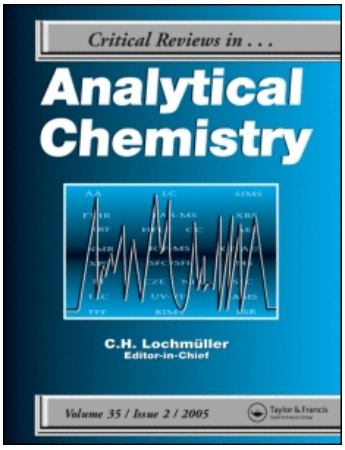

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Coupling Continuous Sample Treatment Systems to Capillary Electophoresis

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ABSTRACT: The state of the art and prospects for the combined use of continuous sample treatment systems and custom-made and commercially available capillary electrophoresis equipment is presented and discussed. Sample treatment in this separation technique is of great practical relevance by virtue of its inherent shortcomings, which include low sensitivity, small sample volumes, and a marked influence of matrix components. The main technical types of coupling (in-line, on-line, and mixed) are dealt with systematically and compared to manually implemented approaches in order to derive practical conclusions with a view to developing new technical applications and facilitate use by routine laboratories.

KEY WORDS: capillary electrophoresis, sample treatment, automation.

I. INTRODUCTION

Capillary electrophoresis (CE) is a highly efficient and flexible analytical separation technique; over time, it has become a serious competitor for gas (GC), liquid (LC), and supercritical column chromatographies (SFC). An impressive number of books, reviews and experimental papers have been published on the topic in the last 10 years,[1–4] and a substantial variety of commercially available equipment is by now available for use in routine laboratories.[5] Both are conclusive proofs of the high potential of CE in Analytical Chemistry. However, only in a small fraction of reported CE separations are the difficulties of sample preparation considered. Such an essential step of chemical measurement processes in general, is even more crucial in this separation technique owing to a number of factors including:

1. The need to insert very small sample volumes (in the nanoliter region) which can detract from such important analytical features as precision and representativeness
2. The typically low sensitivity of continuous detection systems; and
3. The strong influence of the sample matrix components (e.g., salt content, macromolecules, etc.), which can disturb CE separations reversibly or irreversibly.

One way of avoiding or minimizing these inherent difficulties and their strong effects on the quality of analytical results is by considering sample preparation a key part of CE processes.

We should thus focus CE research and development not only on improved separation efficiency, detection, etc., but also on constructing reliable, robust systems in order to establish solid links between real samples and CE equipment. The latter should evolve from custom-built set-ups to commercially available sample preparation modules for easy coupling to CE systems in order to avoid manual implementation of off-line sample preparation

procedures, which are tedious, time-consuming, the source of major random and systematic errors, and of hazards to the analyst and the environment.

The automation, miniaturization, and simplification of the chemical measurement process, particularly preliminary operations, are among the main goals of today's Analytical Chemistry. So far, efforts have materialized in important advances such as multisample off-line treatment modules, continuous flow systems, sensors, screening systems, direct introduction of solid samples, adaptation of industrial processes to laboratory scale (e.g., supercritical fluid extraction, lyophilization), process analyzers, etc.[6]

The special features of capillary electrophoresis require careful adaptation of such advances so as to make automated and miniaturized sample treatment modules compatible with custom-made and commercially available CE equipment.

Hydrodynamic continuous-flow systems (whether or not they involve separation technique) are among the most powerful tools for the automatic implementation of the preliminary operations of the analytical process, i.e., for establishing a reliable link between the unsampled, unmeasured, untreated real sample and the instrument.[7,8]

This paper presents and discusses the most relevant recent advances in continuous sample treatment coupled to CE equipment. The interface between these two analytical systems is the crucial technical aspect and as such the main subject matter of this review. The advantages and shortcomings of the coupled analytical systems are dealt with in a systematic manner in reviewing the state of the art and prospects for this approach, which is essential with a view to consolidating CE as an analytical tool for solving real analytical problems.

II. CONTINUOUS-FLOW SYSTEMS

Under the generic designation "continuous flow systems" (CFS), this papers considers several approaches, almost all of which use liquids (organic solvents, water, mixtures) as carriers, so they are of hydrodynamic nature. These systems encompass those based on injection of the sample (or reagent) and those relying on continuous sample introduction by aspiration. They can operate at both low and high pressures. The latter resemble liquid chromatographic systems. The potential of (CFS) for automatic implementation of the preliminary operations of chemical measurement processes in order to link real samples with measuring instruments was recently reviewed by the authors.[9]

CFS can be used to automate, simplify and miniaturize various preliminary operations including measurement of sample volume; insertion of modifiers, solvents, reagents (e.g., to derivatize analytes or interferents); mixing; dilution; etc. However, one of the most fruitful uses of CFS is for implementing of reliable non-chromatographic continuous separation techniques[10] such as liquid-liquid (LLE), liquid-solid (SPE) and solid-liquid (leaching) extraction; dialysis; gas-diffusion; precipitation; etc. They decisively enhance the performance of CFS through indirectly increased sensitivity (from analyte preconcentration) and selectivity (from sample clean-up), and the ability to perform other operations such as solvent changover. The advantages of CFS relative to manually implemented off-line preliminary operations include are as follows: (1) ease of incorporation into automatic analytical systems; (2) diminished hazards (e.g., those arising from handling toxic solvents); (3) increased precision resulting from avoidance of the typical errors produced by "human factors", (4) markedly increased throughput; (5) dramatically decreased sample and reagent consumption; and (6) lower analytical costs.

It is interesting to note that CFS applications involving liquid chromatographic components (pumps, injectors) are frequently referred to as "chromatographic separations", which is incorrect because the purpose is not purely "chromatographic" but rather the separation-determination of a group of analytes,

even though sample treatment remains the principal objective.

Based on Figure 1, CFS can be implemented by using capillary electrophoresis (mainly isotacophoretic) systems and low-pressure or high-pressure flow systems. Low-pressure approaches (e.g., FIA) have some advantages such as simplicity, flexibility for implementing a variety of operations in addition to the separation, low cost, etc.; however, they also have some disadvantages such as the limited duration of pump tubes. Their coupling to CE has recently aroused the interest of several groups in recognition of the increasing importance of this separation technique and of the need to facilitate its use for automated the analysis of real samples by routine laboratories.

The main goal of CFS is to avoid or minimize the manual implementation of preliminary operations in chemical measurements processes involving CF (i.e., the direct introduction of untreated real samples). As can be seen in Figure 2, left, there are two general approaches, namely, indirect introduction of untreated samples, which is the more interesting, and introduction of manually pre-treated samples.

III. INTERFACES

Hybrid analytical systems are among the most important advances in Analytical Chemistry in the last two decades. Custom-built set-ups have evolved to commercially available systems, which are the most conclusive proofs of reliability. The most fruitful combinations benefit from positive synergistic effects that afford analytical goals unrealizable by the coupled systems in isolation. The greatest constraint to further achievements in this context is the need to establish a reliable link (an interface) between the two systems.

Although CFS and CE are hydrodynamic in nature, a number of technical hindrances make them theoretically incompatible. The performance of a CFS-CE coupled system is strongly dependent on the interface, between the two, which must allow at least the following (see Figure 2): (1) to introduce (in principle, in a discrete way) moving treated sample plugs or continuously treated sample streams from the CFS, taking into account that in both conventional CE introduction modes (electrokinetic and hydrodynamic), the end of the capillary is fist dipped in the sample solution and then lifted; (2) considerably reduce the treated sample volume (from the milliliter or microliter to the nanoliter level) in a robust, reproducible, a representative way; (3) to maintain an uninterrupted high electrical field; (4) to make the flow-rates of the two systems coupled (CFS and CE) compatible; and (5) to avoid irreversible technical changes in the CE equipment in order not to restrict their use to combined operation (this is of high practical interest for routine laboratories with a view to using the CE equipment in other, more conventional applications).

In addition to the off-line approach based on collection of the treated sample at the end of the CFS and (manual) transport of a portion to the autosampler of modern CE equipment, CFS and CE equipment can be coupled in various ways, namely, in-line (on-column), on-line, and on-line/off-line. This paper is organized on the basis of their differences.

The nature of the interface between CFS and CE is dictated by the latter, which can be a homemade set-up or a commercially available system affordable to routine laboratories. Figure 2 depicts the different types of interface. An appropriate interface is easy to design and develop for in-line and on-line coupled CFS/custom CE set-ups. More complicated is to design and use a reliable interface between CFS and commercially available CE equipment owing to their low flexibility; the two systems can be connected in-line, on-line, or in a mixed manner (on-line/off-line).

IV. OFF-LINE APPROACHES

The most simple way of combining sample treatment systems and CE is by introduc-

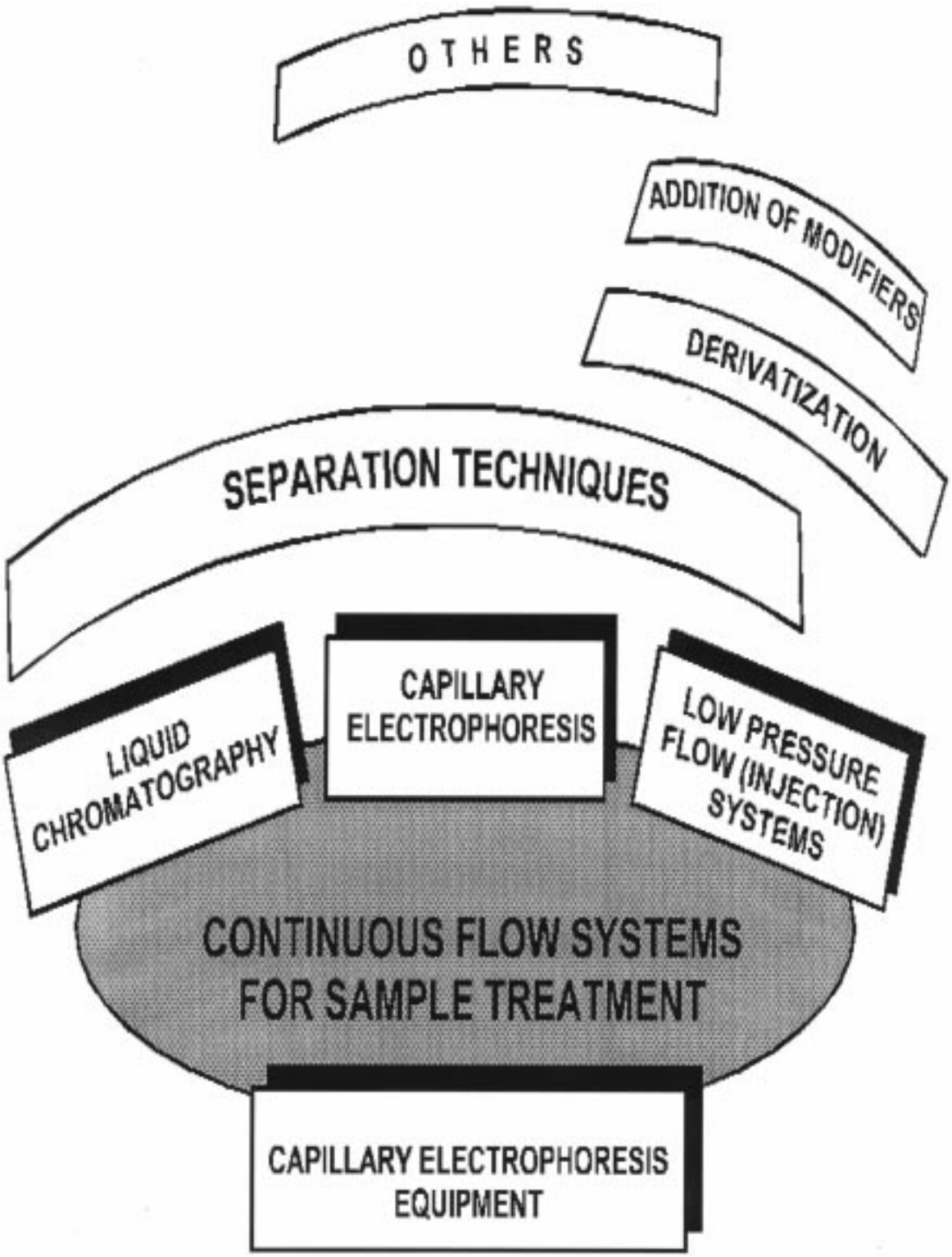

FIGURE 1. Ways of implementing sample treatment as coupled to CE, using hydrodynamic continuous-flow systems, and main operations can that be performed. Note that CE can also be used to implement preparation steps.

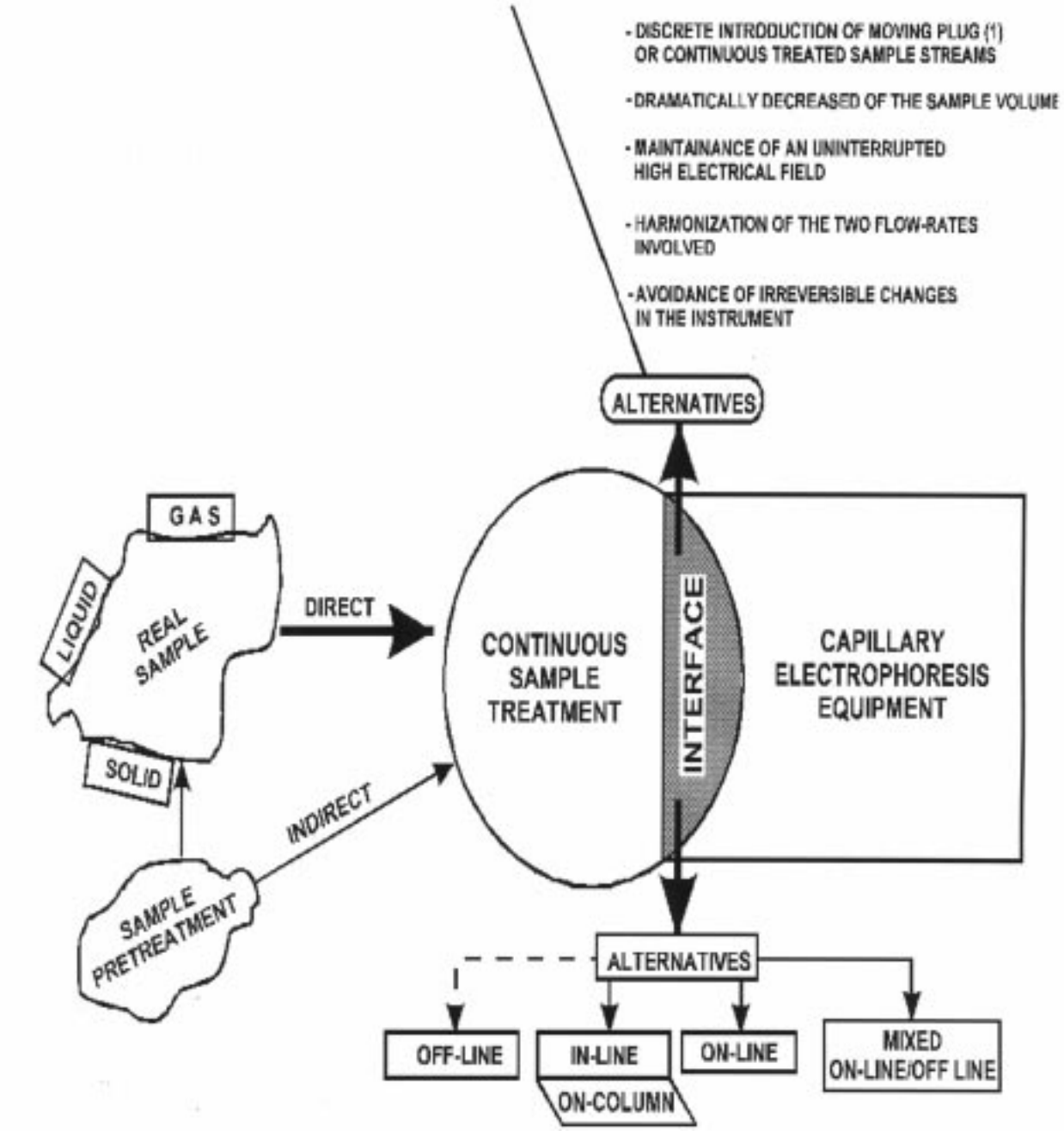

FIGURE 2. Functions and technical alternatives for interfaces between continuous flow sample treatment systems and capillary electrophoresis. (For details, see text.)

ing the sample into the module, collecting a volume of treated sample at the end of the system and transferring it to an autosampler vial of the electrophoretic equipment by means of a micropipette, for example. In fact, there is no interface, though, in some cases, the same autosampler can be used sequentially in each individual system.

Although it is the worse option, the off-line use of CFS also offers several advantages over conventional discrete sample preparation systems, particularly when used to implement continuous separation techniques. There are many reported applications of this approach relating off-line preliminary operations such as solid-phase extraction,[11–13] centrifugation,[12] ion exchange,[14] filtration,[15] and dilution.[14] Recently, Weber et al. developed a new solid-phase microextraction (SPME) system for treating samples prior to CE separation.[16]

The main drawbacks of these off-line systems are limited automatability, slowness, relatively high reagent and solvent consumption, and decressed reproducibility. All these problems can be circunverted or minimized by using alternative.

V. IN-LINE COUPLING

In-line arrangements are also called "on-column" approaches because sample treatment takes place in the capillary where CE separation is effected.

There are two main types of integrated sample preparation/analyte separation systems. One has no interface. The untreated sample is introduced into the capillary and the two steps are sequentially performed by using sophisticated buffer-run programmes. The main purposes of the treatment step are analyte concentration, interference removal, and the implementation of (bio)chemical reactions. Such is the case with classical staking procedures,[17–19] field-amplified polarity switching,[20] and in-line combination of isotacophoresis (ITP) and CE.[21–24] The most salient advantage of these approaches is that a single capillary can perform both functions: The main drawbacks are low preconcentration factors poor interference removal (strongly dependent on the particular sample matrix). Recently, Clarke et al.[25] developed an interesting in-line desalting step prior to capillary isoelectric focusing that relies on the salt anions and cations being much smaller and more highly mobile than the analytes or the ampholites; first, a slow voltage ramp is applied to remove small ions and then focusing is accomplished simply by adjusting the voltage applied to the system. On-column derivatizing reactions can be implemented by using reagents that are either injected (before or after the sample) or contained in the running buffer. Enzyme-catalyzed microreactions in the CE capillary have been developed by using both approaches.[26,27]

The second group of in-line (on-column) sample treatment CE couplings involves placing a sorbent at the beginning of the capillary, whether as a solid plug or in a coated precapillary connected to the separation capillary.[28–31] This can be regarded as an intermediate approach between in-line and on-line systems. The analytes are first concentrated and then desorbed. The main problem is that elution must be done with a few nanoliters of buffer to prevent band broadening, which is rather difficult in practice. A mixed off-line/in-line approach was recently reported by Nguyen et al.[32] First, off-line solid-phase microextraction of the analytes in a fiberglass plug is performed and then the fiberglass is manually brought into the CE capillary via an adapter.

VI. ON-LINE COUPLING

The on-line coupling of CFS and CE and their direct or indirect connection via an appropriate interface constitutes an obviously alternative effective thanks to the hydrodynamic nature of both partners. The two processes (sample treatment and analyte separation) take place sequentially in each coupled system. Many of the problems of off-line and in-line approaches are circumvented or mini-

mized which make this approach an interesting technical alternative for the future.

Depending on the characteristics of the CFS that effects the sample treatment, three technical types of on-line CFS-CE couplings can be distinguished, namely, (1) isotacophoresis (ITP)-CE; (2) liquid chromatography (LC)-CE; and (3) low-pressure CFS-CE. The interfaces involved are slightly different. This criterion is used here to describe the main technical aspects of these hybridisystems.

A. On-Line ITP-CE Interfaces

These combinations use two connected capillaries and are thus different from the on-column ITP-CE approaches discussed in part VI. The first ITP capillary allows both concentration of analytes and removal of matrix components, whereas the CZE capillary implements analyte discrimination. Three types of electrolyte are used in the coupled system (leading, terminating, and background), which are connected via the interface that connects where both capillaries (of equal or different internal diameter). One interface outlet is connected to the leading buffer reservoir. Initially, both the leading and the terminal buffer reservoir are connected so ITP takes place. When the analyte plug reaches the interface, once matrix interferents have been removed, an electrical change allows one to interrupt the electrical field through the ITP capillary and simultaneously create a new electrical field between the terminating and separation buffers through the CZE capillary in such a way that the analyte plug is "injected" into it. Two detection systems are used, one to monitor ITP separation and the other to determinae of the separated analytes.

The most simple way of implementing this on-line ITP-CZE coupling was proposed by Kaniansky,[33,34] who used no valves. Foret et al.[35,36] developed an also simple approach based on a variable-volume sample loop connected on-line to the ITP pre-separation capillary via an additional interface; the ITP-CZE interface included a conductivity detector to control the operation of the hybrid system. By using several valves and ITP and CZE capillaries of different internal diameters (500 μm and 50 μm, respectively), Stegehuis et al.[37] developed a coupled system with several practical advantages over previously reported couplings. A similar approach was developed by Hirokawa et al.[38]

B. On-Line LC-CE Interfaces

By using LC components to implement continuous sample treatment systems coupled on-line to CE equipment, several research groups have developed interesting interfaces that meet the requirements of Figure 2. Although the interface varies between applications, Figure 3 provides a general picture of the possibilities. In order to connect the LC outgoing stream after sample treatment and the CE capillary, the interface may include a (micro)injection valve; a device acting both as a flow splitter and a connector is indispensable, however. The electrode of the CE system (generally the anode) can be made from the grounded stainless-steel valves or be placed in the flow splitter. The CE running buffer can be the LC outgoing stream containing the analytes or be introduced via the injection valve or the flow splitter (which is given additional functions in this case). The analytes can be delivered to the CE equipment both in the electrokinetic and in the hydrodynamic injection mode.

Some LC-CE couplings use a (micro) injector to select a microvolume of the outgoing LC stream for transferral to the CE capillary and to introduce the CE running buffer. Ten years ago, Tsuda et al.[39] reported a ceramic rotary-type injector for introducing microvolumes of sample into a CE capillary between the two buffers reservoirs and on the other side of the detector, thus avoiding dipping-in/lifting-out the end for conventional injection; this approach has not been further applied. In 1991, the same group[40] developed a LC-CE inter-

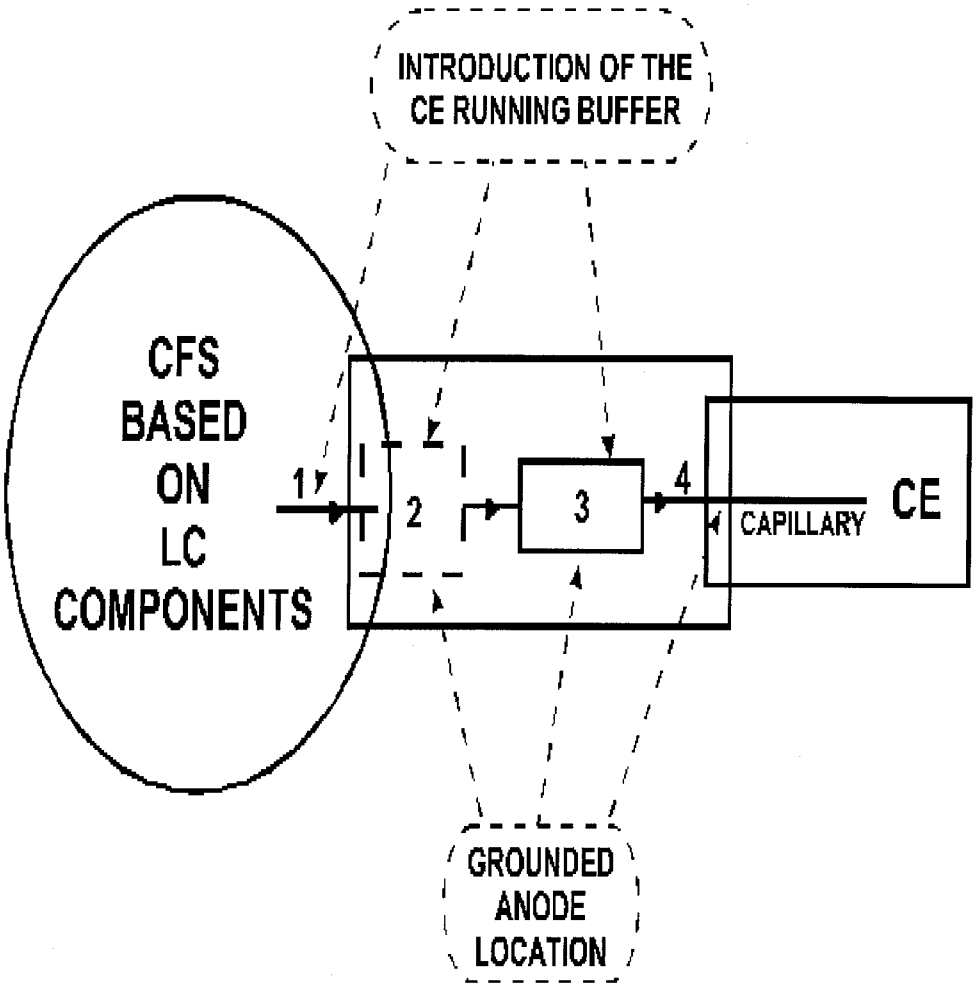

FIGURE 3. General scheme of on-line LC-CE interfaces. 1: LC outgoing (micro)flow; 2: (micro)injector; 3: flow splitter and connector; 4: CE capillary. The different alternatives to introducing the CE running buffer and the location of the (grounded anode) are also shown. (For details, see text.)

face based on an electrically grounded stainless steel injection valve and a split injector (designed for differently circular and rectangular capillaries) inside which one of the electrodes was placed in order to maintain the high electrical field. LC-based CFS involves no sample treatment.

In 1990, Jorgenson et al.[41] reported an on-line interface for two-dimensional LC-CE separation based on a computer-controlled six port injection valve (see Figure 4A): In the "run" position, effluent from the reversed-phase LC column filled the loop while a second pump continuously forced fresh buffer coaxially past the grounded (anode) end of the capillary; a paper wick carrier carried excess buffer away from the valve, thus functioning as a splitter. In the "injection" position, the buffer stream

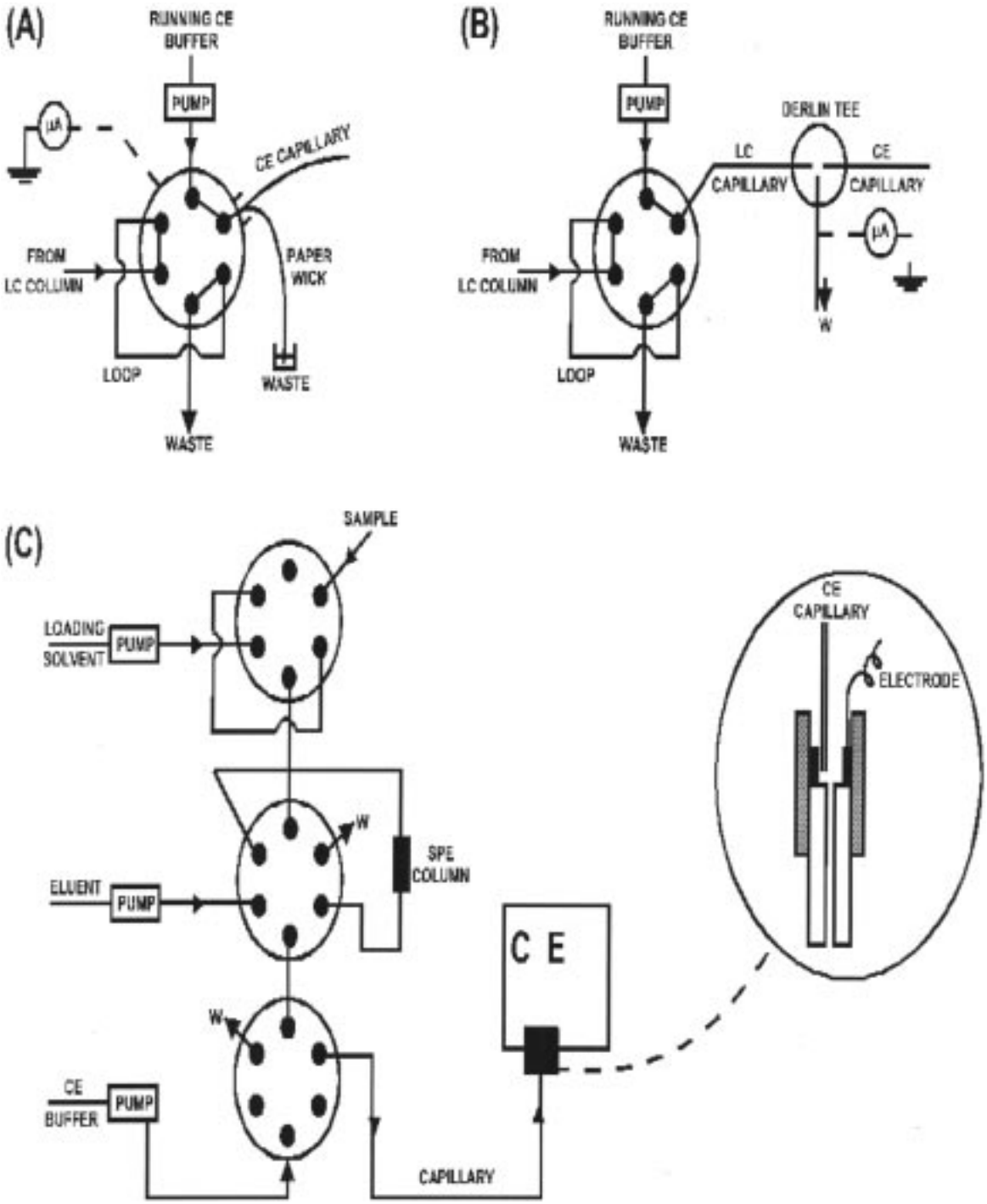

FIGURE 4. On-line LC-CE interfaces based on the combination of rotary injection valves and three types of flow splitter: (1) paper wick (Ref. 41); (2) Delrin T (Ref. 42); and (3) microreservoir (Ref. 43). (For details, see text. Adapted from References 41–43. With permission from the American Chemical Society and Friedr. Vieweg and Sohn.)

flushed the contents of the loop past the end of the CE capillary for electrokinetic injection. The entire valve was held at electrical ground and functioned as the anode of the CE system. These developments can be considered valuable first attempts at improving LC-CE interfaces.

The combination of an injection valve and a flow splitter interface LC and CE was lately approached differently by Jorgenson et al.[42] They use a commercially available Delrin Tee as flow splitter connecting the fused capillary from the injection valve and the CE capillary end, which is located at the center of the device; stainless steel tube serves as both a waste line and as the ground electrode (anode) for the CE system (see Figure 4B). Recently, Brinkman et al.[43] reported a custom-built interface compatible with a commercially available CE instrument to connect it with an SPE sample preparation relying on three LC pumps for loading solvent, the eluent and the CE running buffer, and three LC rotary-type injectors arranged in such a way that the eluted analyte plug is inserted into the CE buffer, which continuously feeds the interface depicted in Figure 4C. This interface is based on a microreservoir that the flowing stream enters from bottom to flush its upper part out to waste. The CE capillary end is located in the interface, near the buffer inlet, but not aligned with it. A platinum electrode is also placed in the microreservoir to maintain an uninterrupted electrical field. Injection of the liquid (CE buffer or eluent) from the interface into the CE capillary is accomplished by applying a backpressure at the other end of the capillary (hydrodynamic injection). In this case, the function of the last injector is simply to insert the eluent from the SPE column located in the loop of the previous valve into the CE running buffer, which differs from the approaches depicted in Figures 4A and 4B.

In vivo CE pharmacokinetics can be implemented on-line by using a mixed microinjector-connector interface linking a microdialysis probe inserted into the animal and the CE equipment.[44] The LC microinjector allows sampling of the dialysate in the CE running buffer and the custom-made connector allows flow conversion (from μL to nL). The connector consists of a reservoir containing the CE running buffer into which two fused capillaries (the transfer line from the microinjector and the CE capillary) are inserted from opposite sides and carefully aligned and fixed at a gap distance of 50 μm. The reservoir also contains the electrode used to apply an interrupted high electrical potential. The running buffer is continuously introduced into the CE capillary by electroendosmotic flow. Only a small fraction of the sample plug is introduced; the remaining plug volume is sent to waste.

Some LC-CE interfaces allow the direct connection of the chromatographic column to the CE capillary, i.e., without the need for a rotary valve to perform microvolume injections. Jorgenson et al.[42] developed a new interface model called "transverse flow-gating interface", which is depicted in Figure 5A. This custom-made device consists of two stainless steel plates (3 in. in diameter, and 0.5 in. thickness) separated by a Teflon gasket (127 μm thickness) with a 1 mm channel cut in it to allow liquid flow between the two plates, which are assembled with six bolts to create a liquid-tight seal. The interface also acts as a grounded electrode. The outlet of the LC column is positioned directly across from the inlet of the CE capillary, which is separated only by the thickness of the Teflon gasket. Normally, a transverse "flush" flow of the buffer enters through the top part of the interface, sweeps through the channel, and exits through the bottom port. This buffer flow carries LC effluent away to waste, thus preventing the sample from reaching the CE capillary. When an injection is desired, the flush flow is halted to allow the LC effluent to be introduced by electrokinetic injection. In this case, the valve does not connect the LC column and flow splitter, so the running buffer is introduced directly into it. This interface has been successfully used in the triple combination LC/CE/MS.[45] Palmarsdóttir et al.[46] developed an interface composed of a microswitching device and a

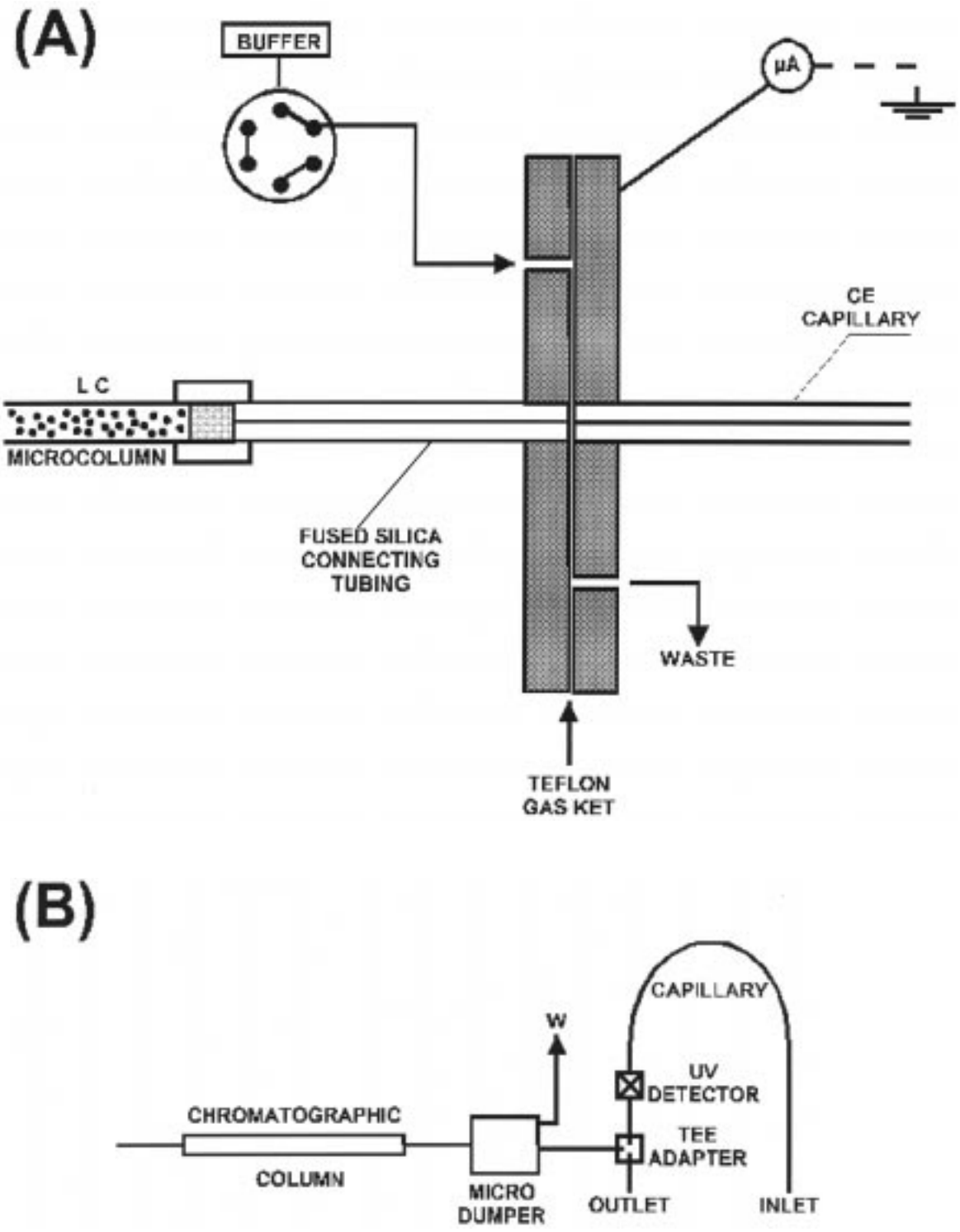

FIGURE 5. On-line LC-CE interfaces involving no rotary injection valve. (A) Transverse flow gating interface (Ref. 42) connecting the LC effluent to the CE capillary end; (B) interface with two components (a switching device and a T adapter) connecting the LC effluent to one point of the CE capillary near its outlet (Ref. 45). (For details, see text. Adapted from References 42 and 45. With permission from the American Chemical Society and Elsevier Science, respectively.)

T adapter (Figure 5B). After clean-up on the chromatographic column, the analyte plug is transferred to the CE capillary by switching the μ-Dumper, which is connected to it through a fused silica tee adapter similar to that shown in Figure 5B. The tee is positioned near the outlet of the capillary, which is manually closed with a PVC stopper while the transfer takes place. In this way, a relatively high sample volume is introduced into the CE capillary, which can be partially or completely filled with the sample fraction. A double stacking procedure is then used for in-line concentration of the analytes in the vicinity of the CE capillary inlet before CE separation and detection take place. The main drawbacks of this approach are as follows: (1) it is not a pure on-line approach owing to the need to manually close one of the ends of the CE capillary during transfer of the analyte fraction; (2) the LC mobile phase must also act as the CE running buffer in the double staking step, which seriously restricts flexibility of use. The double staking procedure was also used by the same research group[47] albeit a mixed approach. The CFS relies on several μ-LC elements to perform a continuous separation in a hollow fiere miniaturized supported liquid membrane (SLM). In fact, the interface is not a continuous device: the outlet of the SLM continuous module is manually connected to the outlet of the CE capillary through a piece of Teflon tubing and disconnected after the extract is transferred, i.e., prior to the double stacking step.

C. On-Line Low-Pressure CFS-CE Interfaces

The implementation of preliminary operations using low-pressure CFS is slightly different from that involving LC components. On the one hand, they are more simple and inexpensive; on the other, they are highly flexible not only for continuous separations (e.g., dialysis, SPE) for automatic development of chemical reactions, addition of modifiers and dilution among others. The coupled CE equipment can be either custom-made and commercially available, and the final injection mode can be electrokinetic or hydrodynamic.

In 1992, Dasgupta et al.[48] developed an interesting CFS-CE interface based on the use of membranes for both sample treatment and introduction into the CE capillary (Figure 6). A sampling jacket connected to the CFS is placed in the middle of the capillary of commercially available EC equipment. The gas or liquid sample flow passes through it and mass transfer takes place through the membrane, which can be porous (for volatile analytes in gases), silicon-rubber permeative (for low ionizable, non-ionic analytes in liquids) or dialysis-type (for low-molecular-weight analytes in liquids). In the former two cases, significant preconcentration is possible that affords attractive limits of detection. The coupled flow system, furnished with one or two 3-way solenoid valves, is quite simple and allows the introduction of gas and liquid samples, air and washing solutions. The main advantages of this interface are that it requires no alteration of the main components or of the usual functioning of the CE equipment, and the great potential for direct introduction of a variety of real samples in different aggregation states.

In Figure 7 are depicted two flow-injection (FIA)-CE interfaces based on an injector which samples a microliter-volume that is transferred to the running buffer. This carrier continuously feeds a custom-made flow splitter-connector to establish a link between the outgoing FIA stream and the CE capillary end. An electrode (anode) is inserted into it to maintain an uninterrupted electrical field. In both cases, a small fraction (a few nanoliters) of the incoming FIA sample plug is introduced electrokinetically into the CE capillary. The waste from the splitter-connector device can be sent directly to waste or passed back to the FIA system.

Recently, Kalberg et al.[49] reported a sample interface of this type (Figure 7A) consisting of a Persplex body. The flowing stream from the FIA module crosses the central part

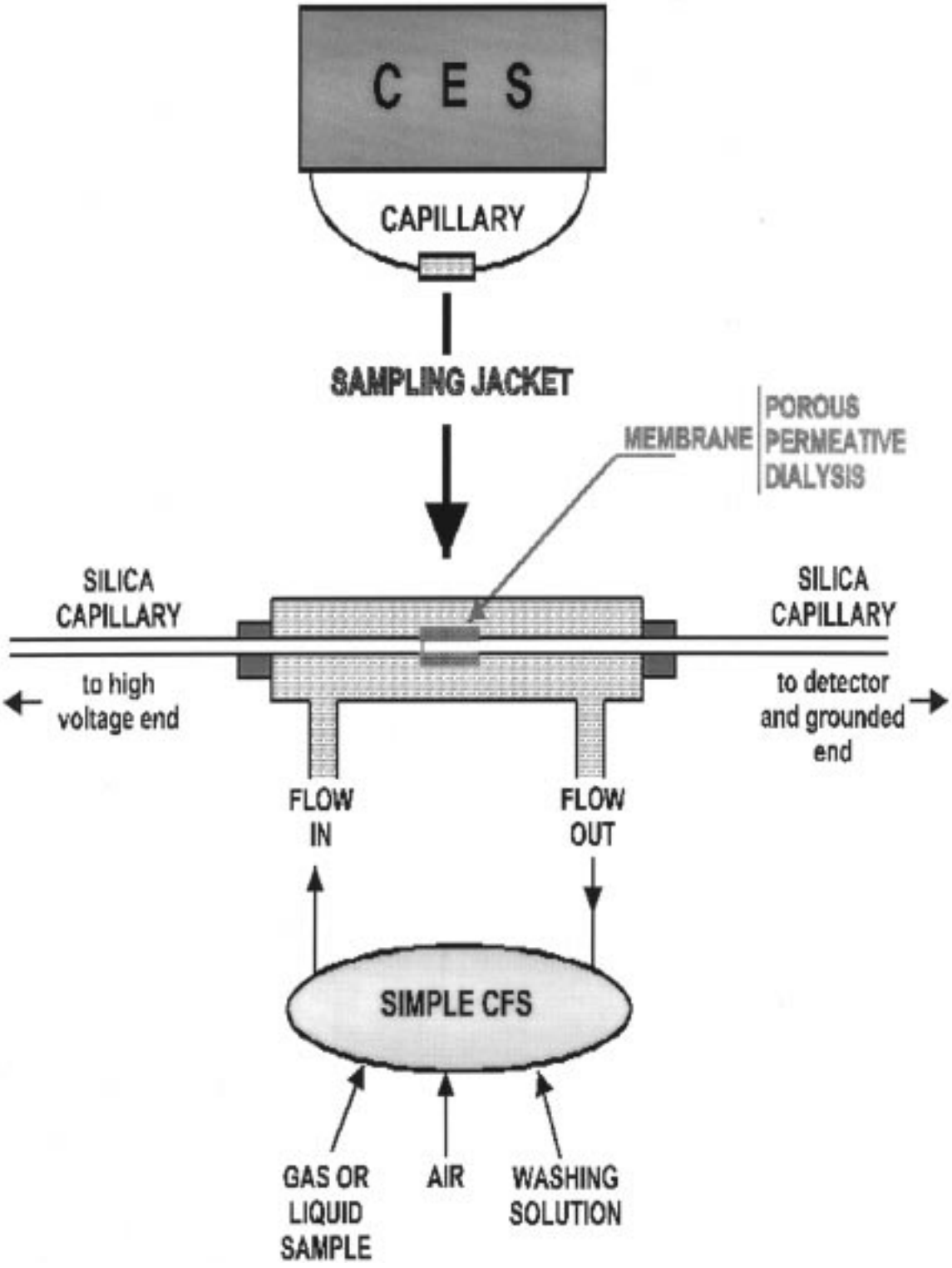

FIGURE 6. Low-pressure on line, CFS-CE coupling based on the insertion of a membrane in the center of the capillary of commercially available electrophoretic equipment. (For details, see text. Adapted from Reference 48. With permission from the American Chemical Society.)

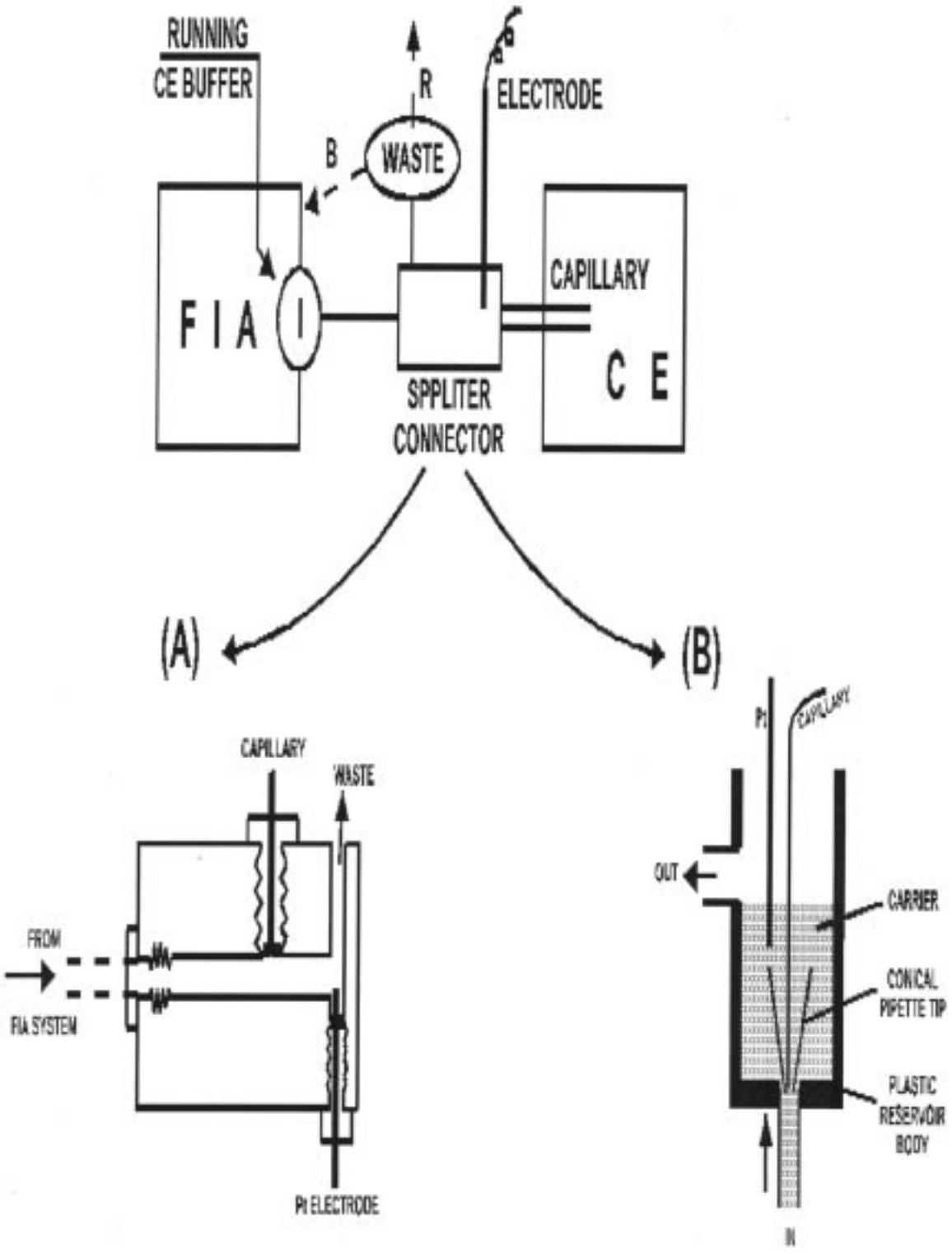

FIGURE 7. On-line FIA-CE coupling based on a mixed injector-flow splitter/connector interface based on a custom-made plastic body (A) or on an adapted plastic vial holder (B). I: Low-pressure injector. (For details, see text. Adapted from References 49 and 50. With permission from the American Chemical Society and Elsevier Science, respectively.)

of it and is sent to waste directly; a platinum electrode (anode) is located at the end of this cylinder. The CE capillary end is inserted normal to this central tube in a tee configuration. Only an infinitesimal portion of the injected sample plug is electrokinetic introduced into the capillary of a custom-made CE set-up. The same research group recently reported[50] an FIA dialysis unit coupled to CE via this type of interface. Liquid samples (acceptor stream) are continuously pumped into the dialyzer while the acceptor stream is allowed to fill the loop of a low-pressure rotary valve. A discrete volume of the acceptor stream is then injected into the CE running buffer, which continuously feeds the interface.

Recently, Fang et al.[51] developed an interface for on-line coupling of an FIA manifold (two pumps and an eight-channel injection valve) to the commercially available CE system depicted in Figure 7B. The interface is a manifold standard plastic vial holder of the autosampler of the CE instrument. A conical pipette tip is located on its bottom. Through it, the flowing stream from the FIA module enters and then exits through a lateral tube back connected to the FIA system in such a way that a constant liquid level is maintained in the vial. The CE capillary is positioned in front of the flowing stream in a jet configuration. A platinum electrode is also included in the interface. A small fraction of the sample plug is introduced into the CE capillary by electrokinetic injection. As in the previous approach, the outgoing FIA carrier acts as the CE running buffer.

In 1993, Ewing et al.[52] developed an innovative CE approach based on the implementation of continuous electrophoretic separations in narrow channels coupled to small-bore capillaries. This technique is carried out using a CE capillary which terminates at the entrance of a rectangular channel. The capillary end is slowly moved back and forth across the entrance to provide continuous transfer of the sample into the channel. Analytes are then separated inside the rectangular channel according to electrophoretic mobility and are detected using a laser-induced fluorescence scheme with two fiber-optic arrays coupled to a photo diode array for quantitation. Recently, the same research group reported a gravity-based FIA system connected on-line with the moving interface to monitor a dynamic chemical environment.[53]

A miniaturized LC mode based on electroosmotic flow (EOF) rather than on conventional high-pressure pumps has been developed and called Capillary Electrochromatography.[54] Dasgupta et al. used the same principle to develop miniaturized FIA systems based on EOF pumping, both with conventional hydrostatic injection at the capillary end[54] and by using a microinjector[55–56] for introducing samples and reagents. Although FIA and CE are combined in these approaches, the aim is absolutely different from that dealt with in this paper, namely, the use of an FIA system for sample preparation coupled to CE equipment.

VII. MIXED ON- LINE/OFF-LINE

Usage of the attributes "on-line" and "off-line" to describe analytical approaches is far from uniform. In some cases, "on-line" characteristic is incorrectly applied to a system in which one of the operations involved is implemented in a discrete way (see, for example, Ref. 47). Furthermore, while the most widely accepted meaning of "off-line" is "discrete", "discontinuous", it is frequently confused with "manually implemented": There are automated off-line operations in many analytical processes.

Thus, the mixed on-line/off-line coupling designates an interface connecting two continuous systems (CFS and CE) that operate in automated, discrete (off-line) manner relative to each other. This coupling, developed by our research group, is based on two types of interface: mechanical and electronic (Figure 8). The mechanical interface consists of a custom-made programmable arm and the autosampler included in commercially avail-

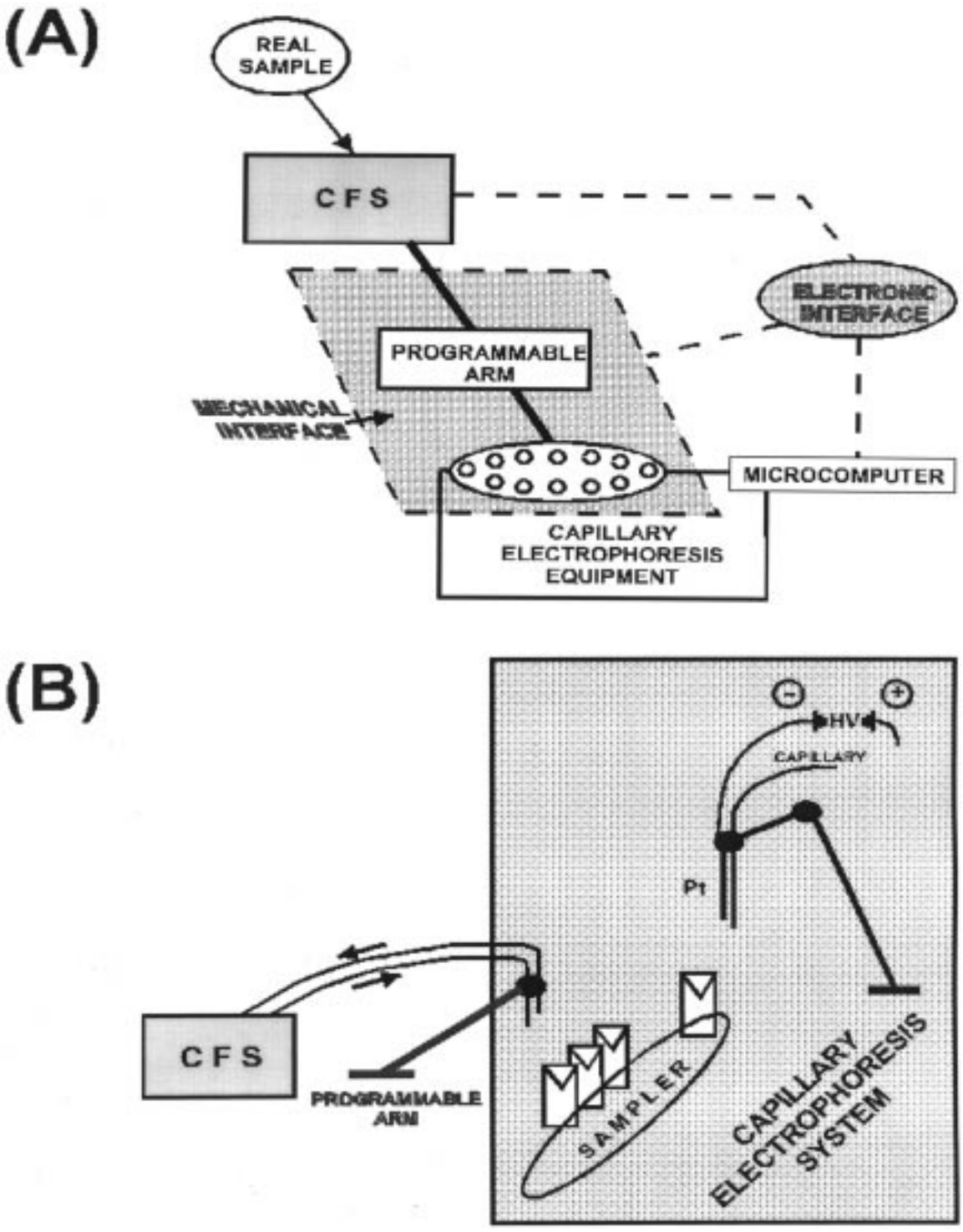

FIGURE 8. On-line/off-line automated coupling of CFS and commercially available CE equipment. (A) mechanical and electronic interfaces; (B) role of the two programmable arms. (For details, see text.)

able CE equipment. The functioning of the coupled elements (CFS, programmable arm, autosampler and electrophoretic system) is controlled by the built-in microprocesor of the instrument via an electronic interface and customized software. As shown in Figure 8B, a vial at two different positions in the autosampler establishes the automated off-line link between the CFS and the capillary end through two programmable arms. The custom-made arm is fitted with two injection needles of different length, both connected to the CFS. The longer one is used to fill the vial and the shorter one to drain it in order to maintain a preset liquid level. While the CFS is working, the needles are down; when at rest, the needles are up. The built-in programmable arm of the CE system having the capillary end and the electrode operates in a conventional way to perform both hydrodynamic and electrokinetic injection.

These approaches has been successfully applied to the direct introduction of both solid (e.g., soils,[57] meat products, tea) and liquid samples (e.g., wines,[58–59] waters[60]). The CFS functions to implement continuous separations such as leaching and SPE, and also to develop preliminary operations such as sample volume measurement, sample conditioning, and analyte derivatization. The fact that this approach is not a pure on-line procedure is offset by several advantages of great practical interest such as: (1) high flexibility that allows one to select the optimal operating conditions for each coupled system without the need to adopt the technical compromises typical of the hyphenated analytical methodologies; (2) minimization or avoidance of manually implemented pretreatment of samples which can be directly introduced into the coupled system; and (3) a broad scope of application for solving real analytical problems.

VIII. FINAL REMARKS

The impact of efficient sample treatment in CE is undeniable. Any attempt at improving the preliminary operations of an analytical process involving CE through automation, miniaturization, and simplification is of great relevance. The use of CFS, implemented by using LC or FIA components coupled to custom-built or commercially available CE equipment, opens up interesting prospects in this context. The principal aim of the coupling is to improve capital (accuracy, representativeness), basic (precision, sensitivity, selectivity), and accessory analytical properties (rapidity, cost-effectiveness, and personnel safety/comfort).

The crucial technical aspect of this symbiosis is the interface, which can connect the two systems in-line, on-line, or in both ways. Despite the interesting, innovative approaches developed so far, the need remains to systematically apply them to real samples in order to fully realize their practical potential. The best proof of their reliability is the increasing development by instrument manufacturers of automatic modules for ready coupling to the existing CE equipment in such a way that real samples can be directly processed with minimal or no pretreatment. This is one of the most important R&D lines to be followed in consolidating CE as a routine tool for analytical laboratories.

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