Electrochemical Detection in Capillary Electrophoresis

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Abstract

Recent advances in the design and application of electrochemical detection (EC) systems in capillary electrophoresis (CE) are reviewed, with the objective of providing the non-electrochemist with a state-of the-art picture of CEEC instrumentation and an overview of the primary analytes and samples for which the technique is best suited. In particular, instrument innovations designed to aid in decoupling the CE and EC systems electrically and in aligning them physically are described in detail. In addition, CEEC applications are summarized for four specific analyte groups: catecholamines, thiols and disulfides, amino acids, and carbohydrates. On this basis, it is clear that EC techniques have reached a stage where they are already having a significant impact on CE usage in selected areas of analysis. Continued developments with respect to new electrode materials and electrode configurations promise to broaden this impact further.

Keywords: Electrochemical detection, capillary electrophoresis,

Introduction

Since its introduction in the early 1980's, capillary electrophoresis (CE) has rapidly become an important instrumental technique suitable, often uniquely, for the separation and analysis of complex mixtures [1-3]. In carrying out such separations, CE's primary strength is its ability to provide extremely high separation efficiencies in short times and to do so with relatively simple instrumentation. These advantages are realized mainly because of the use of small diameter capillaries which enhance heat dissipation and thereby permit the use of high separation voltages. In addition, because the injection volumes required are only in the nanoliter to picoliter range, CE is well suited for the analysis of small amounts of material - which is especially important when only limited quantities of sample are available, such as in analyzing single cells [4]. Finally, the small capillary volume and low flow rates in CE are highly desirable from economic and environmental standpoints as exotic or expensive electrophoresis solutions can be employed at lower cost and with fewer disposal problems than in HPLC.

In order to realize these advantages in practice, detection systems must be available which are both highly sensitive and fully compatible with the small physical scale of CE capillaries. UV visible absorption, which is clearly the most widely used detection approach

in HPLC and would have many attractive features for CE, is restricted in its sensitivity in CE by the short optical pathlengths afforded by the small inside diameters (ID) of the capillaries. Additionally, many interesting analytes do not absorb strongly or at long enough wavelengths and therefore are good candidates for UV-visible detection only after derivatization. Laser-induced fluorescence is currently the CE detection method of choice because of its higher sensitivity but is limited in its applicability to only those analytes possessing strongly fluorescing functionalities. Of course, nonfluorescing compounds may be derivatized with active fluorophores, but such procedures add complexity to the CE analysis and compromise the small-volume capabilities of the technique. Powerful mass spectrometric methods, usually making use of electrospray ionization, have recently begun to be developed for CE detection and undoubtedly will have a significant impact in the future.

Over the past 25 years, electrochemical detection (EC) methods have been developed extensively for use in HPLC and have become well accepted for their ability to provide sensitivity at the pmol level for analytes that are easily oxidized or reduced and selectivity often complementary to that obtained with optically based techniques. However, EC detection was first applied to CE (thus the term CEEC) only in 1987 by Ewing's group [5,6] who began to investigate CEEC for the determination of catecholamine neurotransmitters in individual cells. In view of the fact that these compounds are the prototypical analytes in liquid chromatography with electrochemical detection (LCEC), it was not surprising that they should be the initial focus of CEEC efforts. Subsequently, dozens of papers have appeared both on instrumental developments in CEEC and on a wide range of CEEC applications [7-9]. First and foremost, these studies have served to confirm that the sensitivity and selectivity characteristics long known for EC detection in HPLC are largely transferable into the CE domain. However, it turns out that, in several respects, EC techniques are even better suited for CE than for HPLC. For example, the small scale inherent to CE presents no fundamental problem for EC, as it is relatively straightforward to construct microelectrodes possessing essentially the same 5-100 μm dimensions as CE capillaries. Furthermore, unlike optical detection methods, the performance of EC systems is not compromised by miniaturization as the small currents associated with the microelectrodes used are easily and accurately measured and noise generated at the electrode surface actually decreases with electrode area as fast or faster than does the signal. Finally, EC instrumentation is, as always, relatively inexpensive - especially compared to laser-based fluorescence or mass spectroscopy systems.

Despite these attractions, there are several unique concerns about EC detection that has prevented this approach from becoming a "mainstream" technique widely embraced by electrochemists and non-electrochemists alike. First, there is the question of how the small EC detection potentials and currents are influenced by, and can be shielded or "decoupled" from, the much larger CE voltage and current. Second, the issue of capillary/electrode alignment must be addressed in a manner that provides acceptable analytical performance to be achieved without making the CEEC experiment an unduly complex or burdensome operation to perform. The problem here is that the microelectrode must be placed very close to the capillary opening to ensure maximum interaction with the analyte species and maximum sensitivity. Furthermore, this alignment must be able to be maintained over the course of several experiments and to be reproduced on a daily basis if CEEC is to provide useful and attractive quantitative analysis capabilities. Finally, a critical mass of analytically significant applications must be developed for CEEC. This will require that CEEC-compatible electrodes can be constructed from a variety of different materials in addition to the electrochemist's conventional choice of carbon. This includes noble and transition metal electrodes that often respond to a wider range of analytes than carbon as well as chemically modified electrodes (CMEs) which can be designed to target specific analyte compounds or families.

In most of the CEEC work reported to date, the EC detection has been carried out amperometrically - i.e., by controlling the potential applied to the sensing or working electrode and measuring the faradaic current flowing as a result of analyte oxidation or reduction at this potential. In these experiments, the applied potential is usually held at a constant value throughout the CE experiment; but in specific instances, it can either be scanned continuously over a defined potential window (voltammetric detection) or jumped discontinuously from one potential to another (pulsed amperometric detection). In addition to amperometry, there are also EC detection modes based on potentiometric [10] or conductimetric [11] measurement principles. These detection modes will not be considered here. Rather, we will review recent progress that has been made in amperometric EC detection in each of the three focus areas mentioned above: methods for decoupling CE voltage and current from the EC detection system, capillary/electrode alignment methods, and CEEC electrodes and applications. In addition, we will also briefly comment on a few new and exciting directions that CEEC research is taking. These include the use of new electrode materials, the development of dual electrode systems, and the development of miniaturized CEEC systems.

CEEC instrumentation

In general, EC detection imposes few special restrictions on the design and construction of the CE instrument. The typical CEEC layout, shown in **(Figure 1)**, consists of three principal components: the high voltage CE power supply, the capillary itself, and the EC detection unit, which includes both the sensing electrodes and the associated electronics for potential control and current measurement. Except for the electrodes and the elements introduced for their decoupling and alignment, none of the equipment is different from that used in other CE and EC instrumentation. The EC electronics is simply a potentiostat similar to those developed earlier for use in HPLC and available commercially from a number of vendors. Of course, because of the small size of the working electrodes employed in CEEC, the potentiostat must be able to measure currents in the 10-100 pA range - which is somewhat smaller than what is encountered in analogous HPLC experiments.
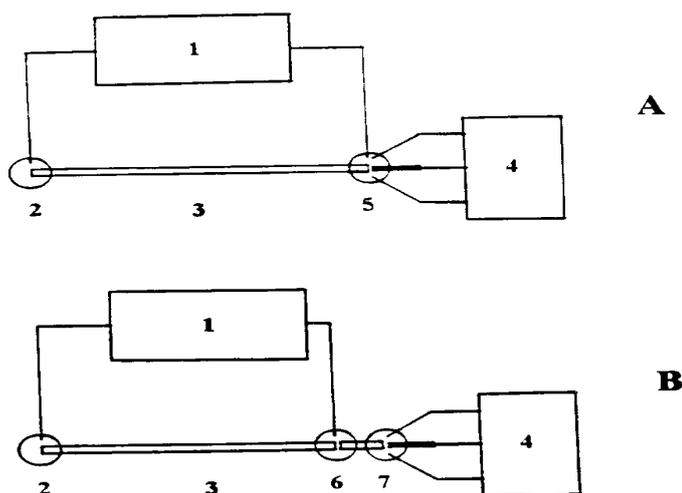

Figure 1. CEEC systems: (A) end-column configuration. (B) off column configuration. (1) High voltage power supply, (2) buffer reservoir, (3) fused silica capillary, (4) potentiostat, (5) cell for end-column electrochemical detection (includes CE cathode. capillary outlet, and working, reference, and auxiliary electrodes), (6) CE anode reservoir for decoupled system (includes CE cathode, separation capillary outlet, and detection capillary inlet), and (7) cell for decoupled electrochemical detection (includes detection capillary outlet and working, reference, and auxiliary electrodes).

The two instrument design elements that are unique to CEEC are those involving capillary/electrode decoupling and alignment. As both of these represent active research areas critical to the development of CEEC, each will be considered in detail below. Before beginning this discussion, it is advisable to define clearly the nomenclature that has evolved to describe the various CEEC detector configurations and, especially, the formats employed for decoupling and alignment purposes. The terminology recently suggested by Zhong and Lunte [12] is consistent with past practices and seems to be the most attractive for this purpose. In this system, the terms "end-column" and "of-column" are used to specify the respective absence and presence of a decoupler. At the same time, a second term - e.g., "on-capillary", "in-capillary", "wall jet", etc. - must be included in order to specify the placement of the electrode vis-a-vis the capillary outlet.

Capillary /electrode decoupling

Successful CEEC requires the accurate maintenance of EC potentials on the order of 1 V or less at sensing electrodes placed in CE fields of 5-30 kV and the recording of EC currents typically pA in magnitude in the presence of pA-level background CE currents. In some cases, it is possible to accomplish this acceptably without specific decoupling measures by simply placing the CE electrodes in solution near the end of the capillary (i.e., end-column format) as illustrated in (Figure lA). Many circumstances require the incorporation of active decoupling measures to isolate the CE and EC functions electrically as in (Figure 1B). In the various forms that these off-column configurations can take, the EC electrodes are still located at the physical end of the capillary; but the CE voltage and current are actually applied before the capillary exit. Some of the most useful of these systems are shown in (**Figure 2**).
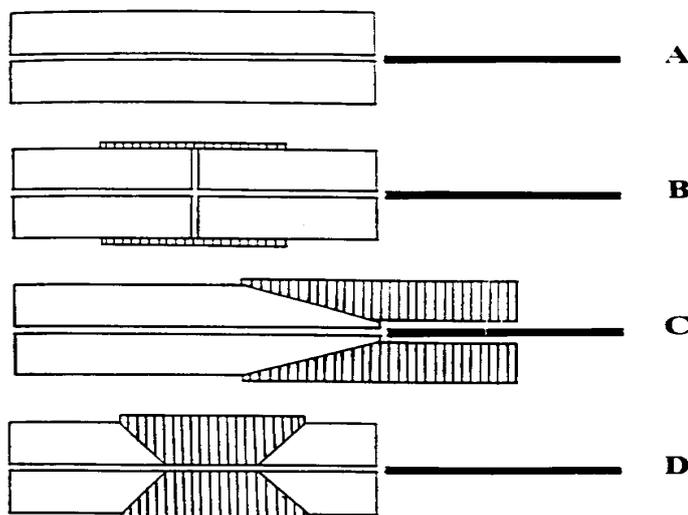

Figure 2. CE decoupling methods: (A) end-column (no decoupling), (B) covered fracture (porous glass, Nafion tubing, Nafion film, etc.), (C) cast Nafion end-column decoupler, and (D) long cast cellulose acetate or Nafion decoupler. The shaded areas represent the actual decoupling elements.

Off-column detection (detection using capillary fractures)

Most CEEC applications reported to date have utilized an off- column detector design. In most cases, the decoupling of the CE and EC systems is accomplished by creating a small opening or fracture in the capillary wall ca. 1 cm before the exit end as shown in Fig. 2B. This

effectively divides the capillary into two sections: a "separation capillary" before the fracture and a "detection capillary" beyond it. As long as the fracture is sufficiently tight, there can be some ion transport and electrical conduction through it without allowing bulk flow of the sample or electrophoresis medium through the capillary to be affected. If the fracture is then immersed in an electrolyte solution where the high voltage CE electrode is also located, the CE voltage and current are dropped across the capillary only up to the point of the fracture and downstream EC electrodes placed at the physical end of the capillary are isolated electrically. Typically, the fracture is created by scoring the capillary with a capillary cutter, covering the scratch with a porous coating to inhibit bulk flow and maintain the physical integrity of the capillary, and then bending the capillary to complete the break. In some cases, connection to the CE electrode is improved by placing the electrode lead under the coating before sealing it around the fracture.

The principal difference in the various decouplers reported thus far resides in the nature of the material used to form the conductive coating on top of the fracture site. In the initial CEEC system of Wallingford and Ewing [8], a porous glass capillary was employed for this purpose. With this material, good separation and detection performance was realized. However, the porous lass used proved difficult to obtain commercially; the fabrication process was painstaking and required a great deal of practice: and the resulting decouplers were fragile and difficult to work with. Subsequently, a number of alternative materials have been employed in place of the porous glass: Nafion tubing [13], cast Nafion films [l4], Teflon tubing [15], Teflon with porous graphite [16], Nafion with porous glass [17], cellulose acetate [18], and palladium tubing [19]. Currently, Nafion appears to be the material of most frequent choice for decoupler use due to its ready availability and reasonably high conductivity. Furthermore, Nafion films can be cast onto any size capillary; and their flexibility lends good physical stability to the capillary joint. Either Nafion tubing of appropriate size can be placed over the decoupling site and sealed in place with epoxy before formation of the fracture [13], or a thin Nafion film can be produced by coating the fracture site with several layers of a Nafion-containing solution that is allowed to cure before use [14].

Typically, such fracture-based decouplers rely on an opening only a few μm wide for making the CE connection and are able to provide good noise suppression only for relatively small CE currents. To improve current shunting capabilities beyond this, the Lunte groups have developed much longer cellulose acetate [20] and Nafion [21] decoupling systems. This was done by inserting the separation and detection capillaries onto a metal wire, forming a thick cellulose acetate or Nafion film over the gap between them, and then removing the support wire. In this manner, it was possible to fabricate 2 mm long decoupling elements (see Fig. 2D) which did not degrade CE efficiency unacceptably but were able to maintain low EC noise levels while shunting CE currents of up to 30 μA. An interesting offshoot of this design was that the CE buffer reservoir created with the longer decoupling joint could also be used as a mixing chamber to optimize the composition of the CE medium for the EC detection that followed. For example, by filling the Nafion decoupler with 1N HCl, the acidity of the pH 6.1 phosphate buffer that gave the best separation of the phenolic acid analytes was reduced on-line to pH 1.8 where the EC detection of these compounds could be carried out much more sensitively [21]. This is potentially a very useful capability as the solution conditions optimum for EC detection is often different from those that give the best CE separation.

The same approach making use of a Nafion film cast over a tungsten wire has also been employed to prepare a decoupler that was actually located at the end of the separation capillary [22]. With this arrangement (see Fig. 2C), the detection capillary common to other decoupling schemes could be omitted. This allowed the favorable noise characteristics of the

longer decoupler to be obtained without decreased separation efficiency resulting from the (laminar flow patterns that normally occur in the detection capillary.

End-column detection (non-decoupled systems)

Regardless of the specific configuration employed, the use of decouplers adds significantly to the complexity of setting up the CEEC instrument. Their fabrication requires considerable time and manipulative skill on the part of the experimenter while, in use, there can be concerns about their fragility and the possibility that they may compromise the separation efficiency obtained. Of course, different decoupler designs may suffer these problems too much different extents. However, it is safe to say that the need for decouplers adds little to the attractiveness of EC as a detection mode for CE.

The alternative - end-column detection (illustrated in Fig. 2A) - was first suggested in 1991 by Huang et al. [23] who showed that a 10 μm carbon fiber electrode placed at the end of a 5 μm ID capillary provided sensitive detection of catechol compounds without substantial noise from the CE high voltage. Subsequently, numerous studies employing a variety of electrodes and analyte species have used end-column detection successfully. The common feature in all the end-column applications is the use of relatively small bore capillaries, usually 25 μm ID or smaller. The idea is that, as the capillary ID is decreased, its electrical resistance increases rapidly. As a consequence, the background current associated with the CE separation decreases proportionately; and the potential field due to the CE voltage drops of more rapidly at the capillary exit. By far the most thorough study of these effects has been carried out by Lu and Cassidy [15] whose survey of several different electrode materials, electrolyte compositions, electrode and capillary sizes showed little extension of the CE field into solution and negligible effect on EC noise for capillaries possessing C 25 μm ID. In the many CE cases, which are compatible with the use of such capillaries, end-column detection represents an effective and easily implemented approach to CEEC.

Capillary/electrode alignment

In order to achieve optimum detection sensitivity in CEEC, it is essential that the capillary outlet and the detection electrode are aligned properly in solution and that this alignment is maintained over the entire analysis period. In view of the fact that the ID of the capillaries used in CE ranges from 5 to 100 μm and the electrodes that have been most often employed in CEEC are carbon fibers or metal wires with diameters of 10 to 50 μm, this requirement is not a trivial one. Typically, the alignment procedure begins with mounting the electrodes on a x,y,z-micropositioner that is used to move the electrode tip as closely as possible to the capillary outlet. The positioning is first monitored visually through a microscope and then further optimized by successively injecting the analyte while systematically varying the electrode position until the placement that produces the greatest current response is identified. Alignment by this approach is time-consuming, difficult for the beginner to master, and difficult to reproduce faithfully on a daily basis. Furthermore, once the initial positioning has been established, any drift of the capillary or electrode over time degrades the alignment and thus lowers the EC currents obtained and decreases their reproducibility. Because of these difficulties, a number of specific alignment strategies have been devised and demonstrated. These have been shown both to simplify the operations involved in establishing the optimum alignment initially and to improve the stability and reproducibility with which it can be maintained. The principal of these approaches are illustrated in **Figure 3** and discussed individually below.
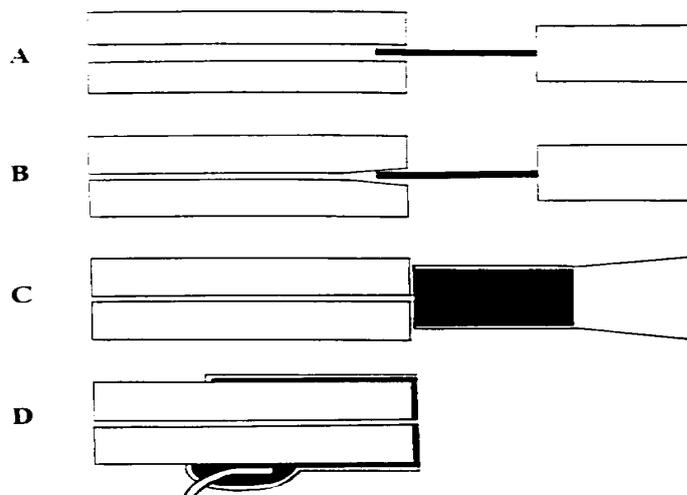
Figure 3. Alignment methods used in CEEC: (A) in-capillary (ICE), (B) optimized in-capillary (O-ICE), (C) wall-jet (WJE) and (D) on-capillary (OCE) configurations.

In-capillary electrodes (electrode insertion)

One of the principal methods employed to alleviate alignment difficulties involves the physical insertion of the EC electrode a short distance into the capillary opening to create what has been termed an "in-capillary" electrode (ICE) configuration. This was in fact the method employed by Wallingford and Ewing [5] in their initial report on CEEC and is accomplished, much as described above, by use of a microscope and a micropositioner. Thus, this approach still involves an exacting and tedious set-up procedure prior to the CEEC experiment. However, once the electrode is successfully placed inside the capillary, the alignment is more or less fixed; and system stability is markedly improved - with uncertainties of 5 % or less typically reported for repeated injections for a given capillary/electrode set-up. Obviously, the electrode must be smaller than the ID of the capillary for this approach to be feasible. Although electrodes as small as 1 µm have been used with 2 µm ID capillaries [6], this requirement generally limits the use of this method to larger capillaries with IDs of at least 50 µm. Furthermore, electrode insertion works well only in decoupled CE systems where the electric field due to the separation voltage has already been terminated and the capillary tip represents a field-free region.

A simple modification of the in-capillary electrode concept, termed "optimized end-column detection", was introduced by Sloss and Ewing [24] in order to address these limitations. In this approach, the fused silica capillary is chemically etched at the detection end by brief exposure to a 50% HF/H_2O solution. This serves to enlarge the capillary opening somewhat (e.g., to 20 µm for a 2 µm ID capillary) and thereby make electrode insertion possible even for a narrow capillary. Also, because of the decreased resistance provided by the larger opening, the electrode is no longer affected significantly by the CE voltage and end-column detection without decoupling is possible.

Wall-jet electrodes

Normally in CEEC, successful alignment depends on the exact placement, relative to one another, of two very small objects, the capillary opening and a microelectrode surface,

both of which are difficult to see clearly with the naked eye. The wall jet electrode (WJE) approach attempts to simplify this task by making one of the objects, the electrode, much larger. This is accomplished by using a flat, disk-shaped electrode in place of the cylindrically shaped wire or fiber microelectrode usually employed in CEEC. As with the microelectrodes above, the larger electrode is aligned with the capillary with the aid of a micropositioning device. However, this operation in practice is only slightly more involved than simply pushing the flat tip of the wall jet electrode up against the capillary outlet and checking the placement by a test injection of analyte. With this design, the electrode is large enough to be seen and worked with relatively easily; and electrode placement with respect to the capillary is far less critical and easier to reproduce over the course of an extended series of CE experiments.

The initial wall jet CEEC system, reported by Ye and Baldwin [25], consisted of a 127 μm diameter Cu disk (actually a 127 μm thick Cu wire whose sides were coated with an insulator so that only the tip was exposed to solution) used in conjunction with a 25 μm ID capillary. Compared to a 300-400 μm long, 25 μm diameter Cu wire electrode (not inserted into the capillary), the WJE gave a slightly better detection limit with only a slight decrease in separation efficiency. Interestingly, the improved detection limits seen with the wall jet configuration was attributed to the fact that the disk electrode actually had a smaller active surface area than the microwire. More important, the WJE system proved much easier to position reproducibly than the other approach. These advantages have been verified in a number of subsequent wall jet CEEC systems employing different electrode materials and capillary/electrode combinations ranging from a 75 μm capillary with a 500 μm electrode [26] to a 13 μm capillary paired with a 90 μm electrode [27].

Capillary/electrode holders

Ideally, it would be desirable to be able to achieve optimum capillary/electrode alignment without any reliance on micropositioners or microscopes. One way to accomplish this is the use of a support apparatus specifically constructed to hold the two elements in place. Thus far, several such alignment devices have been reported [27-30]. In all cases the electrodes are diskshaped, fabricated by sealing a metal wire or carbon fiber into an empty glass capillary of the same dimensions as the separation capillary, and are operated in a wall-jet format. The design suggested by Fermier, Gostkowski, and Colon [27] is especially straightforward as it consists simply of two Plexiglas plates, one of which has been machined to contain a small groove just the right size to accommodate both the capillary and the electrode. Once both of these are inserted into the groove and spaced as desired, the two plates are screwed together to hold everything in place. Reproducible results were obtained not only for repetitive injections (relative standard deviations of 5 %), but also for repeated experiments in which the holder was taken apart and reassembled between injections (8 %).

On-capillary electrodes

The obvious way to avoid alignment issues completely is to incorporate both the capillary and the electrode into a single integrated unit. Very recently, two reports describing fabrication methods for such "on-capillary" electrodes (OCEs) have appeared. In the first, Zhong and Lunte [12] demonstrated the concept by mounting a 25 μm diameter Au wire across the outlet of a 50 μm ID capillary and analyzing catecholamine and glucosamine samples. This was done simply by gluing one end of the wire onto one side of the capillary tip, bending the wire across the capillary opening, and then gluing the other end of the wire to

the other side of the capillary. This arrangement succeeded in stabilizing the electrode alignment while maintaining analytical performance that was comparable to that obtained with other alignment designs. Furthermore, the approach could obviously be extended to fiber or wire electrodes made from materials other than Au. Although a degree of manipulative skill on the part of the experimenter, including some reliance on microscopes and micropositioners, is still required for initial construction of this OCE, the system proved quite rugged in operation, with the useful life of the OCE normally outlasting that of the capillary itself.

Subsequently, Voegel and Baldwin [31] reported a simple and flexible scheme for OCE fabrication. In their approach, a thin conductive Au or Pt coating was deposited on the exit end of the capillary with ordinary metal sputtering equipment, such as that commonly used in electron microscopy and integrated circuit laboratories. The resulting OCEs, operated in the end-column format, gave micromolar detection limits for the test analytes dopamine and catechol and delivered relative standard deviations of only 2-4% for repeated injections even if removed from solution between runs. A real advantage of this approach is that it can be adapted to make a batch of OCEs simultaneously (so far, as many as 21 at one time). Further, the method can be used to create a wide range of different OCEs, either by sputter-coating different metals or by altering the Au and Pt coatings by conventional electrode modification schemes. Examples cited included the formation of an OCE for carbohydrates by electrodeposition of Cu onto Au and the formation of an enzyme OCE for glucose by incorporation of glucose oxidase onto Pt. In a separate study [32], an OCE formed by direct sputtering of Cu was shown to give comparable detection capabilities to wall-jet CEEC systems for the determination of aminoglycoside antibiotics.

CEEC applications

The recent advances in CEEC instrumentation that have made decoupling and alignment operations easier and more effective represent significant developments not so much because of the degree to which they have increased the analytical performance of CEEC equipment but because of their ability to make the technique easier to perform and more accessible to the non-specialist. Accompanying these advances in instrumentation, and also greatly needed for the popularization of CEEC, has been the development of CEEC applications that include a diverse range of important analytes and high light the unique capabilities of EC detection. In this section, we review some of the more interesting of the body of applications that have been recently been reported for CEEC. The discussion below is organized on the basis of the primary analyte groups for which successful CEEC assays have been demonstrated catechol compounds, thiols and disulfides, amino acids, and carbohydrates. Of course, a central issue in the development of any such application is the choice of electrode material carbon, a metal such as Pt, Au, or Cu, or a chemically modified surface specifically designed for the analyte of interest. This selection, which can be a deciding factor in determining CE separation conditions as well as detector design, will therefore be a principal focus of the discussion. The analytical performance obtained in different studies, even with the same analyte and electrode, is extremely difficult to compare directly because of slight but meaningful differences in operating conditions - capillary size, injection volume, applied potential, pH, etc. Consequently, quantitative performance characteristics such as limits of detection will not be emphasized in the discussion.

CEEC of catchall compounds

The catecholamine family of neurotransmitters dopamine, epinephrine, norepinephrine, and catechol have been the analytes most frequently studied by LCEC over the past 20-30 years. Therefore, it is not surprising that these compounds were the first ones on which CEEC efforts were focused 10 years ago and that they remain one of the groups most frequently studied by CEEC today. The reasons for this are related both to the important physiological role of these compounds in the transmission of nerve impulses and to the fact that, by virtue of their facile oxidation at low potentials, they exhibit nearly ideal electrochemical properties for detection purposes.

The electrode material of choice for catechol in LCEC has traditionally been carbon, and this preference has continued in most CEEC examinations of these compounds. Although other electrode materials such as Pt and Au have been employed for catechol analysis, carbon's low background and high stability and resistance to passivation make it clearly the best selection. Detection limits cited with these electrodes have typically been in the 0.01-1 μM range. Furthermore, CE separations of the catechols have generally not been difficult to achieve. Common separation buffers such as pH 6 sodium phosphate or morphilinoethanesulfonic acids have been shown to provide excellent separation of most catechols and catecholamines. Also, micellar kinetic chromatography has been shown to give good separation of many neutral catechol compounds.

In view of this, it is somewhat surprising that, of all the CEEC/catecholamine studies relatively few have focused on in vivo analysis situations. Early on, Ewing's group reported the application of CEEC with carbon fiber microelectrodes to the analysis of snail cell cytoplasm for serotonin, dopamine, and DOPAC [4, 6, 33]. Injections made directly from the cell interior were shown to contain up to μM levels of these compounds. More recently, Hadwieer et al. [34] used a similar electrode set-up to obtain pharmacokinetic data on the catecholamine isoproterenol after intravenous administration of the drug to rats. Samples obtained by microdialysis were analyzed for both isoproterenol enantiomers, which could be separated in a cyclodextrin-containing CE buffer.

CEEC of thiols and disulfides

As in LCEC, sulfur-containing compounds - in particular, thiols and disulfides - have also been a priority target for CEEC analysis. The most intensely investigated of these compounds have been the amino acids cysteine and cystine and the peptides glutathione and glutathione disulfide whose levels in blood are of physiological interest and which have no strongly absorbing or fluorescing functional groups to make optical detection an attractive option. Unlike the catechols, thiols undergo oxidation at carbon electrodes only at potentials too positive to be of much analytical utility; and disulfides generally are not oxidized at all at carbon. Accordingly, much of the effort in devising effective CEEC methods for these compounds has been directed toward the development of alternative electrode systems.

In the initial CEEC work with thiols, O'Shea and Lunte [35] employed an Au/Hg amalgam microelectrode, prepared by dip-coating Hg onto a 50 μm diameter Au wire, to carry out the determination of cysteine and glutathione as well as the thiol-containing pharmaceuticals penacillamine, captopril, and 6-mercaptopurine. Detection at this electrode, which actually consists of the catalytic oxidation of the mercury surface in the presence of thiols, can be performed at applied potentials as low as +0.1 V vs. Ag/AgCl, and, as a result, was both very sensitive and very selective. Additionally, the Au/Hg electrodes are relatively easy to fabricate initially and to regenerate, when necessary, later on.

Unfortunately, disulfides do not react with Hg in a similar fashion to thiols; and detection of these compounds was not possible with a simple Au/Hg electrode system. To circumvent this problem, Lin, Colon, and Zare [36] utilized a dual electrode arrangement in which a second Au/Hg wire was inserted into the capillary upstream from the first through a hole drilled between the decoupler and the capillary exit. In this configuration, the upstream electrode was held at a negative potential sufficient to reduce cystine to cysteine, which could then be detected, as above, at the downstream electrode. With this scheme, cysteine was detected down to the 5 μM level; but because of the low conversion efficiency of the cystine reduction, this compound could be detected only down to a 100 μM concentration.

Two chemically modified electrode (CME) systems have also been reported for CEEC of thiols and related compounds. The first consisted of a carbon fiber bundle whose surface was coated with a mixed-valent ruthenium cyanide film [37]. The resulting CME was capable of oxidizing both thiols and disulfides electrocatalytically and therefore permitted the detection of both species at a single electrode. Unfortunately, the activity of this electrode decreased rapidly under the solution conditions needed to achieve acceptable CE separations. In a subsequent study [20], the electrode stability was increased significantly by insertion of a cellulose acetate mixing chamber into the capillary just before the detector. With this approach, an ideal separation was obtained by using a pH 7 run buffer whose pH and electrolyte composition were then adjusted in the mixer to levels that were more compatible with the EC electrode. The second CME system, reported separately by O'Shea and Lunte [38] and by Huang and Kok [39], employed a different electrocatalyst, cobalt phthalocyanine (CoPc). Slightly different electrode fabrication strategies were used by the two groups (CoPc in carbon paste vs. CoPc in a conductive carbon cement), but similar detection capabilities were obtained. Because CoPc catalyzes the oxidation of thiols only, detection was possible only for these species and not for the disulfides. However, both short-term reproducibility and long-term stability of these CEEC systems were excellent.

CEEC of amino acids

As a group, amino acids are not readily oxidized or reduced at carbon electrodes and are often classified as electroinactive. The exceptions to this classification are tyrosine and tryptophan, which do undergo electro-oxidation at accessible potentials at carbon and other electrodes. This characteristic has not, in general, been taken advantage of in CEEC, although Lunte's group has demonstrated that direct oxidation at carbon fiber electrodes can be used to detect leucine and methionine enkephalins [20], both of which are pentapeptides that contain a tyrosine residue, and to follow the metabolic breakdown of tryptophan administered to rats [40]. Rather, amino acid detection in CEEC has relied on three other approaches: indirect detection at carbon fiber electrodes, detection of electroactive derivatives at carbon electrodes, and direct detection by oxidation at metallic electrodes.

The two different pre-column procedures for amino acids that have been reported to date involve reaction to form either cyano[f]benzoisoindole [13, 41] or dinitrophenyl [42] derivatives. Detectability of both of these derivatives varied somewhat from one amino acid to another, and the operations involved in derivative formation both complicate the analysis operation and increase the amount of sample required to more than the nanoliter level demanded by CE itself. Nevertheless, this methodology was used to analyze samples collected from rat brains for alanine, glutamate, and aspartate [13, 41]. One on-column derivatization procedure reported by Lunte's group involves the formation of electroactive Cu(II) complexes of small peptides upon addition of CuSO, to the CE buffer [43]. This

process, which is similar to the biuret reaction, was useful primarily for polyglycine peptides containing 2-5 amino acid units with other polypeptides giving a much reduced response.

The most promising detection of amino acid compounds in CEEC is provided by the use of metallic Cu electrodes [29, 44-46] which have made possible the direct detection of these analytes with no need for derivatization and only modest sample treatment requirements. Two different electrode mechanisms can be involved in these detection schemes, depending on the solution conditions in effect. In moderately alkaline phosphate buffers, the oxidation occurs by complexation of Cu surface species by the amino acids [44] while, in strongly basic NaOH media, the amino acids appear to be oxidized electrocatalytically [29, 45, 46]. In both cases, all amino acids can be detected - often at or below the μM level. Most important, CEEC at Cu electrodes was shown in these studies to work well for amino acids and small peptides in a variety of sample matrices of practical interest.

CEEC of carbohydrates

Like the catecholamines, carbohydrate compounds have been one of the groups that have received a great deal of attention from CEEC practitioners. This is due to the enormous importance of these compounds, to their poor compatibility with optical-based detection techniques, and to the development and use of metallic electrodes, which are capable of carrying out their oxidation directly at low potentials. Because carbohydrates are not electroactive at carbon, it has been essential to follow the lead of earlier LCEC studies and employ alternative electrode materials such as Au, Cu, and Ni. A feature which all of these metal-based electrode systems share is that they function well for carbohydrates only in high pH conditions - typically 0.1 M NaOH. Although this requirement is a nuisance for LCEC, it actually is quite convenient for CEEC as such strongly basic solutions serve to deprotonate the carbohydrates, which otherwise would be uncharged and have no electrophoretic mobility of their own [47].

With Au electrodes, a pulsed amperometric detection (PAD) mode is required to obtain stable, reproducible results [14, 48, 49]. This avoids the fouling of the electrode surface by carbohydrate oxidation products that occurs with constant potential operation. With Cu and also Ni electrodes, no potential pulsing is required [25, 27, 50, 51]. The advantages of this operating mode are that simpler instrumentation is required and that improved noise characteristics and lower detectability should result. Each of these electrodes, especially Au and Cu, has been used to determine a variety of different carbohydrates in a variety of different kinds of samples. It is notable that not only are simple carbohydrates detected well by EC at these metal electrodes but also oligo- and polysaccharides, carbohydrate derivatives such as alditols, sugar acids, and amino sugars, and carbohydrate conjugates such as glycopeptides and nucleotides. In these cases, the coupling of the high efficiency CE separation with an EC detector of broad selectivity has proven to be ideal. For example, Ye and Baldwin [52] showed that related carbohydrate compounds - e.g., glucose, glucitol, and gluconic, glucaric, and glucuronic acids - are ionized to different degrees in the strongly basic media used and can be easily resolved by CE. Subsequently, Zhou and Baldwin [53] demonstrated the CEEC separation of complex polysaccharide mixtures including maltoses up to maltoheptaose, enzymatically hydrolyzed starch, and M_r 18 000 dextrans.

Some of the more challenging samples, which have been analyzed for carbohydrate content, include human blood and urine. For instance, glucose in blood has been determined by PAD at an Au electrode by O'Shea, Lunte, and LaCourse [14] and by Roberts and Johnson [49]. Very recently, Hong and Baldwin [54] showed that the CEEC response at Cu electrodes

could be used to profile several clinically important metabolic markers in urine. The specific compounds quantitated included glucose, galactose, creatinine, and uric acid; but numerous other carbohydrates, alditols, and amino acids might be profiled by this method as well. Finally, Weber et al. [55] and Weber and Lunte [56] demonstrated several applications of PAD at Au electrodes for the characterization of glycopeptides produced by enzymatic cleavage of glycoproteins. These studies, which made use of the special selectivity of the Au electrode to identify the carbohydrate- containing cleavage products, represent the first successful foray of CEEC into the structural determination of biological macromolecules.

Concluding remarks

From the above discussion, it is clear that EC detection techniques have developed to the stage where they are having a significant impact on CE usage, especially for the determination of analytes that are not well-suited for optically based detection techniques. Nevertheless, CEEC is still not a commercially available option from CE manufacturers and is still the domain primarily of specialists in electrochemistry. Some of the most important factors which have previously discouraged non-electrochemists from more rapidly embracing the EC technique have begun to be satisfactorily addressed by simple but timely innovations in CEEC instrumentation. For example, there has been the development of rugged and easy-to-fabricate decouplers and the increased usage of small ID capillary systems, which avoid decoupling altogether. Likewise, innovations such as on-capillary electrodes, which render obsolete the concept of alignment by microscope and micropositioner, allow one to envision the mass production and sale of capillaries with EC electrodes already on board and ready for use.

If a brief look ahead into the short-term future of CEEC is permitted, several areas in which the next generation of innovations can be expected are relatively easy to predict. First and foremost, one can expect to see a continuation in the application of new electrodes and electrode materials in CEEC. Already, there has been a progression from carbon electrodes to Au and Cu and, most recently, to various CMEs. This trend has culminated in the first reports of CEEC with enzyme-modified electrodes similar to those used in biosensors: electrodes modified with glucose oxidase for glucose detection [31, 45] and with amino acid oxidase for amino acid detection [20]. Second, one should expect to see an increased sophistication in the physical design of CEEC electrodes. While such design improvements in the past have been directed largely toward minimizing alignment diffculties, future work will have a more positive analytical focus. For example, a few dual-electrode CEEC systems have been reported. These include a dual Hg/Au scheme described above for the detection of thiols and disulfides [36]. More provocative is the dual carbon electrode schemes reported by Zhong et al. [57]. These set-ups, which were intended to mimic the dual-parallel and dual-series electrode configurations developed in the 1980s for LCEC, were shown to permit the simultaneous detection of NADH and NAD^+ at electrodes poised at different potentials (dual-parallel) and the determination of catechols in the presence of electrochemically irreversible species such as ascorbic acid (dual-series). Finally, one should expect to see EC detection techniques participate fully in the trend toward microminiaturization of the CE instrument. Many EC electrodes can be fabricated on the scale required for "CE on a chip" devices, but none have as yet been incorporated in them. Recently, Ciucu and Baldwin [58] demonstrated that an Au OCE mounted on a 5 cm long capillary can provide separation and detection of dopamine and hydroquinone in less than 20 s. This is only a first small step toward microminiaturization of the entire CEEC system.

CEEC has come a long way in the first decade of its existence. As a result, the technique appears ready to move into the CE mainstream and become a routine CE detection mode. At the same time, there is still plenty of room for continued research focusing on the development of new electrodes and electrode configurations. One can only conclude that the CEEC's second decade should be even better than its first.

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