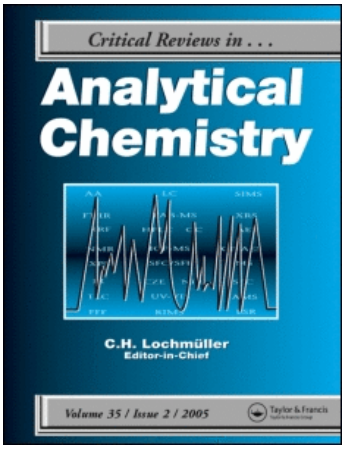

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ELECTROCHEMICAL DETECTION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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I. INTRODUCTION

Modern analytical chemistry must satisfy very diverse demands. Among the most important and simultaneously most difficult tasks are the continuous monitoring of substances (chiefly in industrial and environmental control) and analyses of very complicated organic and inorganic systems, often at trace concentration levels (mainly in clinical analysis, pharmaceutical research and industry, petrochemistry, etc.). For these purposes, suitable detectors must be available. Industrial and laboratory continuous analyzers have a long history. Combination of continuous monitoring with high-performance chromatographic techniques is a much more recent technique.

The increasing requirements on analyses of very complex systems and the great recent progress in instrumentation led to an enormous development in chromatographic techniques, especially gas and high-performance liquid chromatography. Whereas the theory and practice of gas chromatographic separation and detection have progressed to a very advanced level, a rational theoretical background of high-performance liquid chromatography (HPLC) has yet to be developed fully and is mainly based on modification of the relationships derived originally for gas chromatography. Although gas-liquid chromatography (GLC) and liquid-liquid chromatography (LLC), as well as gas-solid chromatography (GSC) and liquid-solid chromatography (LSC), are similar in many respects, substantially stronger intermolecular interactions in condensed mobile phases render the validity of many correlations transferred from gas chromatography to liquid chromatography very dubious.

From the point of view of detection techniques, there are two principal differences between gas and liquid chromatography: (1) the diffusion coefficients of solutes in liquids are four to five orders of magnitude smaller than those in gaseous mixtures. These differences are reflected in the different shapes of the dependence of the column efficiency H (height equivalent to a theoretical plate) on the mobile phase flow rate, u (Figure 1). At low flow rates, longitudinal diffusion, which is proportional to the diffusion coefficient, plays an important role in gas chromatography. In liquid chromatography, longitudinal diffusion is unimportant because of the smaller diffusion coefficients, but slow diffusion in the mobile phase has an adverse effect on mass transport between the stationary and mobile phase (broadening of the elution curve is inversely proportional to the diffusion coefficient). Because of slow diffusion, the adverse effect on the elution curve broadening of dead volumes in the detector and in the pathways connecting the detector with the column is much more pronounced in liquid chromatography than in gas chromatography. (2) Whereas in gas chromatography the properties of the solutes are usually quite different from the properties of the carrier gas, the properties of the solutes and of the mobile phase in liquid chromatography are mostly very similar.

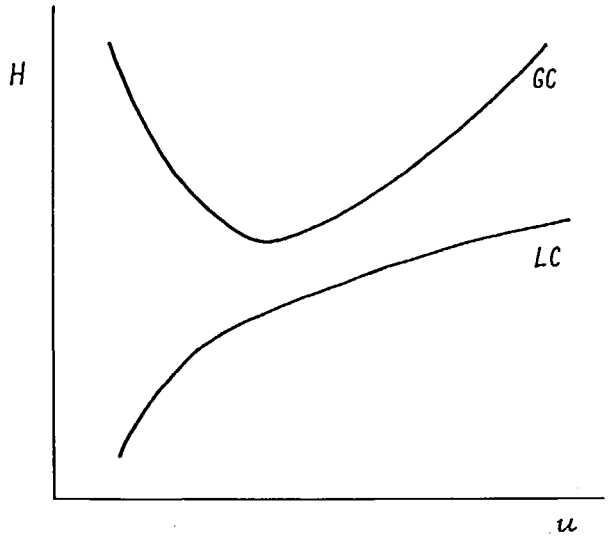


FIGURE 1. Dependence of the height equivalent to a theoretical plate, H, on the mobile-phase linear flow rate, u, for gas (GC) and liquid (LC) chromatography.

Primarily as a consequence of the latter difference, most gas chromatographic detectors are unsuitable for liquid chromatography. An ideal detector should be universal. However, the enormous diversity in the systems to be analyzed makes the construction of a universal detector impossible and it is necessary to employ a number of detectors monitoring various physicochemical properties of the system, the optimal measuring conditions being determined specifically for each system.

Any physical or physicochemical property of the eluate from a chromatographic column which has a known and reproducible relationship to the amount or the concentration of the components to be detected in the eluate can be employed as an analytical property for detection purposes. Either bulk properties, which vary with a change in the composition of the system (e.g., refractive index, thermal conductivity, electric conductance, high-frequency impedance, ionization, etc.), or specific properties, which depend selectively on the concentration (amount) of certain components (the absorbance at a certain wavelength, fluorescence excited by electromagnetic radiation, radioactivity, electrolytic current at a certain electrode potential, etc.) can be measured. The measurement of bulk properties should be universal, but the properties of the solutes must differ substantially from those of the carrier medium to yield sufficiently large signal changes in the detection; therefore, the measurement is mostly less sensitive and is subject to higher noise levels than the measurement of specific properties and is rarely compatible with gradient elution. The measurement of specific properties is usually characterized by a much higher signal-to-noise ratio than the measurement of bulk properties, is less depedent on changes in the composition of the mobile phase (i.e., is better suited for gradient elution), but its application is narrower and is limited by the requirement that the carrier medium yield the lowest possible signal under the given experimental conditions.

Contemporary HPLC mainly uses detectors based on measurement of the absorbance in the UV region, of fluorescence, and of the refractive index. Electrochemical detectors, especially voltammetric and coulometric, have gained importance in recent years. Their applicability is limited to certain groups of substances, but they exhibit detection limits,

sensitivity, reproducibility, and a linear dynamic range for these substances which cannot be matched by other detectors.

The field of HPLC detectors has been reviewed many times (see, e.g., the references 1-9 for detectors, in general, and the references 10-27 for electrochemical detectors). The present review is intended as an up-to-date, critical treatment of the theory, design, and application of electrochemical detectors, including an outline of future trends.

II. PRINCIPAL REQUIREMENTS ON HPLC DETECTORS

A. Theoretical Basis of Detection in Flowing Liquids

As in any other measurement, monitoring in flowing systems is carried out in order to obtain the required information on the test substances as quickly as possible and with as low a decrease in the information content as possible. The theory of detection in flowing liquids should, thus, define criteria in terms of which the detector performance can be evaluated and should yield relationships describing the dependence of the detector performance on the experimental conditions. A general treatment of this problem is very difficult and involves many approximations (see, e.g., the references 5, 28).

As has been stated recently,[28] a suitable theory should be based both on chromatographic concepts and on the elements of systems analysis, developed in chemical engineering, which considers the system as a black box, i.e., studies only the input-output relationships. The basic criterion is peak broadening, which is a decisive factor for the overall speed of obtaining information.

It has been shown[29] that the detector output signal function is a convolution of the concentration input function and the impulse response function of the detecting system. The signal is, thus, given not only by the instantaneous solute concentration, but also by previous concentration values. The response function depends on many experimental parameters and cannot be described accurately. Any description of the peak broadening, thus, involves some sort of approximation.

Response function is related to the static sensitivity, defined as the ratio of the signal change to the concentration change. At a constant concentration level, the static sensitivity is the time integral of the response function from zero to infinity. This integral is difficult or impossible to explicitly calculate and, thus, it is generally replaced by a single value of the sensitivity that can readily be obtained experimentally. This value specifies only the amplitude of the response function and must be supplemented with another parameter expressing the width.

From the chromatographic point of view, standard deviation σ_t is a suitable means of expressing the response curve width. The width of Gaussian peaks at the base equals $4\sigma_t$, is easy to measure, and is not affected much by noise. Therefore, the performance of a flow detector can be described by its sensitivity S and by the value of the peak broadening expressed in terms of standard deviation σ_t.

The overall peak broadening is given by contributions from all parts of the apparatus, e.g., in chromatography from the injection block, the column, the connecting tubing, the detector, and the electronic circuitry. It is important that the variances of systems connected in series are additive,[29-31]

$$\sigma_{t,tot}^2 = \sum_i \alpha_{t,i}^2 \qquad (1)$$

Therefore, the values of σ_t should be as small as possible for all components of the system.

In a chromatographic system, three main sources of peak broadening caused by the detecting device can be distinguished (as differentiated from broadening caused by the chromatographic components of the system):

1. Solute dispersion in the connecting tubes
2. Broadening due to the effective working volume of the detector
3. Broadening caused by the dynamics of the sensor, of the electronic circuitry, and of the recording apparatus.

The solute dispersion in tubes has been treated in several works.[32-34] For a straight, narrow, and long tube with radius r and length l it has been derived that time standard deviation σ_t is given by

$$\sigma_t^2 = t_R \cdot r^2/24\ D \tag{2}$$

where t_R is the residence time of the solute in the tube and D is its diffusion coefficient. If the broadening is expressed in terms of volume standard deviation σ_V,

$$\sigma_V = w \cdot \sigma_t \tag{3}$$

where w is the volume flow rate of the liquid, then Equation 2 assumes the form

$$\sigma_V^2 = \pi\ r^4\ l\ w\ /24\ D \tag{4}$$

Hence, the dispersion increases with increasing flow rate, decreasing diffusion coefficient of the solute, and especially strongly with increasing radius of the tube.

The effect of coiling of the tube on the dispersion has recently been studied,[35-38] especially in connection with flow-injection analysis, and it has been concluded that the dispersion in coiled tubes is smaller than in straight tubes, due to secondary flow of the solution. One should, however, bear in mind that the above conclusions hold reasonably accurately only for long residence times and narrow tubes; otherwise the values predicted are too large.

Broadening due to the effective volume of the detector is difficult to predict[28] because the flow profile is not exactly known. However, a semiquantitative prediction can be made for the two limited cases:[28]

1. When the effective volume equals cell volume V_c and the plug flow profile is predominant, then the output curve is rectangular, with a base of $V_c \cdot w$ and a volume standard deviation of $V_c/12^{1/2}$.
2. If the cell operates as an ideal mixer, then the output function is exponential, with a time constant of V_c/w and a volume standard deviation of V_c. The response of real detectors generally lies between these two extremes and it holds that $\sigma_V = k \cdot V_c$, where k is a numerical factor with a value between 0.3 and 1.

Broadening caused by the electronics is constant in time and can often be predicted from the amplifier time constants, i.e., it can be written that $\sigma_t = C$ or $\sigma_v = w \cdot C$.

Therefore, the total volume standard deviation is given, on the basis of Equation 1, by

$$\sigma_V^2 = \pi \cdot r^4 l\ w/24\ D + k^2\ V_c^2 + w^2 \cdot C^2 \tag{5}$$

To keep the contribution from the detector to the overall peak broadening as small as possible, it is, thus, desirable that the flow rate be low, the connecting tubes are short and narrow, the detector effective volume be small, and the response of electronic circuit be fast. For a more detailed discussion see, e.g., the references 5, 28.

B. Operational Parameters of HPLC Detectors

On the basis of the theory discussed in the previous section, a HPLC detector should meet the following requirements, regardless of the analytical property monitored and of the detection technique used:

1. High sensitivity, defined as the slope of the plot of the signal vs. the solute concentration (amount). This criterion is especially important in HPLC,[5] because of low loading capacities of the columns.
2. Low detection limit c_d, defined as a multiple of an estimate of the standard deviation of the noise, s_V (in voltage),

$$c_d = k \cdot s_V / S \qquad (6)$$

where S is the sensitivity. The value of k is usually 2 to 4, depending on the statistical significance level chosen. (For a discussion of the reliability of the detection limit see also Winefordner and Ward[39]). For attainment of a low detection limit, the noise value is extremely important. Three kinds of noise can be distinguished:[5] (1) high frequency noise, with a frequency much greater than that of the solute peaks. This noise is chiefly caused by the electronic circuitry and can usually be eliminated by filtering. To avoid an adverse effect of filtering on the peak broadening, the time constant of the filter should not exceed a value of about 0.3 σ_t;[28] (2) low frequency noise, whose frequency is comparable with that of the solute peaks. This type of noise affects the results most seriously and cannot be removed in any other way than by an improvement in the detector construction or by using another detector; (3) very low frequency noise, with a frequency much lower than that of the solute peaks. This is usually called the baseline drift. The results are virtually unaffected by a drifting baseline, but frequent adjustment of the baseline complicates the instrument operation.
3. A wide linear dynamic range. If the detector response is expressed by the equation

$$R = S \cdot c^x \qquad (7)$$

then the dynamic range is the concentration interval for which $x \neq 0$, i.e., an interval within which a change in the solute concentration produces a change in the signal. To obtain accurate and precise results it is desirable that the response be linear. Ideally, x should equal unity; in practice, the linear dynamic range is usually defined as[5] the concentration interval within which it holds that $0.98 < x < 1.02$.
4. Good stability and reproducibility of the signal in prolonged measurements
5. Fast response of the sensor, to attain a low time constant for the detector. This constant is defined as the time necessary for the signal to reach 63.2% of the maximum value.[40]
6. Small contribution of the detector and of the connecting tubing to the elution curve broadening. Thus, the tubes must be short, narrow, must not hold up the eluate, and the detector must have the smallest possible dead volume. For a more detailed discussion see, e.g., Hermansson.[41]

In addition to these principal requirements, further criteria affect the detector applicability and performance. For example, it is advantageous if the detector exhibits the same sensitivity for all the solutes detected and if its signal depends as little as possible on the experimental conditions, mainly on the temperature and the mobile-phase flow rate. For the use of gradient elution, it is necessary that the detector signal be independent of the mobile phase composition.

Table 1
ELECTROCHEMICAL METHODS THAT CAN BE USED FOR CONTINUOUS MONITORING OF SUBSTANCES IN FLOWING LIQUIDS

Controlled quantity	Measured quantity	Method
	Methods Based on Electric Properties of Solutions	
—	$\mathcal{H}$	Low-frequency conductometry
—	$Z = f(\mathcal{H} + \epsilon)$	High-frequency impedance measurements
	Electrolytic Methods	
—	$E = f(a)$	Equilibrium potentiometry
E	$I_l = k_l c$	D.c. polarography and steady-state voltammetry
E(t)	$I_{ac} = f(t)$	Nonstationary polarographic and voltammetric methods (a.c., square-wave, pulse, differential pulse)
E	$Q = \int_0^t I\,dt$	Constant-potential coulometry
I	$Q = I \cdot t$	Coulometric titration

Note: For the significance of the symbols see the List of Important Symbols and Abbreviations.

III. ELECTROCHEMICAL DETECTORS

A. Classification

Modern electrochemistry offers a wide selection of methods useful for continuous detection in flowing liquids (see Table 1). A common advantage of electrolytic methods is their high sensitivity, combined with good precision and accuracy of the measurement. All electrolytic methods measure specific properties of the solutes and their selectivity for certain substances can, to a certain extent, be regulated by judicious selection of the experimental conditions. Therefore, electrolytic methods are suitable for construction of specific detectors. Their main disadvantage lies in the high demands placed on the carrier liquid, which must be sufficiently electrically conductive and, under the given experimental conditions, must yield a constant electric signal, which is as low as possible. The application of electrolytic detectors is, thus, limited to systems of electrolytes (either aqueous electrolyte solutions or polar liquids in which a suitable electrolyte can be dissolved). Mixed media can also be used (a nonpolar liquid leaving the chromatographic column is mixed with a suitable electrolyte before the detector). The application range can further be broadened to include various electroinactive substances, if an auxiliary reactor is placed before the detector, in which inactive components react chemically with a suitable electroactive substance present at a constant concentration and monitored by the detector.

The greatest problem in the application of electrolytic detectors is the maintenance of a constant and reproducible electrode-active surface. In contact with a solution, various processes occur at the electrode surface, e.g., adsorption and catalytic effects, redox reactions, ion exchange, etc., which cause continuous changes in the electrochemical properties of the electrode. So far, there is no universal and reliable method for electrode surface renewal (see Section III.C.2.a).

Methods based on the electric properties of solutions monitor the bulk properties of systems and, thus, should permit construction of universal detectors. However, the measurement of low-frequency electric conductance is sufficiently sensitive only for electrolytes dissolved in media with a very low conductance (e.g., ions dissolved in a nonelectrolyte), which is rarely encountered in practice. Nonionic substances exhibit

negligible conductance and with charged species dissolved in an electrolyte relatively small changes in the conductance are measured against a high background value. The measured value is very temperature dependent (a change of temperature by 1° C leads to a signal change of about 2%). Moreover, problems with passivation of the measuring electrodes may be encountered, similar to electrolytic methods. Measurement of the high-frequency impedance removes the problem of electrode passivation, because the electrodes can be placed outside the measuring cell. Moreover, the measurement is truly universal; any change in the composition of the flowing liquid is reflected in a change of the high-frequency impedance. However, high-frequency measurements are very empirical, the relationship between the impedance and the solution composition is complicated and mostly nonlinear, and the sensitivity is usually insufficient for use in HPLC.

A certain advantage of potentiometric, conductometric, and high-frequency impedance detectors is their nondestructive character; polarographic, voltammetric, and coulometric detectors cause changes in the substances detected.

B. Conductometric and High-Frequency Impedance Detectors

Conductometric[42-55] and high-frequency impedance[13,50,56-69] detectors have a long tradition in industrial on-line monitoring. Consequently, they were used in liquid chromatography before the advent of HPLC and were among the first electrochemical detectors tested in HPLC. Their main limitations are summarized in the previous section. An additional disadvantage of high-frequency impedance detectors is the strong temperature dependence of the signal, which usually requires thermostatting within 10^{-3}° C (i.e., one order of magnitude more precisely than for refractive index detectors).

Conductometric detectors are very simple and easy to construct with a small internal volume (less than 1 $\mu\ell$). Inert measuring electrodes, made mostly of platinum or stainless steel, are connected in a Wheatstone bridge powered by a low-frequency a.c. current to suppress electrode polarization and consequent large noise and baseline drift. One of the best conductometric detectors,[52] with an internal volume of less than 0.5 $\mu\ell$, is shown in Figure 2. The authors claim a detection limit of 6×10^{-6} *M* KCl, with a relative standard deviation of 13.7%. The linear dynamic range of conductometric detectors can be wide; e.g., a value of six concentration decades was reported,[54] using a 3-$\mu\ell$ detector with an a.c. square-wave measuring voltage (amplitude, 1 V; frequency, 2.5 kHz) and a logarithmic circuit.

Recently, a bipolar pulse conductometric detector was described.[55] The technique is based on application of two equal successive constant-voltage pulses of opposite polarity to the electrodes and measuring the current at the end of the second pulse. The author claims that this technique permits measurement over a broader conductance range than using a conventional a.c. bridge and suffers less from interferences from the background conductance.

Many conductometric detectors are manufactured commercially, e.g., by Chromatronix, Laboratory Data Control, etc.

High-frequency impedance detectors monitor changes in the capacitance (or, less often, the inductance) of the effluent. Consequently, they are most sensitive for solutes whose permittivities are considerably different from that of the solvent. There are two principal methods of measurement, namely, the impedance bridge method[13,58,63,65] and the monitoring of variations in the resonance frequency.[57,59-62,64,66-68] The metal electrodes are usually placed on the outside walls of the glass-measuring cell. The frequency employed ranges from 1 to 5 kHz[62] to 25 MHz.[67] High values of the frequency are preferable, because they permit the use of low capacitance cells with volumes below 10 $\mu\ell$ without loss in the measuring sensitivity. The extremely pronounced temperature

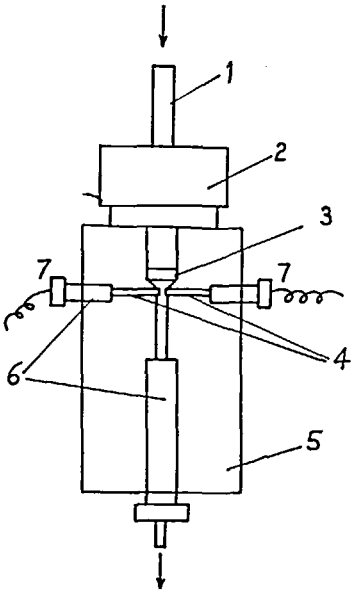


FIGURE 2. A conductometric detector. (1) Inlet, (2) connecting nut, (3) silicone rubber seal, (4) platinum measuring electrodes, (5) organic glass, (6) silicone rubber seals, (7) electrode contacts. (From Tesařík, K. and Kaláb, P., *J. Chromatogr.*, 78, 357 [1973]. With permission.)

dependence of the signal stems chiefly from fluctuations in the permittivity and in the cell dimensions; fluctuations in the oscillator frequency are not as important.[68] The temperature effect is compensated in several ways:

1. A differential cell is used[58] with one compartment for the effluent and the other for the pure mobile phase, the two compartments being separated by one of the metal electrodes.
2. The detector is thermostatted with a high precision.[63]
3. Simple feedback circuits are designed to compensate for temperature fluctuations.[66,68]
4. The effect of temperature fluctuations on the cell dimensions is suppressed by suitable cell design. Some authors recommend concentric tubular electrodes[61] (Figure 3), others a parallel-plate electrode arrangement[68] (Figure 4).

Typical values of the detection limit and the linear dynamic range of high-frequency impedance detectors are 10^{-8} mol/mℓ (benzene in hexane) and four concentration decades, respectively.[57,58]

Recently, interest has somewhat revived in high-frequency impedance detection in HPLC.[63,64,66-69] The authors try to improve the detector performance by better cell construction and by the use of more sophisticated circuitry. An example of such a cell is given in Figure 5.[67] This cell has an effective volume of 5.5 μℓ. Three oscillators are employed in a Franklin Type of circuit, to enable measurement over the whole permittivity range and to prevent locking. The resonance frequency is monitored at an applied frequency of 25 MHz. The authors point out that, to obtain a high sensitivity, the

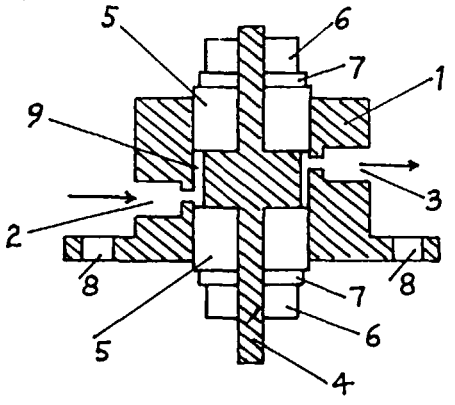


FIGURE 3. A tubular high-frequency impedance detector. (1) Outer electrode (grounded), (2) inlet, (3) outlet, (4) internal electrode, (5) PTFE seals, (6) nuts, (7) supports, (8) holes for fixing bolts, (9) working space. (From Vespalec, R. and Hána, K., *J. Chromatogr.*, 65, 53 [1972]. With permission.)

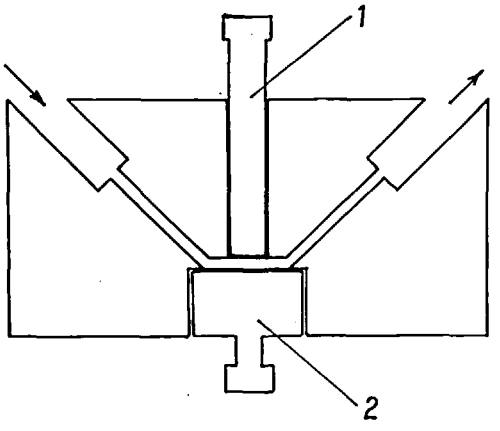


FIGURE 4. A parallel-plate high-frequency impedance detector. (1) Adjustable grounded electrode, (2) fixed electrode. (From Alder, J. F. and Thoër, A., *J. Chromatogr.*, 178, 15 [1979]. With permission.

applied frequency must be high, the cell capacitance small, and stray capacitances must be effectively suppressed.

On the whole, it can be concluded that conductometric and high-frequency impedance detectors are inferior to UV absorbance detectors and their performance in optimal cases can be compared with common refractive index detectors,[69] as also demonstrated in Table 2.[59] They cannot be expected to find wide practical application.

C. Electrolytic Detectors

1. Equilibrium Potentiometric Detectors

Equilibrium potentiometric detectors have many attractive features. It is easy to design a simple potentiometric cell with a small internal volume; the measuring technique and the signal handling are not complicated, atmospheric oxygen, which interferes in

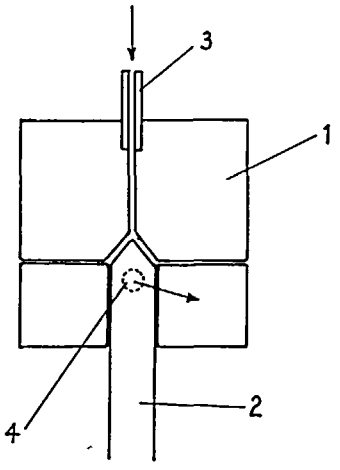


FIGURE 5. A high-frequency impendance detector with a conical electrode arrangement. (1) Grounded electrode, (2) adjustable conical electrode, (3) inlet, (4) outlet. (From Alder, J. F., Drew, P. K. P., and Fielden, P. R., *J. Chromatogr.*, 212, 167 [1981]. With permission.)

Table 2
COMPARISON OF UV ABSORBANCE, REFRACTIVE INDEX, AND HIGH-FREQUENCY CAPACITIES DETECTORS

Detector	Cell volume ($\mu\ell$)	Analytical property	Noise	Sensitivity	Detection limit (mol/s)	Detection limit (mol/mℓ)
Refractive index (Laboratory Data Control)	5.0	Δn	1.43×10^{-7}	2.87×10^{-7}	5.67×10^{-11}	2.67×10^{-8}
Refractive index (Waters Associates)	10.0	Δn	2.03×10^{-7}	4.06×10^{-7}	9.86×10^{-10}	9.13×10^{-8}
UV absorbance opt. path = 1 mm	27.2	A	4.34×10^{-3}	8.68×10^{-3}	4.73×10^{-10}	5.69×10^{-8}
UV absorbance opt. path = 7 mm	49.0	A	0.78×10^{-3}	1.56×10^{-3}	1.21×10^{-11}	1.46×10^{-9}
High-frequency capacitance	11.7	$\Delta\epsilon$	5.90×10^{-6}	11.8×10^{-6}	1.81×10^{-9}	3.38×10^{-7}

From Krejčí, M. and Pospíšilová, N., *J. Chromatogr.*, 73, 105 (1972). With permission.

polarographic and most voltammetric measurements, need not be removed, and the detector signal is virtually independent of the effluent flow rate. The signal depends on temperature (see the Nernst equation), but the dependence is not critical.

However, there are several principal limitations which prevent wide use of potentiometric detectors in HPLC:

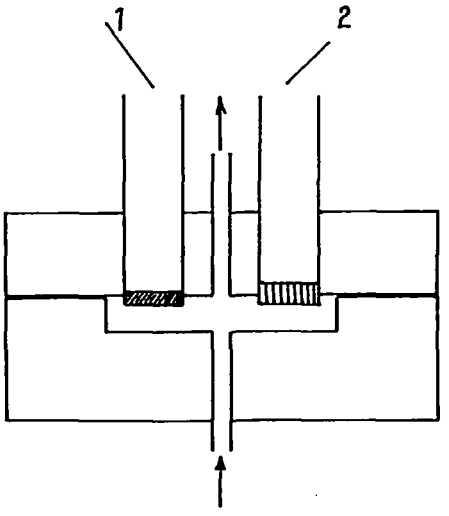


FIGURE 6. A potentiometric detector with an ISE. (1) ISE, (2) reference electrode. (From Deguchi, T., Kuma, T., and Nagai, H., *J. Chromatogr.*, 152, 349 [1978]. With permission.)

1. The measurement of equilibrium potentials of the first kind, second kind, and of redox electrodes is generally insufficiently sensitive and poorly reproducible.
2. Ion-selective electrodes (ISE) are sufficiently sensitive and their potentials exhibit good precision, but their response is too selective and often slow, especially at low solute activities.
3. The mobile phase must have a relatively high and constant ionic strength to yield precise and accurate results[70] and, thus, these detectors can be applied almost exclusively to inorganic systems and gradient elution technique can rarely be used.

The difficulties caused by the high selectivity of ISEs can be somewhat alleviated by using electrodes of poor selectivity (e.g., the nitrate ISE), or by application of auxiliary reactions: (1) electrodes with additional membranes (gas sensors and enzyme electrodes)[70] can be employed, thus, also extending the applicability of the sensor to nonionic substances; (2) preliminary chemical reactions in a reactor placed between the column outlet and the detector inlet[71,477] can be used to convert the eluate components into a species sensed by the electrode; and (3) continuous titration of the eluate components with an ion sensed by an ISE can be employed.[72] Methods 1 to 3 have not yet been used in HPLC practice, but they seem promising. Obviously, the problems with peak broadening will have to be solved first, especially because the rather slow response of most ISEs can hardly be improved.

For the reasons given above, detectors containing ISEs are widely used in on-stream monitoring, but are rarely combined with HPLC.[73-78,477] A typical HPLC detector cell[74] is shown in Figure. 6.

Recently, a differential potentiometric detector was proposed for ionized substances.[79] It contains two chambers separated by an ion-exchanger membrane, the effluent passing through one chamber and the eluent through the other. The membrane potential is monitored by two reference electrodes placed in the chambers. The authors reported a detection limit of about 1 nmol, a linear dynamic range of two to three concentration decades, a relative standard deviation of the measurement of more than 3%, and a time constant of 2 sec.

2. Polarographic, Voltammetric, and Coulometric Detectors

These detectors are by far the most common among all electrochemical detectors. In fact, many authors of works dealing with HPLC electrochemical detection do not distinguish between the terms "polarographic" or "voltammetric" and "electrochemical". These detectors are sensitive, their response is generally rapid, and they can be applied to a relatively broad range of inorganic and organic substances. Many inorganic ions can be reduced or oxidized; among organic substances, those with multiple bonds, reducible or oxidizable functional groups, aromatic systems, etc. are electroactive. The detector sensitivity is approximately the same for substances with similar diffusion coefficients and similar rates of the charge-transfer reactions involving an exchange of the same number of elementary charges. The measurement usually has a good precision, even at very low concentrations of electroactive substances.

However, these detectors also have several serious drawbacks. The most serious limitation, inherent to all electrolytic methods, is the poor reproducibility of the properties of the electrode-active surface due to interactions with the solution, as mentioned in Section III.A. Further, the measured signal is dependent on the hydrodynamic conditions and, consequently, on the mobile phase flow rate. Electrolytic measurement requires the presence of a suitable base electrolyte and the background current depends to a greater or lesser degree on the composition of this electrolyte; hence, electrolytic detectors are often incompatible with gradient elution. When the working potential in aqueous and mixed media is more negative than about +0.5 V (SCE), atmospheric oxygen interferes in the measurement, because of its reduction through hydrogen peroxide to water, and, thus, must be removed from the solution. The detector signal depends on the temperature (a change by 1°C causes a change in the current of about 3%), but this dependence is not as serious as to require thermostatting of the detector in routine work.

Coulometric measurements are especially attractive, because of the high precision and accuracy of the method and its absolute character. When 100% current efficiency is attained, then the amount of depolarizer can be directly and highly accurately calculated from the equation describing Faraday's law, provided that all the substance has been electrochemically converted. However, practical application of this principle to HPLC detection encounters several problems. First, it is mostly difficult to satisfy the condition of 100% current efficiency (side electrode reactions occur). Second, to achieve complete electrolysis during the residence time of the substance in the detector, it is necessary that the working electrode be very large and the mobile-phase flow rate very low, which imposes severe limitations on the cell construction and on the chromatographic separation. On the other hand, the detector signal is independent of the mobile-phase flow rate within the region corresponding to complete electrolysis.

Whereas conductometric, high-frequency impedance, and equilibrium potentiometric detectors are nondestructive, polarographic and voltammetric detectors are partially destructive and coulometric detectors are destructive. This fact can prevent certain applications of these detectors, e.g., in preparative chromatography or in a dual arrangement, i.e., in combination of an electrolytic detector with another detection method.

In constructing and applying electrolytic detectors, three principal problems must be solved:

1. The working electrode material must be selected and the working electrode constructed in such a way that the accessible potential range is suitable for the given purpose, the residual current and noise are sufficiently low and constant, the surface activity of the electrode is reproducible at least over the time of measurement, and the kinetic parameters of the solute electrode reactions are favorable.

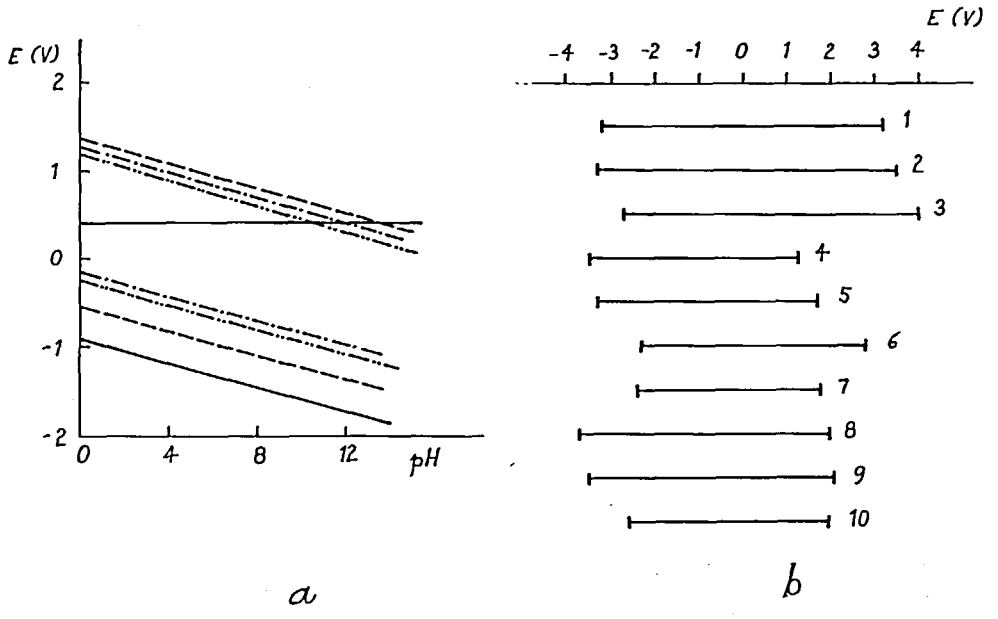


FIGURE 7. Accessible potential ranges of various electrode materials in aqueous solution and of platinum in various nonaqueous solvents.[18,81] (a) Aqueous solution (vs. SCE); ——— Hg, — — — C, —·—· Pt, —··—·· Au; (b) nonaqueous solutions (vs. NHE_{aq}; Pt electrode, base electrolytes: lithium perchlorate (1, 3 to 5, 10) and tetraalkylammonium salts [2, 6, 7 to 9]). (1) Acetonitrile, (2) propylene carbonate, (3) nitromethane, (4) dimethylsulfoxide, (5) dimethylformamide, (6) dichloromethane, (7) dichloroethane, (8) dimethyoxyethane, (9) tetrahydrofuran, (10) acetyl chloride.

2. The measuring cell must be constructed with a dead volume as small as possible and with hydrodynamic conditions permitting the most sensitive and reproducible measurements.
3. A suitable measuring technique must be chosen, from the point of view of the sensitivity of the measurement, reproducibility, selectivity, and the ease of signal handling.

a. Working Electrode Material

Common electrode materials are mercury, several forms of carbon (impregnated graphite, glassy carbon, pyrolytic graphite, carbon paste), platinum, and gold. Less noble materials undergo various side reactions with mobile-phase components and, thus, are generally unsuitable for the detection. Some inert compounds (notably carbides) have been tested but can find use only in special cases (see, e.g., Beauchamp et al.[80]).

The accessible potential ranges of some electrode materials and of platinum in various nonaqueous solutions are shown in Figure 7.[18,81] Because of the high hydrogen overvoltage, mercury electrodes have the widest cathodic potential range. Moreover, the dropping mercury electrode (DME) has a great advantage in the periodical renewal of the active surface, so that passivation effects are considerably suppressed. From the point of view of the detector construction (see Section III.C.2.b), the use of thin-film mercury electrodes is advantageous. However, one must bear in mind that the main advantage of the dropping mercury electrode, the periodical renewal of the electrode-active surface, is lost and that deposition of mercury on noble metals (Pt, Au) produces an amalgam electrode, not a pure mercury electrode (for details see, e.g., Vydra et al.[82]). Amalgam electrodes generally exhibit lower hydrogen overvoltage, i.e., have a narrower cathodic

Table 3
THE ACCESSIBLE POTENTIAL RANGES OF SOME ELECTRODES (AQUEOUS BUFFER, pH 4.5)

Electrode	Potential limit (V/SCE)	
	Cathodic	Anodic
Glassy carbon	−0.8	+1.2
Pyrolytic graphite	−1.5	+1.2
Wax-impregnated graphite	−0.6	+1.2
Graphite paste (nujol)	−1.6	+1.1
Platinum	−0.5	+1.2
Mercury	−2.0	+0.4
Mercury film (Pt or C support)	−1.0	+0.4

From Štulík, K. and Pacáková, V., *J. Chromatogr.*, 208, 269 (1981). With permission.

potential range (see Table 3), and there is always the danger of side reactions of the solutes detected with the metal dissolved in mercury. Deposition of mercury on carbon (see, e.g., Štulíková[83] and Štulíková and Štulík[84]) produces an array of microscopic mercury droplets, not a continuous film, which can be mechanically damaged at higher flow rates. The hydrogen overvoltage at such electrodes is also rather low, due to evolution of hydrogen on the carbon surface not covered by mercury. The residual current at mercury electrodes is small and reproducible. However, the main limitation of the use of mercury electrodes is the anodic dissolution of mercury at rather negative potentials (about +0.4 V [SCE] in media not containing substances that form complexes of precipitates with mercury ions). Therefore, mercury electrodes cannot be used for many oxidation processes, i.e., for the detection of most organic compounds.

The behavior of carbon and platinum electrodes more or less approaches that of an ideal inert redox electrode. The anodic potential limit is given by the electrolytic decomposition of the base electrolyte or of the solvent[80,81] and in neutral aqueous solutions amounts to about +1.2 V (SCE). These electrodes, therefore, permit measurements in the anodic region, but their cathodic potential range is narrower than with dropping mercury electrode due to hydrogen evolution in aqueous solutions. It is narrowest for platinum and widest for carbon paste[81] (see Table 3). (One should, of course, bear in mind that the potential range in aqueous solutions shifts in dependance on the pH.) For a detailed discussion of the electrochemical properties of various carbonaceous materials, see, e.g., Štulíková and Štulík[84] and for a review of the electrochemical behavior of glassy carbon, e.g., van der Linden and Dieker.[85]

Nonaqueous solutions can be employed in voltammetric measurements, provided that a base electrolyte can be dissolved in the medium at a concentration sufficiently high to maintain the resistance at an acceptable level (not greater than about 10^5 Ω) and for effective suppression of the migration current.

Residual currents at solid electrodes are generally higher, have a poorer reproducibility, and are subject to a greater noise level than those at mercury electrodes, chiefly due to surface inhomogeneity of the electrodes and to their surface oxidation at positive

potentials. The performance of platinum,[81] glassy carbon,[81,86-90] pyrolytic graphite,[91] and carbon paste[81,87,89,90,92-95] electrodes has been studied and the conclusions can be summarized as follows (see also Table 5). Carbon paste electrodes are superior to the other electrodes in having a lower residual current and noise, being very cheap and easy to prepare and replace. The performance of glassy carbon, pyrolytic graphite, and platinum electrodes strongly depends on the quality of the electrode surface polishing. Moreover, glassy carbon is rather sensitive to passage of higher currents and to aging, which lead to its recrystallization to graphite and a great deterioration in its performance.[84] Impregnation of glassy carbon and pyrolytic graphite with ceresine wax sometimes improves the reproducibility of the residual current and noise.[86,91] Because of a favorable signal-to-noise ratio, carbon paste electrodes exhibit very low detection limits, although electrode reactions on the paste are generally slower than on well-polished platinum or glassy carbon[84] (see also Table 5).

However, classical carbon pastes prepared by homogenization of finely pulverized graphite with a liquid diluent, such as nujol or α-bromonaphthalene (for a more detailed description see, e.g., the references[81,84]), have serious drawbacks. It is difficult to make the electrode surface sufficiently planar and smooth. The cathodic residual current is high, due to the reduction of traces of oxygen adsorbed on and absorbed in the paste diluent. Pastes cannot be used for work with organic mobile phases because of dissolution of the paste diluent, and even in aqueous solutions bleeding of the diluent limits the lifetime of the paste to a maximum of a few days.[81,90] Moreover, the paste is not sufficiently resistant to mechanical damage especially at higher mobile-phase flow rates.[81]

Therefore, most promising seem to be carbon paste electrodes with a solid matrix, which retain the advantages of classical pastes, but are mechanically strong, can be polished, and resist many organic solvents, so that their lifetime is at least 1 month.[87,95] A number of such electrodes have been described and their applicability depends to a certain extent on the type of mobile phase and on the solutes.[87] Good results have been obtained using carbon powder mixed with high molecular weight waxes,[19,94] polyethylene[87] (a 1:1 mixture), polypropylene[96] (carbon-to-matrix ratio, 2:1), Kel-F (25% C, 75% Kel-F[97] or 15% C, 85% Kel-F[98,99]), polyvinyl chloride[95] (carbon-to-matrix ratio, 17:1), and a 1:1 mixture of chloroprene rubber and alkylphenol resin[95] (carbon-to-matrix ratio, 1.1:1). Surprisingly, PTFE[87] and silicone rubber[95] have been found unsuitable, as the electrodes prepared with them have a very low sensitivity.

As mentioned above, the greatest problem is attainment of long-term measuring reproducibility with solid and stationary mercury electrodes, especially in the presence of surface-active substances. Unfortunately, there is no general and reliable method for regeneration of the electrode-active surface. Mechanical polishing of solid electrodes is most effective, but is tedious and requires dismantling of the detector. The electrode surface can sometimes be freed of adsorbed substances chemically, by treatment with mineral acids, reductants, oxidants, etc. This method is not as reliable as mechanical polishing and also can be done only in the intervals between measurements. Electrochemical cleaning of the electrode-active surface can sometimes be achieved by cyclically polarizing the electrode at suitable potentials. Electrochemical cleaning can be carried out continuously during the measurement by polarizing the electrode alternately by measuring and cleaning pulses.[100] However, one must bear in mind that electrochemical cleaning procedures are by no means general, the cleaning potential values must be determined specifically for each case, and the method often fails.

Nevertheless, it is often possible to measure with solid electrodes for long-time intervals (at least several weeks), with a brief repolishing of the electrode surface once a day, without an appreciable deterioration in the measuring sensitivity and reproducibility.

The working electrode material is the main limiting factor in further development of electrochemical methods, in general, and, thus, also of electrochemical detection in HPLC. One of the promising trends is the use of chemically modified electrodes (see, e.g., Delaney[101] and Delaney and Warren[102]). Another is the application of microvoltammetric electrodes (e.g., carbon fibers).[103]

b. Detector Design

The construction of the measuring cell must permit attainment of a smallest possible effective volume, of defined and advantageous hydrodynamic conditions, and of the lowest possible impedance between the electrodes. Three-electrode measuring systems are mostly used to suppress the effect of the ohmic drop, especially in solutions with low conductivity. For these reasons it is also desirable that the auxiliary and reference electrodes be placed as close as possible to the working electrode; this actually is the most intricate problem in detector design.

Some hydrodynamic systems that can be used in polarographic, voltammetric, and coulometric detectors are characterized in Table 4.

The most common solid electrode detectors are based on the planar thin-layer hydrodynamic system[80,81,88,91-95,116-139] (Figures 8A and 8B) and the wall-jet system[80,81,89,95,114,115,134,140-150] (Figures 9A and 9B). The construction of some detectors permits their use in both the thin-layer and wall-jet system[80,81,95,151] (for an example see Figure 10). A dual-electrode thin-layer detector has also been described.[126,152,479] The dual arrangement can also be used to suppress the background current by passing the eluate over one working electrode and the pure mobile phase over the other (i.e., by working in a differential mode).[479] These detectors attain detection limits in the subnanogram range, have a wide linear dynamic range (3 to 5 concentration decades), and yield very reproducible values. Measuring cell volumes of less than 1 $\mu\ell$ can readily be attained; by decreasing the spacer thickness, the linear flow rate, and, consequently, also the measuring sensitivity is increased. However, this increase in the sensitivity has a limited practical importance, because the noise rapidly increases with decreasing cell thickness. The quality of polishing of the walls of the working space is decisive; the better the walls are polished, the thinner the spacer that can be used without the noise becoming too large.[81]

A decrease in the cell thickness also leads to a rapid increase in the electric resistance between the electrodes, to a consequent increase in the ohmic drop, and to nonuniform polarization of the working electrode.[19,153] From this point of view the cell type depicted in Figure 8B is preferable to that shown in Figure 8A, because the distance between the electrodes is much shorter. Typical electric resistances are $5 \times 10^5\ \Omega$ for the cell given in Figure 8A and $7 \times 10^4\ \Omega$ for that in Figure 8B[153] (using an aqueous citrate-phosphate buffer, whose resistance in a macroscopic conductometric cell is a few hundreds of ohms). Hence, it seems that the best electrode arrangement is that with the auxiliary electrode opposite the working electrode.[19] However, the products of the reaction on the auxiliary electrode may then interfere in the reaction on the working electrode, especially with very thin cells and low flow rates; therefore, the best position for the auxiliary electrode is down stream from the working electrode[95] or together with the reference electrode, in a compartment placed opposite the working electrode and separated from the effluent stream by a low electric resistance membrane which is impermeable for the auxiliary electrode reaction products.[154] On the other hand, this effect can sometimes be used to increase the sensitivity of the measurement. As shown earlier,[155-158] when a substance which can form a reversible redox system passes between two working electrodes placed opposite one another in a thin-layer cell and the potential of one electrode corresponds to the oxidation and that of the other to the reduction of the redox pair components, then the measuring sensitivity increases by multiple rereductions and reoxidations of the

Table 4
MOST IMPORTANT HYDRODYNAMIC ELECTRODE SYSTEMS

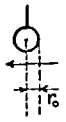
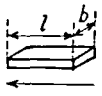
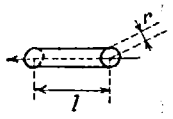
Electrode	Equation for limiting current	Notes	Ref.
Spherical	$I_l = 4\pi r_o nFDc + knr_o^2 D^{2/3} f^{1/2} c$	Turbulent flow; constant k must be determined empirically	104—106
			
Dropping mercury; parallel flow	$I_l = 0.0605nFcD^{2/3}(mt_l)^{4/9}u^{1/3}[1 + 1.86D^{1/3}(mt_l)^{-1/9}u^{-1/3} + 0.00332D^{-1/3}m^{4/9}t_l^{-5/9}u^{-2/3}]$		107
			
Dropping mercury; opposite flow	$I_l = 0.0154nFcD^{1/2}(mt_l)^{1/2}u^{1/2}[1 + 39.3D^{1/2}(mt_l)^{-1/6}u^{-1/2} - 2154D(mt_l)^{-1/3}u^{-1} + 0.0710m^{1/3}t_l^{-2/3}u^{-1}]$		108
			
Dropping mercury; perpendicular flow	$I_l = 0.0178nFcD^{1/2}u^{1/2}(mt_l)^{1/2}[1 + 13.8D^{1/2}(mt_l)^{-1/6}u^{-1/2} + 0.134m^{1/6}t_l^{-1/3}u^{-1/2}]$		109
			
Planar	$I_l = 0.68nFD^{2/3}cbl^{1/2}u^{1/2}\nu^{-1/6}$	Laminar flow	110
			
Tubular	$I_l = 2.01nF\pi D^{2/3}l^{2/3}u^{1/3}r^{2/3}c$	k = 0.33 for laminar flow; k = 1 for turbulent flow	111
			

Table 4 (continued)
MOST IMPORTANT HYDRODYNAMIC ELECTRODE SYSTEMS

Electrode	Equation for limiting current	Notes	Ref.
Cylindrical in a narrow channel	$I_l = 3.22nFc[\phi(a)]^{1/3}[\pi^{2/3}l^{2/3}(r_2 - r_1)D^{2/3}w^{1/3}/\{2(r_2 - r_1)\}^{1/3}(r_2^2 - r_1^2)^{1/3}]$; $\phi(a) = \frac{1-a}{a}\left\{\left[0.5 - \frac{a^2}{(1-a^2)}\ln(1/a)\right] / \left[\left(\frac{1+a^2}{1-a^2}\right)\ln(1/a) - 1\right]\right\}$; $a = r_1/r_2$	Laminar flow	112, 484
Conical	$I_l = 0.77nFAcD^{2/3}u^{1/2}\nu^{-1/6}l^{1/2}$	Laminar flow	113
Wall-jet	$I_l = knFAcD^{2/3}\nu^{-1/6}(u/L)^{1/2}$	Turbulent flow; k is an empirical constant; L is the characteristic dimension of the disk electrode	114
	$I_l = 1.60knFcD^{2/3}\nu^{-5/12}w^{3/4}a^{-1/2}R^{3/4}$		115

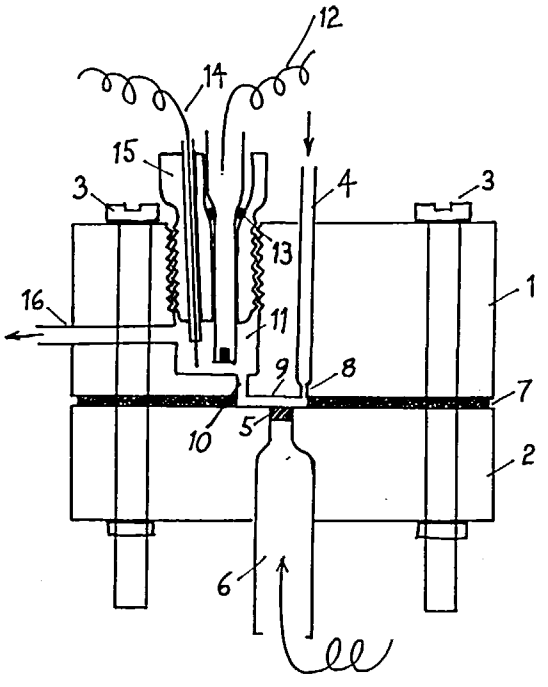
Note: For the significance of the symbols see the List of Important Symbols and Abbreviations.

redox pair components. This principle has actually been applied to thin-layer HPLC detectors.[94,138] However, one should bear in mind that this principle is only applicable to reversible redox systems, with very thin cells and at low flow rate; otherwise the number of electrochemical conversions is too small to cause any appreciable increase in the measuring sensitivity.

Some authors tried to alleviate the problem of a high IR drop by using a thin-layer cell with two carbon electrodes without a reference[136] or by short-circuiting the auxiliary electrode with an external reference electrode.[135] However, in these arrangements control of the working electrode potential is rather uncertain.

In some detectors, stainless steel, platinum, or silver outlet tubes are used as auxiliary and reference electrodes (see, e.g., the references[129,137,144]). This is also useful when the detector should permit fraction collection.[129] For work with nonpolar effluents, a thin-layer detector was adapted so that it allowed for mixing the effluent with a polar nonaqueous electrolyte solution before the working electrode.[128]

Recently, increased attention has been paid to application of capillary columns in HPLC. To avoid elution curve broadening, the detectors used with capillary columns must have especially small volumes. So far, a thin-layer voltammetric detector has been described for this purpose,[159] with an internal volume of about 0.1 $\mu\ell$ (see Figure 11). Another detector for capillary columns, based on the wall-jet principle, will soon be published.[482] For identification purposes it is useful to combine electrochemistry with other methods. A very promising combination seems to be coupling of voltammetry with rapid scan spectrometry. Much information can be found from Rhodes and Kadish.[160] An optically transparent cell of this type, designed for liquid chromatography,[161] is of the thin-layer type, with a volume of 10 to 25 $\mu\ell$, made of plexiglass, with a 120-line-per-inch



A

FIGURE 8. Thin-layer voltammetric detectors. (A) A detector with a separate reference and auxiliary electrode compartment; (1) PTFE detector body, upper part, (2) PTFE detector body, lower part, (3) plastic screws, (4) inlet, (5) working electrode, (6) brass contact, (7) PTFE spacer, (8) inlet PTFE capillary, (9) working space, (10) outlet, (11) reference and auxiliary electrode space, (12) SCE, (13) PTFE O-ring, (14) platinum wire auxiliary electrode, (15) plastic screw, (16) detector outlet. (From Štulík, K. and Pacáková, V., *J. Chromatogr.*, 208, 269 [1981]. With permission.) (B) A detector with a close arrangement of the electrodes; (1) PTFE blocks, (2) PTFE spacer, (3) inlet, (4) outlet, (5) working electrode, (6) auxiliary electrode, (7) reference electrode (Ag wire in a KCl solution), (8) porous glass. (From Štulík, K., Pacáková, V., and Stárková, B., *J. Chromatogr.*, 213, 41 [1981]. With permission.)

gold minigrid working electrode and an auxiliary electrode sandwiched between a quartz plate and a PTFE spacer.

Tubular[87,100,162-166] and cylindrical[112,167] hydrodynamic systems have been used less frequently, although they are theoretically well defined, chiefly because it is rather difficult to attain a small internal volume to maintain a constant geometry of cylindrical electrodes (wires) and to clean the electrodes with tubular systems. A tubular detector[164] whose construction has removed most of these difficulties is depicted in Figure 12. Here the diameter of the tubular working electrode is sufficiently large to enable easy mechanical polishing of the active surface, and the cylindrical auxiliary electrode placed concentrically in the working electrode maintains a small working volume (2.3 $\mu\ell$) and a

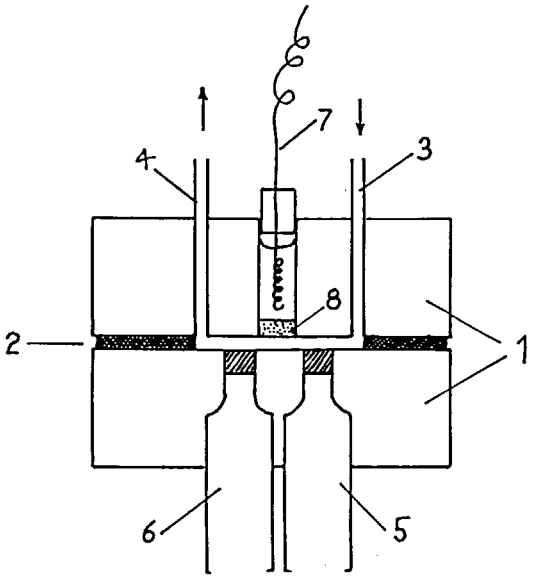


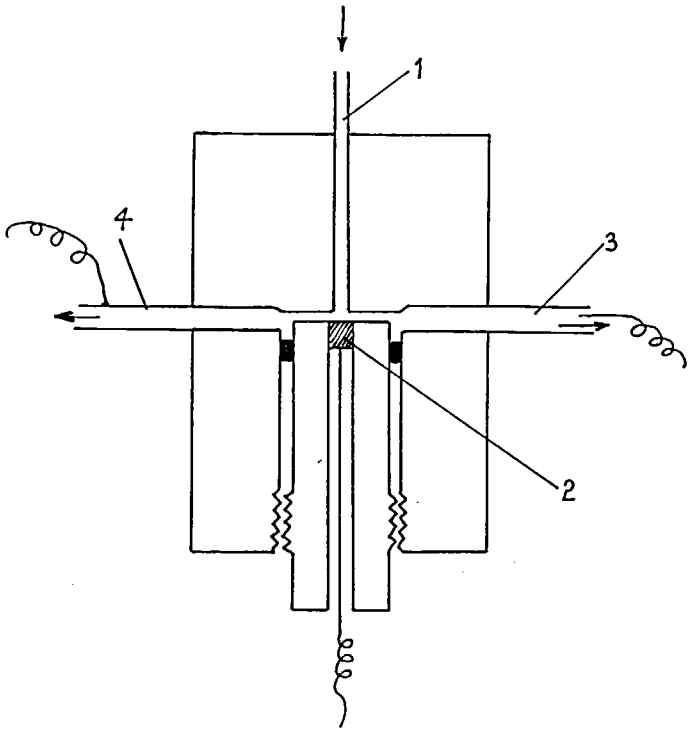
FIGURE 8B

small distance between the working and auxiliary electrode (i.e., a low IR drop); the plastic cap on the auxiliary electrode prevents short-circuiting and interference between the products of reactions on the working and auxiliary electrode. A simple design of a tubular detector with a very small dead volume was reported recently.[166] The working electrode is obtained by drilling a hole with a diameter of 0.3 mm in a 0.5-mm-thick platinum foil, thus, obtaining a working volume of 0.035 $\mu\ell$. The authors claim that the electrode can be mechanically cleaned, but it is difficult to imagine how this can be done to produce a polished and reproducible surface.

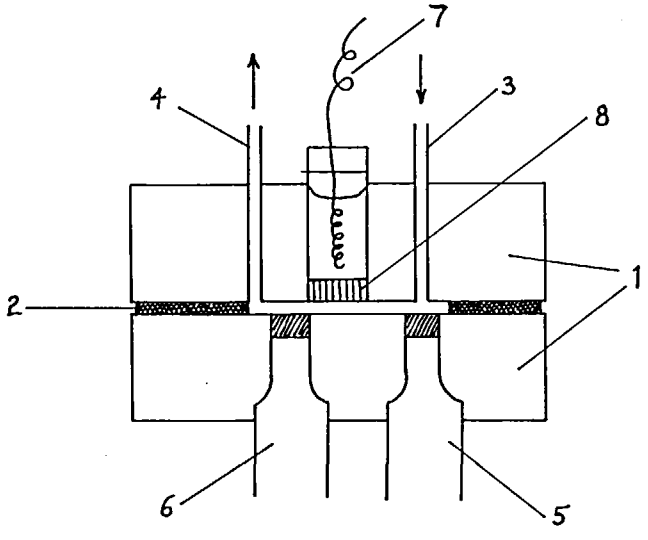
Two cylindrical systems have been described,[112,167] both based on a wire placed in a narrow channel, either platinum[112] or gold.[167] The cell with the gold electrode is shown in Figure 13. A gold wire 5.8 cm long and 0.5 mm in diameter is inserted in a dialysis membrane tube through which the effluent passes. The tube is immersed in a vessel containing an aqueous KCl solution. An SCE reference and a platinum auxiliary electrode are placed in the KCl solution. These designs are very simple and high sensitivity is claimed, but the hydrodynamics are poorly defined, although not as poorly as with wire electrodes placed perpendicularly to the liquid flow.[168,169]

Very many flow-through cells have been constructed, employing dropping mercury electrodes (DMEs).[80,144,170-210] The cells designed before the 70s are, of course, suitable only for classical liquid chromatography, not HPLC. The most difficult problem encountered in the construction is the attainment of a very small working space while retaining reliable function of the DME. Two typical versions of the cell have been developed, employing either a vertical DME (see, e.g., Figure 14)[144] or a horizontal DME (e.g., Figure 15).[198] Internal volume values of down to 1 $\mu\ell$ can be attained.

From the point of view of the signal handling, the current oscillations caused by dropping of the mercury are undesirable and there are two general methods for their suppression: (1) an RC filter can be used; however, the time constant of the cell is then increased and, thus, this approach is less suitable than (2) employing a DME with a very short drop time. This is most easily attained by using a horizontal capillary. For this reason, cells with horizontal capillaries have mostly been preferred (see, e.g., the references[186,198,202-204,206,208]). Recently, very good results were obtained[202,206] using



A



B

FIGURE 9. Wall-jet voltammetric detectors. (A) Detector, (1) inlet, (2) glassy carbon working electrode, (3) reference electrode, (4) auxiliary electrode. (According to Fleet, B. and Little, C. J., *J. Chromatogr. Sci.*, 12, 747 [1974]. With permission.) (B) Detector; (1) PTFE blocks, (2) PTFE spacer, (3) inlet, (4) outlet, (5) working electrode, (6) auxiliary electrode, (7) reference electrode, (8) porous glass. (According to Štulik, K., Pacáková, V., and Stárková, B., *J. Chromatogr.*, 213, 41 [1981]. With permission.)

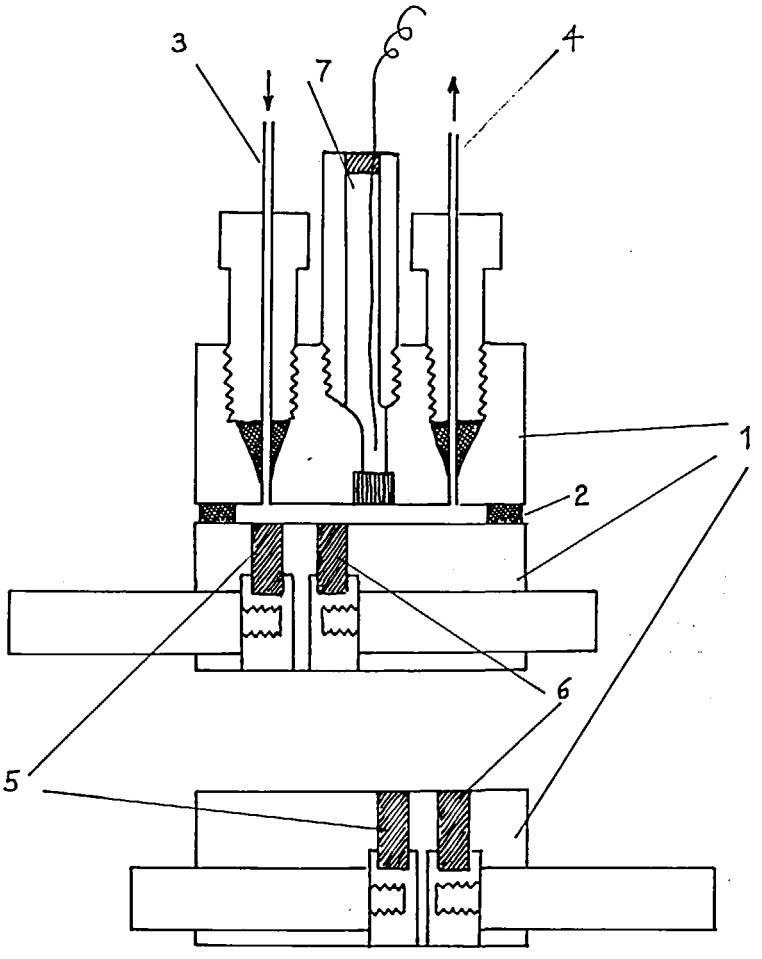


FIGURE 10. A thin-layer/wall-jet detector. (1) PTFE blocks, (2) PTFE spacer, (3) inlet, (4) outlet, (5) working electrode, (6) auxiliary electrode, (7) reference electrode. The thin-layer detector is changed into the wall-jet detector by turning the bottom part of the detector by 180°. (From Podolák, M., Štulík, K., and Pacáková, V. With permission.)

horizontal capillaries conically narrowed at the tip, either with mechanical drop control, using a needle inserted into the capillary,[202] or with the drop time controlled electronically by applying pulses 20 msec long and with an amplitude of up to −1 V to the electrode. The latter method produces drop times of 0.3 to 0.9 sec, well suited for application of pulse-measuring methods. Another interesting method for attaining controlled, short drop times is based[208] on placing a polished plate or piston very close to and opposite the orifice of a horizontal DME, adjusting the distance with a micrometer screw (Figure 16). In this way a small internal volume of the cell is also obtained.

A disadvantage common to all the above methods is the rather complicated construction and the danger of mechanical faults. Construction problems have been coped with very ingeniously in the design of the Princeton Applied Research commercial detector[210] (Figure 17), where a DME is combined with the wall-jet principle. The mobile phase is directed from a vertical jet onto a macroscopic vertical DME placed in a common macroscopic vessel containing a base electrolyte; the effective working volume

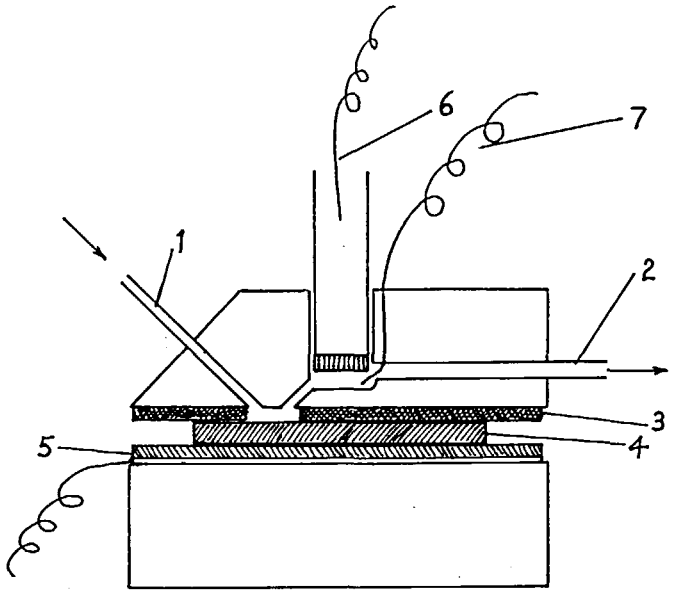


FIGURE 11. A voltammetric detector for use with capillary columns. (1) Inlet, (2) outlet, (3) polyethylene spacer, (4) pyrolytic graphite working electrode, (5) copper plate serving as the contact of the working electrode, (6) reference electrode, (7) auxiliary electrode. (From Hirata, Y., Lin, P. T., Novotný, M., and Wightman, R. M., *J. Chromatogr.*, 181, 287 [1980]. With permission.)

is only about 1 $\mu\ell$. The detector actually consists of a very simple adapter to the PAR commercial polarographic DME.

The construction of a detector with a stationary mercury electrode can be very simple[211] (Figure 18), but the main advantage of the DME, periodic surface renewal, is lacking. The same holds for detectors with mercury film electrode,[139,212,213] with the additional drawback of a narrowed cathodic potential range (see Section III.C.2.a.).

A voltammetric cell which combines the wall-jet principle with a rotating-disk solid electrode has also been constructed[214,478] in an attempt to decrease the diffusion layer thickness at the working electrode and, thus, to improve the measuring sensitivity and to suppress the signal dependence on the liquid flow rate. The authors do report improved sensitivity, without an increase in the noise level, but the cell seems to be rather complicated for use in routine measurements.

For the reasons discussed in Section III.C.2, cells for coulometric measurements[215-228] employ working electrodes with very large active surface areas, have relatively large internal volumes, and require low liquid flow rates. Various hydrodynamic systems have been used, such as a tubular platinum[217,220] or cadmium[221] electrode, silver or platinum gauze,[218,223] a column electrode with granular glassy carbon,[215] or granular amalgamated nickel.[216] As serious difficulties may arise from passivation of large-area electrodes, a cell design with planar working electrodes[226] (Figure 19), which can readily be repolished, seems to be preferable.

Provided that 100% electrolysis yield is attained (i.e., 100% current efficiency and complete electrolysis during the residence time of the substance in the detector), the sensitivity of coulometric detectors is very high, the detection limit lies in the picogram range, the precision is very good, and the linear dynamic range is very wide (up to six concentration decades). Within the range of complete electrolysis, the signal dependence

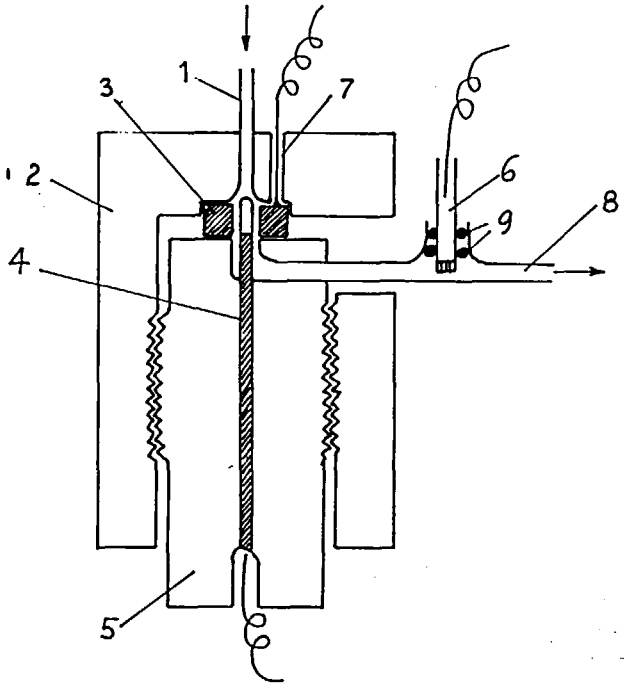


FIGURE 12. A tubular detector. (1) Inlet, (2) PTFE detector body, outer part, (3) platinum tubular working electrode, (4) platinum cylindrical auxiliary electrode with a PTFE insulating cap, (5) PTFE detector body, inner part, (6) reference electrode, (7) channel for the lead to working electrode, (8) glass tube to waste, (9) PTFE O-rings. (From Štulík, K. and Pacáková, V., *J. Chromatogr.*, 192, 135 [1980]. With permission.)

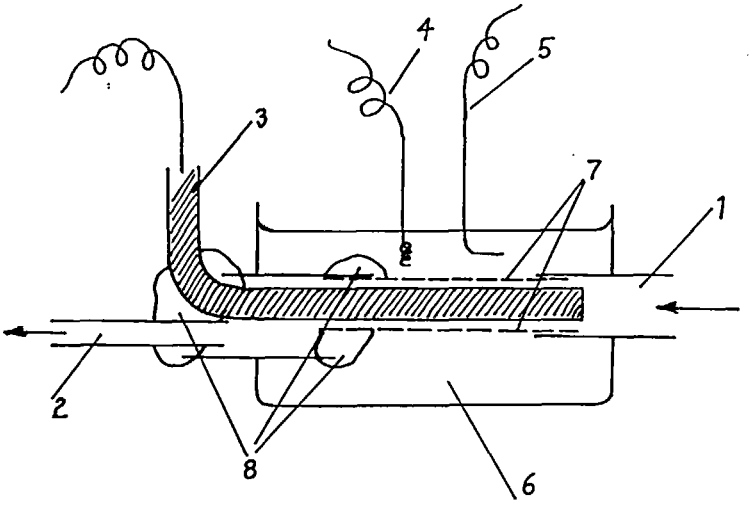


FIGURE 13. A cell with a gold wire electrode placed in a dialysis membrane tube. (1) Inlet, (2) outlet, (3) gold wire working electrode, (4) reference electrode, (5) auxiliary electrode, (6) aqueous KCl solution, (7) dialysis membrane, (8) epoxy resin. (From Rubinson, K. A., Gilbert, T. W., and Mark, H. B., Jr., *Anal. Chem.*, 52, 1549 [1980]. With permission.)

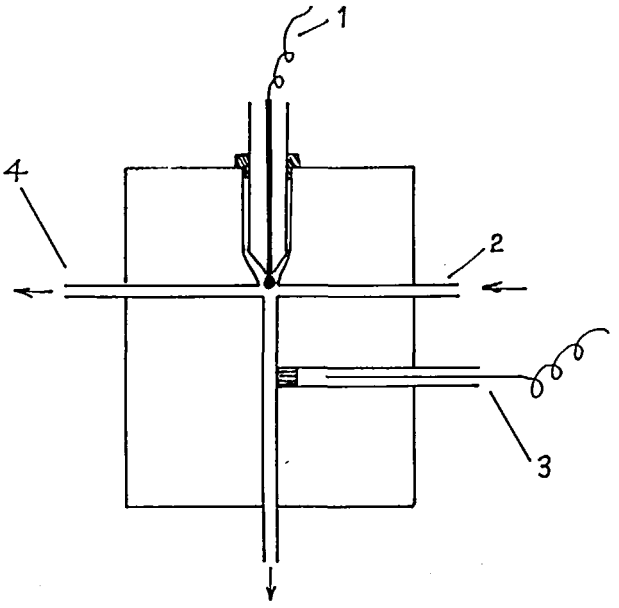


FIGURE 14. A polarographic detector with vertical DME. (1) Indicator electrode, (2) inlet, (3) reference electrode, (4) auxiliary electrode. (From Fleet, B. and Little, C. J., *J. Chromatogr. Sci.*, 12, 747 [1974]. With permission.)

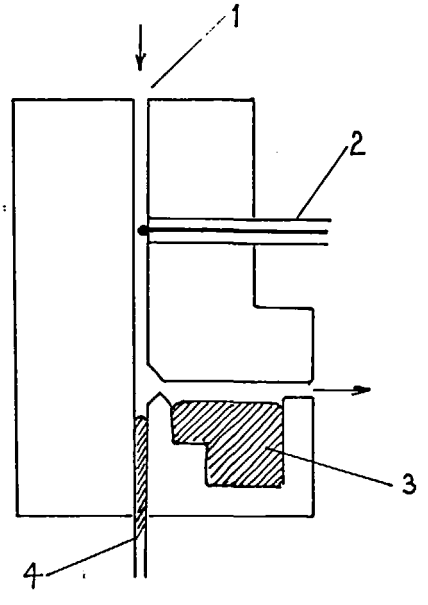


FIGURE 15. A polarographic detector with horizontal DME. (1) Inlet, (2) indicator electrode, (3) mercury pool anode, (4) tube to the mercury reservoir. (From Wasa, T. and Musha, S., *Bull. Chem. Soc. Jpn.*, 48, 2176 [1975]. With permission.)

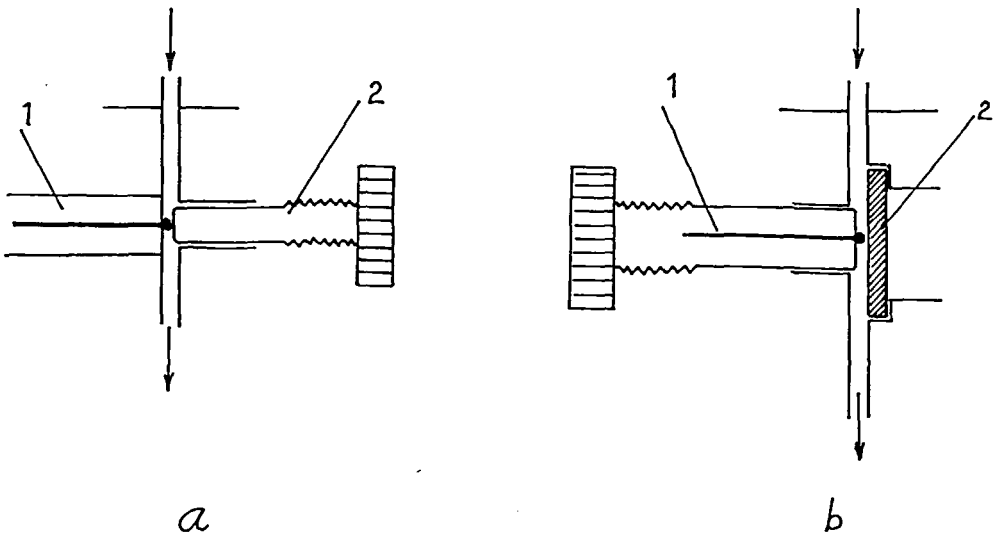


FIGURE 16. Polarographic detectors. (a) Detector with adjustable piston; (1) DME, (2) piston with micrometer screw. (b) Detector with adjustable DME; (1) DME adjustable with micrometer screw, (2) polished plate. (According to Michel, L. and Zatka, A., *Anal. Chim. Acta,* 105, 109 [1979]. With permission.)

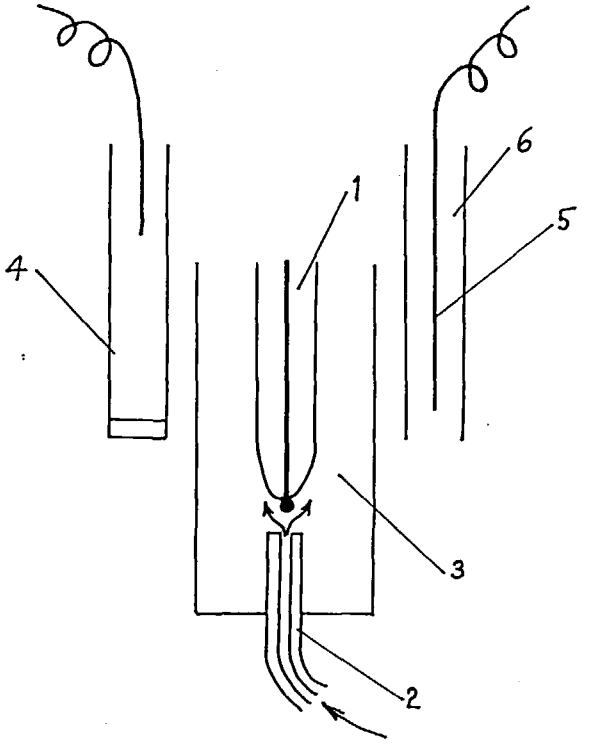


FIGURE 17. Principle of the PAR polarographic detector. (1) DME, (2) jet, (3) base electrolyte, (4) reference electrode, (5) auxiliary electrode, (6) inert gas inlet. (From PAR Model 310 Polarographic Detector, manufacturer's literature, Princeton Applied Research, Princeton, N.J. With permission.)

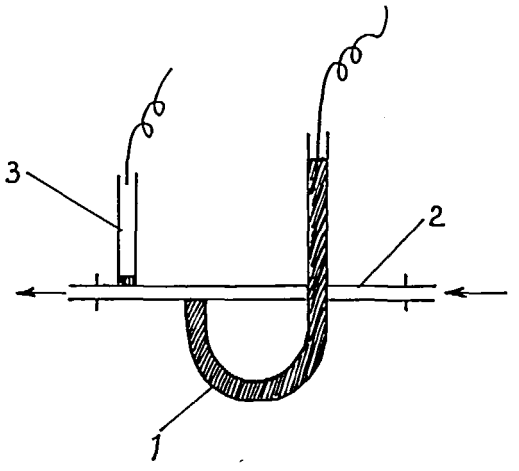


FIGURE 18. A polarographic detector with a stationary mercury electrode. (1) Indicator electrode, (2) eluate channel, (3) reference electrode. (From Rabenstein, D. L. and Saetre, R., *Anal. Chem.*, 49, 1036 [1977]. With permission.)

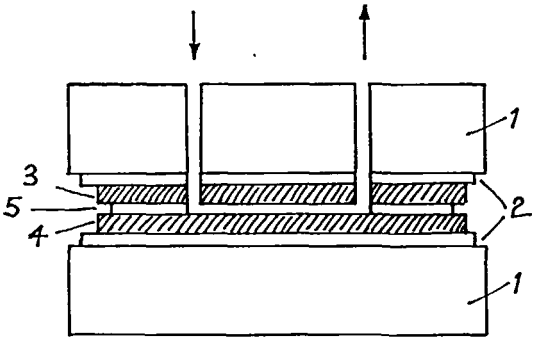


FIGURE 19. A coulometric detector with large-area planar glassy carbon electrodes. (1) Detector body, (2) PTFE insulating foils, (3 and 4) working electrodes, (5) spacer. (From Lankelma, J. and Poppe, H., *J. Chromatogr.*, 125, 375 [1976]. With permission.)

on the flow rate and composition of the liquid is less critical than with voltammetric detectors. On the other hand, the large volume of the detector may lead to broadening of the elution curve and the detector is destructive, thus, preventing fraction collection or placing of another detector at its outlet. A semiintegral cell with a stationary mercury electrode, in which the current semiintegral at a potential step or a potential sweep is recorded, is an intermediate between voltammetric and coulometric cells.[480]

Coulometric cells are well suited for use as precolumn or predetector reactors to improve the sensitivity and, especially, the selectivity and application range of voltammetric detectors. For this purpose a dual coulometric-voltammetric cell has been proposed.[154] The potential of a granulated glassy carbon coulometric electrode is more positive than that of a carbon paste voltammetric electrode, so that certain electroactive components are removed from the effluent before they reach the voltammetric detector

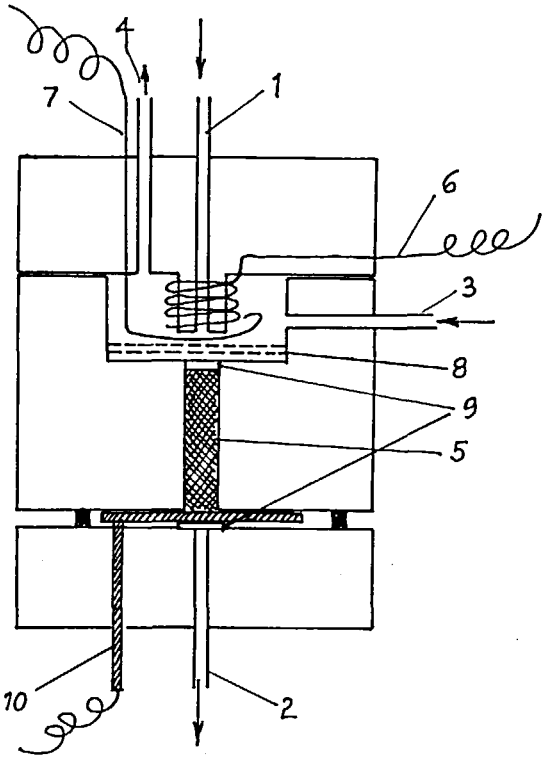


FIGURE 20. A precolumn coulometric cell. (1) Mobile phase inlet, (2) mobile phase outlet, (3 and 4) inlet and outlet of 0.1 *M* KCl, respectively, (5) powdered reticulated vitreous carbon working electrode, (6) reference electrode, (7) auxiliary electrode, (8) cation-exchanger washer, (9) PTFE screen, (10) platinum contact of the working electrode. (From Schieffer, G. W., *Anal. Chem.*, 53, 126 [1981]. With permission.)

(the commercial Bioanalytical Systems detector or a detector of the author's own construction[154] with the reference and auxiliary electrodes placed against the working electrode and separated by a cation-exchange membrane). Another dual cell[229] contains an amalgamated silver powder column electrode for the reducton of cystine and related compounds in the effluent to cysteine, which is then voltammetrically detected on a mercury pool electrode.

The original precolumn coulometric cells for electrochemical derivatization required stopping of the effluent after the sample injection to allow the electrochemical reaction to proceed to completion[230,231] and used a glassy carbon tubular[230] or a porous graphite[231] working electrode. A newer precolumn cell[232] (Figure 20) with a column working electrode of powder reticulated vitreous carbon attains 100% electrolysis efficiency without stopping of the flow. A similar approach has also been taken to improve the signal-to-noise ratio with polarographic detectors.[233] A cell containing a porous silver electrode is placed in the chromatographic system, in which traces of oxygen, metals, and reducible organic impurities are reduced. The authors report that the residual detector current decreases 100-fold and the noise tenfold in comparison with the use of a mobile phase deaerated by the passage of nitrogen alone.

On the whole, predetector and precolumn electrochemical cells may be very important

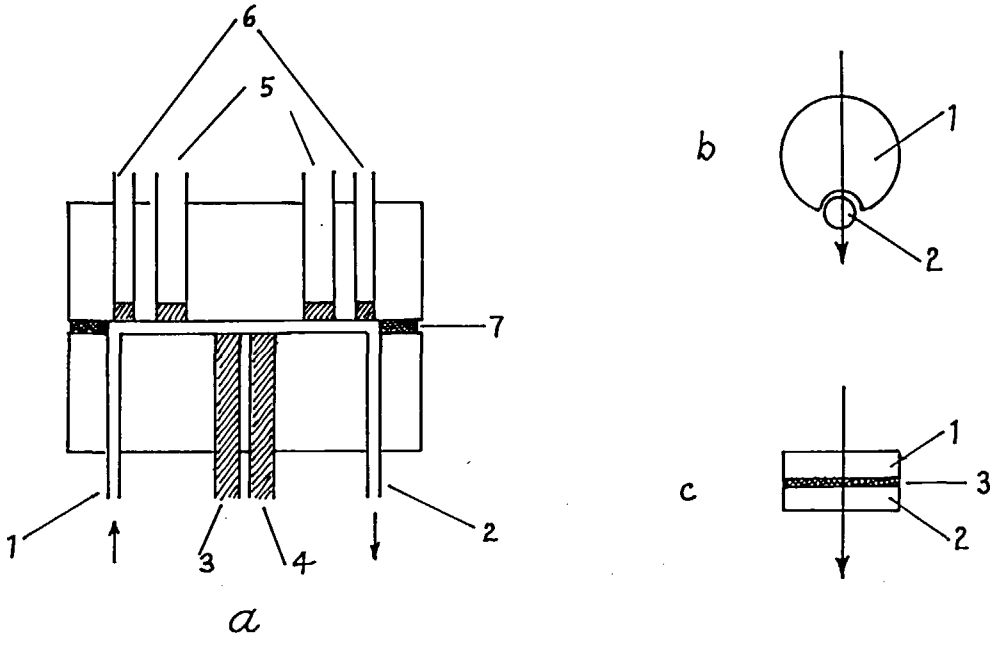


FIGURE 21. A dual electrode cell. (a) Overall view; (1) inlet, (2) outlet, (3) generator electrode, (4) detector electrode, (5) reference electrode, (6) auxiliary electrode, (7) spacer. (b) Detailed view of the generator (1) and detector (2) electrodes in the "horseshoe" arrangement. (c) Detailed view of the generator (1) and detector (2) electrodes in the "sandwich" arrangement; (3) polyethylene spacer. (From Mac Crehan, W. A. and Durst, R. A., *Anal. Chem.*, 53, 1700 [1981]. With permission.)

for widening the application range of electrochemical detectors, but one should always bear in mind that they may significantly contribute to broadening of the elution curves. This danger is suppressed somewhat by the construction of a dual electrode cell[234] (Figure 21) containing a generation electrode (amalgamated silver), placed close to a detector electrode (either amalgamated silver or glassy carbon). The selectivity of the measurement may further be improved by modulating the generation electrode potential.

c. Measuring Techniques

The simplest and by far the most common measuring technique involves monitoring of the limiting current (or the charge in coulometric detectors) at a constant electrode potential. Nonstationary measuring techniques (square-wave, normal pulse, and differential pulse voltammetry) have been introduced in an attempt to (1) improve the sensitivity and selectivity of the measurements, (2) eliminate the dependence of the signal on the liquid flow rate, and (3) suppress the adsorption of substances on the electrode leading to the electrode passivation. So far, the results have been rather ambiguous.

Except for a single paper,[168] all the authors[80,81,88,93,139,164,201,206,235,236] have found that square-wave, normal pulse, and differential pulse measurements do not improve the sensitivity of measurements and that the detection limit is usually higher than that obtained using d.c. voltammetry. Moreover, it has been shown[81,88,139,164] that the background current and noise are higher in pulse and diffential pulse measurements than in d.c. methods.

Some authors[88,93] claim that in pulse measurements the signal is virtually independent of the liquid flow rate; however, this effect was not observed in other works.[81,164] It seems

probable that increased measuring sensitivity and decreased dependence of the signal on the medium flow rate, due to a decreased diffusion layer thickness in pulse methods, can become significant only at very low flow rates, where the diffusion layer in d.c. measurements is thick. At higher flow rates, the liquid movement apparently leads to an even greater and more reproducible decrease in the diffusion layer thickness than application of a nonstationary measuring technique. This explanation seems more probable than the assumption that the reversibility of the electrode reactions is lower in flowing systems than in a quiescent solution.[206]

There are two advantages of some nonstationary methods that can be considered unambiguous. The first is a certain decrease in the adsorption of substances on the electrode,[88,100,139,235] however, this effect is not general and need not always be encountered. The other is an improvement in the selectivity compared with the d.c. measurement of square-wave[201,235] and differential pulse[88,89,93,139] measurements (but not of normal pulse voltammetry[88]). The improvement in the selectivity strongly depends on the choice of the pulse amplitude, which must be an optimal compromise from the point of view of the signal-to-noise ratio and the selectivity, as the selectivity improves with the decreasing amplitude while both the signal and the noise decrease.[139] The advantage of improved selectivity can also be obtained in another way.[237] When a dual electrode voltammetric cell is employed, the two working electrodes are maintained at somewhat different potentials close to the half-wave potential of the particular systems, and the difference signal is obtained by subtracting the two electrode signals; then a similar effect is obtained as in differential pulse measurements with a pulse amplitude analogous to the potential difference between the two electrodes, but without a decrease in the sensitivity.

There is renewed interest in tensammetric techniques in electroanalytical chemistry, which is also reflected in a paper[187] describing a HPLC detector based on the a.c. polarographic monitoring of variations in the electric double-layer capacity of a DME due to adsorption of substances present in the eluate.

On the whole, it can be concluded that for most purposes sampled d.c. measurement, possibly combined with cleaning pulses,[100] is optimal.

The problem of identification of substances eluted from a chromatographic column is often encountered in practice. This problem can be tackled by combining electrochemical detection with other, chiefly spectrometric, techniques, discussed in Section III.C.2.b in connection with the spectroelectrochemical cell, or by fraction collection after the detector and subsequent analysis. Another promising possibility was described recently.[235] Rapid scan square-wave polarography with computer accumulation and handling of the data was used with PAR commercial detectors. The potential was scanned over 500 mV every 2 sec, with a pulse amplitude of 10 mV and a frequency of 100 Hz. The data, containing 102 current samples, were then handled by a program that subtrated the background, filtered the noise, and looked for the position and height of the peak. Three-dimensional polarograms were, thus, obtained (Figure 22), which can be used for identification. However, it seems that electrochemical detectors will chiefly be important for their simplicity, sensitivity, and measuring reproducibility, rather than for their use for identification purposes, because they will hardly be able to compete with the liquid chromatography-mass spectrometry combination once it has been developed to a level comparable with the present GC-MS technique.

d. Comparison of Detector Performance with the Theory

The equations for the limiting currents in various hydrodynamic systems are given in Table 4 and were recently summarized and discussed by Hanekamp and van Nieuwkerk.[238] The qualitative conclusions following from this discussion are as follows:[238]

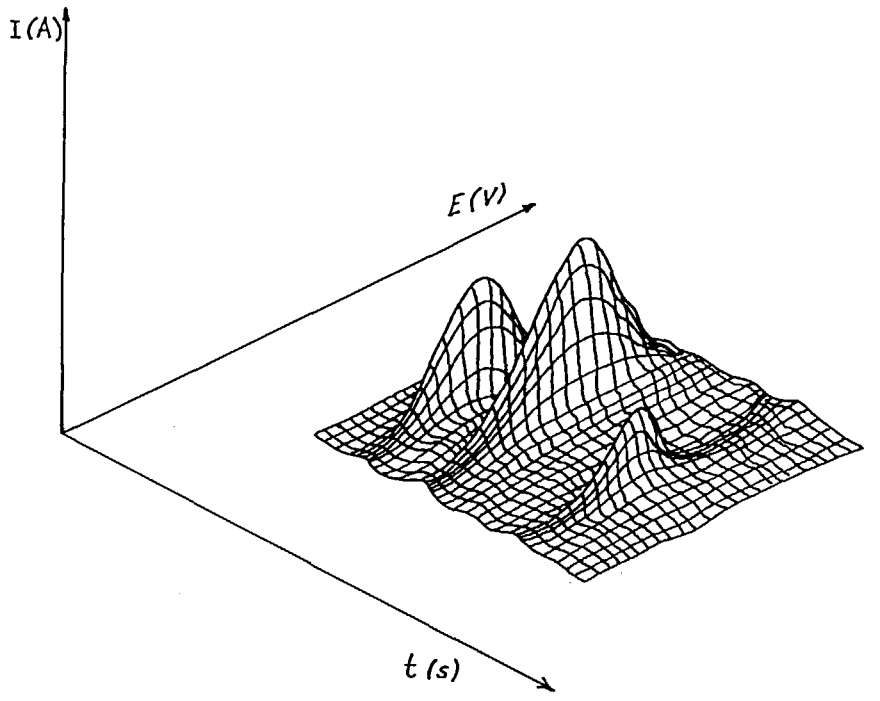


FIGURE 22. Three-dimensional polarogram obtained by rapid-scan square-wave technique. (From Samuelsson, R., O'Dea, J., and Osteryoung, J., *Anal. Chem.*, 52, 2215 [1980]. With permission.)

1. The highest sensitivity of measurement with polarographic detectors is attained when the flows of the solution and of the mercury are perpendicular and the highest signal-to-noise ratio is obtained with rapidly dropping electrodes (small drops).
2. With solid-electrode detectors, the highest signal-to-noise ratios are obtained with short and narrow tubular electrodes, in thin-layer detectors with short and wide electrodes in a narrow channel, and in wall-jet cells with electrodes of a small radius.

Typical parameters of some voltammetric and polarographic detectors are summarized in Table 5. When the actual performance of detectors is compared with the values predicted from the equations given in Table 4 (see, e.g., Štulík and Pacáková[81] for thin-layer, wall-jet, and tubular hydrodynamic systems), then it is found (Figure 23) that there is a significant difference. This difference is apparently caused by several factors:[81] (1) an uncertainty in the numerical values substituted into the equations (chiefly the values of the solute diffusion coefficients and of empirical constants); (2) specific effects of the electrode material (adsorption, passivation, and other deviations from the behavior of an ideal inert redox electrode); (3) deviations from laminar flow in the systems where this is assumed; (4) the effect of a very small working space, i.e., negation of the assumption that the thickness of the hydrodynamic boundary layer is negligible compared with the cell dimensions, under which the equations given in Table 4 have been derived.

The contribution from factors (3) and (4) strongly depends on the quality of polishing of the electrode surface and of the walls of the working space and on the precision with which the detector was fabricated.

Table 5
PARAMETERS OF SOME POLAROGRAPHIC AND VOLTAMMETRIC DETECTORS

Detector	Residual current (μA)	Detection limit (ng)	Lin. dynamic range (ng)	Calibration curve parameters slope (μA/μg); corr coefficient	Time constant (sec)	Cell volume (μℓ)	Ref.
Polarogr., HMDE	—	15[a]	100—30,000	0.993; 1.000	2.2[b]	8	202
Polarogr., conical HMDE	—	3[a]	10—30,000	0.529; 0.999	2.3[b]	8	202
Polarogr., conical HMDE[c]	—	3[a]	10—25,000	0.274; 1.000	0.6[b]	8	202
Thin-layer (glassy C)	2.4—5.6	0.5—1.0[d]	1—5,000	2.6; 0.999	0.75[e]	0.65	81
Thin-layer (C paste)	0.1—0.2	0.5—1.0[d]	1—5,000	1.17; 0.997	1.7[e]	0.65	81
Wall-jet (glassy C)	10	0.3[d]	0.3—3,000	2.3; 0.998	1.0[e]	0.35	81
Wall-jet (C paste)	0.18	0.03—0.1[d]	0.03—3,000	2.25; 0.958	0.86[e]	0.35	81
Metrohm EA 1096 (wall-jet, glassy C)	—	0.4[a]	10—8,000	6.345; 0.999	0.3[b]	1.3	239
Tubular (Pt)	3—10	0.3[d]	0.3—2,500	1.3; 0.998	1.7[e]	2.3	81

[a] Nitrobenzene.
[b] Flow rate, 1 mℓ/min.
[c] Wall-jet system.
[d] Adrenaline.
[e] Flow rate, 0.3 mℓ/min.

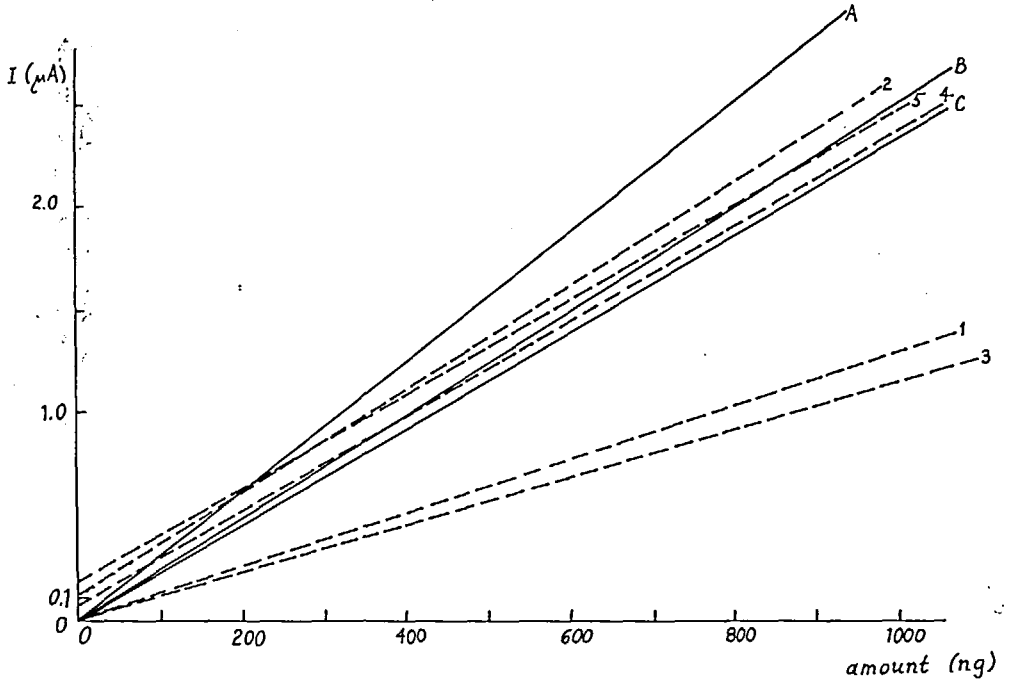


FIGURE 23. Experimental and theoretical calibration curves. (A, B, and C) Theoretical curves for the thin-layer, wall-jet, and tubular detector, respectively; experimental curves: (1) tubular detector (Pt), (2) thin-layer detector (glassy C), (3) thin-layer detector (C paste), (4) wall-jet detector (glassy C), (5) wall-jet detector (C paste). Epinephrine in a citrate/phosphate buffer, +0.8 V (SCE), 0.3 mℓ/min. (From Štulík, K. and Pacáková, V., *J. Chromatogr.*, 208, 269 [1981]. With permission.)

Wall-jet detectors exhibit better agreement with the theory than other types, because the theory assumes turbulent flow, which is apparently the predominant parameter determining the detector performance. This theory[114,115] has been experimentally verified by a number of authors[140-143,145-149] and it has been concluded that the optimal parameters involve a working electrode diameter smaller than that of the jet, rigorously concentric placement of the jet and the electrode, and the shortest possible distance of the jet orifice from the electrode surface (of the order of tenths of a millimeter).

An improvement in the theory of other detector types requires a more rigorous treatment of flow electrolysis in a very limited space, which, admittedly, is a very difficult problem. A first step in this direction is the work[96,481] treating the current controlled by mass transport in a rectangular channel containing a planar electrode, assuming laminar flow and neglecting longitudinal diffusion. The authors have shown that it is necessary to evaluate the expression

$$k = \frac{D\,b\,l}{w\,h} \tag{8}$$

where D is the solute diffusion coefficient, b is the channel width and l its length, w is the volume flow rate, and h is the channel height, whether the mode of operation is predominantly voltammetric or coulometric.

For $k \ll 0.3337$, the operation is voltammetric and the current is given by the relationship derived earlier,[240]

$$I = 1.467\,n\,F\,c\,\left(\frac{D\,l\,b}{h}\right)^{2/3} w^{1/3} \tag{9}$$

For $k > 0.3337$, the cell behavior is partly coulometric, described by the equation

$$I = n\,F\,c\,w\left[1 - 0.3992\exp\left\{2.505\,(0.3337 - k)\right\}\right] \tag{10}$$

and for $k \gg 0.3337$ it is coulometric, given by the equation

$$I = n\,F\,w\,c\,(0.2671 + \frac{D\,l\,b}{w\,h}) \tag{11}$$

From the equations derived it follows that the signal-to-noise ratio increases with decreasing electrode surface area A and channel height h, but is independent of the electrode shape. The optimal value of h for a d.c. measurement in a cell with volume V is given by the equation

$$h = (V\,D/0.42\,\bar{w})^{1/2} \tag{12}$$

where $\bar{w}$ is the mean volume flow rate.

D. Electrokinetic Detector

The electrokinetic effect can also be used for detection of components in eluates.[241] On passage of liquids of low conductivity carrying electric charge through a capillary or a porous bed, electric currents are generated from sorption equilibria and the formation of an electric double layer, which depend on the composition of the flowing liquid. The detector based on this principle is nonselective and nondestructive. The measured signal does not depend on the temperature, but depends on the flow rate. It has been shown[242]

that the current also depends on any changes on the solid phase surface which need not affect the mass equilibria involved. A detection limit of down to 0.01 ppm was reported.[242]

IV. COMPARISON OF ELECTROCHEMICAL DETECTORS WITH OTHER HPLC DETECTORS

As can be seen in Table 6, only the voltammetric, polarographic, and coulometric detectors can successfully compete with the best HPLC detectors based on other principles. Their main advantages are a high sensitivity combined with good measuring precision and a broad linear dynamic range.

V. APPLICATION OF ELECTROCHEMICAL DETECTORS

Electrochemical detectors were used in chromatography even before the advent of HPLC, for example, for the determination of inorganic ions (e.g.[176,192]), amino acids (e.g.[44,177]), and other organic compounds (e.g.[45,145,185,187,246,247]). However, the real importance of electrochemical detectors has only been recognized recently in combination with HPLC techniques (LCEC), where the advantages discussed above can be fully utilized.

The most common applications of electrochemical detectors in chromatography are in the analysis of aromatic amines and phenolic compounds of biological, pharmaceutical, and environmental importance. The great majority of practical determinations involve the anodic oxidation at solid electrodes with voltammetric or coulometric detection. However, various sulfur- nitroso-, and nitrocompounds can be reduced at DME, Hg pool, or Au amalgam and, therefore, detected with polarographic detectors. For previous reviews of the applications of electrochemical detectors in HPLC, see examples in references.[14,15,20,248-251]

In the following sections the applications of electrochemical detectors are summarized and typical examples of procedures for important determinations are given in Table 7.

A. Tyrosine Metabolites

1. Catecholamines

The most important application of liquid chromatography with electrochemical detection is the determination of biogenic amines and their metabolites.[117,129,131,248,250-253,473,474] In this group, the most important to neurochemists are catecholamines, e.g., epinephrine (E), norepinephrine (NE), and dopamine (DA). They can easily be oxidized at +0.7 V (Ag/AgCl) and can, therefore, be detected with electrochemical detectors. Urinary, serum (or plasma), and tissue catecholamines can be determined. Whereas urinary catecholamines reflect the overall activity of the sympathetic and central nervous system over a given period of time, serum (or plasma) catecholamines represent temporary changes that occur in the sympathetic nervous system and, thus, permit the monitoring of certain diseases. Analysis of brain tissue enables endogenous catecholamines to be studied.

The concentrations of catecholamines in a healthy individual are very low: NE is present in a few hundred picograms per milliliter plasma or serum, E and DA approximately ten times lower. Moreover, they occur in complex matrices whose analyses by convential methods are very tedious. Prior to LCEC they were usually isolated using simple procedures.[250] Catecholamines are selectively adsorbed on alumina; they form stable complexes with alumina at high pH values that allow their preconcentration prior to liquid chromatography and removal of the undesirable

Table 6
COMPARISON OF THE CHARACTERISTICS OF SOME LIQUID CHROMATOGRAPHIC DETECTORS

	Detector										
	Voltammetric[81,202,239]										
Parameter	Polarographic	Thin-layer	Wall-jet	Coulometric[212]	Low-frequency conductance[52,54]	High-frequency impedance[57,58,61,63,67]	Potentiometric	Electro-kinetic[241]	UV-Photo-metric[243]	Refrac-tometric[244]	Fluorescence[245]
Analytical property	Current (A)			Charge (C)	Specific conductance (S)	Permitivity	Eq. potential (V)	Current (A)	Absorbance	Refr. index	Fluorescence
Lin. dynam. range	10^5	10^5	10^5	10^6	10^6	10^4—10^5	10^4	10^2—10^3	10^4	10^1—10^4	10^3—10^6
Noise (signal units)	10^{-9}	10^{-9}	10^{-9}	10^{-9}—10^{-10}	10^{-6}—10^{-8}	10^{-5}—5×10^{-7}	10^{-3}	10^{-14}	2×10^{-4}	2×10^{-7}—5×10^{-8}	—
Min detect. conc (g/mℓ)	10^{-9}—10^{-10}	10^{-10}	10^{-10}	10^{-10}	10^{-7}—10^{-8}	10^{-6}—10^{-7}	10^{-7}	10^{-7} (g/s)	10^{-10}	10^{-7}	10^{-10}
Cellvolume ($\mu\ell$)	>1	<1	<1	30—50	0.5—3.0	2—10	1—5	—	10	3—10	3—20
Selectivity	Select.	Select.	Select.	Select.	Select.	Nonselect.	Specific	Nonselective	Selective	Nonselective	Selective
Temp dependence	Small	Small	Small	Small	Small	Great	Small	Small	Small	Large	Small
Dependence on flow rate	Yes	Yes	Yes	No, in certain range	No	No	No	No	No	No	No
Applicability to gradient elution	Limited	Limited	Limited	Limited	No	No	No	No	Yes	No	Yes

Table 7
APPLICATIONS OF ELECTROCHEMICAL DETECTORS IN HPLC

Substance	HPLC conditions (Stationary and mobile phases)	Detection conditions	Detection limit	Ref.
Catecholamines and related compounds	Various stationary and mobile phases	C paste, +0.7 V	10 pg—1 ng	253
Catecholamines in urine	C_{18} stationary phase, citrate/phosphate buffer, octyl sulphate	C paste	1 μg/ℓ	124
Catecholamines in brain	Vydac SC, citrate-phosphate buffer	C paste, +0.5 V	10—20 pg	276
C-methyl derivatives of catecholamines in urine	μBondapac C_{18}, citrate-phosphate buffer	C paste	20 μg/ℓ	123
HVA, DOPAC, DA	Nucleosil C_{18}, citrate buffer, pH 4.25, 8% methanol + hexyl sulphate	C paste, +0.6 V	—	328
Tryptophan metabolites in urine, serum, plasma, brain tissue, CSF	μBondapac C_{18}, citrate-phosphate buffer, methanol	C paste, +0.5—+1.0 V	1—6 ng/mℓ	335
Tryptophan metabolites	ODS—Hypersil, KNO_3, or KH_2PO_4 + methanol (gradient elution)	Glassy C, +1.2 V	2 pmol	475
L-5-hydroxytryptophan decarboxylase activity	ODS-T Yanapak, phosphate buffer + 10% methanol	Glassy C, +0:8 V	0.1 pmol	359
Phenolic compounds in water	Various stationary phases, water-acetonitrile—H_2SO_4	Polymeric C paste, +1.2 V	0.8—2 ppb	87
Halogenated anilines	Zorbax ODS, phosphate buffer + acetonitrile	C paste, +1.1 V	<1 ng	387
Benzidine and related compounds in waste water	LiChrosorb RP-2, acetonitrile-water	Polymeric C paste, +0.7 V	Sub—ppb	384
Nitrosamines	μBondapak Phenyl, phosphate buffer, pH 3.5	PAR SMDE	0.11 μ*M* (normal pulse), 0.06 μ*M* (DPP)	389
Ascorbic acid in animal tissue	Partisil 10 SAX, acetate buffer, pH 4.6	C paste, +0.75 V	0.25 ng/mℓ	399
Uric acid in amniotic fluid	C_{18} stationary phase, phosphate buffer + acetonitrile (gradient elution)	Glassy C, +0.8 V	—	401
Benzodiazepines	Spherosorb ODS, acetate-methanol-water	Hg pool, −0.93—−1.3 V	3 ng	89
Ethylene-thiourea and related S-compounds	Supelco LC-18, 0.1 *M* KNO_3 pH 3	DME, +0.2 V	1—15 ng	434
Organo-metallic compounds (Hg, Sb, Pb)	Spherosorb ODS, LiChrosorb NH_2, acetate buffer-methanol, pH 5.5	Hg film(Au)	0.1 ng ($[CH_3]_4Pb^+$)	213
Narcotic alkaloids	μBondapak C_{18}, methanol-water + $(CH_3)_4N^+OH^-$, pH 6.1	C paste, +0.8 V	1 ng	453
Thyroid hormones	μBondapak C_{18}, H_3PO_4-methanol	Isotropic C, +0.8 V	<1 ng	135
Peroxides	Zorbax BP ODS, methanol-water + $LiClO_4$	Hg film(Au)	10 ng	463
Carbamate pesticides	μBondapak C_{18}, water-acetonitrile, acetate buffer, pH 6	Polymeric C paste, +1.1 V	40—150 pg	97
Arylhydroxylamines (derivatization to a hydroxy-urea)	μBondapak C_{18}, methanol-water-acetate acid	Glassy C, +0.38 V	10^{-8} *M*	469

components by washing the alumina with an alkali. The catecholamines can then be desorbed using a dilute acid. Similar complexes are formed by catecholamines with boric acid.

A great deal of attention has been paid to the optimization of chromatographic conditions for the separation of catecholamines.[253-256] Either ion exchangers or chemically bonded phases with mobile phases containing water and an ion-pairing agent, such as sodium dodecyl sulfate or octyl sulfate, have permitted the best separation[248,256] (see also Figure 24).

Catecholamines were determined in urine[124,225,257-261] with a detection limit of 1 $\mu g/\ell$ and relative standard deviation of 10%.[124] The main advantages of urinary catecholamine assays with LCEC are the speed of the analysis (50 samples per day can be analyzed), high selectivity, and sensitivity.[124]

Many papers deal with the determination of catecholamines in plasma or serum[32,94,225,262-269,271-274] and with therapeutic monitoring of catecholamines in serum.[262,270,274] Plasma catecholamines can be determined with a detection limit of 0.1 pmol with interassay relative standard deviation of 15% at a plasma catecholamine level approximately 1 n*M*.[269] Good correlation with the radioenzymatic method was found.[269,273,275] For concentrations below 100 pg/m ℓ, the radioenzymatic method is more reliable.[275]

Various tissue samples were analyzed for catecholamine content. Brain tissue represents a relatively simple matrix, so that it can be directly injected with sonification and centrifugation.[276] However, the actual determination is usually preceded by the adsorption on alumina described above. The LCEC method has been applied to the determination of catecholamines in brain tissue,[11,92,127,248,252,272,276-308] to brain mapping,[289,291,295,309,310] to monitoring of catecholamine biosynthesis in brain,[278,311] and to the determination of catecholamines in other tissues.[292,312,313]

A greater sensitivity than with glassy carbon for low-level catecholamine determinations has been achieved with carbon paste.[473] A high degree of correlation has been found between the results of catecholamine determinations by LCEC and by fluorimetric method.[308] The LCEC method for brain DA and serotonin was compared with a GC-MS assay.[314] Results with comparable sensitivity were obtained; an advantage of LCEC over GC-MS lies in the fact that there is no need for derivatization and the instrumentation is simpler. For the comparison of various methods used for the analysis of catecholamines see also Table 8.[253,315]

2. *O-Methyl Derivatives of Catecholamines*

Metanephrine, normetanephrine, and 3-methoxytyramine can be isolated on weak cation exchange columns.[250] Separation from catecholamines can be achieved by washing with boric acid. The catecholamines form soluble complexes and are washed out; the methyl derivatives remain in the column. Another possibility is oxidative degradation and elution of the catecholamines by a base.

O-methyl derivatives of catecholamines were determined by HPLC with electrochemical detection in urine[123,260,316,317] at concentrations down to 20 $\mu g/\ell$ [123] or 0.25 ng.[317] From their concentrations in urine some information can be obtained on various types of tumor diseases and some kinds of hypertension.

3. *Acid and Neutral Metabolites of Tyrosine*

Study of the metabolism of catecholamines, namely, neurotransmitters NE and DA, yields important information on many physiological functions and also provides a good estimate of the actual catecholamine turnover in the brain. The major metabolites from dopamine catabolism are acidic 3,4-dihydroxyphenylacetic acid (DOPAC) and

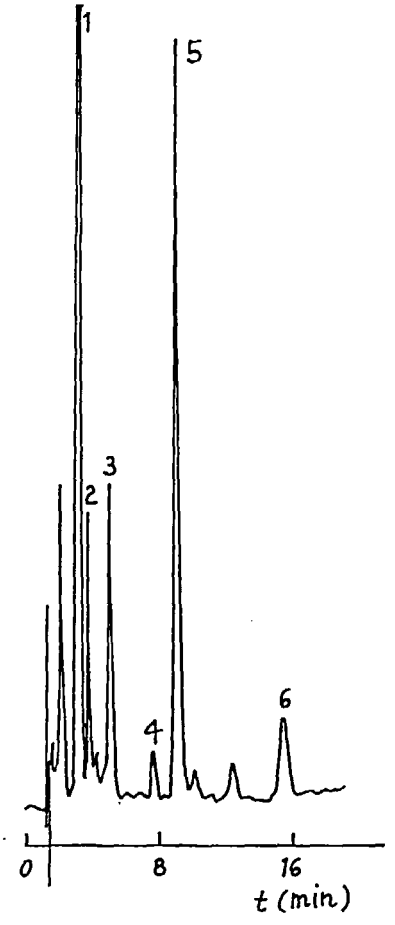


FIGURE 24. LCEC determination of plasma catecholamines. (1) Uric acid, (2) NE, (3) DOPA, (4) E, (5) 3,4-dihydroxybenzylamine, (6) DA. Conditions: C-18 chemically bonded stationary phase with a mixture to monochloroacetate buffer, EDTA and sodium octyl sulfate as the mobile phase, flow rate, 2.2 mℓ/min, C paste, +0.65 V. (From LCEC Application Note No. 14, Bioanal. Systems Inc., W. Laffayette, Ind. With permission.)

Table 8
COMPARISON OF THE SENSITIVITY OF VARIOUS METHODS FOR CATECHOLAMINES ANALYSIS[253,315]

	Detection limit (ng)			
		Fluorescence		
Catecholamine	UV	Natural	*o*-Phthaladehyde derivative	Electro-chemical
Epinephrine	1.2—5	0.3—2	—	0.025—0.15
Norepinephrine	1.4—5	0.3—2	0.075	0.025—0.05
Dopamine	1.25—5	0.3—5	0.13	0.025—0.10

homovanillic acid (HVA); norepinephrine metabolism yields neutral 3-methoxy-4-hydroxyphenyl ethylene glycol (MHPG) and 3,4-di-hydroxyphenylethyleneglycol (DHPG). Another major metabolite of NE and E, vanilmandelic acid (VMA), is of a little interest for the determination of the NE activity, but can be useful in the diagnosis of some diseases.

Both acid and neutral metabolites can be extracted into a polar solvent and the extract directly injected into a LCEC system. HVA and DOPAC can be determined in the tissue[318] by homogenizing it with dilute $HClO_4$, centrifuging, extracting the supernatant with diethylether, drying, and dissolving the residue in dilute HAc. Both acid and neutral metabolites were determined in urine,[260,319-327] in brain tissue,[296,298,318,328-330,472] and in cerebrospinal fluid.[331-334]

B. Tryptophan Metabolites

From the point of view of neurochemistry, the determination of tryptophan (TP) and its metabolites, serotonin (5-hydroxytryptamine, 5-HT), 5-hydroxytryptophan (5-HTP), and 5-hydroxyindoleacetic acid (5-HIAA) is important. TP and 5-HTP can be isolated from the tissue using a strong cation-exchange resin (e.g., Dowex AG-50), isolation of serotonin can be achieved with a weak cation-exchange resin (Amberlite CG-50), and 5-HIAA can be isolated on the Sephadex G-10 gel.[250] When two or more tryptophan metabolites are to be determined, isolation on Amberlite, then on Dowex, and finally on Sephadex is carried out. In the determination in brain tissue, small extraction columns have been used[335] for purification of the sample; in this procedure, the sample volume increased and, thus, the sensitivity of the determination decreased. By replacing this extraction column with a precolumn sample preconcentration system,[336,337] the sensitivity of the detection was increased almost 100 times. The applied detector potential is +1.0 V (Ag/AgCl) for TP and +0.5 V for 5-HT, 5-HTP, and 5-HIAA.

The applications involve the determination of tryptophan metabolites in urine,[335,338] in serum and plasma,[335,336,339,340] in brain tissue,[127,236,280,301,337,341-347,352] in cerebrospinal fluid,[335] and in other biological materials.[348,349,351] Further, serotonin metabolites[350] and serotonin with octopamine[355] were determined by LCEC. A detailed procedure has been given for the determination of tryptophan and its metabolites in blood, urine, cerebrospinal fluid, and tissue samples such as whole brain, brain parts, and endocrine glands.[335]

The LCEC method has been compared with UV detection and superiority of EC over UV has been demonstrated.[475] It follows from the comparison of electrochemical detection with fluorimetric[353,354] that the determination of 5-HTP, 5-HIAA, and HVA is more sensitive with LCEC and that of tryptophan and serotonin less.[344]

C. Enzyme Activity

The methods used for the determination of tyrosine and tryptophan metabolites can be modified so that the activity of the related enzymes is measured. A problem encountered in enzyme analysis lies in the necessity of using an excess of substrate to ensure that the reaction is zero order. The amount of substrate is extremely large compared with the enzymatically generated product monitored and can interfere in its HPLC analysis. For example, with dopamine-β-hydroxylase and dopamine as the substrate, norepinephrine is generated. The separation of NE from DA is not difficult but time consuming; one way of solving this problem is to use two short columns instead of one long and after the elution of NE to switch the eluate to the waste.[250,356]

The following systems have been studied using LCEC: tyrosine hydroxylase activity,[292,357,358] L-DOPA decarboxylase,[292] 5-hydroxytryptophan decarboxylase (monitoring 5-HT),[359] L-DOPA and 5-HTP decarboxylase in rat striatum,[360] L-amino

acid decarboxylase (with L-DOPA and 5-HTP as substrates),[361] inhibition of aromatic L-amino acid decarboxylase by α-difluoromethyldopa,[362] tyrosine hydroxylase distribution,[363] determination of pyridoxal-5'-phosphate as a cofactor in the enzymatic reaction of tyrosine apodecarboxylase with L-DOPA as a substrate,[364] catechol-*O*-methyltransferase,[317,365] also in various biological media using dopamine as the substrate,[366] norepinephrine *N*-methyl-transferase inhibition,[282] dopamine-β-hydroxylase activity,[356,367] alcohol dehydrogenase for blood alcohol determination,[368] and phenylethanolamine *N*-methyl-transferase.[369]

The LCEC method has several advantages for the measurement of enzyme activities. The high precision at low concentrations and easy handling and control of the reaction mixture make it especially well suited for accurate kinetic studies.

D. Phenolic Compounds

Most important phenolic compounds, in addition to catecholamine and tryptophan metabolites discussed in Sections V.A and B, are those of environmental interest, including chlorinated biphenols, hydroxylated biphenyls, chlorinated phenols and naphthols, aminophenols, and antioxidants (various alkylphenols).

Phenols are easily oxidized at carbon electrodes in the potential range +0.7 to +1.1 V (Ag/AgCl) and can, therefore, be determined by LCEC. The lowest detection limits (3 to 15 pg) have been attained when using graphite-Kel-F electrodes.[99] Hydroquinone and catechol have been analyzed as impurities in phenol,[370] trace concentrations of phenolic compounds have been determined in water,[87,99,165] and phenolic residues[371] and 2-phenylphenol in orange rind[372] have also been analyzed. Further, trace amounts of aminophenols,[373] isomeric aminophenols,[130,137] phenolic antioxidants,[374,375] and natural phenolic compounds[122,152,376-380,474] have been determined.

In comparison with UV detection, the LCEC system provides higher sensitivity[384] and, thus, better separation. Compared with gas chromatography with electron-capture detection for chlorinated compounds, where a similar sensitivity is attained, LCEC does not require time-consuming clean-up procedures.

E. Nitrogen-Containing Compounds

1. Aromatic Amines

Aromatic amines have been extensively studied because of their carcinogenic nature. Some of them are important industrial compounds and some are formed from carbamate pesticides as degradation products.

Most anilines undergo one-electron oxidation in aqueous weakly acidic media and some of them, e.g., benzidines, two-electron oxidation to imine. Using LCEC, aromatic carcinogens,[381] aniline-derived carbamates (detection limit of 3 ng),[382] benzidine and its metabolites in urine,[19,383] and its derivatives in waste waters[384,385] have been determined. Chlorinated anilines have been analyzed in urine with both EC[483] and UV detection[386,387] and a detection limit of 2 to 3 pmol has been found for EC. A determination of chlorinated anilines in the air has also been described.[388]

2. Nitrosamines

Among other N-containing compounds of environmental interest, N-nitrosamines can be determined with polarographic detection.[389-391] DPP seems to be more sensitive than normal d.c. techniques (30% higher sensitivity; detection limit of 0.06 μM for DPP).[389]

3. Nitrocompounds

Nitrocompounds are readily reduced and, thus, polarographic detectors can be employed for the determination of nitrobenzene,[185,392] nitrotoluenes,[181,182] nitro-

phenols,[247,392] trace amounts of nitroglycerine and trinitrotoluene,[393] and other nitrocompounds.[184,191]

F. Organic Acids

1. Ascorbic Acid

Analysis for ascorbic acid is important in the food and pharmaceutical industries and in studies of the biochemical role of vitamin C in metabolism. Ascorbic acid is easily oxidized at +0.6 V (Ag/AgCl) and can, therefore, be determined by LCEC.[117,133,249,394,395] Because of this low oxidation potential, there are few interferences and little sample preparation is needed. The other advantages are the speed of analysis and a greater sensitivity than with UV detection (subpicogram amounts can be determined).[117] Care must be taken during the analysis of ascorbic acid because of its instability; standard solutions must be prepared in cold $HClO_4$ with EDTA and nitrogen must be passed through the mobile phase during the analysis.

Ascorbic acid has been determined in foodstuffs[395,396] (see Figure 25), in pharmaceuticals and body fluids,[396] in plasma,[397,398] in urine,[398] in brain tissues,[471] and in the tissues of marine animals.[399]

2. Uric acid

Uric acid can be determined by LCEC[117,249,476] under the same conditions as ascorbic acid (see also Figure 25). The electrochemical oxidation of a number of *N*-methylated uric acids at pyrolytic graphite and gold electrodes was compared with their enzymatic oxidation and an identical mechanism was found.[347] In comparison with radiolabeled carbon assay, the LCEC was found to be more sensitive, precise, and convenient.[400]

The applications involve the determination of uric acid in plasma[397] and serum[120] (detection limit of 1 pg), a determination of fluid uric acid levels,[401] determination of uric acid in cereal products,[402] monitoring of the renal handling of urate,[403] quantitation of the renal uric synthesis,[404] a study of urate excretion,[400] and urate tubular transport[405] from the kidney.

3. Other Organic Acids

Other applications involve a determination of oxalic acid,[236,406] carboxylic acids (coulometric determination),[218,219] conductometric determination of free organic acids and other acidic compounds[407] (detection limit 1 to 10 mg/ℓ), indole-3-acetic acid in plant tissue,[408] lactic and pyruvic acids in plasma,[320] bile acids,[187,409] fatty acids (high-frequency impedance),[64] isomeric phthalic acids (ISE)[73] and amino acids,[144,148] high-frequency impedance,[67] ISE,[78] 3-methyl-hydrofolic acid in plasma and CSF[410] (coulometry with detection limit of $2 \times 10^{-9} M$), and 2,5-dihydroxyphenylacetic acid in serum and urine.[122]

G. Drugs and Their Metabolites

The sensitivity and selectivity of LCEC make the method attractive, also, for analysis of drugs and their metabolites. Both oxidative and reductive detection were used. In the reduction (DME,[205,411] Hg pool,[412,413] amalgamated gold electrode[19]) the detection limits are not as low as in the oxidation due to the reduction of O_2, H^+, and trace metals.[19]

The following drugs have been determined in pharmaceutical preparations, body fluids, or tissues: acetaminophen,[119,231,415,416] β-cetoterine,[125] 8-hydroxycarteolol,[378] perphenazine and fluphenazine (coulometrically),[225] theophilline,[417,418] tocopherols,[419] morphine,[454] apomorphine,[420] phenolic sympathomimetic stimulants,[118,259] tricyclic antidepressants and their 2-hydroxy metabolites,[421] phenothiazine compounds,[422] benzodiazepines,[19,89] chlorodiazepoxide and its metabolite,[411,423] diazepam,[423] ni-

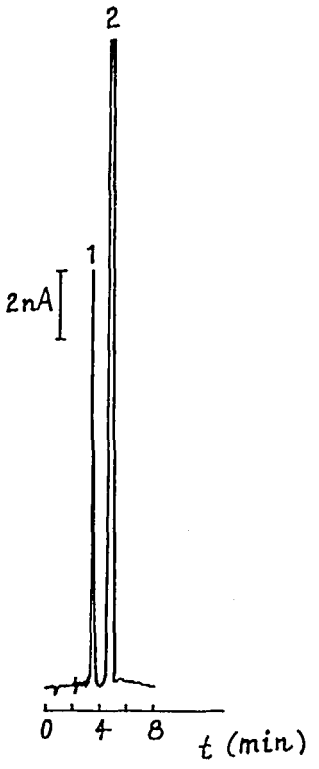


FIGURE 25. LCEC determination of ascorbic acid in milk. (1) Ascorbic acid (10 ng), (2) uric acid. Conditions: C-18 chemically bonded stationary phase with a mixture of sodium acetate, EDTA and octyl amine as the mobile phase, flow rate, 1.7 mℓ/min, glassy C, +0.6 V. (From Heiliger, F., *Curr. Sep.*, 2, 4 [1980]. With permission.)

trazepam,[205] 5-methyltetrahydrofolic acid,[410] mepindolol,[424] penicillamine,[412,413] amoxicillin,[425] paracetamol,[426] ubiquinones,[419,427] phylloquinone,[419] diethylstilbestrol,[428] methotrexate,[429] enkephalin catabolism,[430] sulfinalol hydrochloride,[414] procarbazine hydrochloride,[90] *p*-aminohippuric acid,[431] and others.[432,433]

H. Sulfur-Containing Compounds

Several sulfur-containing compounds are electrochemically active and can be analyzed by LCEC. Mercury electrodes are mostly used, either DME[194,196,197,434] or Hg pool, e.g., see references.[211,229] Carbon paste electrodes have also been used for sulfur compounds,[435] but their cathodic potential range is limited and adsorption of the compounds analyzed on their surface has been observed.[435]

Applications involve the determination of thiols, isocyanates, aliphatic thioureas and thioamides,[435] ethylene thiourea and related sulfur compounds in urine,[434] thiourea herbicides,[204] pesticides such as parathion and methyl parathion,[194,196,197] biologically active sulfhydril-containing compounds, such as glutathione and related compounds in blood and tissue,[211,436,437] glutathione and cysteine in fruit,[438] cysteine in plasma and urine,[439] homocysteine in plasma,[439] cystine and related compounds,[229] and penicillamine in blood and urine[413] and in plasma and albumin solutions.[412]

The results obtained with an electrochemical detector were compared with UV detection; the LCEC was found to be more sensitive (sub ppm range).[435]

I. Inorganic and Organometallic Substances

Determinations of inorganic ions form a minor part of the applications of electrochemical detectors in liquid chromatography. Cations of metals, such as Cu, Cd, Pb, Zn, Fe, Ni, Co, Yt, and the rare earths, were determined using polarographic[173,176,189,192,392,440,441] (a detection limit of 2 ng has been achieved with a DME[441]), voltammetric,[116,299] coulometric,[217,218,220,223,442,443] conductometric,[444] and ion-selective potentiometric detectors.[76]

Electrochemical detectors have also been used for the determination of inorganic anions, namely, voltammetric,[163,221] coulometric,[218,224,228] and ion-selective potentiometric.[35,73,75,77,79,445]

Various organometallic compounds (organomercury, -tin, -lead, -antimony) have been determined in water or in biological species on an amalgamated gold electrode[139,213,234,446,447] with a detection limit for CH_3Hg^+ of 2 ppb or 1×10^{-8} mol/ℓ (DPP measurement).[446]

J. Other Applications

1. Alkaloids

Among these compounds, tetrahydroisoquinoline alkaloids in biological materials,[257,377,379,448,449] an isoquinoline alkaloid formulation,[450] tetrahydropapaveroline, and tetrahydrobarberine alkaloids[365] have been analyzed. Ergot peptide alkaloids[452] and narcotic alkaloids[134,453,454] have been further determined. Morphine has been analyzed in serum in concentrations down to 1 ng/mℓ with a relative standard deviation of 5 to 6%.[454] Alkaloids have also been determined using high-frequency impedance detection.[64]

2. Hormones, Steroids

Thyroid hormones,[135] trace concentrations of phenolic growth-promoting hormones in meat,[455] estrogenic growth-promoting hormones,[456] estriols in urine,[457,458] catechol estrogens,[459-461] testosteroids,[186] and corticosteroids[462] have been determined.

3. Other Systems

Various other compounds were determined by LCEC: pesticides,[97,194,382] herbicides,[197,204] vitamins,[206] peroxides,[463] alcohols (high-frequency impedance measurement),[67] phosphorus ethers, phosphates, etc. (high-frequency impedance),[68] surfactants (with DME),[199] natural products in cocoa,[464] roquefortine,[465] sugars, and other organic compounds (coulometric determination).[218]

K. Electroinactive Substances

The application range of electrochemical detectors can be extended by conversion of electroinactive substances into electrochemically active ones. Either precolumn or postcolumn reactions can be used (see References 35, 71, 466, 467). The derivatives usually strongly absorb in the UV region so that a dual EC-UV system of detectors can be used.[468,469] The original substances to be determined can also be stabilized by derivatization.[469]

Precolumn derivatization was used for the conversion of amino acids into phenylazobenzenesulfonyl chloride derivatives;[467] fatty acids, bile acids, and prostaglandins were converted into 4'-hydroxy-anilides by reaction with 4-aminophenol in the presence of 2-bromo-1-methylpyridinium iodide and triethylamine.[409]

Postcolumn reaction with electrochemically generated bromine was used for the derivatization of unsaturated compounds.[470] By measuring the excess Br_2, a detection

limit of less than 10 pmol (for one double bond) was achieved. Arylhydroxylamines were converted into hydroxyurea with *p*-dimethylaminophenylisocyanate,[469] monomeric aromatic and aliphatic isocyanates were derivatized with 1-(2-methoxyphenyl)piperazine,[468] and carbonyl compounds were converted into semicarbazone derivatives.[71,144]

For a bibliography on recent applications see Reference 485.

VI. LIST OF IMPORTANT SYMBOLS AND ABBREVIATIONS

A — electrode surface area
a — activity
b — width
C — constant
c — concentration
c_d — detection limit
D — diffusion coefficient
E — potential
E(t) — potential as a periodic function of time
F — Faraday constant
H — height equivalent to a theoretical plate
h — height
I — electric current
I_{ac} — a.c. component of electrolytic current
I_l — limiting electrolytic current
k — proportionality constant
k_I — diffusion current constant
l — length
m — mercury flow rate
n — number of electrons exchanged
Q — electric charge
R — response; electric resistance
r — radius
S — sensitivity
s — standard deviation estimate
t — time
t_R — residence time
t_l — mercury electrode drop time
u — linear flow rate
V — volume
V_c — cell volume
w — volume flow rate
Z — impedance
ϵ — permittivity
$\mathcal{H}$ — specific conductance
ν — kinematic viscosity
σ — standard deviation
σ_t — time standard deviation
σ_V — volume standard deviation

CSF — cerebrospinal fluid
DHPG — 3,4-dihydroxyphenylethyleneglycol
DME — dropping mercury electrode
DOPAC — 3,4-dihydroxyphenylacetic acid

DPP — differential pulse polarography
E — epinephrine
EC — electrochemical
EDTA — ethylenediaminetetraacetic acid
HAc — acetic acid
5-HIAA — 5-hydroxyindoleacetic acid
HMDE — horizontal dropping mercury electrode
5-HT — 5-hydroxytyramine (serotonin)
5-HTP — 5-hydroxytryptophan
HVA — homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid)
ISE — ion-selective electrode
LCEC — liquid chromatography with electrochemical detection
L-DOPA — 3,4-dihydroxyphenylalanine
MHPG — 3-methoxy-4-hydroxyphenylethyleneglycol
NE — norepinephrine
NHE — normal hydrogen electrode
SCE — saturated calomel electrode
SMDE — static mercury drop electrode
TP — tryptophan
VMA — vanilmandelic acid (4-hydroxy-3-methoxymandelic acid)

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