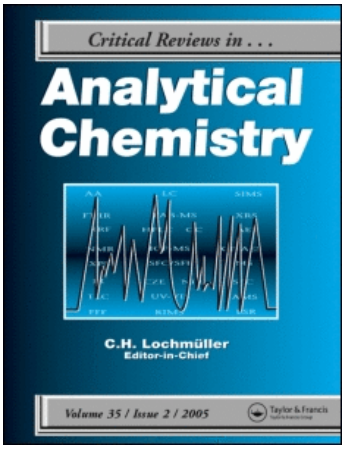


Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Application of Liquid Chromatography/Electrochemistry in Pharmaceutical and Biochemical Analysis: A Critical Review

Donna M. Radzik[a]; Susan M. Lunte[b]
[a] Analytical Chemistry Research Marion Laboratories Inc., Kansas City, Missouri [b] Center for Bioanalytical Research University of Kansas, Kansas

To cite this Article Radzik, Donna M. and Lunte, Susan M.(1989) 'Application of Liquid Chromatography/Electrochemistry in Pharmaceutical and Biochemical Analysis: A Critical Review', Critical Reviews in Analytical Chemistry, 20: 5, 317 — 358
To link to this Article: DOI: 10.1080/10408348908050070
URL: http://dx.doi.org/10.1080/10408348908050070

APPLICATION OF LIQUID CHROMATOGRAPHY/ELECTROCHEMISTRY IN PHARMACEUTICAL AND BIOCHEMICAL ANALYSIS: A CRITICAL REVIEW

Authors: **Donna M. Radzik**
Analytical Chemistry Research
Marion Laboratories, Inc.
Kansas City, Missouri

Susan M. Lunte
Center for Bioanalytical Research
University of Kansas
Lawrence, Kansas

Referee: Dennis C. Johnson
Department of Chemistry
Iowa State University
Ames, Iowa

I. INTRODUCTION

In the last 20 years, research efforts have increased the number of separation and detection techniques available for the analyses encountered in the development of pharmaceutical compounds. From a holistic point of view, the analytical effort required in the study of a bioactive moiety involves development of analytics to evaluate not just to determine and analyze the compound of interest, but, just as important, the effect the compound has on the biological system into which it is introduced. Therefore, in evaluating an analytical technique such as liquid chromatography/electrochemistry (LCEC) for its applicability to the study of bioactive compounds, it usually becomes apparent that techniques useful to the scientist studying a drug's metabolism are also useful to the scientist studying the physical and chemical properties of the drug.

The object of using chromatography for separating substances in complex samples is really to overcome the inability of the detector to distinguish these substances individually. If the ideal detector were available, there would be no reason to employ chromatography for analytical purposes. The chance of such a detector being developed is highly unlikely. Optical spectra and voltammetric curves, for example, tend to "spread out" to such a degree that the response for a component is likely to overlap with those of many other components. The most sophisticated deconvolution techniques currently available are usually applicable only to the study of very simple well-defined systems; in complex samples, a separation step is almost always needed.

LCEC is best described as an electrochemical (EC) technique augmented by chromatography. Chromatography and electrochemistry are both analytical tools that are heterogeneous and require an understanding of the mass transport of molecules. Experimentally, the combination provides a powerful approach for trace determinations and is the fastest growing electroanalytical technique. Its rapid development and acceptance among a wide range of researchers is directly correlated to a need for more sensitive and selective detection schemes. Of course, other elegant schemes have enjoyed success in this same period, including gas and liquid chromatography coupled with mass spectrometry and liquid chromatography with UV/vis photodiode array and fluorescence detection. Each of these techniques attempts to address the need for an inexpensive, easy-to-use, selective, and sensitive detection technique.

They all succeed and fail at various levels to meet these requirements. Indeed, an aspect often neglected in evaluating the utility of an analytical instrument is the information gained in relation to its ease of use.

The focus of this review is on applications of LCEC in the fields of pharmaceutical and biochemical analysis. Several excellent reviews dealing with fundamental and technical aspects of LCEC have become available recently.[1-4]

II. ELECTROCHEMICAL DETECTION FOR LIQUID CHROMATOGRAPHY

A. Applicability of LCEC

The basic principles of LCEC as an electroanalytical technique have been reviewed in excellent treatments by Shoup,[1] Kissinger,[2] Johnson et al.,[3] and Weber.[4] As in spectroscopic methods, molecular structure is the primary determinant for the electroactivity of an analyte. The accessibility of various filled and unfilled molecular orbitals ultimately determines the thermodynamics and kinetics of the electrode process. Just as in spectroscopy, there is a great body of empirical information that can be drawn upon to predict the behavior of individual compounds. In examining a candidate drug, metabolite, or related bioactive components, several key questions must be answered.

What functional groups are present? Does the parent structure permit delocalization of the added positive or negative charge? Are there substituents present in the molecule that enhance or detract from electroactivity? Is the redox reaction pH-dependent? What is the solubility? All of these factors are important considerations in assessing electroactivity for LCEC or any other EC technique. Among the many electroactive organic compounds, the following classes of substances are frequently ideal candidates for LCEC: phenols (especially hydroquinones and catechols), aromatic amines, thiols, nitro compounds, and quinones. There are other classes that are electroactive, as well as some unique compounds, such as ascorbic acid, uric acid, phenothiazines, and NADH. Organo-metallics such as *cis*-platinum anticancer agents are also amenable to electrochemical detection, along with other inorganic species, such as iodide and bisulfite, which may be present in bulk or formulated drug substances.

Pre- and post-column derivatizations are now being utilized to make some compounds better candidates for LCEC. Chemical, EC, enzymatic, and photolytic derivatizations in conjunction with both oxidative and reductive detection schemes are being used to determine species with normally unfavorable redox properties.

B. EC Techniques and Theory

Cyclic voltammetry (CV) rapidly provides useful preliminary information needed for the study of an electroactive compound. CV may also be used to evaluate the mechanism of redox reactions and subsequent chemical reactions.[5] As the UV absorbance spectrum is used to choose an optimum wavelength for detection, the cyclic voltammogram is used to help choose an optimum potential for detection. This convenient experiment duplicates those conditions (electrode material, electrolyte, etc.) found in a LCEC detector cell.

EC detection for liquid chromatography (LC) is in almost every case based on controlled potential amperometry. A predetermined potential difference is applied between the reference and working electrode(s) which is very dependent on the restrictions imparted by the LC working conditions. Since reverse-phase LC conditions (aqueous, pH 2 to 8) are most widely used, a typical example is an applied potential between $+1.3$ and -1.2 V (using a glassy carbon electrode material and a Ag/AgCl reference electrode). The applied potential, however, is highly dependent upon the redox behavior of the compound to be detected and varies due to experimental conditions (Table 1). Simply stated, the applied potential serves as the driving force for the EC reaction. As the potential of the working electrode (see Figure 1)

Table 1
OPERATIONAL CONSIDERATIONS FOR LCEC

Selected electrode materials	**Consider**
Glassy carbon	Analyte of interest
Carbon paste	Desired operation potential
Gold	Mobile phase composition
Mercury/gold amalgam	EC detector design
Platinum	
Nickel	
EC detector design	**Consider**
Thin-layer flowcell	Analyte of interest
Coulometric (porous) flowcell	Choice of electrode material
Wall-jet transducer	Electrode configurations
Tubular electrode	Ease of set up and use
Polarographic (dropping mercury electrode)	Effective detector cell
Wire electrode	Volume (microbore?)
	Commercially available?
Mobile phase components	**Consider**
Solvents	pH
Water	Desired operation potential
Alcohols	Chromatography
Dimethylsulfoxide	Analyte of interest
Dimethylformamide	Purity
Acetonitrile	Fouling of electrode
Tetrahydrofuran	Background noise
Buffer components/electrolytes	Electrode material
Phosphates	Detection method
Citrate	(Special electrode, thin-layer, pulsed amperometric,
Acetates	pre/post column derivatization?)
Perchlorate	
Additives	
Ion-pair reagents	
Chloride salts	
Hydroxides	
Surfactants	
Complexometric agents	

relative to the reference becomes more positive, the surface becomes a better oxidant (electron sink). Likewise, as the working electrode becomes more negative, the surface becomes a better reductant. Since the rate of conversion in the EC reaction (mol/s) is proportional to the instantaneous concentration at the electrode surface (i.e., the reaction is "mass transport-limited"), the current measured will be directly related to the amount of compound plotted as a function of time. Electrode current can be useful for reactions occurring below the limiting rate of the mass-transport processes. In some cases, the direct proportionality between electrode current and surface concentration will hold true in kinetically limited reactions. This conversion at an electrode surface is a function of Faraday's law (Equation 1).

$$Q = nFN \tag{1}$$

In this statement Q is the number of coulombs used to convert N moles of a substance, with a gain or loss of n electrons needed for the conversion of 1 mol of material; F is Faraday's constant, 96,484.6 C/Equivalents. Current, i, is the rate at which material is converted, so that Equation 1 can be written as follows:

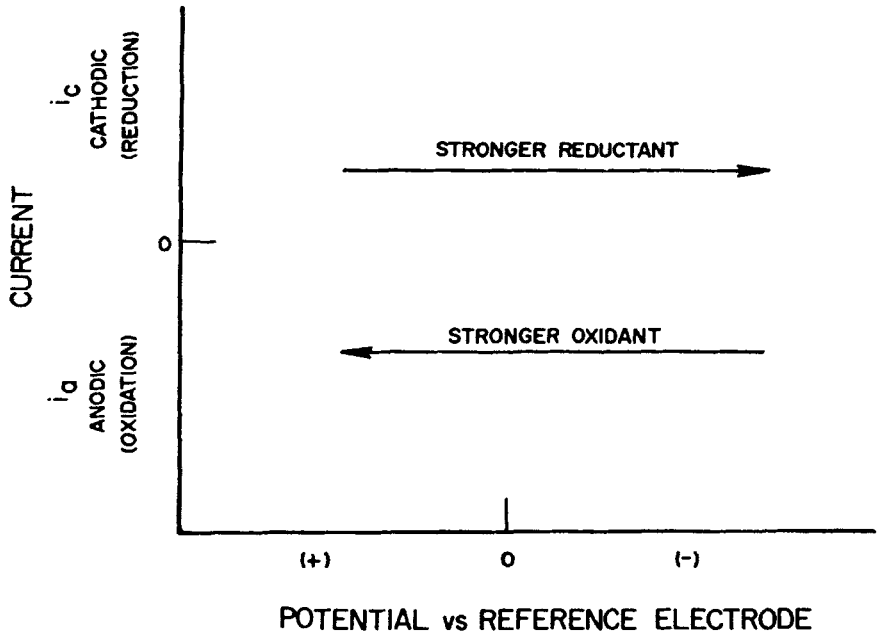


FIGURE 1. Action of applied potential as the driving force in an electrochemical reaction.

$$i = \frac{dQ}{dt} = nF\frac{dN}{dt} \tag{2}$$

This is the theoretical basis for all amperometric and coulometric-based cell designs. If chromatographic conditions (see Table 1) are carefully controlled, then EC detection is quite precise. Quantitative data can be obtained at the picomole level (total injected amount) for many compounds.

In the thin-layer amperometric transducer (Figure 2) conversion efficiency (% of reactant that actually reacts while passing over the electrode) will be limited, since only those molecules adjacent to the electrode surface are reacted, typically being only 3 to 30% under normal operating conditions. Frequently, the amount reacted is on the order of 10^{-15} mol. For example, in the case of a molecule with a mol wt of 200 undergoing a two-electron transfer, only 5×10^{-13} g of sample might be converted into product for quantitation at a signal-to-noise (S/N) ratio of 5. This reaction for 10^{-15} mol, reacting over a period of 2 s ($n = 2$, $F = 10^5$) produces an average of 0.1 nA!

Initially, it would seem worthwhile to increase the electrode surface area and thereby increase the conversion efficiency of analyte. This is the basis of the development of the "coulometric" detectors for LCEC. In contrast to a thin-layer cell design, in a coulometric cell the column eluent passes through a low-dead volume flow-cell packed with an electrode material, where conversion efficiency approaches 100%. Unfortunately, it has been determined that the conversion efficiencies of both the analyte and the background electrolyte are increased in such systems, and a concomitant improvement in S/N is not realized. Analogously, ultra-small electrodes pose S/N problems from outside sources, notably, the electronic circuitry used in the detector potentiostat. Working with the low currents encountered at small electrodes requires the ultimate in low-noise electronics. Environmental influences can also be problematic with small electrodes if proper grounding and shielding are ignored.

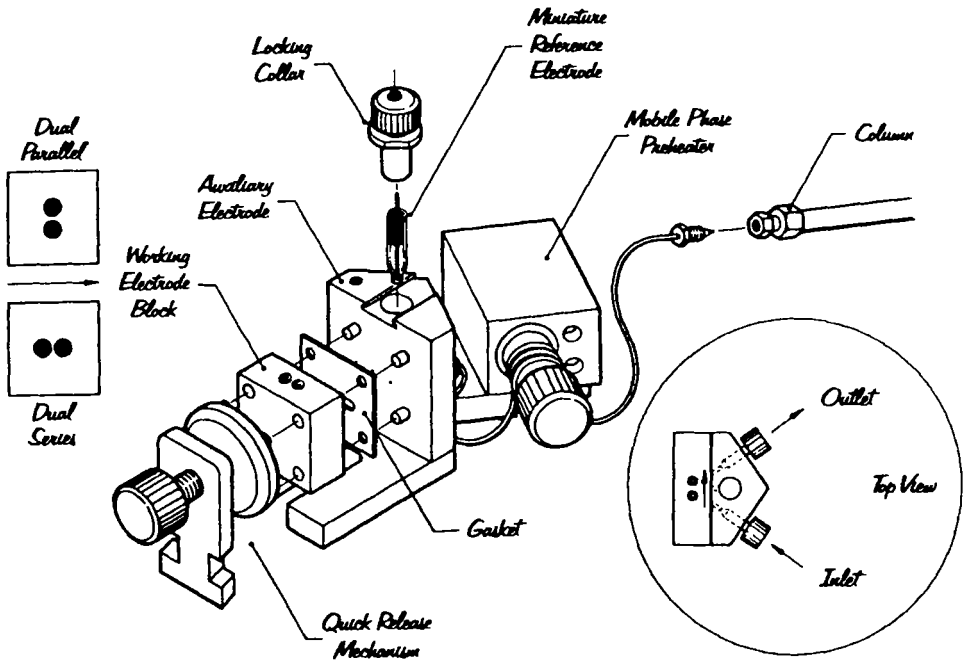


FIGURE 2. Thin-layer amperometric transducer and illustration of the dual-parallel and dual-series mode of electrochemical detection. (Reproduced with permission of Bioanalytical Systems, Inc.)

C. Selectivity in LCEC

Using LC in conjunction with an amperometric detector offers the advantages of efficient separation on microparticulate packing materials and the low detection limits (sub-picomole range) and selectivity of EC techniques. While the present-day LC column technology is at a point where one may quickly achieve excellent separation of closely related substances, this is often not sufficient. This is especially true when a determination involves a biological matrix, such as blood or urine. Often selectivity must be enhanced by a cleanup step prior to injecting material on the column. This typically consists of one or more liquid-liquid or liquid-solid extractions carried out in a batch mode.

The selectivity of the column is also commonly augmented by the detector. This is a real strength of EC detection schemes. While a "universal detector" could be useful in solving some simple problems, a "selective detector" is much more applicable in difficult determinations. The EC detector is a tunable device that permits enhancement of selectivity by changing the applied potential. The choice of electrode material, derivatizing agent, electrode configuration, and mobile-phase composition can also influence selectivity. To fully understand how this works, it is important to be familiar with "hydrodynamic voltammetry", a technique that is also the basis for amperometric and coulometric titrations.[6]

Since the solution is moving, the products of the electrode reaction are rapidly swept away from the electrode and therefore are not readily determined by reversing the potential scan. Hydrodynamic voltammograms (HDV) are generally developed by making repeated injections of a standard solution and stepping the potential of the detector between these injections. Chromatographically assisted HDVs may be used in tandem with a stationary CV experiment to determine the optimum detector operating potential. In addition to matching retention times, these current-potential curves (HDVs) can be compared. In this way, identities of peaks are confirmed based on the chromatographic and electrochemical properties of a compound.

An LCEC detector should have an active volume (that volume of the cell where the

detection is taking place) which is small relative to the volume occupied by the concentration zone passing from the column. The detector should also respond rapidly in order to accurately represent the shape of the concentration profile. LC peaks with volumes well below 100 nl are becoming quite common. Often the peak width is measured in seconds. Obviously, not all LCEC analyzers need to be used with such highly efficient columns. Nevertheless, the future clearly points to the need for EC flow cells with very small dead volumes and clean flow dynamics. It is generally desirable that the flow cell volume be adjustable and that the response time of the electronics be tunable over a wide range. The most popular EC detector cells are thin-layer devices in which the active electrode(s) is embedded in the wall of a channel formed by two blocks pressed around a thin gasket (Figure 2). The active volume of such cells is typically between 0.1 and 1 μl and therefore the shape of the chromatographic concentration profile is not disturbed even for the most efficient commercially available "high speed" or "microbore" columns.

The choice of the working electrode is critical to successful LCEC operation. Obviously, the surface should be physically and chemically inert to the mobile phase at the chosen applied potential. Two electrode surfaces have found greatest utility: carbon and mercury. The most versatile choice is glassy carbon. It has excellent chemical resistance to nearly any solvent used in LC and may be used over a wide potential range. Mercury provides an extended negative potential range, but is very limited in the positive direction. Conventional dropping mercury electrodes are not amenable to the low-dead volume thin-layer design, where conditions approaching laminar flow are achieved. A better alternative is to employ a mercury film on a polished gold substrate. The mercury is better than glassy carbon when dealing with substances difficult to reduce. It is also the electrode of choice for many sulfur-containing compounds.

Platinum, gold, and nickel also have shown utility in several compound-specific studies. However, the uses of these electrode materials have been somewhat stifled due to the chemical nature of these materials. Reactivity at the surface of these electrodes is a complicated combination of irreproducible surface chemistry and adsorption of compounds (mobile phase and analyte constituents) which degrades electrode performance during the course of its use. Recently, Johnson and co-workers have developed a triple-pulse amperometric detector, whereby the electrode surface may be rapidly recycled.[7] Reactions of alcohols and carbohydrates at the electrode active sites during this process may be described as follows in Equations 3 to 7, where E equals electrode active site and A, A′ and A″ = the analyte and its various oxidation states

$$E + A \rightarrow E - A \quad \text{(adsorption)} \tag{3}$$

$$E - A \rightarrow E - A' + ne \quad \text{(oxidation of H atoms from surface-catalyzed dehydrogenation)} \tag{4}$$

$$E - A' \rightarrow E + A' \quad \text{(slow desorption of oxidation product)} \tag{5}$$

$$E - A' \rightarrow E - A'' + n'e \quad \text{(secondary anodic reactions)} \tag{6}$$

$$E - A'' \rightarrow E + A'' \quad \text{(rapid desorption)} \tag{7}$$

A rapid adsorption of analyte to the electrode surface occurs at a fixed potential (Equation 3). The potential at the electrode is then stepped to a higher potential to oxidize hydrogen atoms removed from the adsorbed analyte (Equation 4); this is the step where current is measured for analytical purposes. By stepping to a second, excessively high potential the adsorbed analyte is catalytically desorbed from the electrode surface (Equations 5 to 7); the

potential of the electrode is then stepped back down to its original value for the next measurement. The entire triple-pulse sequence occurs on the order of 600 to 1000 msec, indicating that approximately 100 analytically useful measurements may be taken on the peak eluting in a 1-min time envelope. This detection method is now available commercially and has expanded both the type of electrode materials that can be used for LCEC and the range of analytes that can be determined, being especially directed at the determination of various carbohydrates following separation by anion exchange chromatography.[8]

The simultaneous use of two (or more) working electrodes greatly improves both the qualitative and quantitative aspects of an LCEC experiment.[9,10] Electrodes of the same or different materials, shapes, and surface areas may be used, and the electrode potentials may be independently controlled (Figure 2).

In the "parallel mode" the compounds eluting from the column pass over each electrode at the same time. The following applications are quite useful.

1. The ratio of currents monitored at each electrode can provide confirmation of peak identity and purity.
2. Oxidations and reductions can be carried out simultaneously. This saves time and enhances selectivity. This can be ideal for compounds in several different redox states.
3. Signals from low and high anodic (or cathodic) potential reactions can be recorded simultaneously, providing both greater selectivity and wider applicability in a single experiment.

In the "series mode" the working electrode block is rotated 90° in relation to the flow stream. Products of the upstream electrode reaction can be detected downstream. If an oxidation is carried out upstream, a reduction is accomplished downstream and vice versa. The following applications are popular:

1. The ratio of currents monitored at each electrode can provide confirmation of peak identity and purity.
2. Selectivity is enhanced at the downstream electrode because compounds that undergo chemically irreversible reactions upstream are discriminated against.
3. The upstream electrode can "derivatize" compounds to enhance detectability at the downstream electrode. Overall selectivity and detection limits can be greatly improved.
4. Dissolved oxygen can be discriminated against, simplifying LCEC detection of compounds that ordinarily would require mobile phase deoxygenation (e.g., nitro compounds).
5. "Common mode" currents can be discriminated against by taking the difference between the two signals.[9]

More recent reports[11] have described the use of potential scanning techniques with a dual-electrode detector. Both series and parallel dual-electrode LCEC already provide many opportunities for study of neurochemistry and xenobiotic metabolism because of the wide range of redox properties involved. Voltammetric-amperometric detection is achieved using the dual-electrode transducer in the series configuration by scanning the potential at the upstream electrode while maintaining a constant potential at the downstream electrode. The downstream electrode is used to monitor the redox reaction that occurred at the upstream electrode without the excess charging current, which would normally be viewed as noise associated with scanning the potential. In this way, the voltammetric information obtained for compounds in the bulk CV experiment may be obtained on a chromatographic time and quantity scale, while maintaining the detection limits possible with amperometric detection.

The scope of compounds that are amenable to EC detection has been increased by use of

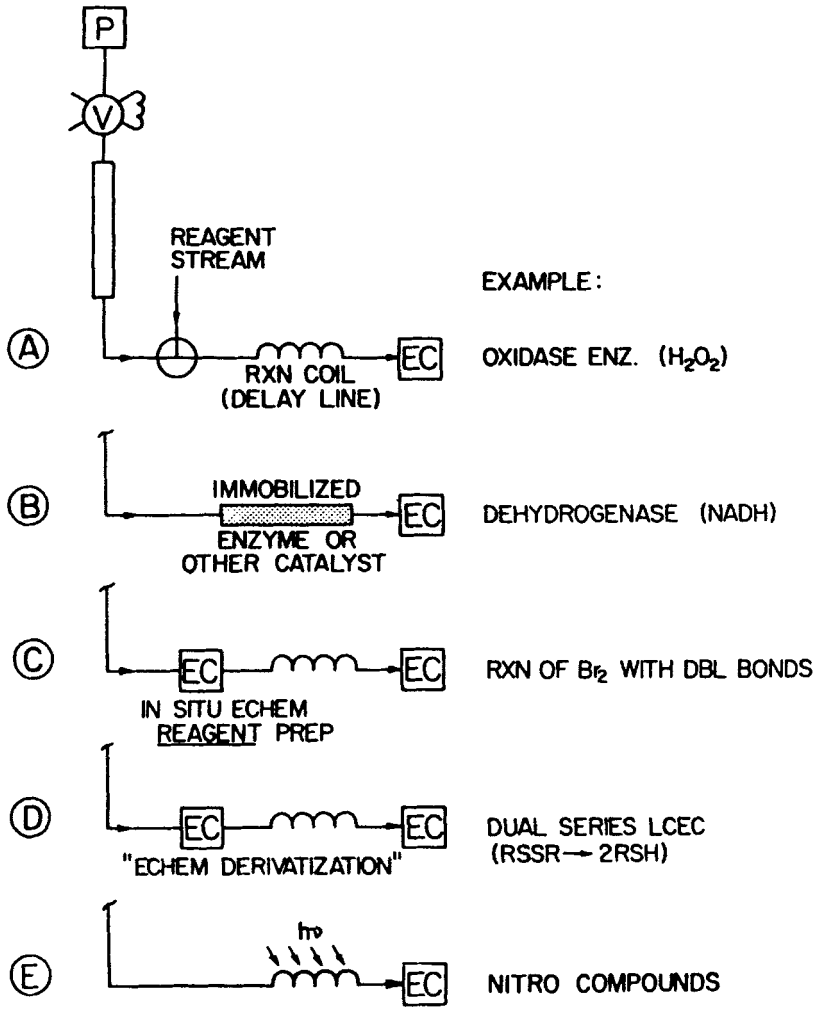


FIGURE 3. Configurations for post-column reactions with electrochemical analyzers. Various combinations of these five arrangements have also been used.

several pre/post-column reaction schemes (see Figure 3). Such configurations have been developed to (1) derivatize the analyte of interest to allow for lower limits of detection, or (2) cause formation of a new chemical species, which is then detected with a response proportional to the analyte of interest. This has become extremely important in the low-level determination of peptides and amino acids, which is discussed in more depth in Section IV.C.

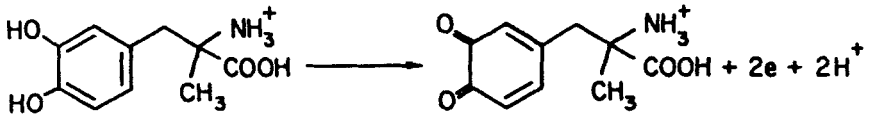
III. ELECTROACTIVE DRUGS AND REACTION SCHEMES

Among the many electroactive substances that frequently become analytes for LCEC are phenols (especially hydroquinones and catechols), aromatic amines, thiols, nitro compounds, quinones, and some unique compounds such as ascorbic acid, uric acid, phenothiazines, and NADH. Some organo-metallics are also amenable to EC detection, along with inorganic species such as iodide and bisulfite.

A. Oxidative Applications

1. Phenolic Substances

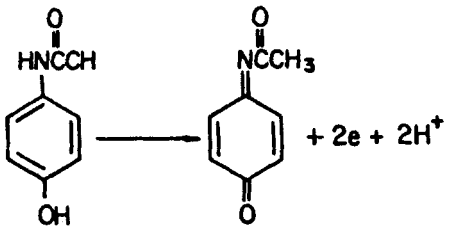
These are for the most part readily oxidized at a carbon electrode. The oxidation potentials for phenols vary widely with structure, and some (hydroquinones and catechols) are far more easily oxidized than others. Many compounds of pharmacological interest (catecholamines, pharmaceuticals) and industrial interest (antioxidants, antimicrobials, agricultural chemicals) are phenolic, and trace determinations based on LCEC are now quite popular. Aldomet® (α-methyldopa) is a hydroquinone (a catechol) representative of the more easily oxidized phenols. It undergoes a clean two-electron oxidation to an *o*-quinone:



(8)

2. Aromatic Amines

Like phenols, aromatic amines are oxidized at a graphite electrode over a wide range of oxidation potentials. Some compounds (phenylenediamines, benzidines, and aminophenols) are ideal candidates due to their very low oxidation potentials. The analgesic acetaminophen is an ideal example of an aminophenol that readily oxidizes to a quinoneimine:



(9)

3. Thiols ("Sulfhydryls" or "Mercaptans")

These compounds are very easily oxidized to disulfides in solution, but this thermodynamically very favorable redox reaction occurs only very slowly at most electrode surfaces (e.g., glassy carbon). Therefore, LCEC methods, for thiols usually depend on the unique behavior of these compounds at a mercury electrode surface at about +0.10 V (a very low potential). The reaction involves formation of a stable complex between the thiol and mercury. In a formal sense, the mercury and not the thiol is oxidized:

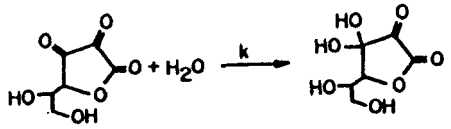
$$2RSH + Hg \rightarrow Hg(RS)_2 + 2e + 2H^+ \quad (10)$$

Thiols are very susceptible to oxidation by dissolved oxygen. It is therefore not surprising that samples and mobile phases must be deoxygenated to achieve good precision and the lowest detection limits. This requires a chromatograph free of Teflon® tubing.

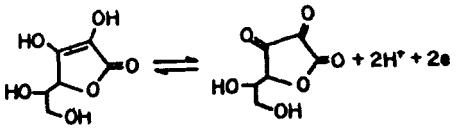
This approach has been used to determine the amino acid cysteine, the tripeptide glutathione, and the pharmaceuticals penicillamine and captopril. Besides thiols, many other sulfur-containing compounds are good candidates for LCEC.

4. Miscellaneous Oxidizable Compounds

A number of unique substances have been studied by oxidative LCEC. Ascorbic acid is easily detected with excellent selectivity in very complex biological samples:



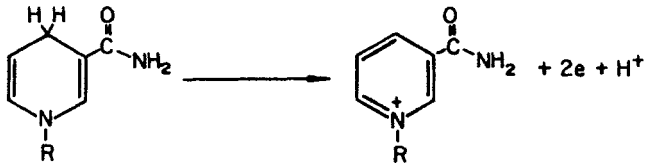
(11)



(12)

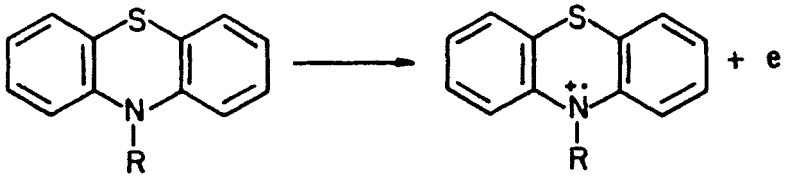
Low detection limits require the use of a deoxygenated mobile phase.

Similarly, uric acid is readily detected in biological materials. The important enzyme cofactor, NADH, is readily oxidized at carbon electrodes and provides a unique opportunity for enzyme immunoassays coupled to LCEC:



(13)

LCEC is also uniquely applicable to some heterocycles of pharmacologic interest (phenothiazines, imipramine). Phenothiazines undergo a very clean, one-electron oxidation to a cation radical:

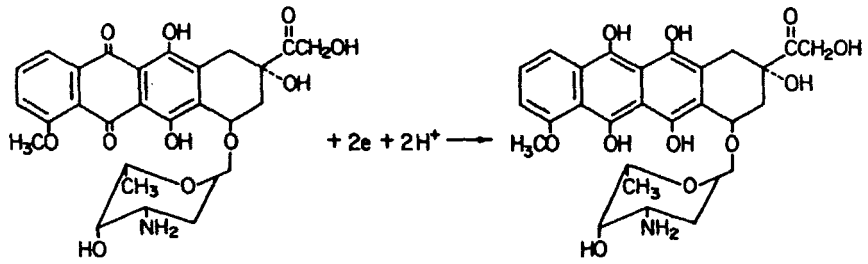


(14)

B. Reductive Applications

1. Quinones

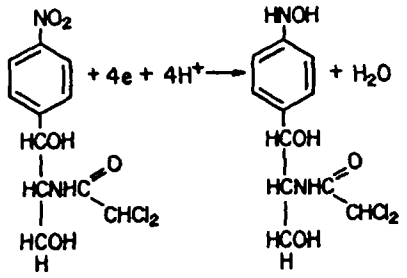
These are among the best-behaved organic compounds to undergo redox reactions in aqueous solutions. There are a reasonably large number of synthetic and natural products containing the quinone moiety, and many of these are excellent candidates for selective determination by LCEC. Unfortunately, some of the most important of these compounds (vitamin K) are extremely hydrophobic due to the presence of long hydrocarbon side chains and are therefore quite difficult to study by reverse-phase LC. A number of pharmacologic agents used in chemotherapy are quinones. For example, pharmacokinetic studies of the antibiotic doxorubicin can benefit from the following electrochemical reaction for use in an LCEC method:



(15)

2. Nitro (and Nitroso) Aromatic Compounds

These have been among the most extensively investigated by both organic and analytical electrochemists. Aromatic nitro and nitroso compounds are very readily reduced at both carbon and mercury electrodes, but other compounds, such as nitrate esters, nitramine, nitrosamines, and nitrosoureas, are often good candidates as well. The selectivity of EC detection is extremely good in many biological and environmental samples because the nitro group is rare in nature, and few other organic compounds are so easily reduced. A good example is the popular antibiotic chloramphenicol, which can be easily determined in blood using a four-electron reduction to the corresponding hydroxylamine:



(16)

Reagents containing the aromatic nitro group have frequently been used to derivatize amines, aldehydes, and carboxylic acids, etc. to improve their characteristics for determination by absorption spectroscopy. The same or closely related reagents are now being used to provide an electrochemically active functionality for these types of compounds.[12] Although it is true that aldehydes and ketones can be electrochemically reduced, and alkyl amines and carboxylic acids can be electrochemically oxidized, the potential energy required to initiate these well-known reactions is at present far too great to permit development of a successful LCEC trace determination procedure without derivatization. This subject is covered in some detail later in this review.

3. Miscellaneous Reducible Compounds

A number of organometallic compounds show promise for LCEC studies, and a few have already been examined in detail. Highly conjugated organic compounds, such as α,β-unsaturated ketones and imines, are occasionally good candidates, but at this time, ultraviolet (UV) detectors frequently outperform EC detectors for such systems. To date, there have been relatively few reports on LCEC studies of metal ions in bulk pharmaceuticals or pharmacologically active metal complexes, compared to wholly organic species. The disulfides derived from *in vivo* oxidation of thiol-based drugs can be detected by EC reaction of the disulfide to the thiol and detection of the latter by oxidation, as previously described. This approach uses the dual-series arrangement with two mercury film electrodes.

IV. APPLICATIONS IN PHARMACEUTICAL AND BIOCHEMICAL RESEARCH

A. Introduction

A great number of analytes of interest to researchers in all aspects of the pharmaceutical industry have been determined using LCEC. In an evaluation of UV vs. amperometric detection for LC, Musch et al.[13,14] reported minimum detection concentrations (EC vs. absorbance detection) for 94 different drugs. Classes of pharmacologic compounds included local anesthetics, antipyretics, tricyclic antidepressants, sulfonamides, sex hormones, beta-adrenoceptor blocking agents, phenothiazines, alkaloids, diuretics, and penicillins. These papers made an attempt to develop a set of ground rules by which EC detection for LC

could be chosen when developing LC methodology for an analyte. The scope of the papers describing an "expert system" for choosing EC detection as a detection method was narrowed to single mobile phase, electrode material (glassy carbon) and the oxidative mode (+0.7 to +1.2 V). This is somewhat akin to evaluating UV detection for pharmacologic compounds between 230 and 260 nm. Such limitations may narrow down the number of possible applications for the technique. The chemistry of the developed system must encompass the analyte of interest, the electrode material, the applied potential, and the mobile phase characteristics. EC detection was found to give enhanced selectivity and sensitivity advantages for a relatively broad range of pharmacologic compounds.

One of the greatest merits of LCEC is that the enhanced selectivity and improved detection limits allow for a simplification of sample cleanup procedures in many biological applications. The ramifications of this are additional time and cost savings to the laboratory. This has led investigators to pursue LCEC as a method of choice for many compounds. Since new applications of LCEC are appearing daily, an attempt to touch on each individual application would be a monumental task. This review will focus on emerging LCEC techniques and/or applications in the development of newer classes of pharmacologically active agents. Several excellent supplemental reviews are available which treat LCEC applications for individual drug classes and substances[15,16] and practical aspects in evaluation electrochemistry as a detection technique of choice in more detail.[1-4]

B. Bulk and Formulated Drug Analysis

The initial development of a pharmaceutical agent usually involves the development and characterization of the bulk reagent. Although this is not an area in which LCEC is typically applied, recent applications have begun to demonstrate its utility, especially in the determination of the purity of reference standards. This issue is becoming more important as most current LC methods rely on a single or perhaps dual UV wavelength detection to assign bulk drug purity. Auxiliary methods must be utilized to confirm the absence or presence of non-UV-absorbing materials, which may elute during the normal course of a chromatographic method.

LC followed by controlled potential coulometry has been utilized to determine the purity of electroactive drugs, such as acetaminophen.[17] A coulometric detector made of crushed reticulated vitreous carbon was used, and the number of coulombs generated were counted. By relating the number of coulombs generated in the EC reaction to that which is predicted by theory, an assignment of purity may be made using this method. The Faraday, 96,484.6 C/equivalent becomes a universal standard. Such a technique may be limited by several factors.

1. One must assume that the chromatographic method separates all electroactive components from the analyte of interest.
2. When injecting a known amount of material onto a column it is assumed that the analyte of interest will elute as a single band, and that none of the analyte is irreversibly bound to the column packing material.
3. The electrode material and EC characteristics of the species must be well defined and reproducible enough to measure bulk batches in the same manner weeks or even years apart.
4. The apparent (observed) value of n must be a constant that is independent of concentration.

The utility of the EC detector as a screen for electroactive impurities also has been demonstrated.[18] When investigating the purity of a bulk drug that is not electroactive, the presence of electroactive impurities can provide important information about the causes of

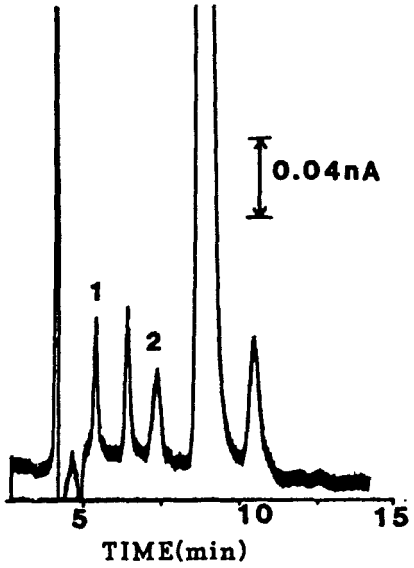


FIGURE 4. Chromatogram respresenting the determination of DNA near the detection limit: (1) guanine, (2) adenine. The original solution contained 1.0 ng/ml of *E. coli* DNA before hydrolysis. Injection volume: 100 ul. (Reprinted from Kafil, J. B., Cheng, H.-Y., and Last, T. A., *Anal. Chem.*, 58, 285, 1986. With permission.)

contamination or the route of degradation. The combination of UV and EC detection is becoming routine in many cases like this. Often the UV detection is used to quantitate major components, while EC selectively detects the low-level impurities.

An important new area of pharmaceutical research is the development of biotechnology products. The lack of previous background information or therapeutic applications have led to stringent restrictions on trace levels of impurities in these bulk drug substances. Prior to their use for therapeutic applications, recombinant DNA products must be evaluated for trace levels of nucleic acids derived from recombinant bacterial chromosome and plasmid. Previous techniques for such low-level determinations have been time consuming, laborious, and prone to experimental error. Kafil at al. have developed a highly sensitive and precise method for quantitation of DNA/RNA fragments.[19,20] The method is based on hydrolysis of nucleic acids followed by LCEC. Previous investigations have indicated the utility of EC detection for the quantitation of guanine nucleotides.[21] Using a dual-electrode cell with a metal oxide electrode as catalyst generator and a glassy carbon electrode as detector, this method allows for quantitation of nucleic acids on the 50-pg level and DNA to the 100-pg level. Figure 4 illustrates the determination of DNA near the detection limit. The method allows for the determination of RNA in the presence of DNA in complex recombinant DNA and provides qualitative information on the adenine/guanine ratio. With the rapid expansion of biotechnology, it appears that the selectivity and sensitivity of LCEC will be applicable to bulk substances, as well as to determinations in biological samples.

Many smaller anionic species such as CN^-, HS^-, Br^-, I^-, $S_2O_3^-$, and SCN^- may be determined in bulk drugs using LCEC. Simultaneous determination of cyanide, sulfide, iodide, and bromide by ion chromatography followed by electrochemical detection at a silver electrode has been demonstrated.[22-24] Such rapid and sensitive determinations are especially applicable to bulk drug and formulated drug preparations, where meta-bisulfite is sometimes added as an antioxidant, and the other species are considered contaminants.

Table 2
SELECTION OF REAGENTS FOR THE PRECOLUMN DERIVATIZATION OF AMINO ACIDS

Reagent	Analyte	EC detection mode	Ref.
Trinitrobenzene sulfonic acid	Amines; amino acids; γ-aminobutyric acid	Reductive	12,29,37
2,4-Dinitrofluorobenzene	Amines; amino acids	Reductive	12
2-Chloro-3,5-dinitropyridine	Amines; amino acids	Reductive	12
3,6-Dinitrophthalic anhydride	Peptides	Reductive	30,39
2-Carboxy-4,6-dinitro fluorobenzene	Peptides	Reductive	30
o-Phthalaldehyde	Amines; amino acids, peptides	Oxidative	31,32,40, 41,43
Phenylisothiocyanate	Amino acids	Oxidative	33
p-N,N-dimethylamino phenylisothiocyanate	Amino acids	Oxidative	34
N-(4-anilinophenyl)-isomaleimide	Amino acids	Oxidative	30
Naphthalene dialdehyde	Amino acids; peptides	Oxidative	28,36,38

C. Amino Acids by LCEC

Excellent reviews of the detection of amino acids and peptides by LCEC are included in the articles by Krull et al.[25] and Johnson et al.[3] This section briefly summarizes work done in three specific areas. These are (1) direct detection, (2) derivatization for reductive detection, and (3) derivatization for oxidative detection. Table 2 shows a representative selection of reagents for precolumn derivatization of amino acids.

1. Direct Detection

Amino acids and peptides containing tyrosine have been detected directly using a glassy carbon electrode at +0.90 V. White used LCEC to detect several biologically important peptides that contain tyrosine residues.[26] These included leu-enkephalin and oxytocin. Santer and Frick also have used this approach for the detection of several important neuropeptides.[27]

Amino acids have also been detected at platinum electrodes using pulsed electrocatalytic detection. This method, developed by Johnson and co-workers, has been discussed in a previous section. Using PAD for amino acids, Polta reported detection limits of 23 ng for hydroxyproline and 13 ng for glycine.[44]

An EC detector based on nickel oxide has also been used to detect amino acids directly. This detector is based on the reduction of nickel in the presence of the analyte to produce a radical species.[45] The reaction sequence is as follows

$$Ni + 2OH^- \rightarrow 2e^- + Ni(OH)_2 \quad (17)$$

$$- le^- + Ni(OH)_2 + OH^- \rightarrow NiOOH + H_2O$$

$$NiOOH + \text{analyte(ad)} \rightarrow Ni(OH)_2 + \text{radical} \quad (18)$$

$$\text{Radical} \rightarrow \text{Products} \quad (19)$$

The nickel electrode is maintained in the +3 oxidation state by applying a potential of +0.49 V vs. SCE. As the sample passes over the electrode, the amine is oxidized to a radical species and the nickel is reduced. The resulting current is proportional to the concentration. Detection limits for glycine using this detector in combination with flow injection analysis are in the low nanogram range.

2. Derivatization (Reductive Mode)

Most derivatives that have been synthesized for LCEC analysis of amino acids are detected in the reductive mode. Several nitroaromatic derivatives have been investigated, with trinitrobenzosulfonic acid being the most popular. In theory, these compounds give up to six electrons per nitro group and therefore should be very sensitive reagents. In practice, the trinitrobenzoic acid derivatives yield 12 electrons and not the predicted 18 at useful LCEC potentials.[28] However, this still allows as little as 310 fmol of the amino acid to be detected.

Another advantage of the trinitro derivative is that peptides can also be derivatized without a significant loss of sensitivity. On glassy carbon, the detection limit for TNB-GABA was 1 pmol (S/N = 8).[29] The only disadvantage of this method is the electrode potential necessary for this analysis. At −0.85 V, oxygen must be eliminated from the mobile phase or otherwise there will be excessive background in the system. The chemicals dinitrobenzene and dinitropyridine have also been investigated as derivatizing agents for amino acids; however, the detection limits are not as good as for the trinitro compound. Meek found that the dinitrophthalic anhydride was electroactive at a moderate reduction potential (−0.24 V) and afforded detection limits of approximately 1 pmol injected.[30]

3. Derivatization (Oxidative Mode)

In the oxidative mode, two commercially available amino acid-derivatizing reagents produce electroactive derivatives. The OPA derivatives are electroactive and can be detected at a modest oxidation potential (+0.70 V), with detection limits in the 30 to 150 fmol range with isocratic elution. The oxidation of the isoindole is believed to involve two electrons.[31,32] As shown in Figure 5, 22 derivatized amino acids have been separated on high-speed columns, with detection in the picomole range.

Phenylisothiocyanate also produces a derivative that is electroactive at a potential of +0.85 V (15). Using *N*,*N*-dimethylaminophenylisothiocyanate, Mananchi et al. were able to detect amino acids in the low picomole range.[34]

Shimada et al.[35] found that *N*-(4-anilinophenyl)isomaleimide produces a derivative with amino acids which also is easily oxidized (0.60 V). Detection limits were 13 pg at a S/N of 2.

An analog of OPA, namely, naphthalene dialdehyde, produces an electroactive isoindole derivative of amino acids. The cyanobenzo(f)isoindole (CBI) derivative of glycine has been detected electrochemically using flow injection analysis and LC in the low femtomole range.[28,36]

Krull et al. have used LC/photolysis-EC to detect the dinitrobenzene derivatives of amino acids under oxidative conditions. The nitroaromatic is reduced by the photolysis and is then detected by oxidative LCEC.[3]

A more extensive review of the use of derivatizing agents for the detection of amino acids using LCEC can be found in Reference 25.

D. Determination of Biogenic Amines

The influence of a drug on a disease state or the metabolic functions of an organism is an important aspect in the determination of drug efficacy. The first widespread use of LCEC was for the determination of biogenic amines, as well as a host of other important phenolics in various biological media.[46]

Currently, developments in chromatographic technology have enhanced the manner in which biogenic amines are determined using EC detection. Microbore techniques are distinguished by the low-dead volume needed in the chromatographic system (especially the detection system) and the low flow rates used. Thin-layer EC cells have especially low-dead volumes (less than 0.3 μl) and therefore are ideal detectors for this method.[47,48] The efficiency of the surface reaction on an amperometric detector increases with decreasing flowrates and,

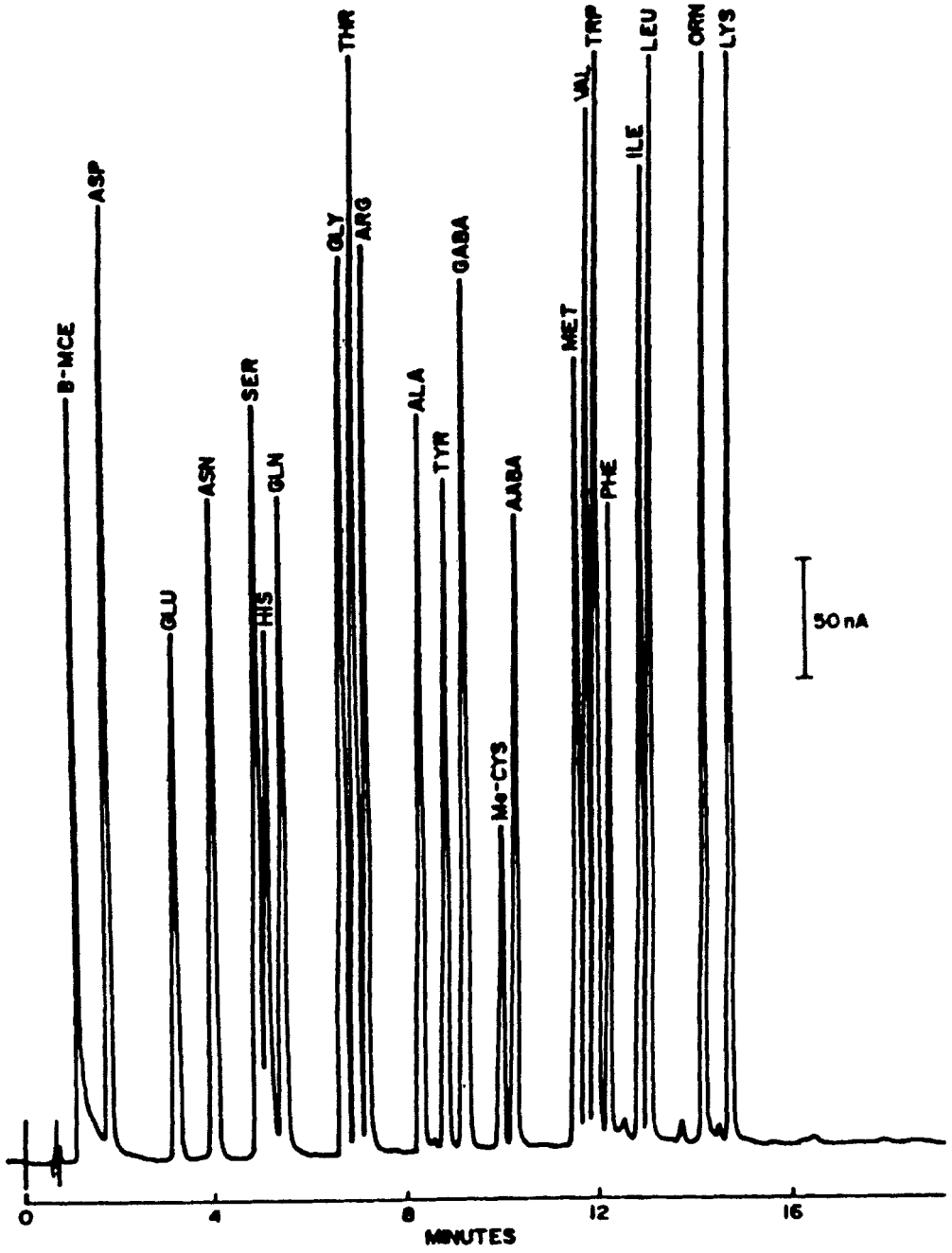


FIGURE 5. High-speed chromatographic separation of OPA/B-MCE derivatives of 22 amino acids under gradient conditions. 113 to 258 pmol of each derivative was injected. (Reprinted from Allison, L. A., Mayer, G. S., and Shoup, R. E., *Anal. Chem.*, 56, 1089, 1984. With permission. Copyright 1986 American Chemical Society.)

since microbore columns allow for elution of much more concentrated segments, detection limits for some biogenic amines have been reported to be in the range of 1 to 5 fmol.[48] With a cartridge column technique (columns ranging anywhere from 1 to 5 cm in length) and a modified thin-layer cell with fast electronic current amplifiers, a series of 18 biogenic amines and metabolites can be separated in 4 to 7 min following extraction from brain tissue.[49]

The use of derivatization schemes have broadened the scope of biogenic amines that can

be detected by LCEC. Naito et al.[50] reported the determination of kynurenine in serum by LCEC after enzymatic conversion to 3-hydroxykynurenine. Kynurenine is converted in the presence of the mitochondrial fraction of cellular components and NADPH. The detection level is as low as 1 pmol. Histamine[51] and β-phenylethylamine[52] both have been determined at low levels following derivatization.

Future use of LCEC in the neurochemistry area will be linked to development of improved sampling technology. As in the study of all metabolic transformations, whether they are endogenous or exogenous analytes, the ideal technique would be to monitor changes *in vivo* using a totally noninvasive technique. Some strides are being made in this area using *in vivo* voltammetry; however, since compounds may exhibit similar electrochemical properties, interference among analytes is a strong possibility. Microdialysis is a new methodology that allows for the monitoring of chemical events in the extracellular space where chemical transmission takes place.[39-55] The perfusing fluid in this technique is circulating inside a semipermeable membrane (instead of freely in the tissue) in effect acting as a synthetic "blood vessel". Fluid is pumped through the probe at a slow rate (1 to 10 μl/min), and dialysate from the extracellular media is transported directly to the LCEC system. Since the system is used with awake, unrestrained animals, a true profile of metabolic changes over time may be realized. A great deal of physiologically important substances may be determined in the perfusate and the comparison of *in vitro* techniques with *in vivo* experiments is a real possibility. Since a great number of exogenous substances such as drugs also enter into the extracellular liquid, microdialysis should also find application in xenobiotic metabolism studies. By uniting microdialysis with LCEC, the number of analytes that may be determined far exceeds that attainable using other *in vivo* techniques such as biosensors.

E. Thiols and Disulfides

As described in Section III, there are some functional groups that are uniquely suited to the LCEC approach. Thiols (mercaptans, sulfhydryls) and the corresponding disulfides form some of the most important redox couples in biochemistry (e.g., glutathione and glutathione disulfide). An excellent review on the determination of thiols and related compounds by HPLC is found in Reference 56. This review contains information on applications of LCEC for the determination of thiols and disulfides.

There are relatively few thiol- or disulfide-containing drugs; however, captopril and penicillamine have received considerable attention in recent years. Such compounds can be very difficult to determine due to their instability in the presence of dissolved oxygen. This problem is more often a consideration in establishing a lower limit of detection than is the instrumentation. With proper sample handling, LCEC would appear to be the method of choice for these compounds. Table 3 gives some examples of the application of LCEC to the determination of thiols, disulfides, and thioethers.

1. Thiols

Most thiols have to be derivatized to be detected by HPLC with UV detection.[86] Unlike UV detection methods, LCEC can be used to detect thiols in biological samples directly. The most popular electrochemical detector for thiols is one which was introduced by Rabenstein and Saetre.[68] This detector takes advantage of the fact that mercury oxidizes at a lower potential in the presence of thiols. The reaction used to detect the thiols is as follows:

$$2RSH + Hg \rightarrow Hg(SR)_2 + 2e^- + 2H^+ \quad (20)$$

In the original papers, a mercury pool set at a potential of +0.1 V was used to detect thiols as they eluted from the column. At this potential only thiols and other compounds that form complexes with mercury are detected. Saetre and Rabenstein used this detector for the

Table 3
LCEC OF THIOLS, DISULFIDES, AND THIOETHERS

Compound	Electrode	Ref.
Glutathione	Hg(Au)	59—66
	Hg pool	67—69
	Carbon	70—72
Cysteine	Hg(Au)	59—61,65,66,73
	Hg	67,68
	Au	74
	Carbon	70
Glutathione disulfide	Dual Hg(Au)	59,61,63,65,66
Cystine	Dual Hg(Au)	59,62,63,65,66,73
D-Penicillamine	Hg(Au)	57,62,63,73
	Au	74,75
	Hg(pool)	76,77
N-Acetylcysteine	Hg(Au)	78
	Hg(pool)	78
	Au	74
	Carbon	79
	Bromine	78
Homocysteine	Hg(Au)	61,62,66,67
	Carbon	70
	Dual Hg(Au)	66
Cysteinylglycine	Hg(Au)	59,66
Cysteinylglycine disulfide	Dual Hg(Au)	59,66
Cysteine-glutathione mixed disulfide	Dual Hg(Au)	59,66
Cysteine-homocysteine mixed disulfide	Dual Hg(Au)	66
GSH-Homocysteine mixed disulfide	Dual Hg(Au)	66
a-Glutamyl cysteine	Hg(Au)	59
Protein bound	Hg(Au)	64,65
Captopril	Hg(Au)	58
Cysteamine	Au	74
WR33278	Hg(Au)	80
Thioglucose	Au	74
N-Acetylpenicillamine	Au	74
2-Mercaptopropyl-glycine	Au	74
Dithiothreitol	Au	74
Ranitidine	Bromine	81
Antibioticum	Bromine	81
Methionine	Carbon	82
6-Mercaptopurine	Carbon	83
6-Thioguanine	Carbon	83
Phenothiazine	Carbon	84,85
Thioxanthine	Carbon	84

determination of cysteine, homocysteine, glutathione, and penicillamine in a variety of biological samples, including serum, urine, and plasma.[57,67,69] Using the mercury pool electrode, less than 10 pmol of glutathione could be detected. This reaction can also take place at a gold electrode that has been amalgamated with mercury. Perret and Drury used an Hg(Au) electrode set at +0.07 V for the detection of captopril in plasma and urine.[58]

Carbon paste electrodes have been used to detect glutathione in brain and liver samples at a potential of +1.00 V vs. Ag/AgCl.[72] The disadvantage of detecting glutathione with a carbon electrode is that due to the high potential needed, the detector is less selective than is the mercury-based detector. Electrode pretreatment has been used to enhance the electrochemical response of the electrode for glutathione. Iriyama et al. pretreated a glassy carbon electrode by placing it in 0.2 *M* phosphate buffer (pH = 6.5) and applying a potential of

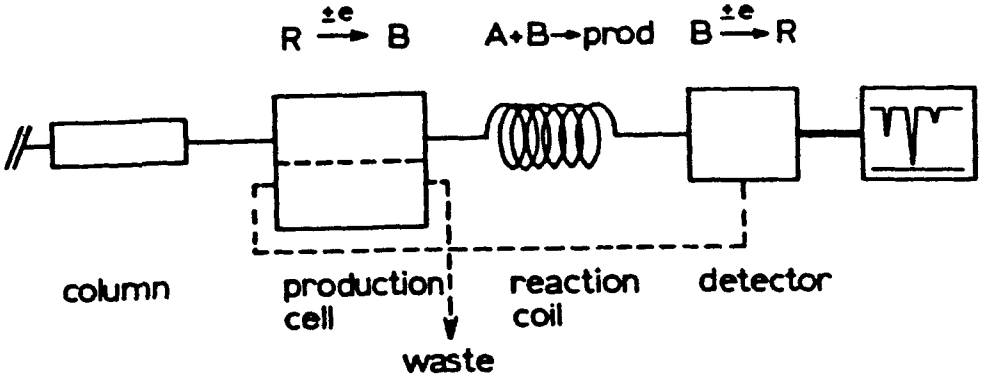


FIGURE 6. Electrochemical reagent production with downstream amperometric detection. (A) Analyte; (B) reagent (Iodine); (R) precursor of the reagent (Iodide). (Reprinted from Kok, W. Th., Halvax, J. J., and Frei, R. N., *J. Chromatogr.*, 352, 27, 1986. With permission. Copyright 1986, Elsevier Science Publishers.)

+1.9 V for 2 min.[71] They claimed that they could detect as little as 5 ng of glutathione at +1.1 V using this method. However, considering the high background current associated with this potential, as well as the detection limits that are reported, there does not appear to be any advantage for this method over the use of mercury or carbon paste electrodes.

Halbert and Baldwin have lowered the potential for oxidation of GSH at a glassy carbon electrode by modifying it with cobalt-phthalocyanine.[70] They report detection limits of about 3 pmol using this electrode at +0.75 V vs. Ag/AgCl. The electrode was stable over 100 separations, retaining 85 to 90% of its initial current response. Detector response was also linear over the range of 5 to 200 pmol injected. The electrode was more selective than direct detection using glassy carbon. However, at this potential other easily oxidized compounds such as ascorbic acid, uric acid, phenols, and aromatic amines can still interfere.

Gold and platinum electrodes also have been used for the determination of thiols in biological samples. Perret and Rudge reported the detection of D-penicillamine, thiomalate, and captopril using a gold electrode at +0.8 V vs. Ag/AgCl.[74] Kreuzig and Frank used a gold electrode set at +0.8 V for the determination of D-penicillamine in plasma and urine.[75] Rudge maintains that the gold electrode is more rugged for routine analysis than the Au/Hg amalgam.[56]

Kok et al.[78] compared three methods of detection for *N*-acetylcysteine. The performance of the dropping mercury electrode and gold/mercury amalgamated electrode were compared to a detector utilizing the on-line generation of iodine as an oxidant. The latter detector detects thiols by reaction with iodine. Iodine is generated by oxidizing potassium iodide (present in the mobile phase) to iodine in a production cell that is followed by a reaction coil (Figure 6). In the coil, the iodine reacts with the thiol of interest by the following reaction:

$$2RSH + I_2 \rightarrow RSSR + 2H^+ + 2I^- \quad (21)$$

Unreacted I_2 is reduced at the downstream electrode and the resulting current measured. The decrease in current is proportional to the amount of RSH present in the sample.

Kok found that the DME had the best precision, with only 1% variance. However, it was not as sensitive as the two other methods. The Hg(Au)-based detector was more sensitive, but the detector response was less reproducible (7% for *n*-acetyl-cysteine). The detector based on iodide generation was as sensitive as the Hg(Au), but had higher noise. It was, however, reported to be more precise than the Hg(Au)-based detector.

Overall, based on its selectivity and ease of preparation, the Hg(Au) electrode is probably

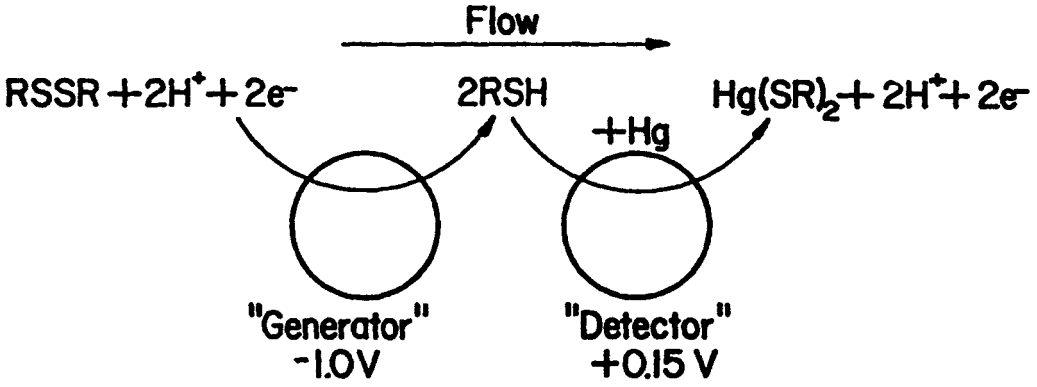


FIGURE 7. Schematic twin Hg/Au series electrode arrangement for conversion of disulfides to thiols on-line for subsequent downstream measurement of thiols.

the best choice if thiols are the only compounds to be determined. If other electroactive components are of interest, carbon or gold electrodes can be utilized.

2. Disulfides

Disulfides traditionally are measured following reduction to the thiol. In fact, many of the disulfides measured by LCEC have been measured using a single electrode following chemical reduction. EC reduction has also been employed. Rabenstein and Saetre used a mercury pool electrode to reduce penicillamine disulfide to its corresponding thiol, which was then chromatographed and subsequently detected at a mercury pool electrode.[57]

A dual electrode detector designed by Eggli and Asper allowed both thiols and disulfides to be detected as they eluted from the chromatographic column.[73] The first electrode consisted of a column of amalgamated silver grains set at a potential of -1.1 V. At this electrode, the disulfides are reduced to the corresponding thiols. The second electrode consisted of a mercury pool electrode set at 0.00 V. Thiols generated by the first electrode, as well as endogenous thiols in solution, were detected at this electrode. The oxidized (RSSR) and reduced forms (RSH) of the thiol were separated chromatographically.

A more elegant version of this detector was designed by Allison and Shoup.[63] Two gold/mercury amalgam electrodes are used for the detection of thiols and disulfides. A diagram of this detector is shown in Figure 7. As in the previous design, the first electrode is set at a reducing potential of -1.00 V, and the second electrode is set at $+0.15$ V vs. Ag/AgCl. With both detectors on, thiols and disulfides are detected as they elute from the chromatographic column. One advantage of this detector is that the thiols can be determined independently of the disulfides by turning the generator electrode off (see Figure 8). Additionally, if a chemical reagent such as *N*-ethylmaleimide (NEM) is used prior to injection, the disulfides can be determined independently of thiols.[66] This method can be used in the identification of unknown thiols and disulfides, as well as for verifying peak purity. The dual-electrode detector, which is commercially available, has been used extensively for the determination of thiols and disulfides in biological samples. These include glutathione, cysteine, and their disulfides in liver, kidney, and urine samples,[59,60,64,87-88] and the radioprotector WR2721 in blood and urine.[80]

One interesting application of the dual electrode detector is the measurement of protein, protein-bound, and nonprotein thiols in tissues. Dupuy and Szabo used LCEC to measure each of these quantities.[65] Nonprotein thiols and disulfides were measured in the supernatant following acid precipitation of the protein and centrifugation. The protein pellet was then divided into two fractions. The first fraction underwent reduction with borohydride, followed

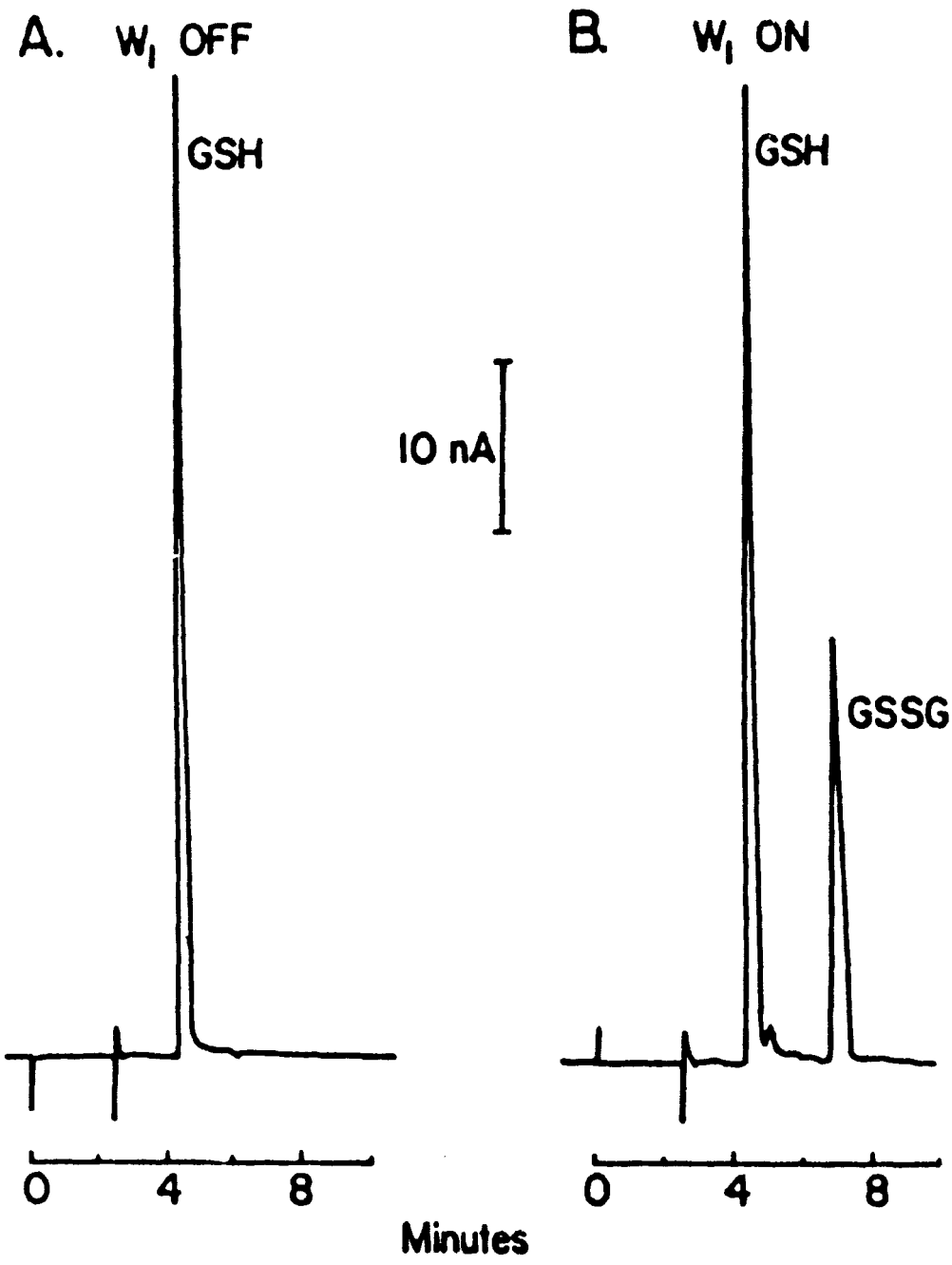


FIGURE 8. (A) Detection of the thiol glutathione with only the downstream electrode on; (B) simultaneous determination of glutathione and the disulfide of glutathione using the dual series configuration with the upstream electrode now on. Mobile phase is deoxygenated by N_2 purging. (Reprinted from Eggli, R. and Asper, R., *Anal. Chim. Acta*, 101, 253, 1978. With permission. Copyright 1983, American Chemical Society.)

by LCEC analysis. The LCEC analysis of this fraction provides a number for the protein-bound sulfhydryls. Acid hydrolysis of the second fraction made it possible to calculate the amount of protein sulfhydryls present in the sample.

3. Thioethers

Thioethers do not form complexes with mercury and are therefore not detected by the mercury pool or Hg(Au) electrode. However, thioethers can be detected using the post-column reactor system described by Kok and Frei by using bromine instead of iodine as the oxidant.[81] Both ratinidine and antibioticum have been detected by this method.

Methionine has been detected directly using an electrochemically pretreated glassy carbon electrode. However, this was at an extreme potential (+1.7 V). Special modifications of the equipment had to be made in order to subtract the large background current. Detection limits were in the nmole range.[82]

F. Determination of Other Endogenous Compounds

Other endogenous analytes have been determined using LCEC techniques, including prostaglandins,[89] uric acid, and NADH.[90] Pterins, a class of nitrogen-containing heterocyclics, may be determined simultaneously in many biological fluids using parallel-adjacent dual electrodes.[91] In this way, the various oxidation states in which these compounds exist can be detected in a single injection. Peak confirmation was made for these species based on the ratio of responses at the EC detector. Such a current-ratio technique has been used for identification of guanine nucleotides.[21]

A number of vitamins have been determined using LCEC. An excellent review on all methods for ascorbic acid (vitamin C) determinations, including those involving LC with EC detection, has been compiled by Pachla et al.[92]

α-Tocopherol (vitamin E) has been determined in plasma using LCEC. Due to the hydrophobicity of this compound, a mobile phase consisting of 0.1% pyridine, methanol, and 0.05 *M* sodium perchlorate was used for the reverse-phase separation.[93] The detection limit for alpha-tocopherol was approximately 0.6 ng at a S/N of 10 with an applied potential of +0.70 V.

Lang et al.[94] were able to determine a variety of biologically significant hydroquinones by LCEC. In this case, a mobile phase containing 10% methanol, 90% reagent alcohol, and 20 m*M* lithium perchlorate was employed. Several tocopherols (delta, gamma, and alpha), and two ubiquinols were detected using a glassy carbon electrode set at +500 mV. Tocopherols and ubiquinols were determined in liver, muscle, blood, and plasma using LCEC, while the ubiquinones were determined using UV detection.[94]

Vitamin K was determined in plasma by three separate methods, all based on electrochemistry.[95] Two of the methods used dual electrode LCEC coupled with reverse-phase chromatography, the third combined electrochemistry with fluorescence. The mobile phase consisted of 7.5% water in methanol with sodium perchlorate as the electrolyte. The first electrode was "coulometric" and reduced the quinone to the corresponding hydroquinone. If the coulometric electrode was used as the detector, detection limits were approximately 250 pg for vitamin K. However, if a second electrode was added to oxidize the hydroquinone to the quinone, detection limits of 150 pg could be obtained. The reoxidation could be done either coulometrically or amperometrically. In the case of coulometric detection, the selectivity was not as good even though the detection limits were lower. Overall, the authors decided the best method was a combination of coulomtric reduction followed by fluorescence detection.

Carbohydrates may be determined at nickel (III) oxide,[96] platinum,[7,97] or gold electrodes.[98,99] A triple pulse amperometric detector has recently commercialized[100] the detection method pioneered by Johnson and Hughes.[7,97] This method gives detection limits as low as 50 nmol injected for some carbohydrates following separation on an anion exchange column (see Figure 9). One drawback of the technique is that the exact oxidation reaction mechanism is not well defined, and a high pH must be utilized. The optimum is 13, although in some cases the pH may be around 11. If the separation or sample considerations require a pH

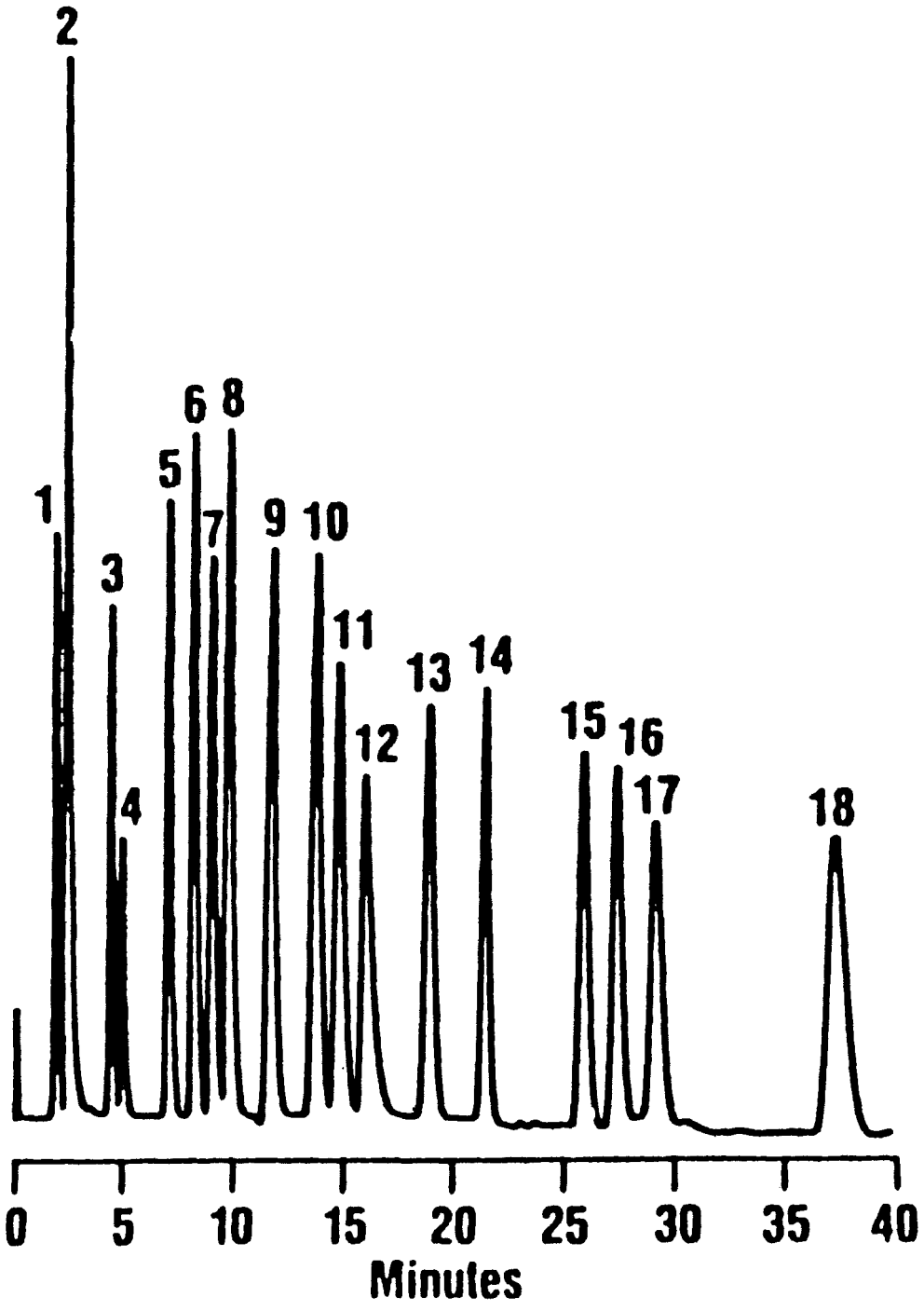


FIGURE 9. Determination of 18 carbohydrates by gradient elution with pulsed amperometric detection at a gold electrode. (1) Inositol (15 ppm); (2) Sorbitol (40 ppm); (3) Fucose (25 ppm); (4) Deoxyribose (25 ppm); (5) Deoxyglucose (20 ppm); (6) Arabinose (25 ppm); (7) Rhamnose (25 ppm); (8) Galactose (25 ppm); (9) Glucose (25 ppm); (10) Xylose (25 ppm); (11) Mannose (25 ppm); (12) Fructose (25 ppm); (13) Melibiose (25 ppm); (14) Isomaltose (25 ppm); (15) Gentiobiose (25 ppm); (16) Cellobiose (25 pm); (17) Turanose (50 ppm); (18) Maltose (50 ppm). (Reproduced with permission of Dionex, Inc.)

Table 4
DETERMINATION OF FLAVONOIDS AND PHENOLIC ACIDS BY LCEC

Phenolic compound	Ref.
Cinnamic acids	101,102
Benzoic acids	101
Flavanols	103,106,107,109
Flavonols	106
Flavones	104
Flavanones	105
Phytoestrogens	110

lower than 11, then sodium hydroxide may be added post-column. Post-column addition of reagent is not that difficult to accomplish, and this new electrochemical method to detect carbohydrates is much more selective and sensitive than refractive index detection.

G. FLAVONOIDS AND PHENOLIC ACIDS

The flavonoids and phenolic acids are important plant constituents that are synthesized from phenylalanine in the plant. UV detection is normally used for these compounds; however, several papers have been published in which LCEC was utilized.

The primary advantage of LCEC in the case of plant phenolics is selectivity. These compounds can be identified in a complex matrix of UV-absorbing material. Other UV-absorbing compounds, such as caffeine, do not interfere in the analysis. LCEC has been used extensively in food and beverage analysis. Phenolic acids have been detected in beer, chocolate, wine, and sunflower seeds.[101-104] Flavonoids have been detected in wine, chocolate, orange juice, grapefruit juice, and forage.[105-108]

Using dual-electrode LCEC, Roston and Kissinger were able to selectively detect the dihydroxylated, or chemically reversible, phenolic acids in a beer sample.[101] The identity of these compounds could also be further verified using dual-electrode LCEC in the parallel configuration. Alternatively, both collection efficiencies and current ratios can be used to classify an unknown phenolic compound based on its phenolic substitution.[106] A combination of UV data and EC data was used to classify flavonoid compounds found in wine and grape juice.

Table 4 gives some examples of the uses of LCEC in the analysis of plant secondary metabolites in a variety of samples.

H. Therapeutic Drug Monitoring and Determination of Drugs in Biological Matrices

The routine use of LCEC in the specific detection of pharmacologically active substances in biological samples is as widespread as its use in determination of biogenic amines. This includes blood, plasma, urine, organ tissues, and subcellular fractions (e.g., microsomes). The advantages of sensitivity and specificity have led to further applications, such as the study of the metabolism of biologically active compounds, which is discussed in more detail in Section VI. Lavrich and Kissinger[16] have reviewed LCEC as it relates specifically to therapeutic drug monitoring. In most cases, if a compound is electroactive, it will be detected selectively and generally with better detection limits than those found for UV detection. Since, in essence, the same detection principle has been applied for most pharmacologically active substances (buffered mobile phase followed by detection at a glassy carbon electrode),

only the more unique applications for some specific classes of compounds will be discussed here.

1. Analgesics

As a group, analgesics have been the drugs most widely studied by LCEC techniques. The most widely studied of this group have been acetaminophen,[111-113] salicylates,[114] codeine,[115-117] and morphine.[113,115]

LCEC has been investigated in more detail recently as a method for opiate alkaloids of abuse, including heroin, cocaine, and related compounds.[118,119] In the development of a general method,[119] it is the aliphatic tertiary nitrogen atom common to these compounds, rather than a phenolic moiety, which is reacted anodically at a glassy carbon electrode at +1.2 V vs. Ag/AgCl. Detection limits were reported to be in the nanogram range for morphine, heroin, and cocaine.

Ciramadol and dezocine are synthetic opioid analgesics of the agonist-antagonist type and have phenolic moieties that allow selective detection in plasma at the 10 μg/ml level.[120] In a similar manner, the narcotic analgesic ketobemidene (l-[4-(3-hydroxyphenyl)-1-methyl-4-piperidyl]-1-propanone) is another new narcotic analgesic and phenolic that is amenable to EC detection at the 1 ng/ml level in plasma.[121]

2. Antibiotics

Traditionally, antibiotics have been analyzed by some form of bioassay without specificity, which allows interferences from both active metabolites and other antibiotics. Methods employing LC with UV detection suffer from poor detection limits and extensive sample preparation. While many antibiotics are not electrochemically active, several elegant applications have been developed, including the photolytic derivatization of β-lactams.[122]

Chloramphenicol is an antibiotic that can be assayed conveniently by LCEC at negative potentials at mercury film electrodes, like other compounds containing an aromatic nitro group. Since there are very few reducible compounds found naturally in blood, this determination is highly selective.[16] Abou-Khalil et al.[123] recently reported determination of chloramphenicol and four analgesics using both oxidation and reduction modes at a glassy carbon electrode, as well as UV detection (see Figure 10). Although the ease of use of EC detection was demonstrated in this paper, a time savings could have been gained by using a dual-electrode approach. Using a series EC detector, with the upstream electrode set at −0.85 V (reducing the $-NO_2$ to −NHOH) and the downstream electrode set at +0.50 V (oxidizing the -NHOH to −NO), the determination could have been completed without need for mobile phase deoxygenation. Likewise, a determination of amino-chloramphenicol and chloramphenicol, which required separate determinations using a single electrode (once with the electrode poised at −0.85 V and then at +0.95 V), could be simplified. Using a parallel -adjacent dual cell, both potentials could be monitored simultaneously (although in this case deoxygenation of the mobile phase would be needed).

Both trimethoprim and sulfonamides can be detected utilizing LCEC methodology at a greater sensitivity than is possible with UV detection, although the applied potential is high, generally about +1.1 to +1.2 V. Tetracyclines have several moieties that make them amenable to electrochemical detection (typically at about +0.60 V).[124] Other antibiotics determined have included erythromycin and related macrolide antibiotics,[125-127] enviroxime,[128] and amoxicillin.[129] Several anticancer drugs have been determined and their mode of action studied with electrochemistry. These applications are considered in the next section on chemotherapeutic agents.

3. Chemotherapeutic Agents

Many of the diverse chemical agents used to treat various cancers have favorable redox properties for very selective LCEC methods. Cis-platinum complexes have been widely

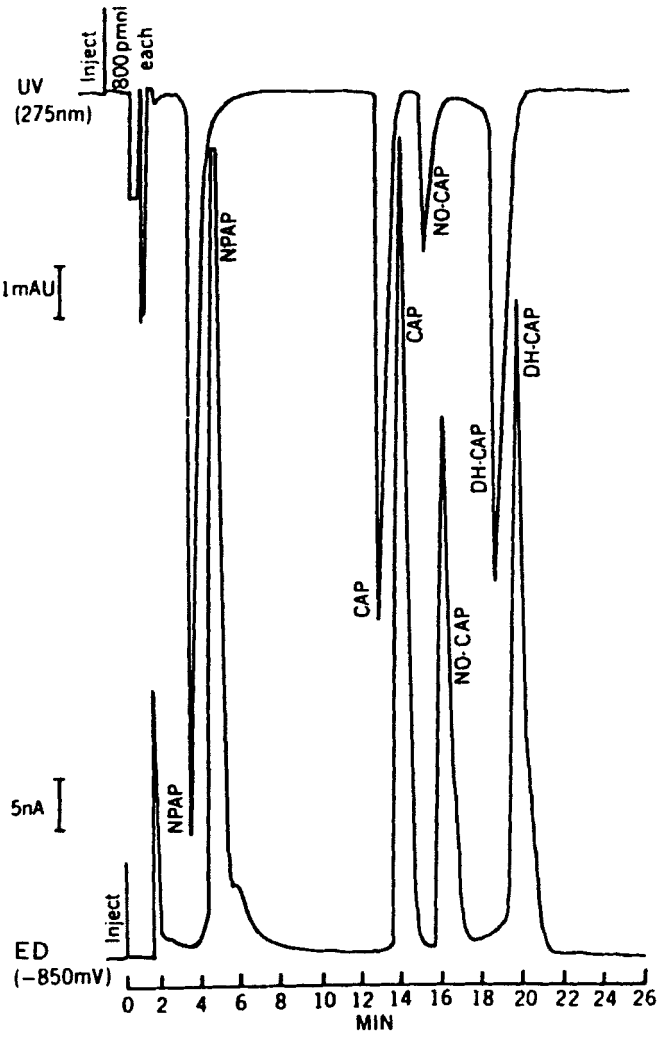


FIGURE 10. HPLC separation and dual detection of a standard mixture of (NPAP) Nitrophenylaminopropanedione; (CAP) Chloramphenicol; (NO-CAP) Nitrosochloramphenicol; (DH-CAP) Dehydrochloramphenicol. (Reprinted from Abou-Khalil, S., Abou-Khalil, W. H., Masoud, A. N., and Yunis, A. A., *J. Chromatogr.*, 417, 11, 1987. With permission. Copyright 1987, Elsevier Science Publishers.)

studied by LCEC with various degrees of success. These species have been detected following LC at dropping-mercury and hanging-mercury drop electrodes (using differential pulse amperometry), thin-layer Au/Hg electrodes, glassy carbon, and by a halide-catalyzed oxidation platinum electrode.[130-135] Although detection limits tend to be very dependent upon the ligands associated with the Pt (thus altering oxidation states and molecular geometry) and are also higher (typically in the micromolar range) than those usually expected with EC detection, most of these species lack significant UV activity, and LCEC provides an excellent method for their determination.

Many anticancer drugs are based on a quinone or hydroquinone structure. This makes them very amenable to electrochemical detection. Table 5 gives some of the quinone anticancer drugs and their metabolites that have been detected using LCEC.

The anthracycline antibiotics, adriamycin and daunomycin, can be detected by either

Table 5
ANTICANCER DRUGS

Compound	Electrode	Potential	Ref.
Doxorubicin (adriamycin)	GC	+0.80	136,137,142
	GC	+0.65	140
Daunorubicin (daunomycin)	GC	+0.80	136,142
	GC	+0.65	140
Teniposide (VM 26)	GC	+0.75	140
Etoposide (VP 16)	GC	+0.75	140
4′-Epidoxorubicin	GC	+0.80	143
Anthracene-dicarboxaldehyde bis hydrazone (bisantrene, ADCA)	GC	+0.65	140
	GC	+0.75	144
Mitomycin C	Hg	−0.60	138
Daunorubinol	GC	+0.65	136,140
7-Deoxy-daunorubicinol-aglycone	GC	+0.65	136,140
Daunorubicin aglycone	GC	+0.65	136,140
7-deoxydanorubicin-aglycone			
4′-deoxydoxorubicin	GC	+0.80	142
3′-deamino-3′-(3-cyano-4-morphol) doxorubicin			
4-Demethoxydaunorubicin	GC	+0.80	142
1-Napthol (napthoquinone)	GC	−0.40	139
Mitoxantrone (novantrone)	GC	+0.75	144

oxidative or reductive LCEC due to the presence of both a quinone and hydroquinone moiety. Akpofure et al. have detected daunomycin and adriamycin and their metabolites in serum and plasma using oxidative LCEC at a potential of +0.65 V.[136] The detection limits using electrochemical detection were comparable with those of fluorescence detection (2 ng on-column).

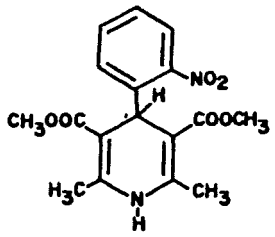
Riley at al.[137] compared the use of coulometric and amperometric detection for doxorubicin. They found that amperometric detection was more selective than coulometric detection. The detection limits for the two types of EC detectors were in agreement. However, with coulometric detection some interfering peaks were produced that made quantitation of the metabolites more difficult. These peaks could not be eliminated with a guard cell. One important point made in this paper was that although doxorubicin is reported to oxidize at a lower potential (+0.35 V) using the coulometric detector than with the amperometric detector (+0.80 V), this is a consequence only of the choice of two different reference electrodes. The selectivity is not increased using the coulometric cell.

Mitomycin C has been detected by reducing the quinone group present on the molecule using a mercury drop electrode.[138] The authors found that the hanging mercury drop electrode (HMDE) offered a much better S/N ratio than did the static mercury drop electrode. This led to better detection limits using HMDE (ca. 250 pg) as compared to the SMDE (15 ng). A small drop size (50 mg of Hg) also decreased the amount of noise produced.

1-Naphthol has been proposed as an anticancer agent. This compound is oxidized to naphthoquinone *in vivo*. The quinone has been detected using reductive LCEC *in vitro*.[139] Other common anticancer drugs such as methotrexate[145] and procarbazine hydrochloride[146] are also easily determined with LCEC.

4. Additional Applications

A large number of diverse pharmaceuticals are nitrogen heterocycles, and are unique both from pharmacological and electrochemical points of view. The calcium antagonist nifedipine is a good example. The heterocyclic ring can undergo a two-electrón oxidation (as per NADH), and the ring bearing the nitro group can be easily reduced.



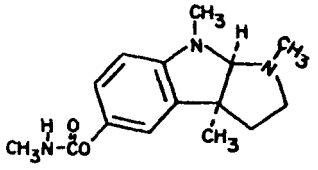
(22)

Nifedipine

Nifedepine and its metabolites have been determined with LCEC using both approaches.[147,148]

Physostigmine is another unique structure that acts as a rather strong cholinesterase inhibitor. The use of series electrodes for its determination in mouse liver microsomes and plasma[149] is based on the oxidation of physostigmine at +1.0 V.

The oxidized form rapidly hydrolyzes



(23)

Physostigmine

to form a quinoneimine product that can be reduced (two electrons) at +0.1 V (see Figure 11). This scheme allows for a detection limit of 0.5 ng/ml for a 2-ml plasma sample.

Theoharides et al.[150] have demonstrated the utility of LCEC for the determination of the antimalarial drug artesunic acid (ARTS) and its metabolite dihydroqinghaosu (DQHS). ARTS is a synthetic derivative of the sesquinterpene lactone endoperoxide qinghaosu (artemisinin) (see Figure 12). Detection of the compounds, which have little UV activity, was accomplished using EC detection in the reductive mode at an applied potential of −0.8 V using a Hg/Au thin-layer cell (see Figure 13). This system allowed for the determination of the compounds of interest in biological matrices and for a study of the conversion of ARTS to dihydroquinghaosu *in vitro*. The greatest difficulty encountered in the method was the continued deoxygenation of the mobile phase.

Another interesting application of LCEC for determination of compounds in biological matrices includes determination of some retinoid radicals.[151] In this case, the LCEC data were correlated with data obtained by LC-ESR to compare the relative information content. In incubations of retinoic acid and nitrosobenzene to generate radical species, the LCEC technique was found to be much more sensitive but much less specific than LC-ESR. The authors concluded that LCEC and LC-ESR are complementary methods suitable for the study of radical species.

Purdy and Bedard[152] have demonstrated the utility of surfactant-mediated gradient elution for the determination of serum thyroid hormones. Gradient LC methods have been shown to be compatible with EC detection; however, the electrical double layer tends to be affected, causing background shifts analagous to those seen in LCUV from absorbance and refractive index changes. In the thyroid hormone determination, cyclohexylaminopropane sulfonic acid (which is capable of causing a large change in retention over a concentration range) was used to alter retention times significantly for these compounds. The chromatographic profiles obtained using this technique did not appear to allow for unambiguous quantitation of the thyroid hormones.

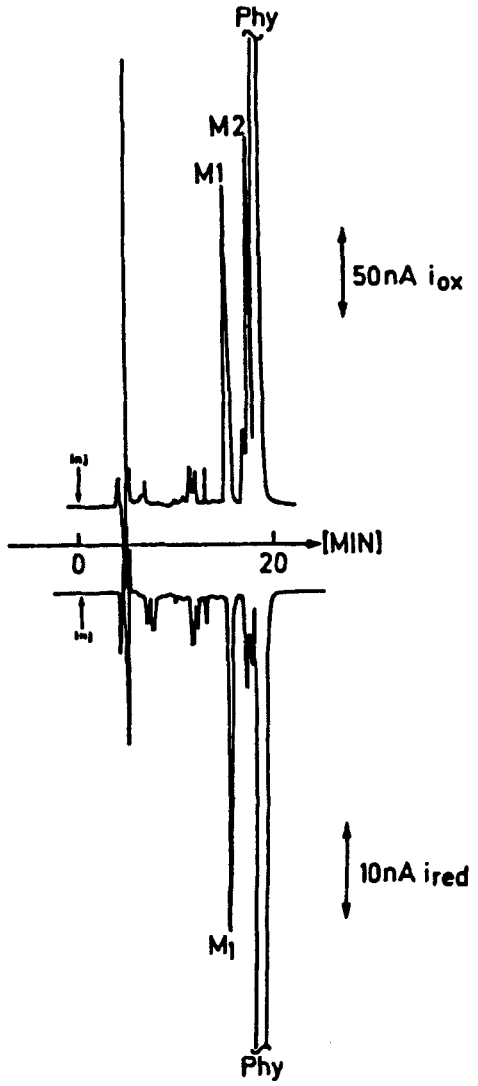


FIGURE 11. Physostigmine in mouse liver microsomal incubation. Series dual detection with $E_1 = +1.0$ V and $E_2 = -0.2$ V. After chromatography, the physostigmine is oxidized (iox) to allow formation of the quinoneimine, which is reduced (i_{red}) at the downstream electrode. (Reprinted from Isaksson, K. and Kissinger, P. T., *J. Chromatogr.*, 419, 165, 1987. With permission. Copyright 1987, American Chemical Society.)

Table 6 illustrates some of the more recent uses of LCEC in the determination of a broad range of pharmacologically active species.

V. APPLICATIONS TO THE STUDY OF XENOBIOTIC METABOLISM

A. Overview

The metabolism of drugs and other xenobiotic compounds (those species which serve no nutritional or biochemical role) is frequently ideal to study from an electrochemical perspective because the most interesting pathways (from a toxicological viewpoint) often lead to products with lower redox potentials than the initial reactant. This means that minor drug

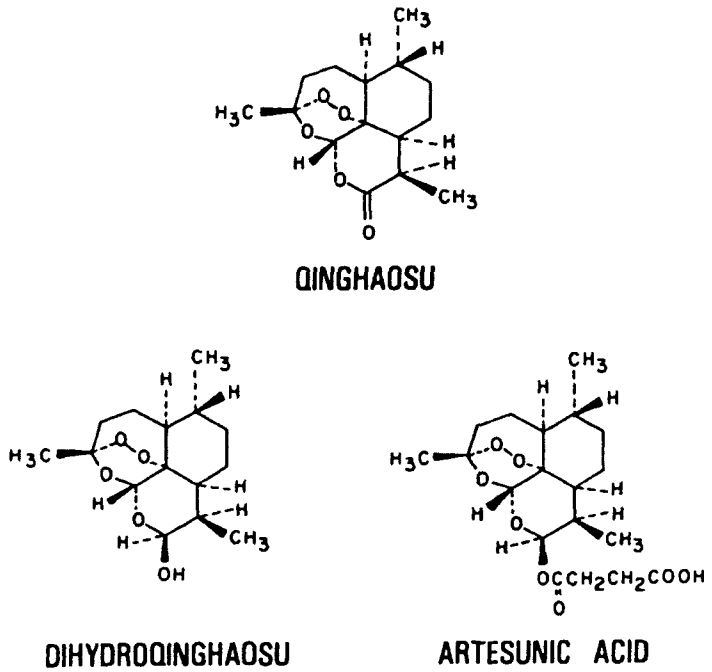


FIGURE 12. Structure of qinghaosu, dihydroqinghaosu, and artesunic acid. (Reprinted from Zhou, Z. M., Anders, J. C., Chung, H., and Theoharides, A. D., *J. Chromatogr.*, 414, 77, 1987. With permission. Copyright 1987, Elsevier Scientific Publishers.)

metabolites can often be detected by LCEC at lower concentrations than the drug itself. Due to the complex nature of the samples required to follow such biological transformations and the extremely low levels at which both drug and metabolites are often present, this makes LCEC is an excellent approach to this challenging analytical problem. The development of an LCEC method for such a task will ultimately allow rapid trace determinations of analyte at varying levels of system complexity. The ability to determine the rate of reaction, as well as the chemical nature and yields of specific products, can also provide information on the mechanism of metabolism. These procedures can then be applied to the subsequent reactions of activated metabolites with target nucleophiles that may lead to toxicity.

LCEC is particularly suited to study the transformation of a drug and also the metabolism of that drug's metabolite. Dual-electrode amperometric detectors have several advantages in this determination. The parallel-adjacent mode may be utilized to confirm peak identity based on the EC characteristics of a compound[164,165] and also to monitor two redox states simultaneously.[91] The series configuration has been used to determine important endogenous thiols[56,66-73,86-88] and to identify metabolites based on their electrochemical reversibility.[166] This section describes applications of LCEC in the important areas of drug metabolism.

B. Determination of Primary and Secondary Metabolites

Drug-metabolizing enzymes generally catalyze the biotransformation of lipophilic aryl compounds to more polar, water-soluble products that are more readily excreted. The metabolism of these tissue-penetrating, lipophilic species are categorized by two phases. Phase I reactions (which include the mixed-function oxidations such as cytochrome P-450) catabolize the molecules by oxidation, reduction, and hydrolysis. These reactions introduce polar reactive groups to the molecule, which increase water solubility and create a species more reactive toward the second metabolic phase. In phase II, the xenobiotic or its phase I products

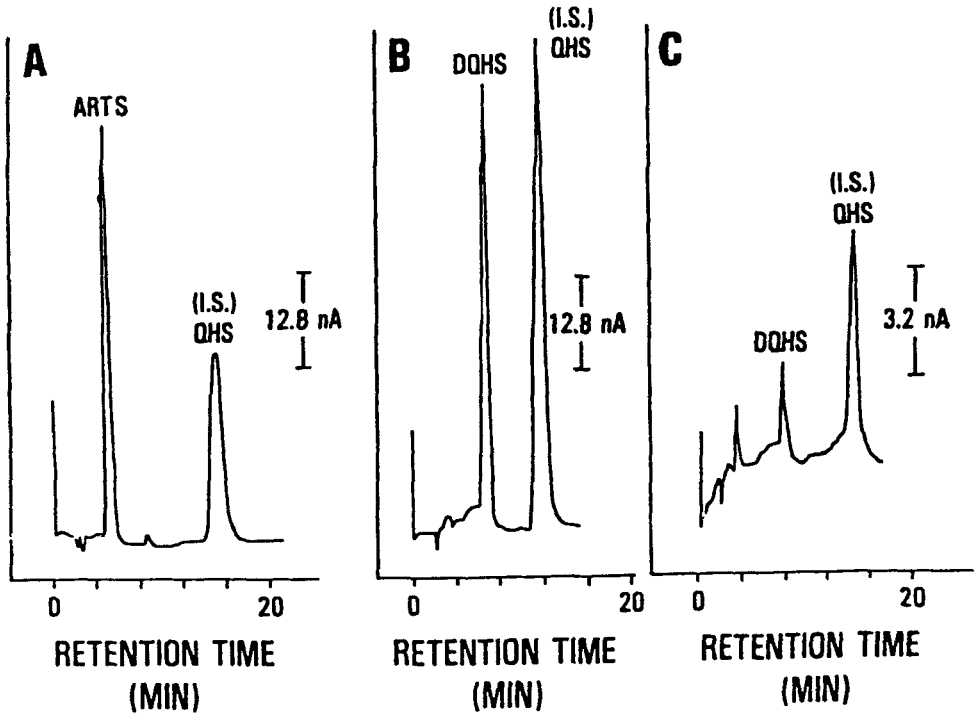


FIGURE 13. Chromatograms of human blood spiked with (A) Artesunic acid (10 ug per 0.5 ml); (B) Dihydroqinghaosu (2.5 ug per 0.5 ml; (C) Dihydroqinghaosu (200 ng/ml). (Reprinted from Zhou, Z. M., Anders, J. C., Chung, H., and Theoharides, A. D., *J. Chromatogr.*, 414, 77, 1987. With permission. Copyright 1987, Elsevier Scientific Publishers.)

Table 6
RECENT APPLICATION OF LCEC TO DRUG SUBSTANCES

Compound	Action	LCEC technique	Ref.
Amodiaquine	Antimalarial	+0.80 V, A	153
Minoxidil	Vasodilator	+0.30 V and +0.80 V, B	154
Fenoldopam	Vasodilator	+0.65 V and 0.90 V, A	155
Enoximone	Cardiotonic	A	156
Piroximone	Cardiotonic	A	156
Resorcinol	Kenatolytic	+1.0 V, A	157
Dopazinol	Anti-parkinson	+0.92 V, A	158
Dosulepin	Antidepressant	+1.3 V, A	159
Piriprost potassium	Leukotriene inhibitor	+0.90 V, A	160
THC-COOH, cannabis metabolite	Psychoactive	+1.2 V, A	161,162
Chlorprothixene	Antipsychotic	+1.0 V, A	163

Note: A = glassy carbon, thin-layer ampeometric detector. B = glassy carbon, coulometric detector.

combine with endogenous substrates (glucuronate, sulfate, acetate, glycine, gluthathione, etc.), making them more readily excretable. In general, the phase I reactions that make many aryl-based aromatic drugs more readily excretable also make them electroactive.

Evidence has been building that indicates that certain aryl xenobiotics may undergo direct two electron oxidations *in vivo* (see Figure 14). Such conversion may be mediated by the mixed-function oxidase and other enzyme systems. This electron withdrawal supersedes the normal oxygen insertion mechanisms and creates electrophilic species. A case in point is

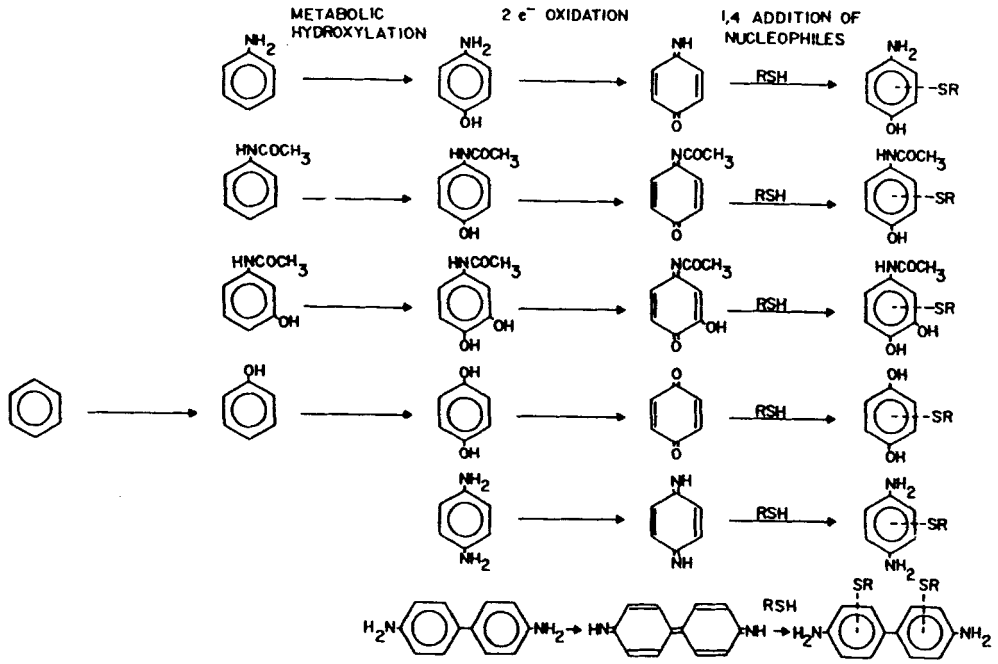


FIGURE 14. Reactions of some aryl xenobiotics, including acetaminophen (4-hydroxyacetanilide), benzene, and phenol.

the formation of the quinoid *N*-acetyl-*p*-quinoneimine from acetaminophen.[167] This compound and similar species undergo rapid Michael-type additions with endogenous nucleophiles. Some enzyme systems, such as peroxidase, catalase, and prostaglandin H synthetase, oxidize via one-electron withdrawals in the same manner that an analyte is reacted at an electrode surface. With such mimicry between EC and metabolic reactions, it is not surprising that LCEC is readily applicable to the determination of primary (phase I) and secondary to (phase II) metabolites and that it may also lend insight to the metabolic transformations that are occurring.

LCEC has been applied to the determination of metabolites from several sources of varying levels of system complexity. With pure enzyme incubations and subcellular fraction incubations (microsomes), those substances needed for conjugation are missing; however, this matrix makes the determination of initial metabolic products easy. Higher levels of complexity include tissue slices, perfusates, urine, and plasma, in all of which LCEC has shown utility because of enhanced selectivity and sensitivity advantages.[167-187] Dual-electrode detection has allowed scientists to perform EC experiments on injected solutions to characterize metabolites.[149,155,164,171,175] Since even small changes in chemical structure can influence the potential at which a compound will be oxidized and/or reduced, this information can be used to characterize both drug metabolites and endogenous species. The parallel-adjacent detection scheme allows confirmation of peak identity and, more importantly, evaluation of peak homogeneity in these difficult matrices.[164,175] The series detector has, as previously described, been most often used as a post-column reactor followed by an EC detector. For the identification and quantitation of metabolites, tentative structural assignments may be made based on the reversibility of the EC reaction. In the determination of fenoldopam (a renal vasodilator) and its metabolites, series detection was used to determine catechol sulfate and methoxy conjugates.[155] The conjugates oxidized at a potential of +0.9 V, which did not allow sufficient sensitivity for determination. However, at this potential, the conjugate is cleaved to form an *o*-quinone that is determined by reduction at the second

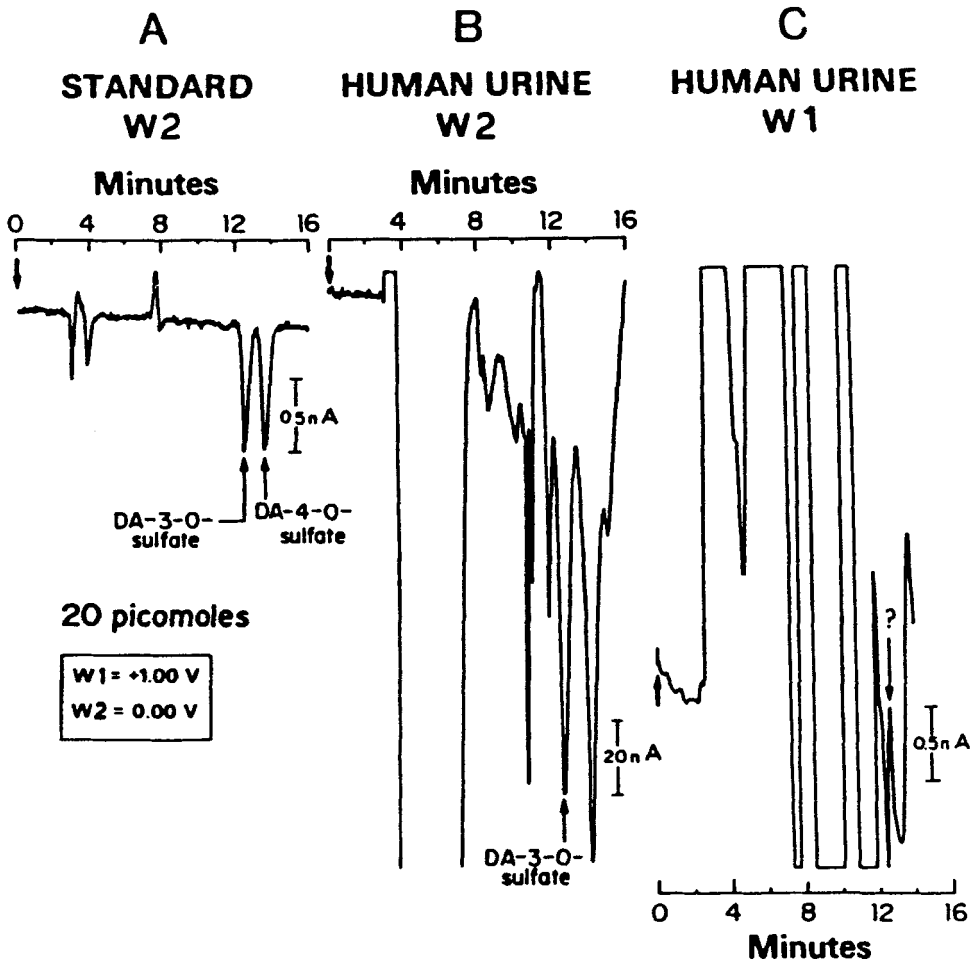


FIGURE 15. Series dual-electrode chromatograms for 20 µl of human urine injected directly, $W_1 = +1.00$ V; $W_2 = 0.00$ V. (A) Standard solution containing 20 pmol of each dopamine-sulfate isomer. Responses monitored at W_2; human urine sample injected and responses monitored at W_2 (B) and W_1 (C). (Reprinted from Elchisak, M. A., *J. Chromatogr.*, 264, 119, 1983. With permission. Copyright 1983, Elsevier Scientific Publishers.)

electrode at a potential of 0.0 V. Elchisiak has used a similar approach to determine sulfates and catechol sulfates of biogenic amines, as illustrated in Figure 15.[171] This technique should have general utility for drugs or xenobiotics in which one phenolic group is conjugated.

The ability to perform experiments as described above on the chromatographic time scale makes LCEC an invaluable resource for the scientist involved in xenobiotic metabolism studies. Table 7 summarizes various problems relating to xenobiotic metabolism in which LCEC has shown utility in providing qualitative and quantitative information.

VI. FUTURE DIRECTIONS

The applications of LCEC will continue to grow in several directions. The sheer number of electroactive pharmacologic agents and those which will certainly be synthesized in the future guarantee this. Microbore LC is currently in a growth period and will probably gain greater popularity in areas where development efforts for a pharmaceutical are very sample limited. Such a case may be in the development of modified monoclonal antibodies. Biotechnology projects where a monoclonal antibody is being utilized as a drug delivery system

Table 7
SELECTED APPLICATIONS OF LCEC IN THE STUDY OF XENOBIOTIC METABOLISM

Compound	Method/utility	Ref.
Acetaminophen and related compounds	Thin-layer LCEC,[a] single and dual; determination of phase I and phase II metabolites, *in vivo* and *in vitro;* characterization of compounds based on electrochemical properties; study fundamental metabolic activation mechanism	111,112,167,168,180
Benzene, naphthalene, phenol, quinones	Thin-layer LCEC,[a] single and dual; as above, insights into mechanism of activation and detoxification as pertains to hydrocarbons	139,149,157,164,169,179,181, 186,187
Benzidine aniline, azo dye metabolites; 2-Amino-4-(5-nitro-2-furyl) thiazole	Thin-layer LCEC,[a] single and dual; as above, insights into mechanism of activation and detoxification as pertains to N-substituted aromatic amines and nitroaromatics	170,174,175,183-185
Fenoldopam	Thin-layer LCEC,[a] dual; determine phase II metabolites formed *in vivo;* characterization based on electrochemical properties	155
Glutathione cysteine	Thin-layer LCEC,[b] single and dual; determine role as a nucleophile, a reducing agent, or as a substrate for glutathione peroxidase	59-74,86-88
Estradiol	Thin-layer LCEC,[a] single; determination of phase I and phase II metabolites	173,182
Aniline, phenol, salicylate	Thin-layer LCEC;[a] determination of hydroxyl free radical formation, *in vivo* and *in vitro*	176,177

[a] Electrode material is glassy carbon.
[b] Electrode material is Hg/Au amalgam.

for an extremely small amount of a potent drug to a specific organ site may be well suited to such micro-column techniques for determination of the free drug. Since EC detection is compatible with such small-bore techniques[1] and provides the sensitivity and selectivity advantages that are needed, the combination will probably see use in such areas.

The EC detector is being developed in ways that are directed toward uses in biological applications. As described in Section IV, elegant pre- and post-column derivatization methods are being developed to extend the applicability of the LCEC technique. Just as it has become relatively easy to determine thiols at what is essentially a modified electrode surface, other materials are being evaluated for such specific tasks. This ranges from the development by Baldwin's group of an electrode for determination of the smaller oxalic acid and alpha-ketoacid molecules (at cobalt phthalocyanine modified carbon paste surfaces)[188] to determination of the relatively large ferro- and ferricytochrome c molecules (at 4,4′-dithiodipyridine modified gold electrodes).[189] One might imagine that some day electrodes may be easily and rapidly modified for specific analyses, perhaps eliminating the column completely. Other developments of this genre include coating electrodes with a permselective polymeric film, which allows for detection based upon a size exclusion principle.[190] Selectivity is improved as small eluting molecules are detected, whereas large molecules are

excluded. This could prevent rapid electrode fouling by endogenous compounds, reduce the amount of sample clean-up needed, and provide qualitative information on approximate molecular size of unknown compounds eluting. Techniques such as multielectrode and pulsed amperometric detection should continue to grow in the near future, as will the applicability of newer electrode materials. Such advances will, no doubt, enhance the ability of the researcher to evaluate the many facets of pharmacological and biochemical research.

ACKNOWLEDGMENT

The authors wish to thank Chris Gesner for all her assistance in the preparation of this manuscript.

REFERENCES

1. **Shoup, R. E.,** Liquid chromatography/electrochemistry, *High-Performance Liq. Chromatogr.*, 4, 91, 1986.
2. **Kissinger, P. T.,** Electrochemical detection in liquid chromatography and flow injection analysis, in *Laboratory Techniques in Electroanalytical Chemistry,* Kissinger, P. T. and Heineman, W. R., Eds., Marcel Dekker, New York, 1984, 611.
3. **Johnson, D. C., Weber, S. G., Bond, A. M., Wightman, R. M., Shoup, R. E., and Krull, I. S.,** Electroanalytical voltammetry in flowing solutions, *Anal. Chim. Acta,* 180, 187, 1986.
4. **Weber, S.,** Detection based on electrical and electrochemical measurements, in *Detectors for Liquid Chromatography,* Yeung, E., Ed., John Wiley & Sons, New York, 1986, 229.
5. **Heineman, W. R. and Kissinger, P. T.,** Cyclic voltammetry, *J. Chem. Educ.*, 60, 702, 1983.
6. **Kissinger, P. T.,** *Anal. Chem.*, 49, 447A, 1977.
7. **Hughes, S. and Johnson, D. C.,** Amperometric detection of simple carbohydrates at platinum electrodes in alkaline solutions by application of a triple-pulse potential waveform, *Anal. Chim. Acta.*, 132, 111, 1981.
8. **Edwards, P. and Haak, K. K.,** A pulsed amperometric detector for ion chromatography, *Am. Lab.*, 15, April 1983.
9. **Lunte, C. E., Shoup, R. E., and Kissinger, P. T.,** Difference mode detection with thin-layer dual-electrode liquid chromatography/electrochemistry, *Anal. Chem.*, 57, 1541, 1985.
10. **Roston, D. A., Shoup, R. E., and Kissinger, P. T.,** Liquid chromatography/electrochemistry: thin-layer multiple electrode detection, *Anal. Chem.*, 54, 1417A, 1982.
11. **Lunte, C. E., Ridgway, T. H., and Heineman, W. R.,** Voltammetric-amperometric dual-electrode detection for flow injection analysis and liquid chromatography, *Anal. Chem.*, 59, 761, 1987.
12. **Jacobs, W. A. and Kissinger, P. T.,** Nitroaromatic reagents for determination of amines and amino acids by liquid chromatography/electrochemistry, *J. Liq. Chromatogr.*, 5, 881, 1982.
13. **Musch, G., De Smet, M., and Massart, D. L.,** Expert system for pharmaceutical analysis. I. Selection of the detection system in high-performance liquid chromatographic analysis: UV versus amperometric detection, *J. Chromatog.*, 348, 97, 1985.
14. **Musch, G. and Massart, D. L.,** Expert system for pharmaceutical analysis. II. Relative contribution of and rule validation for amperometric detection (oxidative mode), *J. Chromatogr.*, 370, 1, 1986.
15. **Radzik, D. M. and Kissinger, P. T.,** Liquid chromatography/electrochemistry in pharmaceutical analysis, in *HPLC in Pharmaceutical Analysis,* Fong, G. W. and Lam, S. L., Eds., Marcel Dekker, in press.
16. **Lavrich, C. and Kissinger, P. T.,** Liquid chromatography-electrochemistry: potential utility for therapeutic drug monitoring, in *Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography,* Wong, S. H. Y., Ed., Marcel Dekker, New York, 1985, 191.
17. **Schieffer, G. W.,** High-performance liquid chromatography-controlled potential coulometry as an absolute method for determining the purity of reference standards, *Anal. Chem.*, 57, 968, 1985.
18. **Radzik, D. M.,** Application of liquid chromatography/electrochemistry in pharmaceutical development, in Pittsburgh Conf. Expo. Anal. Chem. Appl. Spectrosc. Abstr., Abstr. No. 963, 1986.
19. **Kafil, J. B., Cheng, H.-Y., and Last, T. A.,** Quantitation of nucleic acids at the picogram level using high-performance liquid chromatography with electrochemical detection, *Anal. Chem.*, 58, 285, 1986.
20. **Kafil, J. B. and Last, T. A.,** Liquid chromatography with metal oxide/glassy carbon electrode as a detector for quantitation of nucleic acids, in Int. Electroanal. Symp. Abstr., Abstr. No. 33, 1986, 110.

21. **Yamamoto, T., Shimizu, H., Kato, T., and Nagatsu, T.,** Simultaneous determination of guanine nucleotides in rat brain by high performance liquid chromatography with dual-electrochemical detection, *Anal. Biochem.*, 142, 395, 1984.
22. **Rocklin, R. D. and Johnson, E. L.,** Determination of cyanide, sulfide, iodide, and bromide by ion chromatography with electrochemical detection, *Anal. Chem.*, 55, 4, 1983.
23. **Bond, A. M., Heritage, I. D., Wallace, G. G., and McCormick, M. J.,** Simultaneous determination of free sulfide and cyanide by ion chromatography with electrochemical detection, *Anal. Chem.*, 54, 582, 1982.
24. **Lookabaugh, M., Krull, I. S., and LaCourse, W. R.,** Determination of iodide and thiosulfate by paired-ion, reversed-phase high-performance liquid chromatography with ultra-violet absorbance, electrochemical, and conductimetric detection, *J. Chromatogr.*, 387, 301, 1987.
25. **Krull, I. S., Selavka, C. M., Duda, C., and Jacobs, W.,** Derivatization and post-column reactions for improved detection in liquid chromatography/electrochemistry, *J. Liq. Chromatogr.*, 8, 2845, 1985.
26. **White, M. V.,** High performance liquid chromatography of tyrosine-related peptides with electrochemical detection, *J. Chromatogr.*, 262, 420, 1983.
27. **Sauter, A. and Frick, W.,** Determination of neuropeptides in discrete regions of the rat brain by high performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 297, 215, 1984.
28. **Kuwana, T.,** High sensitivity analysis of peptides, in Japanese-United States Congr. Pharm. Sci. Abstr., 1987, 35.
29. **Caudill, W. J., Houck, G. P., and Wightman, R. M.,** Determination of gamma-aminobutyric acid by liquid chromatography with electrochemical detection, *J. Chromatog.*, 227, 331, 1982.
30. **Meek, J. L.,** Derivatizing reagents for high-performance liquid chromatography detection of peptides at the picomole level, *J. Chromatogr.*, 266, 401, 1983.
31. **Joseph, M. H. and Davies, P.,** Electrochemical activity of *O*-phthalaldehyde-mercaptoethanol derivatives of amino acids, application to high-performance liquid chromatographic determination of amino acids in plasma and other biological materials, *J. Chromatogr.*, 277, 125, 1983.
32. **Allison, L. A., Mayer, G. S., and Shoup, R. E.,** *O*-Phthalaldehyde derivatives of amines for high-speed liquid chromatography/electrochemistry, *Anal. Chem.*, 56, 1089, 1984.
33. **Granberg, R. R.,** High-resolution analysis of PITC-derivatized amino acids with UV and electrochemical detection, *L.C. Mag.*, 2, 776, 1984.
34. **Mahachi, T. J., Carlson, R. M., and Poe, D. P.,** *p-N,N*-dimethylamino-phenylisothiocyanate as an electroactive label for high performance liquid chromatographic determination of amino acids, *J. Liq. Chromatogr.*, 298, 297, 1984.
35. **Shimada, K., Tanaka, M., and Nambara, T.,** New derivatization of amines for high performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 280, 271, 1983.
36. **Wang, C. C. R.,** Cyclic Voltammetric and Flow Injection Analysis of CBI-alanine for LCEC, Master's thesis, University of Kansas, Lawrence, 1987.
37. **Caudill, W. L., Papach, L. A., and Wightman, R. M.,** Measurement of brain GABA with LCEC, *Curr. Sep.*, 4, 59, 1982.
38. **Lunte, S. M., Wang, C. R., Mohubbut. T., Hu, I. F., and Kuwana, T.,** Determination of CBI-derivatized amino acids by liquid chromatography/electrochemistry, in 12th Int. Symp. Column Liq. Chromatogr. (HPLC '88) Abstr., AMP-110, 1988.
39. **Xie, K.-H., Xie, Colgan, S., and Krull, I. S.,** Solid phase reactions for derivatization of HPLC (HPLC-SPR), *J. Liq. Chromatogr.*, 6, 125, 1983.
40. **Leroy, P., Nicolas, A., and Moreau, A.,** Electrochemical detection of sympathomimetric drugs following precolumn *O*-phthalaldehyde derivatization and reversed-phase HPLC, *J. Chromatogr.*, 282, 561, 1983.
41. **Jacobs, W. A.,** *O*-Phthalaldehyde derivatization of amino acids and peptides for LCEC, *Curr. Sep.*, 7, 39, 1986.
42. **Caudill, W. L. and Wightman, R. M.,** Trinitrobenzenesulfonic acid: a chromophore, electrophore, and pre-column-derivatizing agent for high performance liquid chromatography of alkylamines, *Anal. Chim. Acta*, 141, 269, 1982.
43. **Harsing, L. G., Nagashima, H., Vizi, E. S., and Duncalf, D.,** Electrochemical determination of histamine derivatized with *O*-phthalaldehyde and 2-mercaptoethanol, *J. Chromatogr.*, 383, 19, 1986.
44. **Polta, J. A. and Johnson, D. C.,** The direct electrochemical detection of amino acids at a platinum electrode in an alkaline chromatographic effluent, *J. Liq. Chromatogr.*, 6, 1727, 1983.
45. **Hui, B. S. and Huber, C. O.,** Amperometric detection of amines and amino acids in flow injection systems with a nickel oxide electrode, *Anal. Chim. Acta.*, 134, 211, 1982.
46. **Shoup, R. E.,** Recent Reports on Liquid Chromatography with Electrochemical detection, Bioanalytical Systems, W. Lafayette, IN, 1982.
47. **Goto, M., Nakamura, T., and Ishii, D.,** Micro high-performance liquid chromatographic system with micro precolumn and dual electrochemical detector for direct injection analysis of catecholamines in body fluids, *J. Chromatogr.*, 226, 33, 1981.

48. **Caliguri, E. J. and Mefford, I. N.,** Femtogram detection limits for biogenic amines using microbore HPLC with electrochemical detection, *Brain Res.*, 296, 156, 1984.
49. **Lin, P. Y. T., Bulawa, M. C., Wang, P., Lin, L., Scott, J., and Blank, C. L.,** The determination of catecholamines, indoleamines, metabolites, and related enzymatic activities using three micron liquid chromatography columns, *J. Liq. Chromatogr.*, 7, 509, 1984.
50. **Naito, J., Ishiguro, J., Murazumi, T., and Morimoto, M.,** Determination of kynurenine in serum by high-performance liquid chromatography after enzymatic conversion to 3-hydroxykynurenine, *Anal. Biochem.*, 161, 16, 1987.
51. **Harsing, L. G., Nagashima, H., Vizi, E. S., and Duncalf, D.,** Electrochemical determination of histamine derivatized with *O*-phthalaldehyde and 2-mercaptoethanol, *J. Chromatogr.*, 383, 19, 1986.
52. **Gusovsky, F., Jacobson, K. A., Kirk, K. L., Marshall, T., and Linnoila, M.,** New high-performance liquid chromatographic procedure for the detection and quantification of β-phenylethylamine, *J. Chromatogr.*, 415, 124, 1987.
53. **Ungersted, V.,** *Measurement of Neurotransmitter Release In Vivo,* Marsden, C. A., Ed., John Wiley & Sons, New York, 1984, chap. 4.
54. **Sharp, T., Zetterstrom, T., Series, H. G., Carlsson, A., Grahame-Smith, D. G., and Ungerstedt, V.,** HPLC-EC analysis of catechols and indoles in rat brain dialysates, *Life Sci.*, 41, 869, 1987.
55. **Sharp, T., Carlsson, A., Zetterström, T., Lundstroem, K., and Ungerstedt, V.,** Rapid measurement of dopamine release using brain dialysis combined with microbore HPLC, *Ann. N.Y. Acad. Sci.*, 473, 512, 1986.
56. **Perret, D. and Rudge, S. R.,** The determination of thiols and related compounds using high-performance liquid chromatography, *J. Pharm. Biomed. Anal.*, 3, 3, 1985.
57. **Saetre, R. and Rabenstein, D. L.,** Determination of penicillamine in blood and urine by high performance liquid chromatography, *Anal. Chem.*, 50, 276, 1978.
58. **Perret, D. and Drury, P. L.,** The determination of captopril in physiological fluids using high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 5, 97, 1982.
59. **Stein, A., Dills, R. L., and Klaussen, C. D.,** High-performance liquid chromatographic analysis of glutathione and its thiol and disulfide degradation products, *J. Chromatogr.*, 381, 259, 1986.
60. **Lunte, S. M. and Kissinger, P. T.,** The use of liquid chromatography with dual-electrode electrochemical detection in the investigation of glutathione oxidation during benzene metabolism, *J. Chromatogr.*, 371, 579, 1984.
61. **Demaster, E. G., Shirata, F. N., Redfern, B., Goon, D. J. W., and Nagasawa, H. T.,** Analysis of hepatic-reduced glutathione, cysteine, and homocysteine by cation-exchange high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 308, 83, 1984.
62. **Allison, L. A., Keddington, J., and Shoup, R. E.,** Liquid chromatographic behavior of biological thiols and the corresponding disulfides, *J. Liq. Chromatogr.*, 6, 1785, 1983.
63. **Allison, L. A. and Shoup, R. E.,** Dual-electrode liquid chromatography detector for thiols and disulfides, *Anal. Chem.*, 55, 8, 1983.
64. **Jensen, C. B., Grossman, S. J., and Jollow, D. J.,** Improved method for the determination of cellular thiols, disulfides, and protein-mixed disulfides using HPLC with electrochemical detection, *Adv. Exp. Med. Biol.*, 197, 407, 1986.
65. **Dubry, D. and Szabo, S.,** Measurement of tissue sulfhydryls and disulfides in tissue protein and nonprotein fractions by high-performance liquid chromatography using electrochemical detection, *J. Liq. Chromatogr.*, 10, 107, 1987.
66. **Lunte, S. M. and Kissinger, P. T.,** Detection of thiols and disulfides in liver samples using liquid chromatography/electrochemistry, *J. Liq. Chromatogr.*, 8, 691, 1985.
67. **Saetre, R. and Rabenstein, D. L.,** Determination of cysteine in plasma and urine and homocysteine in plasma by high-pressure liquid chromatography, *Anal. Biochem.*, 90, 684, 1978.
68. **Rabenstein, D. L. and Saetre, R.,** Mercury-based electrochemical detector for liquid chromatography for the detection of glutathione and other sulfur-containing compounds, *Anal. Chem.*, 49, 1036, 1977.
69. **Rabenstein, D. L. and Saetre, R.,** Analysis for glutathione in blood by high-performance liquid chromatography, *Clin. Chem.*, 24, 1140, 1978.
70. **Halbert, M. K. and Baldwin, R. P.,** Determination of cysteine and glutathione in plasma and blood by liquid chromatography with electrochemical detection using a chemically modified electrode containing cobalt phthalocyanine, *J. Chromatogr.*, 345, 43, 1985.
71. **Iriyama, K., Iwamoto, T., and Yoshiura, M.,** Liquid chromatographic determination of glutathione with electrochemically pretreated glassy carbon electrodes, *J. Liq. Chromatogr.*, 9, 955, 1986.
72. **Mefford, I. and Adams, R. N.,** Determination of reduced glutathione in guinea pig and rat tissue by HPLC with electrochemical detection, *Life Sci.*, 23, 1167, 1978.
73. **Eggli, R. and Asper, R.,** Electrochemical flow-through detector for the determination of cystine and related compounds, *Anal. Chim. Acta,* 101, 253, 1978.

74. **Perret, D. and Rudge, S.,** Problems associated with the high-performance liquid chromatography of thiols, *J. Chromatogr.*, 294, 380, 1984.
75. **Kreuzig, F. and Frank, J.,** Rapid automated determination of D-penicillamine in plasma and urine by ion exchange high performance liquid chromatography with electrochemical detection using a gold electrode, *J. Chromatogr.*, 218, 615, 1981.
76. **Russel, A. S., Saetre, R., Davis, P., and Rabenstein, D. L.,** A rapid sensitive technique to assay penicillamine levels in blood and urine, *J. Rheumatol.*, 6, 15, 1979.
77. **Butler, M., Carruthers, G., Harth, M., Freemand, D., Percy, J., and Rabenstein, D.,** Pharmacokinetics of reduced D-penicillamine in patients with rheumatoid arthritis, *Arth. Rheum.*, 25, 111, 1982.
78. **Kok, W. T., Halvax, J. J., and Frei, R. N.,** Comparison of electrochemical detection methods in liquid chromatography for the detection of *N*-acetyl cysteine in plasma, *J. Chromatogr.*, 352, 27, 1986.
79. **Halbert, M. K. and Baldwin, R. P.,** Electrocatalytic and analytical response of cobalt phthalocyanine containing carbon paste electrodes toward sulfhydryl compounds, *Anal. Chem.*, 57, 591, 1985.
80. **Shaw, L. M. and Bonner, H. S.,** Detection and measurement of the disulfide WR 3327B (NH2[CH2]3NHCH2CH2S-)2 in blood and tissues, *J. Liq. Chromatogr.*, 10, 439, 1987.
81. **Kok, W. Th., Halrax, J. J., Voogt, W. H., Brinkman, T. H., and Frei, R. W.,** Detection of thioethers of pharmaceutical importance by liquid chromatography with on-line generated bromine, *Anal. Chem.*, 57, 2580, 1986.
82. **Iriyama, K., Yoshiura, M., and Iwamoto, T.,** A method for the determination of methionine in human serum by high-performance liquid chromatography with electrochemical detection, *J. Liq. Chromatogr.*, 9, 2955, 1986.
83. **Halbert, M. K. and Baldwin, R. P.,** Amperometric detection of thiopurines in blood plasma with a colbalt-phthalocyanine chemically modified electrode after liquid chromatography, *Anal. Chim. Acta.*, 187, 89, 1986.
84. **Curry, S. H., Brown, E. A., Hu, O. Y.-P., and Perrin, J. H.,** Liquid chromatographic assay of phenothiazine, thioxanthene, and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J. Chromatogr.*, 231, 361, 1982.
85. **Stoll, A. L., Baldessarini, R. J., Cohen, B. M., and Finkelstein, S. P.,** Assay of plasma thioridazine and metabolites by high-performance liquid chromatography with amperometric detection, *J. Chromatogr.*, 307, 457, 1984.
86. **Reed, D. J., Babson, J. R., Beatty, P. W., Brodie, A. E., Ellis, W. W., and Potter, D. W.,** High-performance liquid chromatography analysis of nanomole levels of glutathione, glutathione disulfide and related thiols and disulfides, *Anal. Biochem.*, 106, 55, 1982.
87. **Richie, J. P. and Lang, C. A.,** The determination of glutathione, cyst(e)ine, and other thiols and disulfides in biological samples using high performance liquid chromatography with dual electrochemical detection, *Anal. Biochem.*, 163, 9, 1987.
88. **Lunte, S. M.,** Determination of thiols and disulfides in urine by dual electrode liquid chromatography/electrochemistry, *Curr. Sep.*, 6, 30, 1984.
89. **Bond, A. M., Heritage, J. D., Wallace, G. G., and McCormick, M. J.,** Simultaneous determination of free sulfide and cyanide by ion chromatography with electrochemical detection, *Anal. Chem.*, 54, 582, 1982.
90. **Shoup, R. E.,** Recent Reports on Liquid Chromatography with Electrochemical Detection, Bioanalytical Systems, W. Lafayette, IN, 1982.
91. **Lunte, C. E., and Kissinger, P. T.,** Determination of pterins in biological samples by liquid chromatography/electrochemistry with a dual-electrode detector, *Anal. Chem.*, 55, 1458, 1983.
92. **Pachla, L. A., Reynolds, D. L., and Kissinger, P. T.,** Analytical methods for determining ascorbic acid in biological samples, food products, and pharmaceuticals, *J. Off. Assoc. Anal. Chemists*, 68, 1, 1985.
93. **Vanderwounde, M., Claeys, M., and De Leeuw,** Determination of α-tocopherol in human plasma by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 311, 176, 1984.
94. **Lang, J. K., Gohil, K., and Packer, L.,** Simultaneous determination of tocopherols, ubiquinonels and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions, *Anal. Biochem.*, 157, 106, 1986.
95. **Langenberg, J. P. and Tjaden, U. R.,** Determination of (endogenous) vitamin K in human plasma by reverse-phase high-performance liquid chromatography using fluorometric detection after post-column electrochemical reduction, comparison with ultraviolet, single and dual electrochemical detection, *J. Chromatogr.*, 305, 61, 1984.
96. **Reim, R. E. and Van Effen, R. M.,** Determination of carbohydrates by liquid chromatography with oxidation at a nickel (III) oxide electrode, *Anal. Chem.*, 58, 34203, 1986.
97. **Hughes, S. and Johnson, D. C.,** High-performance liquid chromatographic separation with triple-pulse amperometric detection of carbohydrates in beverages, *J. Agric. Food Chem.*, 30, 712, 1982.

98. **Rocklin, R. D. and Pohl, C. A.,** Determination of carbohydrates with pulsed amperometric detection, *J. Liq. Chromatogr.*, 6, 1577, 1983.
99. **Rocklin, R. D.,** Working-electrode materials, *L.C. Mag.*, 2, 588, 1984.
100. **Edwards, P. and Haak, K. K.,** A pulsed amperometric detector for ion chromatography, *Am. Lab.*, 15, April 1983.
101. **Roston, D. A. and Kissinger, P. T.,** Identification of phenolic constituents in commercial beverages by Liquid chromatography with electrochemical detection, *Anal. Chem.*, 53, 1695, 1981.
102. **Felice, L. C., King, W. P., and Kissinger, P. T.,** A new liquid chromatography approach to plant phenolics. Application to the determination of chlorogenic acid in sunflower meal, *J. Agric. Food Chem.*, 24, 380, 1976.
103. **Kenyhercz, T. M. and Kissinger, P. T.,** Determination of selected acidic, neutral, and basic natural products in cocoa beans and processed cocoa, liquid chromatography with electrochemical detection, *Lyoydia*, 41, 130, 1978.
104. **Sonntag, G. and Kral, K.,** Bestimmung von Hesperidin in Orangesaften und grangelimonaden mit einem Amperometrischen Detecktor nach Trennung Durch Hochdruck-Flussigkeits-Chromatographie, *Fresenius Z. Anal. Chem.*, 309, 109, 1981.
105. **Frank, E., Kainz, G., and Sonntag, G.,** Bestimmung von Naringin in Grapefruit-Saften durch HPLC mit Electrochemischer Detektian, *Ernaehrung*, 8, 195, 1984.
106. **Lunte, S. M.,** Structural classification of flavonoids in beverages by liquid chromatography with ultraviolet and electrochemical detection, *J. Chromatogr.*, 384, 371, 1987.
107. **Lunte, S. M., Blankenship, K. D., and Read, S. A.,** Detection and identification of procyanidins and flavanols in wine by dual electrode liquid chromatography-electrochemistry, *The Analyst*, 113, 99, 1988.
108. **Concialini, V., Chiavari, G., and Galletti, G.,** Electrochemical detection of forage phenolics, *The Analyst*, 113, 91, 1988.
109. **Chiavari, G., Vitali, P., and Galletti, G.,** Electrochemical detection in the high-performance liquid chromatography of polyphenols (vegetable tannins), *J. Chromatogr.*, 329, 426, 1987.
110. **Setchell, K. D. R., Welsh, M. B., and Lim, C. K.,** High-performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical, and thermospray mass spectrometric detection, *J. Chromatogr.*, 386, 315, 1987.
111. **Miner, D. J. and Kissinger, P. T.,** Trace determination of acetaminophen in serum, *J. Pharm. Sci.*, 68, 96, 1979.
112. **Munson, J. W., Weierstall, R., and Kostenlauder, H. B.,** Determination of acetaminophen in plasma by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 145, 328, 1978.
113. **Todd, R. D., Muldoon, S. M., and Watson, R. L.,** Determination of morphine in cerebrospinal fluid and plasma by HPLC with electrochemical detection, *J. Chromatogr.*, 232, 101, 1982.
114. **Meinsma, D. A., Radzik, D. M., and Kissinger, P. T.,** Determination of common analgesics in serum and urine by liquid chromatography/electrochemistry, *J. Chromatogr.*, 6, 2311, 1983.
115. **Wallace, J. E., Harris, S. C., and Peek, M. W.,** Determination of morphine by liquid chromatography with electrochemical detection, *Anal. Chem.*, 52, 1328, 1980.
116. **Shak, J. and Mason, W. D.,** Analysis of codeine and its metabolites in human plasma by HPLC with electrochemical detection, *Anal. Lett.*, 20, 881, 1987.
117. **Meinsma, D. A. and Kissinger, P. T.,** Chromatographic and electrochemical investigations of codeine, *Curr. Sep.*, 6, 42, 1985.
118. **Wilson, T. D.,** Structural influences on the amperometric detection of opiates in high-performance liquid chromatography, *J. Chromatogr.*, 301, 39, 1984.
119. **Schwartz, R. S. and David, K. O.,** Liquid chromatography of opium alkaloids, heroin, cocaine, and related compounds using electrochemical detection, *Anal. Chem.*, 57, 1362, 1985.
120. **Loeniskar, A. and Greenblatt, D. J.,** Determination of ciramadol and dezocine, two new analgesics, by high-performance liquid chromatography using electrochemical detection, *J. Chromatogr.*, 374, 215, 1986.
121. **Hynming, P.-A., Anderson, P., Bondesson, V., and Boreus, L. O.,** Determination of ketobemidine in plasma by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 375, 207, 1986.
122. **Krull, I. S., Selavka, C. M., Nelson, R. J., Bratin, K., and Lurie, D.,** Trace analysis for drugs and biologically active materials in commercial preparations, illicit formulations, and biological fluids using LC-photolysis-electrochemical detection (LC-hv-EC), in Abstr. 1985 Electoanal. Symp., Bioanalytical Systems, W. Lafayette, IN, 1985, 87.
123. **Abou-Khalil, S., Abou-Khalil, W. H., Masoud, A. N., and Yunis, A. A.,** High-performance liquid chromatographic determination of chloramphenicol and four analogues using reductive and oxidative electrochemical and ultraviolet detection, *J. Chromatogr.*, 417, 111, 1987.
124. **Alawi, M. A. and Ruessel, H. A.,** A simple, sensitive, and specific HPLC amperometric screening method for trace levels of sulphonamides in liver, kidney, and muscle tissues, *Chromatographia*, 14, 704, 1981.

125. **Croteau, D., Vallee, F., Bergeson, M. G., and LeBel, M.,** High-performance liquid chromatographic assay of erythromycin and its esters using electrochemical detection, *J. Chromatogr.*, 419, 205, 1987.
126. **Duthu, G. S.,** Assay of erythromycin from human serum by high-performance liquid chromatography with electrochemical detection, *J. Liq. Chromatogr.*, 7, 1023, 1984.
127. **Chen, M. L. and Chiou, W. L.,** Analysis of erythromycin in biological fluids by HPLC with electrochemical detection, *J. Chromatogr.*, 278, 91, 1983.
128. **Bopp, R. J. and Miner, D. J.,** Determination of enviroxime in a variety of biological matrixes by liquid chromatography with electrochemical detection, *J. Pharm. Sci.*, 71, 1402, 1982.
129. **Brooks, M. A., Hackman, M. R., and Mazzo, D. J.,** Determination of amoxicillin by high-performance liquid chromatography with amperometric detection, *J. Chromatogr.*, 210, 531, 1981.
130. **Parsons, P. J., Morrison, P. F., and LeRoy, A. F.,** Determination of platinum-containing drugs in human plasma by liquid chromatography with reductive electrochemical detection, *J. Chromatogr.*, 385, 323, 1987.
131. **Bannister, S. J., Sternson, L. A., and Repta, A. J.,** Evaluation of reductive amperometric detection in the liquid chromatographic determination of antineoplastic platinum complexes, *J. Chromatogr.*, 273, 301, 1983.
132. **Richmond, W. N. and Baldwin, R. P.,** Chloride-assisted electrochemical detection of *cis*-dichlorodiammine-platinum (II) after liquid chromatography, *Anal. Chim. Acta.*, 154, 133, 1983.
133. **Elferink, F., Van Der Vijgh, W. J. F., and Pinedo, H. M.,** Analysis of antitumour [1,1-bis(aminomethyl)cyclohexane]platinum (II) complexes derived from spiroplatin by high-performance liquid chromatography with differential pulse amperometric detection, *J. Chromatogr.*, 320, 379, 1985.
134. **Krull, I. S., Ding, X.-D., Braverman, S., Selavka, C., Hochberg, F., and Sternson, L. A.,** Trace analysis for *cis*-platinum anticancer drugs via LCEC, *J. Chromatogr. Sci.*, 21, 166, 1983.
135. **Van Der Vijgh, W. J. F., Van der Lee, H. B. J., Postma, G. J., and Pinedo, H. M.,** Highly sensitive differential pulse amperometric detection of second generation anti-tumor platinum compounds in HPLC effluents, *Chromatographia*, 17, 333, 1983.
136. **Akpofure, C., Riley, C. A., Sinkule, J. A., and Evans, W. E.,** Quantitation of daunorubicin and its metabolites by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 232, 377, 1982.
137. **Riley, C. M., Runyan, A. K., and Graham-Pole, J.,** Determination of doxorubicin in plasma and urine by high-performance liquid chromatography with electrochemical detection (HPLC-EC), application of the clinical pharmacokinetics of doxorubicin in patients with osteogenic sarcoma, *Anal. Lett.*, 20, 97, 1987.
138. **Tjaden, U. R., Landenberg, J. P., Ensing, K., and Van Bennekom, W. P.,** Determination of mitomycin C in plasma, serum, and urine by high-performance liquid chromatography with ultraviolet and electrochemical detection, *J. Chromatogr.*, 232, 355, 1982.
139. **Fluck, D. S., Rappaport, S. M., Eastmond, D. A., and Smith, M. T.,** Conversion of 1-naphthol to naphthoquinone metabolites by rat liver microsomes, demonstration by high-performance liquid chromatography with reductive electrochemical detection, *Arch. Biochem. Biophys.*, 235, 351, 1984.
140. **Sinkule, J. A., Akpofure, C., and Evans, W. E.,** Detection of anticancer drugs and their metabolites by LCEC Methods, *Curr. Sep.*, 4, 68, 1982.
141. **Van Oort, W. J., den Hartigh, J., and Driebergen, R. J.,** Optimization of an electrochemical detector using a static mercury drop electrode in high-performance liquid chromatography. Analysis of the anticancer agent mitomycin C in plasma, in *Electrochemical Detectors*, Ryan, T. H., Ed., Plenum Press, New York, 1984, 71.
142. **Riley, C. M. and Runyan, A. K.,** High-performance liquid chromatography of anthracycline antibiotics with electrochemical detection. Application to the chemical pharmacokinetics of 4′-deoxydoxorubicin, *J. Pharm. Biomed. Anal.*, 5, 33, 1987.
143. **Kotake, A. N., Vogel, N. J., Larson, R. A., and Choporis, N.,** New high-performance liquid chromatographic assay for plasma doxorubicin, *J. Chromatogr.*, 337, 194, 1985.
144. **Choi, K. E., Sinkule, J. A., Han, D. S., McGrath, S. C., Daly, K. M., and Larson, R. A.,** High-performance liquid chromatographic assay for mitoxantrone in plasma using electrochemical detection, *J. Chromatogr.*, 420, 81, 1987.
145. **Palmisano, F., Cataldi, T. R. I., and Zambonin, P. G.,** Determination of the antineoplastic agent methotrexate in body fluids by HPLC with electrochemical detection, *J. Chromatogr.*, 344, 249, 1985.
146. **Rucki, R. J., Ross, A., and Moros, S. A.,** Application of an electrochemical detector to the determination of procarbazine hydrochloride by high-performance liquid chromatography, *J. Chromatogr.*, 190, 359, 1980.
147. **Suzuki, H., Fujiwara, S., Kondo, S., and Sugimoto, I.,** Determination of nifedipine in human plasma by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 341, 341, 1985.
148. **Bratin, K. and Kissinger, P. T.,** Photochemical decomposition of nifedipine, *Curr. Sep.*, 4, 4, 1982.

149. **Isaksson, K. and Kissinger, P. T.,** Metabolism of physostigmine in mouse liver microsomal incubations studied by liquid chromatography with dual-electrode amperometric detection, *J. Chromatogr.*, 419, 165, 1987.
150. **Zhou, Z. M., Anders, J. C., Chung, H., and Theoharides, A. D.,** Analysis of artesunic acid and dihydroqinghoasu in blood by high-performance liquid chromatography with reductive electrochemical detection, *J. Chromatogr.*, 414, 77, 1987.
151. **Iwahashi, H., Negoro, Y., Ikeda, A., and Kido, R.,** Detection of some retinoid radicals using high-performance liquid chromatography with electron spin responance spectroscopy or electrochemical detection, *J. Chromatogr.*, 391, 199, 1987.
152. **Bedard, P. R. and Purdy, W. C.,** Surfactant-mediated gradient elution with electrochemical detection for the assay of serum thyroid hormones, *J. Liq. Chromatogr.*, 9, 1971, 1986.
153. **Mount, D. L., Patchen, L. C., Nguyen-Dinh, P., Barber, A., Schwartz, I. K., and Churchill, F. C.,** Sensitive analysis of blood for amodiaquine and three metabolites by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 383, 375, 1986.
154. **Carrun, G., Abernethy, D. R., Sadhukan, M., and Wright, C. E., III,** Minoxidil analysis in human plasma using high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 381, 127, 1986.
155. **Boppana, V. K., Heineman, F. C., Lynn, R. K., Randolph, W. C., and Ziemniak, J. A.,** Determination of fenoldopam (SK&F 82526) and its metabolites in human plasma and urine by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 317, 463, 1984.
156. **Housmyer, G. L. and Lewis, R. L.,** Electroanalytical characteristics of enoximone and piroximone at carbon or mercury electrodes, in 1986 Int. Electroanal. Symp. Abstr., Bioanalytical Systems, W. Lafayette, IN, 1986, 173.
157. **Palmisano, F., Guerrieri, A., and Zanbonin, P. G.,** Determination of free resorcinol in human serum and urine by high-performance liquid chromatography with amperometric detection, *Ann. Chim.*, 76, 491, 1986.
158. **Mazzo, D. J., Forbes, K. A., and Brooks, M. A.,** High performance liquid chromatography with ultraviolet or electrochemical detection for the determination of dopazinol in a pharmaceutical formulation, *J. Chromatogr.*, 367, 405, 1986.
159. **Shibanoki, S., Imamura, Y., Arakawa, Y., and Ishikawa, K.,** Determination of dosulepin and its metabolite: application of high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 415, 365, 1987.
160. **Lewis, R. C. and Schwende, F. J.,** A specific LCEC assay for piriprost potassium in dog plasma, in 1985 Int. Electroanal. Symp. Abstr., Bioanalytical Systems, W. Lafayette, IN, 1985, 120.
161. **Bourquin, D. and Brenneisen, R.,** Confirmation of cannabis abuse by the determination of 11-nor-DELTA-9-tetrahydrocannabinol-9-carboxylic acid in urine with HPLC and electrochemical detection, *J. Chromatogr.*, 414, 187, 1987.
162. **Karlsson, L.,** Direct injection of urine on a HPLC column-switching system for determination of DELTA-9-tetrahydrocannabinol acid with both ultraviolet and electrochemical detection, *J. Chromatogr.*, 417, 309, 1987.
163. **Brooks, M. A., DiDonato, G., and Blumenthal, H. P.,** Determination of chlorprothixene and its sulfoxide metabolite in plasma by HPLC with ultraviolet and amperometric detection, *J. Chromatogr.*, 337, 351, 1985.
164. **Lunte, S. M. and Kissinger, P. T.,** Detection and identification of sulfhydryl conjugates of *p*-benzoquinone in microsomal incubation of benzene and phenol, *Chem. Biol. Interac.*, 47, 195, 1983.
165. **Mayer, G. S. and Shoup, R. E.,** Simultaneous multiple-electrode liquid chromatographic-electrochemical assay for catecholamines, indole amines, and metabolites in brain tissue, *J. Chromatogr.*, 255, 533, 1983.
166. **Goto, M., Nakamura, T., and Ishii, D.,** Micro HPLC System with micro precolumn and dual electrochemical detector for direct injection analysis of catecholamines in body fluids, *J. Chromatogr.*, 226, 33, 1981.
167. **Miner, D. J. and Kissinger, P. T.,** Evidence for the involvement of *N*-acetyl-*p*-quinoneimine in acetaminophen metabolism, *Biochem. Pharmacol.*, 28, 3285, 1979.
168. **Preddy, C. R., Miner, D. J., Meinsma, D. A., and Kissinger, P. T.,** Electrochemical studies of the kinetics and mechanism of *N*-acetyl-*p*-quinoneimine hydrolysis, *Curr. Sep.*, 6, 57, 1985.
169. **Roston, D. A. and Kissinger, P. T.,** Isolation and identification of benzene metabolites *in vitro* with liquid chromatography/electrochemistry, *Anal. Chem.*, 54, 1798, 1982.
170. **Rice, J. R. and Kissinger, P. T.,** Cooxidation of benzidine by horseradish peroxidase and subsequent formation of possible thioether conjugates of benzidine, *Biochem. Biophys. Res. Commun.*, 104, 1312, 1982.
171. **Elchisak, M. A.,** Determination of dopamine sulfate and norepinephrine-o-sulfate and serotonin-o-sulfate by high-performance liquid chromatography using dual electrode electro-chemical detection, *J. Chromatogr.*, 264, 119, 1983.

172. **Shimosato, K., Tomita, M., and Ijiri, I.,** Rapid determination of *p*-hydroxylated methamphetamine metabolites by column liquid chromatography/electrochemistry, *J. Chromatogr.*, 377, 279, 1986.
173. **Shimada, K., Xie, F., and Nambara, T.,** Separation and determination of 4-hydroxyestriol monoglucuronides and monosulphates in biological fluids by high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 378, 17, 1986.
174. **Radzik, D. M. and Kissinger, P. T.,** Determination of aniline and metabolites produced in vitro by liquid chromatography/electrochemistry, *Anal. Biochem.*, 140, 74, 1984.
175. **Radzik, D. M., Brodbelt, J., and Kissinger, P. T.,** Determination of toxic azo dye metabolites *in vitro* by liquid chromatography/electrochemistry with a dual-electrode detector, *Anal. Chem.*, 56, 1984.
176. **Floyd, R. A., Henderson, R., Watson, J. J., and Wong, P. K.,** Use of salicylate with high pressure liquid chromatography and electrochemical detection (LCED) as a sensitive measure of hydroxyl free radicals in adriamycin-treated rats, *J. Free Radicals Biol. Med.*, 2, 13, 1986.
177. **Radzik, D. M., Roston, D. A., and Kissinger, P. T.,** Determination of hydroxylated aromatic compounds produced via superoxide-dependent formation of hydroxyl radicals by liquid chromatography-electrochemistry, *Anal. Biochem.*, 131, 458, 1983.
178. **McKenna, K., Kraske, P. J., and Brajter-Toth, A.,** Metabolism of thiopurine drugs: electrochemical and mass spectrometric insights, in 1986 Int. Electroanal. Symp. Abstr., Bioanalytical Systems, W. Lafayette, IN, 1986, 174.
179. **Lang, J. K. and Packer, L.,** Quantitative determination of vitamin E and oxidized and reduced coenzyme Q by HPLC with in-line ultraviolet and electrochemical detection, *J. Chromatogr.*, 385, 109, 1987.
180. **Rottero, A. E. and Kissinger, P. T.,** Detection and identification of three thioether conjugates of 2-hydroxyacetanilide by liquid chromatography/electrochemistry, *Biomed. Chromatogr.*, 2, 24, 1987.
181. **McMurtrey, K. D. and Welch, C. J.,** Metabolism studies of polycyclic mutagens and carcinogens using liquid chromatography with ultraviolet and electrochemical detection, *J. Liq. Chromatogr.*, 9, 2749, 1986.
182. **Bunyagidi, C. and McLachlan, J. A.,** Determination of estradiol 2- and 4-hydroxylase activities in mouse liver microsomes by liquid chromatography/electrochemistry, in 1986 Int. Electro Anal. Symp. Abstr., W. Lafayette, IN, 1986, 157.
183. **Rice, J. R.,** Studies on the Determination and Metabolism of Toxic Aromatic Amines, Ph.D. thesis, Purdue University, W. Lafayette, IN, 1982.
184. **Radzik, D. M.,** Investigations in the Determinations and Metabolism of Aromatic Amines by Liquid Chromatography and Electrochemistry, Ph.D. thesis, Purdue University, W. Lafayette, IN, 1984.
185. **Rice, J. R., Zenger, T. V., and Davis, B. B.,** Prostaglandin synthase-dependent cooxidation and aromatic amine carcinogenesis, in *Arachidonic Acid Metabolism and Tumor Initiation*, Marnett, L. J., Ed., Martinus Nijhoff, Boston, 1985, 125.
186. **Rappaport, S. M., Jin, Z. L., and Xu, X. B.,** High-performance liquid chromatography with reductive electrochemical detection of mutagenic nitro-substituted polynuclear aromatic hydrocarbons in diesel exhausts, *J. Chromatogr.*, 240, 145, 1982.
187. **Smith, M. T., Fluck, D. S., Eastmond, D. A., and Rappaport, S. M.,** Detection of quinone metabolites by HPLC with reductive electrochemical detection, *Life Chem. Rep.*, 3, 250, 1985.
188. **Santos, L. M. and Baldwin, R. P.,** Profiling of oxalic acid and α-keto acids in blood and urine by liquid chromatography with electrochemical detection at a chemically modified electrode, *J. Chromatogr.*, 414, 161, 1987.
189. **Schlager, J. W. and Baldwin, R. P.,** Liquid chromatography/electrochemical detection of ferro- and ferricytochrome C at a chemically modified gold electrode, *J. Chromatogr.*, 390, 379, 1987.
190. **Hutchins-Kumar, L. D., Wang, J., and Tuzhi, P.,** Parallel dual-electrode detection based on size exclusion for liquid chromatography/electrochemistry, *Anal. Chem.*, 58, 1019, 1986.