishing between commercially available CE equipment and those not commercially available and focusing on the latter. Interfaced CE approaches clearly expand the field of application of the CE and connect to the real world in routine analytical laboratories, avoiding the drawbacks and limitations of electrophoretic separations.

**Keywords:** commercially available capillary electrophoresis equipment; coupling; interfaced devices; interfaced sample preparation; non-commercially interfaced detection modes.

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**Introduction**

Since the first publications on capillary electrophoresis (CE) in 1979 (Mikkers et al. 1979) and 1981 (Jorgenson and Lukacs 1981), this technique has received considerable attention in analytical chemistry. The popularity and acceptance of CE can be explained by its capacity to rapidly determine many analytes at high resolution with low sample and electrolyte consumption. In 1989, Beckman Instruments introduced the first fully automated CE instrument with ultraviolet-visible (UV-Vis) detector to the scientific community. This instrument constitutes a powerful alternative to the more established liquid chromatography (LC) techniques. As it will be discussed, CE presents interesting characteristics as an instrumental separation technique but also has important limitations.

The possibility of combining CE with other equipment and analytical techniques clearly expands the viability and applicability of the electrophoretic separations. In a broad sense, both arrangements dealing with sample preparation and additional detectors to the one conventionally included in commercially available CE equipment (UV-Vis) can be considered as hyphenated approaches. To achieve practical applications, sample preparation must be systematically considered in CE works. Batch procedures are commonly used, involving a human interface to perform the different steps before to the electrophoretic separation. The possibility of automating and simplifying the human participation in sample preparation is an important issue (Santos et al. 2006a,b). In this way, two additional approaches can be followed to achieve this goal. Thus, some devices integrated into commercially available CE equipment can perform sample preparation in a direct way without human manipulation or any additional interface (hardware). The integrated sample treatment device can be located in the CE capillary, in the sample vial, or in an auxiliary part of the equipment, such as the replenishment system. Another alternative, of high interest as an interfaced CE system, is the use of coupled devices that imply minimal human participation but require additional hardware and software. Coupled methodologies, therefore, involving dedicated interfaces can be arranged at-line, in-line, or on-line. In-line coupled methodologies are compatible with on-capillary integrated approaches.

Detection is the other key way to meet analytical requirements in CE analyses. It is a reality that, in many
cases, an appropriate sample preparation (cleanup and preconcentration processes mainly) allows use of the UV-Vis detector conventionally included in commercial CE for applied methods. In many other cases this possibility is not enough to meet the analytical requirements. In these cases, commercially interfaced detectors can be used. The common choice is laser-induced fluorescence (LIF) and mass spectrometry (MS) detectors. The use of fluorescence detection causes an increase in sensitivity with respect to UV-Vis absorption. LIF detection is one of the most sensitive detection modes in CE. Using this detection system with some different applications has been reported. The disadvantage of this detector is the cost and the limited use of a particular laser for exciting the analytes at a specific wavelength. These are the reasons why other non-commercialized alternatives have been developed for performing fluorimetric detection in CE. Mass spectrometry is one of the most attractive and powerful detection techniques in CE due to its sensitivity, universality, and the structural information it provides. Its main drawback is the high cost of mass spectrometers along with Interferences. CE-MS requires the direct coupling of the ionization method to liquid-phase separation techniques to allow MS detection. During the past few years, improvements have been made in interface reliability and reproducibility. Thus, electrospray ionization (ESI) is the most used approach to developed routine analytical methods based on a CE-ESI-MS combination. However, the adoption of this interfaced CE technique has been relatively slow due to the various challenges in achieving stability, reproducibility, and sensitivity over long-term operation.

Within the non-commercially interfaced detector, chemiluminescence is an effective detection system for CE because of its high sensitivity and selectivity. This detection system uses a simple, inexpensive optical unit that requires no light source – which avoids stray light and source instability problems – and provides low background noise and excellent sensitivity, comparable to that of LIF. One of the interface designs most commonly used in CE with chemiluminescence detection consists of an electrophoretic capillary, a reagent capillary and a reaction capillary held in place by a tee connector (Dadoo et al. 1992). Electrochemical detectors have become increasingly important in CE in recent years. In contrast to optical detection where the response of the detector is dependent on path length, cell volumes can be made very small with no decrease in sensitivity. The lack of commercially available instruments reduces the diffusion of this detection technique in CE. In addition, the difficulty in solving the influence of the strong electric field used in electrophoretic separation is another important challenge for a practical application of CE systems with electrochemical detection. Other possibilities for analyte detection in CE are refractive index, radioactivity, novel optical detection techniques based on phosphorescence, and vibrational spectroscopies (infrared and Raman), or nuclear magnetic resonance. Many of these are the consequence of intense research work achieved in the last years, and they are far from immediate use in routine laboratories.

The objective of this review is to give an overview of the instrumentation, performance, and application of some sample preparation and non-commercially interface detection modes to CE equipment. For further information about integration of specified sample treatments and commercially interfaced CE detectors, readers should consult more specialized books and publications (Marina et al. 2005, Santos et al. 2006a,b, Arce et al. 2009, Rios et al. 2009, De Kort et al. 2013).

Intrinsic capabilities and weak points of commercially available CE equipment

CE is a powerful technique for the separation and determination of a wide variety of compounds in many types of samples. This technique is characterized by interesting advantages, such as high efficiency in the separation (resolution); flexibility in the application, as it can combine electrophoretic and chromatographic separation mechanisms; low cost of the analyses, as very low amounts of samples and buffers/solvents are required; low consumption of sample, a few nanoliters makes CE the separation technique of choice in research studies where samples are only available in very limited amounts (e.g., studies of biological tissues and fluids); wide field of application (pharmaceutical, bioanalytical, environmental, food, agricultural, industrial areas); and low environmental effects (“green analytical chemistry”). Conversely, CE presents some important problems for its implementation in analytical routine laboratories (Piñero et al. 2014). Thus, for instance, lack of sensitivity, not very good reproducibility when real samples are analyzed, and finally, the problems produced by the interferences, particularly for complex sample matrices. For common UV-Vis detection included in commercially available CE equipment, the small inner diameter of the CE capillary is an important problem, as it provides a short optical path length, leading to relatively poor concentration sensitivities. This fact results in concentration-based detection limits typically
two orders of magnitude higher than those obtained by UV-Vis detection used in liquid chromatography. Moreover, the selectivity is very low, as many compounds absorb in the low UV region. Bulk absorbance from samples and background electrolytes can produce interference for the analyte detection or it can produce high background signals or unstable baselines. Of course, there are different ways for introducing potential improvements with respect to these drawbacks. Some examples are using an appropriated sample preparation (cleanup) to avoid matrix interferences; incorporating the preconcentration of the analytes by conventional procedures (including the preconcentration in the capillary); or using stacking or sweeping techniques for improving the sensitivity, as well as the use of more sensitive detectors; and, finally, improving reproducibility through assuring better stability of the electrical field and by automating the whole process.

It is interesting to compare CE with other instrumental separation techniques, mainly liquid chromatography (LC). CE presents well recognized advantages. Thus, short periods for separation are usually involved in CE, providing a high throughput in some cases (parallel analysis is also possible). Conversely, miniaturization is very difficult to achieve in liquid chromatography (μHPLC is exclusively associated to the miniaturization of part of the equipment, e.g., diameter of the capillary or injection system), but it is a good trend and recognized alternative for CE separations. In fact, μTAS or “lab-on-a-chip” approaches are commonly based on electrophoretic separations (CE in microchips). The common option to use an electrokinetic injection sample mode is characteristic for CE, which allows the accurate introduction of very small sample sizes (lower than HPLC) without the participation of any mechanic device (injection valves). The reproducibility of this physical phenomenon (not involving any mechanical movement) avoids the need to use an internal standard, as it is required in chromatographic methods. Another interesting difference with LC is the generation, under specific conditions, of an electroosmotic flow (EOF) as a second driving force (in addition to the electrophoretic mobility of the analytes), which originates a liquid flow in the capillary in the presence of an electric field. Therefore, by including a stationary phase in the capillary, an electrochromatographic process (CEC, capillary electrochromatography) can be carried out without the use of a chromatographic pump. The capability of combined electrophoretic and chromatographic processes using commercially available CE equipment is a very remarkable feature that opens separation possibilities.

After the previous discussion, it can be concluded that CE is a very useful alternative for performing analytical separations. Nevertheless, the reality is that CE has not replaced HPLC in routine analytical laboratories. HPLC is well-established and an older technique than CE, and many official and standard analytical methods were developed using HPLC. It is very difficult to replace these routine methods. Conversely, teaching programs rarely included CE or, at least, not at the same level of LC. Hence, the expertise and the training in CE work are difficult to find in graduated students. Additionally, the weak points of C (previously summarized, but also coming from the need of capillary regeneration steps in CE, and the worst reproducibility and the worst intrinsic sensitivity of CE with respect LC), introduce important challenges for many applications. As stated, there are some ways to solve these problems. In this way, the use of CE interfaced arrangements, involving other equipments in some cases, can be a clear alternative that achieves the interesting advantages and intrinsic capabilities CE can offer.

**Interfaced systems for sample preparation**

Sample processing is a continuous labor in routine analytical laboratories. In fact, preparation of complex samples is often labor intensive and time consuming. Therefore, the simplification of such operations is an objective in routine analyses. In this context, an interface for coupling sample treatment devices to CE provides an elegant and effective way for automating sample treatment. The effective on-line coupling sample preparation and CE enables several advantages, such as high-speed analysis due to the simplification of the process, high efficiency, high selectivity when tailored systems are designed, and low cost of operation due to the extremely low solvent consumption. As discussed in previous articles (Santos et al. 2006a,b, Arce et al. 2009), to integrate or to couple sample-treatment devices into commercial CE equipment, it is important to take into account some key instrumental aspects: the way to apply the high voltage in the CE equipment; how the capillary is assembled in the equipment; which vials are used; where the autosampler is located and how it works; and which additional devices are present in the equipment.

Electroosmotic flow is one of the driving forces in CE. This phenomenon arises from the presence of surface charges on capillary walls. The result is a net flow of buffer solution in the direction of the negative electrode. Electroosmotic flow is quite robust and occurs at a rate of 0.5–4 nL/s, depending on the buffer pH. One other important factor is the small inner diameter of the capillary,
which affords the use of very low volumes of samples and reagents, and also effective coupling. The ability to couple sample treatment devices to CE equipment is limited by the following factors, all of which need careful consideration:

i. Compatibility of hydrodynamic flow in the processing device with electroosmotic flow in the capillary.
ii. Compatibility of the high flow rates typically used in processing devices with the low rates of electroosmotic flow in a CE system.
iii. Compatibility of the sample plug coming from the processing device with the small sample volume to be introduced in the CE capillary.
iv. Compatibility of the sample composition with the electrophoretic system.
v. Decoupling of the high voltages and currents applied to the electrophoretic separation system and the flow processing device.

In the literature, three coupling methodologies have been reported:

i. At-line coupling: there is no physical connection between the flow stream coming from the sample-treatment device and the capillary. It usually involves a robotic interface with access to the vials of the autosampler, allowing a fraction of the treated sample to be introduced into the CE vial, so that, finally, this fraction is analyzed in the normal way.

ii. On-line coupling: involves a physical connection and contact between the capillary and the flow stream coming from the sample-treatment device. Such coupling is performed via a transfer line (split-flow interface), and it connects the analytical device with the electrophoretic capillary throughout the analytical process.

iii. In-line coupling: complete integration between sample preparation and CE equipment. Such integration normally is located in the electrophoretic capillary and involves the injection of the interface or unit in the electrophoretic capillary.

In general, coupled systems are the most commonplace. Interested readers can find an interesting review about sample treatment devices used in the combination with commercially available CE equipment by Santos et al. (2006a, b). Table 1 summarizes different sample treatment devices in-line, on-line, and at-line coupled to commercial CE equipment, and it correlates them with the analytes and samples analyzed. Some selected coupled systems and their interfaces are described in the following, as well as their more salient uses.

### At-line coupled systems

At-line coupling of sample treatment device to commercial CE equipment can be performed using a robotic arm interface (Arce et al. 2000) or the modification of the replenishment system of the CE equipment (Simonet et al. 2005). The use of robotic interface requires an electronic interface and software to synchronize the movement of the robotic interface with the movement of autosampler. Figure 1 represents a scheme of the coupling of this robotic interface with CE equipment. The needles of the programmable robotic arm can be positioned in two ways with respect to the autosampler vials. While the sample treatment processing device is working, the needles are down, and the treated sample is prepared and transferred to the CE vial. When sample treatment processing device stops, the needles are lifted, and the CE analysis is started by moving the previously filled sample vial to the position where the capillary end and the electrode are located. The programmable arm can be fitted with one or two needles that can be identical or different in length – the longer one being used to fill the autosampler vials and the shorter one to drain them to maintain a preset liquid level. When a single needle is used, a constant, preset volume is delivered to each vial (e.g., by using air as the carrier). Several applications using this robotic arm interface were published applying different sample treatment devices, such as flow injection/solid phase extraction (FI/SPE), FI/gas diffusion, and SFE (see Table 2).

Methodologies integrating solid phase extraction (SPE) unit in continuous flow system (CFS) coupled to commercial CE using the programmable arm mode were applied to determination of chlorophenols (Mardones et al. 1999), biogenic amines (Arce et al. 1998a,b,c,d), trimethylamines (Listo et al. 2001), inositol phosphates (Simonet et al. 2003) and inorganic ions (Arce et al. 1997). The online cleanup and/or preconcentration of these analytes were accomplished by using a CFS coupled to the CE equipment. For analysis, samples were pumped through the SPE units to retain the analytes. After the samples were loaded, the SPE units were washed to remove impurities and eluted with appropriate solvents. The eluates, containing the analytes, were inserted in a minivials of the CE autosampler by actuating the programmable arm. Another example is the use of this robotic system for direct determination of trimethylamine and related amines in fish samples (Listo et al. 2001). This arrangement allowed the direct introduction and treatment of these solid samples with a high level of automation. The amine compounds were extracted from fish samples in the gas extraction sampling unit integrated in a CFS coupled to CE, and then
Table 1  Sample-treatment devices coupled at-line, on-line and in-line to commercial CE equipment.

<table>
<thead>
<tr>
<th>Coupling methodology</th>
<th>Analytes</th>
<th>Samples</th>
<th>Sample treatment</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>At-line</td>
<td>Inorganic ions</td>
<td>Water</td>
<td>Preconcentration</td>
<td>Arce et al. 1997</td>
</tr>
<tr>
<td></td>
<td>Polyphenols</td>
<td>Green tea</td>
<td>Microwave extraction/filtration/dilution</td>
<td>Arce et al. 1998b</td>
</tr>
<tr>
<td></td>
<td>Anions</td>
<td>Soil</td>
<td>Filtration</td>
<td>Arce et al. 1998a</td>
</tr>
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<td></td>
<td>Resveratrol, polyphenols</td>
<td>Wines</td>
<td>Clean-up</td>
<td>Arce et al. 1998d</td>
</tr>
<tr>
<td></td>
<td>Biogenic amines</td>
<td>Wines</td>
<td>– Clean-up and preconcentration</td>
<td>Arce et al. 1998c</td>
</tr>
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<td></td>
<td>Chlorophenols</td>
<td>Human urine</td>
<td>Clean-up and preconcentration</td>
<td>Santos et al. 2004a</td>
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<tr>
<td></td>
<td>Cresol and chlorophenol</td>
<td>Human urine</td>
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<td></td>
<td>Pesticides</td>
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<td></td>
<td>Trimethylamine and related</td>
<td>Fish</td>
<td>Gas diffusion</td>
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<td></td>
<td>Nonsteroidal antiinflammatory drugs (NSAIDs)</td>
<td>Human urine and serum</td>
<td>Clean-up and preconcentration</td>
<td>Mardones et al. 2001</td>
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<tr>
<td></td>
<td>Phenolics</td>
<td>Citrus</td>
<td>Preconcentration</td>
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<td></td>
<td>Inositol phosphates</td>
<td>Food</td>
<td>Preconcentration</td>
<td>Simonet et al. 2003</td>
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<td></td>
<td>Sulfonamides</td>
<td>Milk</td>
<td>Precipitation/clean-up</td>
<td>Santos et al. 2005</td>
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<td>On-line</td>
<td>Tetracyclines antibiotics</td>
<td>Soil</td>
<td>Fiber/SPME</td>
<td>Santos et al. 2007</td>
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<tr>
<td></td>
<td>Amines</td>
<td>Water</td>
<td>SI/</td>
<td>Santos et al. 2006b</td>
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<td></td>
<td>Riboflavin vitamins</td>
<td>Food</td>
<td>SFE/clean-up</td>
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<td></td>
<td>Peptides, Amphoteric solutes</td>
<td>Protein samples, cytochrome C digest</td>
<td>Preconcentration</td>
<td>Tempels et al. 2007</td>
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<td></td>
<td>Nonsteroidal antiinflammatory drugs</td>
<td>Serum and urines</td>
<td>Dialysis SPE</td>
<td>Veraart et al. 1998</td>
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<tr>
<td></td>
<td>Tricyclic antidepressants</td>
<td>Serum and urines</td>
<td>Dialysis SPE</td>
<td>Veraart et al. 1999</td>
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<td></td>
<td>Fluoroquinolones</td>
<td>Urines</td>
<td>MEPS</td>
<td>Varaart and Brinkman 2001</td>
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<td>In-line</td>
<td>Heterocyclic aromatic amines</td>
<td>Urine</td>
<td>Micro-SPE</td>
<td>Viberg et al. 2004</td>
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<td></td>
<td>Insulin Derivative</td>
<td>Urine</td>
<td>SPE</td>
<td>Visser et al. 2003</td>
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<td></td>
<td>Nonsteroidal antiinflammatory drugs (NSAIDs)</td>
<td>Serum and urines</td>
<td>SMPE</td>
<td>Guzman 2003</td>
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<td></td>
<td>Peptides</td>
<td>Biological samples</td>
<td>Fritless SPE</td>
<td>Medina et al. 2014</td>
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<td></td>
<td>Nonsteroidal antiinflammatory drugs (NSAIDs)</td>
<td>Human urine</td>
<td>LPME</td>
<td>Nozal et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Nitroimidazoles</td>
<td>Liver</td>
<td>LPME</td>
<td>Nozal et al. 2006</td>
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<td></td>
<td>Bovine Serum Albumin</td>
<td>Serum</td>
<td>Cellulose acetate membrane</td>
<td>Yang et al. 2006</td>
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<td></td>
<td>Chlorophenols</td>
<td>Water</td>
<td>SLM</td>
<td>Almeda et al. 2007</td>
</tr>
<tr>
<td></td>
<td>Formate</td>
<td>Blood</td>
<td>SLM</td>
<td>Pantuckova et al. 2013</td>
</tr>
<tr>
<td></td>
<td>Ochtratoxin</td>
<td>Wine</td>
<td>SLM</td>
<td>Almeda et al. 2008</td>
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<tr>
<td></td>
<td>Aliphatic amines</td>
<td>Water</td>
<td>SPE-replenishment</td>
<td>Santos et al. 2004b</td>
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</tbody>
</table>

they were conditioned in the CFS and injected into the CE vials via a programmable arm.

Supercritical fluid extraction, separation, and detection of cresols and chlorophenols in liquid samples were also achieved by a SFE-CE using this arm interface (Mardones et al. 2000). The mechanical interface used for SFE-CE coupling comprises a customized programmable arm and the autosampler of the CE equipment. This electronic interface enables automatic control of the coupled elements via the built-in microprocessor of the CE equipment. A vial that can be placed at two different positions of the autosampler establishes the link between the rinsing system and the capillary end via two programmable arms. The customized arm is furnished with two injection needles of different lengths; the longer needle is used to fill the vial and the shorter one to drain it to maintain a preset liquid level. The needles are up while the rinsing system is working and down when it is at rest. The programmable arm performs hydrodynamic injections in the conventional manner. The programmable arm was controlled by an electronic interface governed by GW Basic software. The main function of this program was to control the time for insertion of the needles into the CE vial. The determination of these analytes was carried out
automatically without human intervention with good precision and throughput.

As an alternative to programmable robotic arm, sample treatment devices and CE equipment can be coupled at-line via the replenishment system, which is used to empty vials and fill them with fresh buffer in some commercial instruments. This option, proposed for the first time by Santos et al. (2004a), involves disconnecting the replenishment needles from the Teflon tube coming from the replenishment bottles and replacing them with one tube coming from the sample treatment device. This alternative was proposed to automate the monitoring of biogenic amines and sulfonamide residues in milk and wine samples, respectively (Santos et al. 2004a, 2005). For determination of sulfonamides in milk, it combines a screening unit for the total amount of sulfonamide with CE-MS equipment for processing the samples containing a detectable level of sulfonamide. The screening unit consists of CFS to precipitate the proteins connected online to the CE-MS equipment, in which a common characteristic ion of all sulfonamides was monitored with the MS detector by flushing the sample through the capillary. The confirmatory method is based on the purification and preconcentration of sulfonamides in a CFS unit and posterior analysis by CE-MS. The sample treatment unit was also online connected at-line via the replenishment system to the CE-MS equipment.

<table>
<thead>
<tr>
<th>Coupling methodology</th>
<th>Analytes</th>
<th>Samples</th>
<th>Detection</th>
<th>References</th>
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<tr>
<td>On-capillary</td>
<td>B2 and B6 vitamins</td>
<td>Serum</td>
<td>FD</td>
<td>Priego and Luque 2005</td>
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<td>Heterocyclic amines</td>
<td>Meat</td>
<td>FD</td>
<td>De Andrés et al. 2010</td>
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<td></td>
<td>Catecholamines</td>
<td>Urine</td>
<td>FD</td>
<td>Zhu and Kok 1997</td>
</tr>
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<td></td>
<td>Tryptophan; tyrosine; aminoacids</td>
<td>Cerebrospinal fluids</td>
<td>FD</td>
<td>Bayle et al. 2003</td>
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<td>Vitamins</td>
<td>Food</td>
<td>FD</td>
<td>Zougagh and Ríos 2008</td>
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<td>End-capillary</td>
<td>Idolamine, catecholamine</td>
<td>Urine</td>
<td>ED</td>
<td>Park et al. 1995</td>
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<td></td>
<td>Mycotoxins</td>
<td>Maizes</td>
<td>ED</td>
<td>Sanchez et al. 2009</td>
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<td></td>
<td>Maleic hydrazide</td>
<td>Potatoes</td>
<td>ED</td>
<td>Chicharro et al. 2008</td>
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<td></td>
<td>Aminoacids</td>
<td>Tea</td>
<td>ELSD</td>
<td>Bouri et al. 2013a</td>
</tr>
<tr>
<td></td>
<td>Caffeine, carbohydrates</td>
<td>Synthetic samples</td>
<td>ELSD</td>
<td>Bouri et al. 2013b</td>
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<td></td>
<td>Neurotransmitters</td>
<td>Urine</td>
<td>ED</td>
<td>Chicharro et al. 2004</td>
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<td></td>
<td>Proline</td>
<td>Serum</td>
<td>ED</td>
<td>Yuan et al. 2006</td>
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<td></td>
<td>Tripropilamine, proline</td>
<td>Serum</td>
<td>ED</td>
<td>Cao et al. 2002</td>
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</table>
The most salient advantage of at-line coupled systems is that the CE equipment is run in its normal operation mode. This allows samples to be introduced hydrodynamically or electrokinetically into the capillary. It also facilitates other CE operations, such as capillary conditioning.

**On-line coupled systems**

Coupling sample treatment devices integrated in CFS on-line to commercial CE involves inserting the capillary end of the latter in a continuous stream of the former (Figure 2). Because the flow processing device and the electrophoretic system operate at a different flow rate, the two require a split-flow interface for coupling. The split-flow interface was originally developed simultaneously in a vertical configuration by Fang’s group (Fang et al. 2000) and a horizontal configuration by Karlberg (Kuban and Karlberg 1998). Figure 2 compares the two types of interface, which possess a low dead volume and are electrically grounded. Samples and electrolyte solutions are introduced in the electrophoretic capillary by effect of electroosmotic flow and electrophoretic mobility. Hydrodynamic flow should be avoided by ensuring that the liquid level at the interface (viz. the capillary inlet) coincides with that in the capillary outlet. A split-flow interface can be readily constructed from non-conducting polymer materials, such as Teflon, methacrylate, or Plexiglas. Alternatively, a piece of tygon tubing can be used to insert the electrophoretic capillary and attach it with glue. The integrated sample treatment device in CFS is made compatible with the electrophoretic system by grounding the interface. This avoids voltage differences between the interface and flow system. Electrophoretic separation is accomplished by applying a voltage difference to the capillary end. This configuration is highly recommended when an optical detector is used with the electrophoretic system, but scarcely useful with other types of detectors (e.g., mass spectrometers).

In principle, the use of on-line coupled systems linked by a split-flow interface is limited to the electrokinetic introduction of samples. However, this injection mode provides biased results in some cases. This has promoted

![Figure 2](image-url)
the use of hydrodynamic injection instead. Thus, Pu and Fang (1999) used a split-flow cell affording hydrodynamic injection of samples into the CE capillary by electroosmotic flow. The interface included a Nafion joint to connect the CE capillary to the tube of the flow system. An electronic time relay system was used to control switching of the high voltage. Kuban and Karlberg (1998) inserted a valve at the end of a horizontal split-flow interface. Switching the valve off caused the sample to be forced into the capillary. The greatest limitations of this approach arise from the need to control the time during which the valve is on and off and also the pressure generated in the system. One research group (Santos et al. 2006a,b) proposed controlling the pressure by using a valve loop capable of withstanding higher pressures. With commercial CE instruments, a researcher can also connect the pressure system of the equipment (Santos et al. 2006b).

It should be noted that commercial CE equipment affords injection at a low pressure (0.5 psi) with a high precision (0.01 psi). A modified version of the split-flow interface was used to accommodate a typical solid-phase microextraction (SPME) fiber precisely at the capillary end (Santos et al. 2007). Although samples were treated on the fiber and the flow system was only used to couple SPME and CE, they can also be processed at the interface connecting the fiber to the flow system. In this case detection of trace levels of tetracycline from soil samples was demonstrated. Supercritical fluid extraction (SFE) separation and detection of riboflavin vitamins in food samples were also achieved by a SFE-CE using this split-flow interface (Zougagh and Ríos 2008). This method is based on the on-line coupling of a SFE with a continuous flow-CE system with fluorimetric detection (CF-CE-FD). The whole SFE-CF-CE-FD arrangement allowed the automatic treatment of food samples (cleanup of the sample followed by the extraction of the analytes), and the direct introduction of a small volume of the extracted plug to the CE-FD system for the determination of riboflavin (RF) vitamins. Veraart et al. (1998, 1999, 2001) developed online dialysis-SPE-CE systems with a split-flow vial interface for the analysis of amphoteric solutes (Veraart et al. 1998), nonsteroidal anti-inflammatory drugs (Veraart et al. 1999) and tricyclic antidepressants (Veraart and Brinkman 2001) in urine and serum samples. In these works, the dialysis-SPE unit was connected with the CE system via this interface in which the sample is delivered in a vial-like enclosure also containing an electrode and CE capillary separation. This vial interface allows the analytes to flow through to waste, while analytes are injected into the CE capillary by applying a voltage of pressure difference. In this approach, the interface has to be filled with background electrolyte (BGE) prior to starting analysis, because it also serves as the CE inlet vial. The split-flow interface can be used for the on-line coupling of both flow injection and sequential injections systems. Based on a common approach, split-flow interfaces, microsequential injection systems or integrated lab-on-a-valve systems have been coupled to CE. Sequential injection systems have also been on-line coupled via a microvalve allowing the insertion of a constant volume of sample into the capillary (Mai et al. 2012). However, the loop volume is high relative to those typically used in CE work and must be reduced by switching the valve or flushing the sample from the interface to facilitate electrokinetic insertion of a portion of the sample. Another approach for coupling SPE and CE is the use of a valve interface for introducing the eluate to CE capillary via this valve. Detailed information about this valve interface can be found in a previously published review (Tempels et al. 2008). These authors used this interface to on-line coupling of SPE microcolumn with CE-MS for peptides analysis (Tempels et al. 2007). Although the system is very robust, there are some drawbacks. The coupling via a switching valve is quite challenging, and a number of safety recommendations are essential to avoid unintended electrical discharges. In addition, the researchers concluded that it is difficult to accommodate the elution volumes and the CE injection volumes because only 4–5% of the SPE elution volume is injected. To avoid these drawbacks, Morales-Cid et al. (2009) developed an innovative way to integrate SPE, such as microextraction by packed sorbents (MEPS) into commercial CE equipment by modifying the hardware that controlled the fluid/pressure module of the CE equipment. The MEPS was integrated in the outlet region of a commercial CE equipment cartridge to provide easy manipulation and exchange. The robustness of the proposed integration was demonstrated by the design and use of a MEPS-nonaqueous capillary electrophoresis (NACE)-MS method used to determine fluoroquinolones in urine samples.

In-line coupled systems

The integration of the sample treatment devices in-line into commercial equipment can be performed in the electrophoretic capillary, the electrophoretic vial or even in an auxiliary part of the equipment, such as a replenishment system. This integration offers important advantages over coupling methodologies (described following), as there is no need for additional hardware or software. The coupled equipment described in literature includes extraction, filtering, dialysis, membrane, gas extraction and gas
The principal conclusion is that choosing an appropriate flow processing device can facilitate the treatment and analysis of virtually any type of sample (biomedical, pharmaceutical, environmental, food). Readers interested in a more detailed description of the types of devices coupled to CE so far are referred to the article of Marina et al. (2005). The integration of sample treatment device in the CE capillary, is probably the alternative most used to date because of coupling both solid phase extraction (SPE) and liquid phase extraction (LPE) using liquid-supported membranes (LSM) (Kuban and Bocej 2013). SPE has been integrated into the CE capillary by packing the sorbent material in the inlet region of the capillary and using it as a minicartridge (Santos et al. 2006b). For analyte determination, the sample is flushed through the capillary containing the cartridge where the analytes are retained and preconcentrated. Afterward, the sample is removed from the capillary with the electrophoretic buffer, which must avoid the elution of analytes from the minicartridge. Finally, a small plug of eluent is introduced by pressure into the capillary. This plug is moved by EOF through the capillary and, when the plug reaches the minicartridge, analytes are eluted and electrophoretic separation takes place. Some applications include the determination of heterocyclic aromatic amines in food (Viberg et al. 2004), insulin derivatives in biological fluids (Visser et al. 2003), and drugs and peptides in biological samples (Guzman 2003, Medina et al. 2014). More applications using this modality are found in the review by Guzman and Stubbs (2001). The most important shortcoming of this methodology is the need to flush the sample through the capillary, as this can result in adsorption of protein or other macromolecules from the matrix sample onto the capillary wall, disturbing the EOF. In most of these works, the solid packing material was held inside the capillary by two frit structures. However, an alternative packing material is a polymer membrane impregnated with a chromatographic stationary phase (Waterval et al. 2001). The replenishment system, which allows the electrophoretic buffer to be removed from the vial and replaced with fresh buffer, can be also used to perform SPE (see Figures 3A and 4B). For that, a typical minicolumn containing the sorbent material is located between the needles and the two bottles.

![Figure 3](image-url)  
**Figure 3** Schematic description of in-line coupling sample treatment unit to commercial CE equipment using a (A and B) replenishment system, (C) microextraction unit in-capillary (D) microextraction unit in-vial. Reproduced with permission from John Wiley & Sons [Santos et al. 2004a,b, Nozal et al. 2006, 2007].
of the replenishment system (Santos et al. 2004a,b). One of the bottles is used as a waste and the other to provide the eluent. Once again, the minicolumn is inserted in the system and the eluent is located in the bottle, the software of the CE equipment is programmed to ensure sample aspiration, eluent elution and introduction into the vial, and subsequent CE analysis. Perhaps the most important shortcoming of this strategy is that it is impossible to control the flow rate as the replenishment system normally runs at constant pressure. This shortcoming is in part solved by adjusting the column dimensions.

As an alternative to SPE, liquid phase microextraction (LPME) has been coupled in commercial equipment. A simple preconcentration method was based on the formation of a small drop in the inlet region of the capillary. The drop, located inside the sample vial, acts as acceptor phase in the liquid-liquid extraction process. The main shortcoming of this strategy is that the formation of the drop strongly depends on the nature of the liquid and the capabilities of the equipment in applying controlled pressure at the outlet. LPME, based on supported liquid membranes, has also been integrated into the electrophoretic capillary by Nozal et al. (2007). This coupling was achieved by inserting a hollow polypropylene fiber in the inlet region of the capillary (see Figure 3C). The connections between the hollow fiber and the capillary were made by burning the hollow fiber on the capillary. The main difference with integrated SPE is the mode of transportation. In SPE, sample is flushed through the capillary; but, controversially, in LPME analytes migrate through the membrane, so this strategy avoids contact between the sample matrix and the internal capillary wall. The extraction system directly determines non-steroidal anti-inflammatory drugs (NSAIDs) in human urine with no sample pretreatment. This approach, which is simple and useful, can be easily implemented in routine laboratories. It affords a large number of extractions without the need to replace the unit. As with inserting the hollow fiber, Yang et al. (2006) proposed insertion of a cellulose acetate-coated porous membrane. The membrane, located at the end of the capillary, allows the passage of buffer ions but excludes larger protein molecules. The membrane is then used to retain proteins at the end of the capillary. With the retained and preconcentrated proteins, the direction of the electric field is switched to perform separation and detection.

The integration of the sample treatment devices in-line into commercial equipment can also be performed in vial (Nozal et al. 2006, Pantuckova et al. 2013) (see Figure 3D). This modality consists in the assembly of extraction unit directly in the vial containing the sample. The vial containing the microextraction unit was located in the autosampler, and the treated sample was direct analyzed by electrophoresis. The microextraction unit
was designed to permit introduction of the electrode and capillary in the treated sample so that there could be both electrokinetic and hydrodynamic introduction into the capillary. The potential of an in-ivial micro-SLM extraction unit with commercially available CE equipment is the direct determination of nitroimidazoles from pig-liver-tissue homogenates (Nozal et al. 2006). The same coupled system for direct determination of chlorophenols in liquid samples (Almeda et al. 2007) and ochratoxin in wines (Almeda et al. 2008) was also used.

Non-commercially available interfaced detectors

As it was reported in the Introduction, a wide variety of the detection alternatives can be used for CE (Marina et al. 2005). Only a few of them are commercially interfaced detectors (UV-Vis, LIF, and MS). Therefore, this part is focused in other non-commercially interfaced CE detectors. In the case of coupling commercial CE to a non-integrated detector, the most used configurations are on-capillary and end-capillary modes. In the on-capillary mode, the systems are configured to make the detection directly on the separation capillary, whereas in the end-capillary mode, the detectors are connected to the end of the capillary. In this sense, a number of detection methods have been interfaced with commercial CE, including fluorescence, phosphorescence or chemiluminescence (Timperman et al. 1995a,b, De Kort et al. 2013), electrochemical (Chicharro et al. 2002, Wallingford and Ewing 1987, 1988), mass spectrometry (MS) (Santos et al. 2006a,b), inductively coupled plasma-MS (Deng and Chan 2000), Vibrational spectrocometry (Kölhed et al. 2002), nuclear magnetic resonance (NMR) (Johnson and He 1989) and other unconventional detectors, such as evaporating light scattering (ELSD) (Bouri et al. 2013b).

Interfaced CE-FD systems

Several optical designs for fluorescence detection (FD) in CE have been proposed and used during the years, most of which are home-built and comprise various excitation sources and photosensitive detectors. Interested readers can find a recently published review by De Kort et al. (2013) of FD designs, coupled in the on-capillary or end-capillary modes with commercially available CE equipment. In the on-capillary mode, the system configured to make the fluorescent can be detected inside the separation capillary. A good example of a setup using the FD at-line with CE has been developed by Flux (Basel, Switzerland) that is equipped with an external detection cell mounted on the CE cassette, Xenon-mercury lamp as the radiation source, and photomultiplier tube (PMT) with a measurement range of 185–700 nm (for signal acquisition). This external detection uses total internal reflection of emitted fluorescence light to spatially separate the point of analyte excitation from the position where emission light is collected. In this way, the background noise due to scattered excitation light is seriously reduced. Excitation light is focused on the capillary by a patented light cone. After analyte excitation, part of the generated fluorescence emission light is trapped within the capillary walls due to total internal reflection, because the refractive index of fused silica (n=1.458) is higher than that of the aqueous BGE (n=1.333) and air (n=1.000). About 1 cm away from the position of analyte excitation, glycerol (n=1.473) is applied onto the surface of the detection window to match the refractive index of the capillary, so that the emission light exits the capillary and enters a light cone. The cone collimates the emission light and transfers it into an emission fiber, which guides the light toward the detector. The sensitivity of this optical application is comparable to laser-induced fluorescence detectors. Various authors have used this configuration to interface the CE instrument, for determination of B2 and B6 vitamins in serum samples (Priego and Luque 2005), heterocyclic amines in meat samples (De Andrés et al. 2010), and catecholamines and related compounds in urine samples (Zhu and Kok 1997).

In the end-capillary mode, the analytes migrate from the separation capillary before fluorescence detection occurs, thus preventing excitation light scatter and auto-fluorescence from the capillary wall. Timperman et al. (1995a), designed a fluorescence detector interfaced in CE (Figure 4B), that employs a frequency doubled krypton laser operating at 284 nm for excitation, a reflective microscope objective, an imaging spectrograph, a charge coupled detector (CCD), and a sheath-flow cell cuvette. The sheath fluid has a higher flow rate than the capillary flow, which causes elongation and focusing of the capillary to a small volume. The excitation light can be effectively focused onto this volume by use of a lens. The fluorescence emission is collected at 90° to the excitation with an effective microscope objective. In this configuration, neither a Rayleigh filter nor a preexcitation laser-line filter is used, because the sheath flow cell and spectrograph eliminate and separate most of the Rayleigh scattering (Timperman et al. 1995b). Another instrumental configuration using CCD was demonstrated in a two-laser–two-color flow cytometry study (Fuller et al. 1996).
A sheath-flow cell (quartz cuvette) was used as the sample chamber. Excitation of the core stream was achieved by means of a liquid-cooled Ar³ laser and an ArKr⁺ laser operating at 488 nm and 568 nm, respectively. Collection optics included a microscope objective (to eliminate fluorescence from the collection optics) and a series of 500-nm high-pass and 568-nm laser-line rejection filters. The spectrograph (grating 285 grooves mm⁻¹) and CCD were oriented to enable sub-array readout and binning (3 Hz readout, 2×256 binning) while preserving 1024-pixel wavelength information over the 350–800 nm wavelength range. By use of single-particle fluorescence emission spectra, sub-micron particles were individually identified, sized, and distinguished. Park et al. (1995) used the sheath flow cell to interface FD and CE for the separation and fluorescence detection of idolamine and catecholamine.

**Interfaced CE-ED systems**

Electrochemical detection (ED) in CE focuses on the effects of shuffling electrons, ion mobility, and membrane potentials. Modes of interfacing ED to CE included in this review are volammetry at fixed potential (amperometry), because it is by far the most implemented detection of electrochemistry owing to its excellent limit of detections (LODs). However, other ED modes such as conductivity, and potentiometry have various advantages, which are described in other published works (Timerbaev and Buchberger 1999, Huang and Fang 2000, Zemann 2001, Trojanowicz 2009).

Because, the high voltage applied to the separation capillary can lead to serious interferences with detection of the electrochemical signal, the on-capillary detection is rarely used with ED techniques (Timerbaev and Buchberger 1999). Instead, the detection electrode can be positioned at the outlet of the separation capillary (end-capillary). In this arrangement, the high electric field strength is confined to the inside of the separation capillary, whereas outside and at the position of the detection electrode the electric field strength is low and does not significantly interfere with the detection signal. Alternatively, the high electric field can be completely decoupled from the detection electrode by connecting the end of the separation capillary with a short transfer capillary by means of a porous coupling piece surrounded by a carrier electrolyte with the high voltage electrode; the current passes the porous coupling piece, whereas the electroosmotic flow (EOF) generated in the separation capillary carries the analytes through the transfer capillary to the detection electrode (Timerbaev and Buchberger 1999). Wallingford and Ewing (1987) were the first to report the interface for coupling of end-capillary amperometric detection to CE. This system couples two pieces of column together with a section of porous glass capillary, thus forming a joint that is electrically conductive. The joint was immersed in a buffer reservoir along with the ground electrode to allow the separation potential to be applied across only the first section of capillary. The strong electroosmotic flow generated in the first section of capillary serves to force solvent and analyte zones past the joint and through the second section of capillary to the detector. This configuration effectively separates the detector from the high applied voltage via the resistance of the detection capillary. The authors have obtained separation efficiencies on the order of 180,000 theoretical plates for the separation of catechol from catecholamines. Although this interface was initially used with the home-made CE, it was modified and adapted to be used in the commercial CE demonstrated by a wide variety of applications and a large volume of original research publications. In this direction, Chicharro et al. (2002) designed a good end-capillary interface to attach amperometric detector to a commercial CE. This interface, as represented in Figure 5, was mounted by assembling three pieces: two Plexiglas blocks and a PTFE gasket placed between them. Working and reference electrodes were fitted to nuts (1 and 2 in the figure), respectively, by PTFE adapters and could be simply replaced if necessary. The cell operates in an inverse wall-jet arrangement. The inlet-working electrode distance and alignment is quickly and conveniently controlled by nut (marked with 1 in the figure) and by adjusting the upper block with the screws. The electrolyte solutions can easily be changed or renewed with the help of a syringe connected to the drain outlet (marked with 7). Connection, marked with 11 in the figure, allows coupling the vacuum tube (marked with 5) to the equipment vacuum system. The vial could be easily filled and drained with the help of a syringe connected to the position marked with 12 in the figure. The body cell is fitted to this vial with a hollow screw that joins the 13 and 4 positions of the figure. The separation capillary column is inserted inside the detection cell through the hollow screw. A rubber septum fixes the capillary position and ensures the isolation between the vial and the detection cell. No modification was done to adapt the electrochemical cell on the original design of the capillary electrophoresis injection module. Various methodologies, using this interface, for coupling amperometric detection and commercial CE were applied to determination of polyphenols in white wine (Moreno et al. 2011), macrocyclic lactone mycotoxins in maize (Sanchez et al. 2009) and maleic hydrazide in potatoes (Chicharro et al. 2008).
Interfaced CE-ELSD systems

The combination of commercially available CE with ELSD is an interesting possibility, especially for analytes no sensitive to other common detectors used in CE. ELSD can be considered to be an universal detector, as in principle the only analyte characteristic required for response is low volatility compared to the buffer solution. In the bibliography, only two CE-ELSD interfaces have been found, as can be deduced from the papers published on this topic (Szostek and Koropchak 1996, Bouri et al. 2013b). In the first interface designed by Koropchak group, the authors interfaced the ELSD to CE in two approaches. In the first system, the separation capillary was divided in two parts connected by the stainless-steel union. Nafion tubing was glued to the fused silica capillary using epoxy resin. During operation, this Nafion membrane was immersed in a vial containing sulfuric acid, along with the grounding electrode of the CE. The outlet end of the Nafion tubing was glued with epoxy to fused-silica nebulizer capillary. The nebulizer was inserted through a stainless-steel tee and the outer nebulizer capillary. The other arm of the tee was connected to the nebulizer nitrogen gas source. The nebulizer assembly was inserted through the end cap of a spray chamber, which contained a baffle assembly located ~3 mm from the nebulizer tip. The spray chamber was connected to the ELSD using Teflon tubing. The second means of interfacing the CE with the ELSD employed either a PEEK separation capillary or a separation capillary with strong anion-exchange-coated microspheres permanently bonded to the interior walls. In either case, the capillary was directly inserted through the stainless-steel tee into the nebulizer after the last 15 cm of the exit end of the capillary was coated with copper paint containing tungsten wire to match the inner diameter of the capillary. After the paint dried, the wire was removed to leave a tip that
acted as the contact for the ground with the buffer solution of the CE. This coupling is complex and requires a Nafion membrane or other material to ground CE circuit and connect the ELSD system. To solve these problems, Bouri et al. (2013b) proposed a stainless-steel triple-tube nebulizer with some similarities to those used in CE–MS interfaces by the ESI mode (Szostek et al. 1997), which was accommodated in a customized ELSD nebulizer chamber with a stainless head connected to the CE chassis with a ground cable (Figure 6B), to avoid electrical discharges. The triple tube nebulizer consists of a central tube (the CE capillary) surrounded by a second stainless-steel tube, the sheath-liquid tube (see details in Figure 6C). The sheath liquid flows between this tube and the inner CE capillary. Between the sheath-liquid tube and the third outer tube, or gas tube, flows the nebulizing gas controlled by an additional pressure regulator compatible with the CE–ELSD interface that contributes to control the nebulizing process. This arrangement is simple and does not require any special electrical connections between the CE and ELSD instruments, such as the use of Nafion membrane or nebulizer coated with a metal for the grounding of the CE circuit system (Szostek and Koropchak 1996). It is enough to connect a ground cable between the CE chassis and the head of the stainless steel nebulizer chamber (Figure 6A). Our research group recently developed a method based on CE–ELSD for direct determination of underivatized aminoacids in tea samples (Bouri et al. 2013a). The obtained results were positively compared with those obtained with a sofisticated detector, such as MS (Soga and Heiger 2000). It is also expected that CE–ELSD will open new possibilities for the analytical characterization of polymers and biopolymers, macromolecules, nanoparticles, and, in general, big molecules or aggregation of molecules.

Final remarks

There is no doubt that many practical sample preparation problems can be solved by interfacing sample treatment unit to commercial CE, thereby improving the overall efficiency and sensitivity of CE methods. Moreover, this

Figure 6  (A) Scheme of the interface for coupling the CE equipment with the ELSD detector via a stainless steel triple tube nebulizer; (B) picture of the nebulizer accommodated in customized nebulizer chamber and scheme of the different connections; and (C) details of the flow tubes to produce an efficient nebulization. Reprinted with permission from Bouri et al. 2013a,b. Copyright 2014 American Chemical Society.
system presents intrinsic characteristics – low cost, robustness, and ease of operation and automating – that makes it an excellent analytical tool to develop routine analytical methods. The sample treatment units described in this review includes extraction, filtering, dialysis, membrane, gas extraction diffusion units, hollow fibers, and even auxiliary devices. The principal conclusion is that choosing an appropriate sample preparation device can facilitate the treatment and analysis of virtually any type of sample (biochemical, pharmaceutical, environmental, and food). One of the most critical factors in selecting a sample treatment device is compatibility of the chemical composition of the conditioned sample with the electrophoretic analysis to be performed. A number of sample treatment techniques (e.g., SPE, LPE) require the use of organic solvents, such as methanol direct insertion of which into an electrophoretic buffer can result in current interruptions. This problem can be avoided by changing the buffer pH, adding a modifier (e.g., a surfactant to the organic solvent), or supplying the buffer with a small amount of solvent. In this way, at-line coupled systems afford slight modification of treated or conditioned samples by collection into vials containing a modifier. Alternative approaches involve modifying samples within the sample treatment unit. Other influential factors to be considered include the sample volume and dilution by effect of diffusion phenomena occurring in the sample treatment device.

Coupling sample treatment devices to commercial CE systems is of special interest with a view to developing in vivo determinations; this is facilitated by the ability of electrophoretic systems to use small volumes of sample and provide rapid analyses. For example, a microdialysis needle on-line coupled to commercial CE enabled the in vivo monitoring of the evolution of a drug (Wang et al. 2005). In fact, this approach allows the characteristic pharmacokinetic response of a simple animal to the drug to be recorded. One other useful advantage is the ability to analyse special samples such as brain tissue (Ciriacks et al.). In fact, this approach allows the characteristic pharmacokinetic response of a simple animal to the drug to be recorded. One other useful advantage is the ability to analyse special samples such as brain tissue (Ciriacks et al.). In general, the use of sample treatment devices improves the overall efficiency, selectivity, and sensitivity of CE methods. In addition, it facilitates special determinations, such as in vivo pharmacokinetic tests. No doubt, miniaturization of sample treatment devices will help further expand the potential of this combination.

When interfacing non-integrated detection modes in commercially available CE equipments, the properties of an analyte dictate which method is best suited to its detection. For example, with analytes containing fluorophore/electroactive groups, fluorescence/electrochemical detections can be an appropriate choice with a view not only to enhancing sensitivity but also to improving the apparent selectivity through specificity.

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