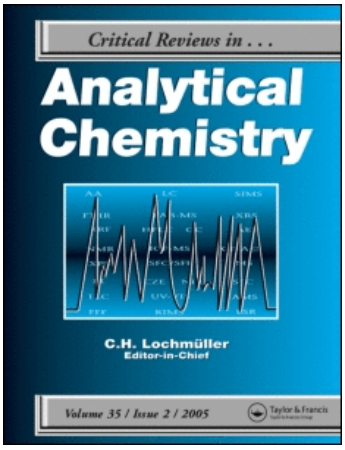

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VOLTAMMETRIC DETERMINATION OF MOLECULES OF BIOLOGICAL, ENVIRONMENTAL, AND PHARMACEUTICAL IMPORTANCE

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I. INTRODUCTION

Voltammetric techniques of analysis, i.e., those based on the measurement of current resulting from an oxidation or reduction at an electrode surface following the application of a potential difference to an electrochemical cell,[1] have assumed an important place in the armory of analytical techniques for the identification and determination of trace concentrations (i.e., 10^2 to 10^{-4} ppm) of many organic, organometallic, and inorganic molecules of environmental significance. Techniques such as normal and differential pulse polarography (NPP and DPP), anodic and cathodic stripping voltammetry (ASV and CSV), adsorptive stripping voltammetry and electrochemical detection (ED) coupled with flow injection analysis (FIA - ED), ion chromatography (IC - ED), and high performance liquid chromatography (HPLC - ED) are particularly notable in this respect. Organic and organometallic molecules of environmental importance as pollutants, drugs of abuse, food additives and contaminants, agrochemicals, etc., as they are contained in a variety of complex biological matrices such as air, plasma, tissue, and soil, can be determined by these voltammetric techniques at trace concentrations usually after the application of sample pretreatment procedures and certain separation techniques. In some cases, it is possible to determine the molecule directly as it exists in the matrix when the electrochemical reaction is particularly selective/specific in the presence of potential interferents. Certain environmentally relevant inorganic molecules, such as oxyanions of S, Se, As, Cr, Mo, and V, can be determined by polarography, cathodic stripping voltammetry, or by on-line electrochemical detection following either IC or HPLC.

It is the purpose of this review (1) to survey the basic principles of these voltammetric techniques as they apply to the molecules in question, (2) to discuss the general voltammetric behavior of the molecules under the particular electrochemical conditions applying, and (3) to make a critical state-of-the-art report on recent applications of voltammetry to the identification and determination of selected organic, organometallic, and inorganic molecules of environmental significance. This report will be subdivided according to the nature of the electroactive functional groups present in the overall molecular structures listed under nitrogen-, sulfur-, oxygen-containing molecules, hydrocarbons, organophosphorus molecules, organometallics, and selected inorganic molecules. Wherever possible, a critical comparison will be made between voltammetric methods and competing analytical methods based primarily on chromatographic and spectroscopic techniques. For an account of the application of voltammetric techniques in practical day-to-day analysis, the reader is referred to Bersier and Bersier's review.[2]

II. THE VOLTAMMETRIC TECHNIQUES

A. Normal Pulse Polarography (NPP) and Differential Pulse Polarography (DPP)

The classical technique of direct current (DC) polarography, invented by Heyrovsky in

the 1920s, involves the measurement of current at the dropping mercury electrode (DME) when a slow linear voltage of, say, 100 mv min^{-1} is applied to the cell. Due to the experimental conditions employed, i.e., quiescent solution and excess of supporting electrolyte, the contributions from convection and migration currents are eliminated so that the observed current arises from the diffusion process. The unique advantages of the DME of renewable, reproducible electrode surface and large negative potential range are supplemented by the fact that well-defined sigmoidal current-voltage curves are generated for each electrode process. The diffusion-controlled limiting current on the plateau of the sigmoidal curve is known as (i_d), i.e., the maximum current at the end of the drop lifetime. This is linearly related to concentration over relatively wide ranges by the Ilkovic Equation 1:

$$i_d = 0.732\ nFCD^{1/2}\ m^{2/3}\ t^{1/6} \tag{1}$$

where n = number of electrons involved in the charge transfer process, F = Faraday, C = concentration of electroactive species in the bulk of solution in mol cm^{-3}, D = diffusion coefficient in $cm^2\ s^{-1}$, m = flow rate of mercury in g s^{-1}, and t = drop line in seconds. The half-wave potential, $E_{1/2}$, is that potential at which the current equals one half of the limiting value, and this parameter is of particular value in the identification of the organic, organometallic, or inorganic molecules. Equation 1 gives only the diffusion-controlled faradic current. The total current also contains a capacitive current which is due to the adsorption of anions or cations at the electrode surface to form a double layer. This double layer has a finite capacitance and therefore a significant current is required to charge the electrode-solution interface to the required potential. It is the existence of this capacitive current which limits the sensitivity of the DC technique to ca. $5 \times 10^{-5}\ M$. The capacitive current is very large at the start of the drop's lifetime, but decreases with the growth of the drop. The faradic current, on the other hand, as defined by Equation 1, increases during the drop's growth. This important difference between the faradic and capactive currents is the basis on which most modern polarographic techniques, such as pulse polarography, have been developed.

Most reductions of metal ions and some electrochemical reactions of organic, organometallic, and inorganic molecules are reversible (i.e., the rate of electron transfer is fast in comparison to mass transport to the electrode surface). Equation 2 describes the shape of the resulting current-potential curve in DC polarography.[3]

$$E = E^\circ + \frac{RT}{nF} \ln\left(\frac{i_d - i}{i}\right)\left(\frac{D_R}{D_o}\right)^{1/2} \tag{2}$$

where i is the current at potential E, i_d is the diffusion-limited current, E° is the standard redox potential of the system $O + ne \rightleftharpoons R$, D_R is the diffusion coefficient of the reduced electroactive molecule, and D_o is the diffusion coefficient of the oxidized electroactive molecule. This equation simplifies to Equation 3:

$$E = E_{1/2} + \frac{RT}{nF} \ln \frac{i_d - i}{i} \tag{3}$$

From Equation 3 it follows that a plot of potential, E vs. $\log \frac{i_d - i}{i}$, should be linear with slope 2.303RT/nF. When $i = \frac{i_d}{2}$, $\log \frac{i_d - i}{i} = O$ and $E = E_{1/2}$. Thus, a plot of this kind is frequently used to assess the reversibility or otherwise of a DC electrode process and to calculate $E_{1/2}$.

However, most electrochemical reactions of the molecules considered in this review are irreversible, i.e., the kinetics of the reaction must be considered as well as the thermodynamics in order to deduce the relationship between current and potential. For the irreversible system $O + ne \rightarrow R$ and using maximum currents at the end of the drop life, a solution is given by Equation 4:

$$E = E^\circ + 0.916 \frac{RT}{\alpha nF} \ln\left(\frac{i_d - i}{i}\right) + \frac{RT}{\alpha nF} \ln 1.359\, k_s \left(\frac{t}{D}\right)^{1/2} \quad (4)$$

where α is the transfer coefficient which denotes the fraction of the potential influencing the rate of electroreduction. For most electrode processes α values lie in the range 0.3 to 0.7 and k_s is the heterogeneous charge transfer rate constant. E, E°, R, T, n, F, i_d, i, t, and D have their usual significance.
Thus,

$$E_{1/2} = E^\circ + \frac{RT}{\alpha nF} \ln 1.359\, k_s \left(\frac{t}{D}\right)^{1/2} \quad (5)$$

and

$$E = E_{1/2} + 0.916 \frac{RT}{\alpha nF} \ln \frac{i_d - i}{i} \quad (6)$$

In Equations 4, 5, and 6, i is no longer the diffusion-controlled value, but is governed by the electron transfer rate. The limiting current i_d is still the diffusion-controlled value. If α is independent of potential, the log plot is linear with a larger scope than in the reversible case. $E_{1/2}$ is also now a function of drop time, t, and the rate constant, k_s, unlike the reversible case. Reversible and irreversible processes can be distinguished on the basis of wave shape, wave position, and drop time dependence. Protons are also involved in many electrode reactions of organic molecules, for example, for the system $O + mH^+ + ne^- \rightarrow R$, Equation 7 can be derived:

$$E_{1/2} = k - 0.059\, m/n \cdot pH \quad (7)$$

where k is a constant standard potential and m is the number of protons involved in the electrode reaction. This illustrates the importance of adequate buffering so that the electrode/solution interface does not undergo any appreciable pH change. It also provides the basis for the use of the $E_{1/2}$-pH dependence as a means of characterization of the electrode process. Detailed discussion of these topics can be found in the literature.[4,5]

The analytical applications of DC polarography at the DME for organic molecules of biological significance have been reviewed.[6-8]

All of the modern variants of the classical DC polarographic technique have improved the sensitivity by eliminating the contribution from the capacitive current due to the double-layer charging of the drop. While there are many techniques available, only a very few have been routinely accepted. Pulse polarography was invented by Barker and co-workers at Harwell, U.K. in the late 1950s. Utilizing the differing time dependences of faradic and charging currents, the normal pulse technique (NPP) imposes a series of pulses of increasing amplitude to successive drops at a preselected time near the end of each drop lifetime. The initially high charging current decays away very rapidly and the residual faradic current is sampled during the final part of the 50- to 60-msec time pulse.

The limiting current in normal pulse polarography, i_d^{NPP}, is given by the Cottrell Equation 8:

$$i_d^{NPP} = nFCA\sqrt{\frac{D}{\pi t_m}} \tag{8}$$

where t_m is the time interval between the pulse application and current measurement and A is the area of the electrode. Symbols n, F, C, and D have their usual significance. For reversible and irreversible systems, analogous i-E equations to those developed for DC polarography can be derived, e.g., Equation 9 for a reversible system.

$$E = E_{1/2} + \frac{RT}{nF} \ln \frac{i_d^{NPP} - i}{i} \tag{9}$$

It is also interesting to compare the diffusion limited currents for pulse (i_d^{NPP}) and DC (i_d) polarography, as in Equation 10:

$$\frac{i_d^{NPP}}{i_d} = \frac{t^{1/6}}{\sqrt{\frac{7}{3}}\, t_m^{1/2}} \tag{10}$$

where t is the drop time in DC polarography and t_m is as defined above. In theory, this ratio is in the range 6 to 7, but in practice it is nearly two orders of magnitude, the additional increase being due to the ability of NPP to discriminate against the capacitive current. Normal pulse polarography (NPP) is extremely useful in analytical applications, since it can respond to both reversible and irreversible processes. A further advantage of the pulse technique is that as the measurement pulse is applied for a small fraction of the total drop time of 0.5 to 5 sec, hence, only a small amount of electroactive material is deposited on the electrode. This therefore means that the pulse technique is far less affected by problems of adsorption than is the DC technique.

While NPP gives a marked improvement in sensitivity over the DC technique, it still gives a sigmoidal-shaped polarogram. A more analytically useful variant is DPP. In this technique, small amplitude (10 to 100 mV) pulses of approximately 60 msec duration, superimposed on a conventional DC ramp voltage, are applied to the DME near to the end of the drop lifetime. The current output is sampled at two time intervals, immediately on the ramp prior to the imposition of the pulse and then again at the end of the pulse (after 40 msec) when the capacitive current has decayed. It is the difference in these two currents that is displayed. As the greatest increase in current for a given voltage increment will occur at the half-wave potential, the i-E curve in DPP has a peak shape.

The theoretical relationship between differential peak current, Δi, and pulse modulation amplitude, ΔE, has been derived.[9] A solution, valid for all values of ΔE, gives, for a reduction process,

$$\Delta i = nFAC\sqrt{\frac{D}{\pi t_m}} \cdot \frac{P_A G^2 - P_A}{G + P_A G^2 + P_A + P_A^2 G} \tag{11}$$

where

$$P_A = \exp \frac{nF}{RT}\left[\frac{E_1 + E_2}{2} - E_{1/2}\right]$$

and

$$G = \exp \frac{nF}{RT}\left[\frac{E_2 - E_1}{2}\right]$$

$E_2 - E_1 = \Delta E$, the pulse amplitude; E_2 = the potential at which current i_2 is measured after application of the pulse; and E_i = the potential at which current i_1 is measured in the absence of the pulse. When $-\Delta E/2 < \frac{RT}{nF}$, Equation 11 simplifies to 12:

$$(\Delta i)_{max} = \frac{n^2F^2AC}{4RT} \cdot \frac{(-\Delta E)}{} \cdot - \sqrt{\frac{D}{\pi t_m}} \qquad (12)$$

For such differential pulse polarograms with very small ΔE values, the peak current potential, Ep, coincides with the half-wave potential $E_{1/2}$. With larger values of ΔE, E_p is related to $E_{1/2}$ by Equation 13:

$$E_p = E_{1/2} - \Delta E/2 \qquad (13)$$

For a reversible process the peak or maximum current, $(\Delta i)_{max}$ is linearly related to concentration C, as in Equation 12. For the irreversible process, $(\Delta i)_{max}$ is also a function of k_s. The current per unit concentration is lower and the peak width broader when compared to those parameters for reversible processes. The presently available theory for irreversible processes is not particularly extensive.

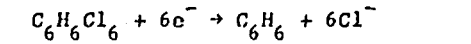
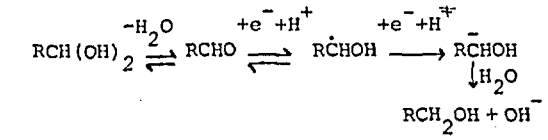
From a consideration of Equation 12, it would appear that DPP is inherently less sensitive than NPP, but in practice it shows a slightly improved sensitivity due to superior resolution of the i-E curves at very low concentrations.

The relatively recent introduction of high-quality three-electrode pulse polarographic equipment has resulted in many applications appearing in the literature, particularly using the differential pulse technique. Linear calibration curves have been reported for a wide range of organic, organometallic, and inorganic molecules of environmental significance, with limits of detection in the range 10^{-7} to 10^{-8} *M*. The later text of this review will deal with these applications in greater detail. Functional groups in these molecules that render them electroactive, principally through reduction processes at the DME, are given in Table 1. Those molecules amenable to oxidation processes at mercury electrodes will be discussed under CSV, and those that oxidize and reduce at nonmercury electrodes, such as glassy carbon, will be discussed under on-line ED.

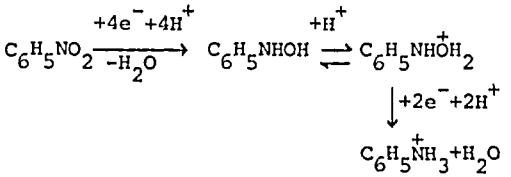
B. Anodic (ASV), Cathodic (CSV), and Adsorptive Stripping Voltammetry (ASV)

Stripping voltammetry is one of the most sensitive analytical techniques and has received a great deal of attention in recent years, especially for environmental monitoring of trace concentrations of metal ions. It is very simple in concept, the technique consisting of a two-stage process: first, a preconcentration step, consisting of controlled electrolytic deposition of the species of interest normally onto a stationary indicator electrode and usually in a stirred solution, for a time-period of several minutes. It is essential that for this deposition step the hydrodynamic parameters be tightly controlled. In practice this means a controlled stirring rate, electrolysis time, and reproducible location of the working electrode in the electrolysis cell. Mathematical treatment has shown that the mercury film electrode (MFE) is capable of more sensitive measurements than the hanging mercury drop electrode (HMDE) under a given set of conditions. It is recommended that for trace analysis at levels down to

Table 1
ELECTROREDUCIBLE BONDS/GROUPS

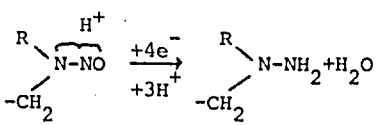
Electroreducible bond/group	Reduction mechanism	Comments
Carbon-carbon bond		
Double bond	$2e^-$ *Process resulting in saturation of the bond*	*Occurs at high negative potentials and can give analytically usable waves in tetraalkylammonium supporting electrolytes dissolved in organic solvents, for example, polynuclear aromatic hydrocarbons are reduced between* -2.00 *and* -2.50 *V (vs. SCE) in 0.17 M* Bu_4NI*/75% dioxan; the reduction potential is made less negative if the bond contains electron-withdrawing groups, for example, in cephalosporins, or the degree of conjugation is increased by the presence of, say, a keto group, for example for cortisone and testosterone, well-defined DPP peaks are obtained for reduction of the carbon-carbon double bond at* -1.77 *and* -1.84 *V (vs. SCE) in 0.1 M* Et_4NClO_4*/50% MeOH*[10]
Triple bond		Reducible if conjugated to an aromatic ring; an unconjugated carbon-carbon triple bond can be rendered electroreducible, as in case of lynestrenol, by heating with 60% H_2SO_4/MeOH[11]
Carbon-halogen bond	Carbon-halogen bond is cleaved, e.g., 	In general, polyhalogenated compounds such as the insecticide, benzene hexachloride (γ-BHC, Lindane), give rise to well-defined DPP peaks allowing their determination at 10^{-7}—10^{-8} *M* concentrations
Carbon-oxygen bond	2e Process generally for aldehydes and ketones, for example, aliphatic aldehydes are reduced by a process that is governed by the rate at which dehydration to the free aldehyde can take place 	Conjugated carbonyl compounds, for example, quinones and benzophenones, reduce at less negative and more analytically usable potentials than nonconjugated ones (e.g., formaldehyde and acetaldehyde); sugars such as ketoses and aldoses are reduced in kinetically controlled processes at relatively negative potentials and give ill-defined peaks on application of DPP

Carbon-nitrogen bond		
Single bond	Reductive splitting occurs in quaternary phenyl and alkyl ammonium salts	Reduction occurs at high negative potentials
Double bond	$2e^-$ Process resulting in saturation of bond	Well-defined DPP peaks produced when group is conjugated to an aromatic nucleus, as with 1,4-benzodiazepines[12]
Nitrogen-nitrogen bond		
Single bond	Reductive cleavage of bond consuming $2e^-$	This process will occur together with saturation of adjacent azomethine groups[13-15]
Double bond	Saturation of bond, in some cases, followed by cleavage to form mixture of amines	The latter process occurs when the group is conjugated to an aromatic one possessing activating substituents, e.g., 4-hydroxyazobenzene;[16] analytical usefulness of DPP peaks of azo dyes lessened by presence of negatively charged sulfonate groups and by binding to proteinaceous matter in complex biological matrices[17]
Nitrogen-oxygen bond	Reduction process is dependent on oxidation state of nitrogen atom, e.g., nitrobenzene	Aromatic nitro-containing molecules can reduce in $2e^-$, $4e^-$, or $6e^-$ processes, depending on the substituents, whereas aliphatic counterparts are reduced in $4e^-$ processes to the hydroxylamine; DPP peaks of aromatic nitro-containing molecules are well defined (with peak widths of 60—70 mV) and can be used for determination down to 10^{-8} *M* in pure solution



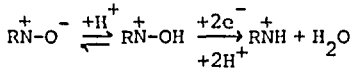
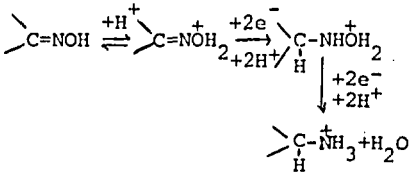
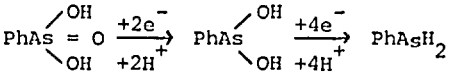
A wide variety of nitro-containing molecules of environmental significance has been subjected to polarographic analysis;[12] aromatic compounds have been derivitized by nitration because of the analytical utility of the electroreduction of this group

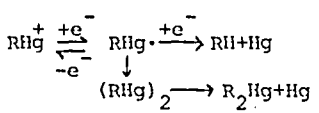
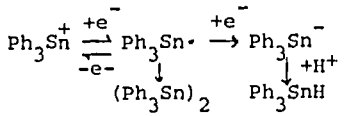
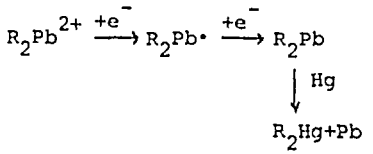
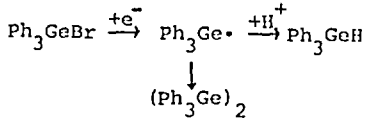
E.g., aliphatic *N*-nitrosamines at pH < pK_t



Alphatic nitroso-containing molecules generally produce ill-defined DPP peaks, whereas aromatic nitroso-containing ones have been observed to give well-defined DPP peaks at less negative potentials[18]

Table 1 (continued)
ELECTROREDUCIBLE BONDS/GROUPS

Electroreducible bond/group	Reduction mechanism	Comments
	E.g., quinoline *N*-oxide at pH < pK_a 	Aliphatic *N*-oxides are reduced at relatively negative potentials; in the absence of extensive conjugation, so are heterocyclic *N*-oxides, such as quinoline *N*-oxide ($E_{1/2}$ = −1.80 V)[19]
	E.g., oximes 	Hydroxylamines are only reduced in the protonated state and generally give ill-defined DPP peaks; aryl hydroxylamines have been reported to give well-defined waves in alkaline media;[20] some aliphatic oximes give relatively small reduction peaks on application of DPP[21]
Carbon-sulfur bond		
Double bond	Can be reduced in a 2e⁻ process involving saturation of bond; further reactions can take place, e.g., elimination of H_2S	Examples are 4-thiouracil[22] and 5,5′ disubstituted thiobarbiturates[23]
Sulfur oxides	Mechanism dependent on oxidation state of sulfur atom	Aromatic *S*-oxides give well-defined DPP peaks corresponding to 2e⁻ processes[24]
Sulfur-sulfur bond	2e⁻ Process resulting in breakage of bond, e.g., R–S–S–R′ + 2e⁻ + 2H⁺ ⇌ 2RSH	Compounds of environmental significance that have been most studied by polarography are the thiuram disulfides, e.g., thiram (tetramethylthiuram disulfide)[12]
Arsenic as in arsenic-containing organometallic molecules	Occurs at the arsenic atom and is dependent on its oxidation state and surrounding substituent groups, e.g., phenylarsonic acid 	Watson's chapter in Reference 8 refers

Mercury as in mercury-containing organometallic molecules	E.g., 	Fouzder and Fleet's chapter in Reference 8 refers
Tin as in tin-containing organometallic molecules	Reduction occurs at tin atom and depends on its oxidation state and surrounding substituent groups, e.g., the triphenyl tin cation 	Fouzder and Fleet's chapter in Reference 8 refers
Lead as in lead-containing organometallic molecules	Reduction occurs at lead atom and depends on its oxidation state and surrounding substituent groups, e.g., dialkyl lead species 	Fouzder and Fleet's chapter in Reference 8 refers
Germanium as in germanium-containing organometallic molecules	Triorganogermanium species are reduced according to 	Fouzder and Fleet's chapter in Reference 8 refers
Zinc, iron and maganese as in metal-containing dithiocarbamate pesticides	For example, zinc in ziram (zinc dimethyldithiocarbamate) in 0.2 *M* NaOH supporting electrolyte can be reduced at -1.42 V	Analytical applications in environmental analysis given in Reference 12
Elements S, Se, As, Cr, Mo, V as they are contained in inorganic oxyanionic molecules with the central element in	Reduction to lower oxidation state(s) of the central element	

low ppb concentrations, the HMDE be used and that the MFE be used for sub-ppb concentrations.[8]

The measurement step consists of electrolytically stripping the deposited species back into solution by imposition of a potential step such as a DC or pulse voltage ramp. The magnitude of the stripping signal is linearly related to the concentration of the species of interest. The technique of anodic stripping voltammetry (ASV) generally refers to stripping with a fast (ca. 100 to 200 mVs^{-1}) voltage sweep from a cathodic to an anodic potential. More recently, the differential pulse technique has become more popular for the stripping process, since it offers a much more effective discrimination against the background charging current and hence an increased sensitivity.[25] Anodic stripping voltammetry has primarily been used for the determination of trace concentrations of toxic heavy metals such as Pb, Cd, Zn, and Hg in a variety of sample matrices, from natural water to body fluids, such as whole blood. Organometallic molecules such as those involving tin as the central metal atom can be determined by ASV[26] following liberation of the metal from the complex or by ASV of the intact complex. Woggon[26] has studied the influence of plating potential and time, the volume of the mercury drop and temperature on the ASV of $RSnCl_3$ (where R = butyl or octyl), $C_6H_5(CH_3)SnCl_2$, $SnCl_4$, and $(C_6H_5CH_2)Sn(O)OH$ and optimized conditions for their analysis in fungicide residues. Booth and Fleet[27] have applied ASV to the stripping of organotin molecules as free radicals which can be stabilized by adsorption on mercury electrodes, following their formation by a $1e^-$ reduction process. An extensive bibliography on ASV and its applications can be found in the book by Vydra et al.[28]

The theory of ASV has been reviewed by Bond[29] and can be summarized as follows: for the electrode process involving deposition of metal ion M^{n+} onto mercury to form a metal amalgam

$$M^{n+} + ne \rightleftharpoons M(Hg) \tag{14}$$

The current flow i(t) at time t is approximated by the Levich equation, i.e.,

$$i(t) = k_1 nFAD_{M^{n+}}^{2/3} \omega^{1/2} \nu^{1/6} C_{M^{n+}}(t) \tag{15}$$

where k_1 = a constant appropriate for the electrode being used for deposition, ω = rate of electrode rotation or solution stirring, ν = kinematic viscosity of the solution, and $C_{M^{n+}}(t)$ = concentration of metal ion in solution at deposition time. Other symbols have ther usual meaning.

By using the symbol m, the mass transfer coefficient, Equation 15 can be rewritten as follows:

$$i = k_1 mnFAC_{M^{n+}} \tag{16}$$

Faraday's law enables the concentration of metal in the amalgam to be calculated. Assuming that a constant current is maintained during deposition (i.e., $C_{M^{n+}}(t)$ does not change appreciably) and that the solution is stirred or electrode rotated at a constant velocity, then the concentration of reduced metal in the mercury $C_{M(Hg)}$ at either a HMDE or MFE is given by Equation 17:

$$C_{M(Hg)} = \frac{it}{nFV} \tag{17}$$

where i is the reduction current, t is the electrolysis time, and V is the volume of mercury in the drop or film. Substitution of Equation 16 into 17,

$$C_{M(Hg)} = \frac{k_2 m C_{M^{n+}} t}{r} \text{ for the HMDE} \quad (18)$$

$$C_{M(Hg)} = \frac{k_3 m C_{M^{n+}} t}{L} \text{ for the MFE} \quad (19)$$

where r is the radius of the mercury drop, L is the thickness of the mercury film, and k_2 and k_3 are constants appropriate for the HMDE and MFE, respectively.

Using an appropriate E-t waveform, such as DC or differential pulse ramp to strip or oxidize the deposited metal from the amalgam, then the resulting peak height i_p for a reversible process at the HMDE is given by

$$i_p = -k_4 n m^{3/2} D_M^{1/2} r v^{1/2} C_{M^{n+}} \quad (20)$$

where k_4 is a numerical constant and v is the scan rate of the DC potential. The peak potential E_p of the stripping curve at the HMDE can be approximated to Equation 21:

$$E_p = E_{1/2} + \frac{1.1\ RT}{nF} \quad (21)$$

where $E_{1/2}$ is the reversible polarographic halfwave potential.

For the MFE, the theory developed by de Vries and van Dalen[30] gives equations for i_p and E_p, i.e.,

$$i_p = -k_5 n m^2 A C_{M^{n+}} vt \quad (22)$$

$$E_p = E_{1/2} + \frac{2.3\ RT}{nF} \log \frac{\delta nFLv}{D_{M^{n+}} RT} \quad (23)$$

where δ is the diffusion layer thickness and the other symbols have their usual meaning.

The technique of CSV has the deposition step carried out electrolytically at anodic potentials and electrolytic stripping by imposition of a cathodic voltage scan. This method has been applied to the determination of a range of anionic species such as Cl^-, Br^-, I^-, CrO_4^{2-}, MoO_4^{2-}, VO_3^-, WO_4^{2-}, etc. and organic molecules such as dithiocarbamate pesticides[31] and organosulfur drugs,[32] all of which form partially insoluble compounds/complexes with mercury at anodic potentials. Metal ions such as Mn(II) and Pb(II) can also be determined by cathodic stripping of their respective molecular oxides on either a carbon paste or rotating platinum electrode.[33]

Brainina[34] has reviewed the theory and applications of film stripping voltammetry for the determination of anions. The relevant electrode reactions are, for a mercury electrode, (1) deposition step:

$$2Hg \rightleftharpoons Hg_2^{2+} + 2e^- \quad (24)$$

$$Hg_2^{2} + 2A^- \rightleftharpoons Hg_2A_2 \downarrow \quad (25)$$

and (2) stripping step:

$$Hg_2A_2 + 2e^- \rightarrow 2Hg + 2A^- \quad (26)$$

Table 2
ELECTROCHEMICAL CONDITIONS FOR STRIPPING VOLTAMMETRY OF ANIONS IN THE FORM OF MERCURY SALTS

Anion	Supporting electrolyte	T(°C)	Deposition potential (volts)	Stripping potential Ep (volts)	Minimum bulk concentration of the anions to give surface deposition (*M*)
Cl^- [34]	0.1 *M* $NaNO_3$	20	+0.35	+0.15	5.10^{-6}
S^{2-} [34]	1 *M* NaOH	20	−0.50	−0.90	5.10^{-8}
CrO_4^{2-} [34]	0.1 *M* $NaNO_3$	20		+0.35	3.10^{-9}
WO_4^{2-} [34]	0.1 *M* $NaNO_3$	20	+0.40	+0.25	4.10^{-7}
MoO_4^{2-} [34]	0.1 *M* $NaNO_3$	20	+0.40	+0.20	1.10^{-6}
VO_3^- [34]	0.1 *M* $NaNO_3$	20	+0.40	+0.25	1.10^{-6}
SO_4^{2-} [34a]		20	−0.50	−0.90	5.10^{-8}
Dithizonate[41]	1 *M* KNO_3	20	+0.10	−0.35	1.10^{-5}
Diethyldithiophosphate[41]	1 *M* KNO_3	20	0.00	−0.30	1.10^{-6}
Oxalate[41]	1 *M* KNO_3	20	+0.35	+0.05	1.10^{-6}
Succinate[41]	1 *M* KNO_3	20	+0.40	0.00	1.10^{-4}

[a] May be determined only after reduction to S^{2-} with Ti(III) in phosphoric acid.

where A^- is the anion removed from solution (assumed for simplicity to be monovalent), and Hg_2A_2 is the sparingly soluble compound forming the film on the electrode surface.

Brainina has also given theoretical consideration to the particular case of irreversible film dissolution. Since in the process of electrodissolving a compound localized on the electrode surface the metal ions being reduced come into contact with the electrode surface, the case may be regarded mathematically as analogous to dissolving the metal from the inert electrode surface. A relationship can then be written for irreversible dissolution of a compound from the electrically active electrode surface, i.e., the peak or maximum current is given by

$$i_p = 0.37 \frac{\alpha n F}{RT} v C_{Hg_2A_2} \quad (27)$$

Since the test substance does not take a direct part in the electrode process, the method may be used to determine electroinactive molecules. Deposition potentials for the mercury salts of selected anions and approximate stripping potentials, E_p, are given in Table 2, together with the minimum anion concentration at which the electrodissolution current, i_p, for an appropriate salt may be determined, with an error not exceeding 20%. In addition, Table 3 further shows the scope of the technique by a listing of selected organic molecules that have been determined by CSV.

In addition to the stripping voltammetric techniques already discussed, an increasing interest has been detected in the literature concerning stripping measurements of organic molecules that are not deposited and preconcentrated on the electrode surface by electrolysis. In particular, adsorptive stripping voltammetry refers to the voltammetric technique in which the analyte is preconcentrated by adsorption onto the indicator electrode followed by voltammetric determination of the concentrated surface molecules. Applications are summarized in Table 4, which illustrates extremely low detection limits for amenable molecules. In addition, the technique can be found extremely selective in that certain molecules can be determined directly in complex matrices, such as urine, without resorting to a separation procedure, for example, adriamycin, chlorpromazine, thiourea, and cimetidine.[46]

Table 3
ORGANIC MOLECULES DETERMINABLE BY CATHODIC STRIPPING VOLTAMMETRY

Molecule	Pretreatment/supporting electrode	Limit of detection	Ref.
Dithiocarbamates	In aqueous solution		31
Thioamides	In biological fluids	2×10^{-8} *M*	32
Selenocystine, cystine, cysteine	In dilute acidic solution	Cystine 1×10^{-8} *M* Cysteine 1×10^{-9} *M*	35
Nucleic acid bases	In borate buffer	10^{-8} *M*	36
Protein disulfide	Determination following degradation in dilute alkali		37
Organic sulfur molecules, porphyrins			37
Penicillins	Alkaline hydrolysis to corresponding penicilloic acid, deposition as copper complex of penicillamine; stripping at −0.40 V	2×10^{-10} *M* After 10 min	38
2-Thiobarbiturates	In aqueous solution	5×10^{-8} *M*	39
2-Mercaptopyridine *N*-oxide	In aqueous solution		40
Cysteine, rubeanic acid, thioanilide, 2-mercaptobenzolthiazide, sodium diethyldithiocarbamate, thiourea	In aqueous solution		42
Drug halides	In dissolution studies	5×10^{-6} *M*	43
Mercaptans	In aqueous solution		44
Thiocyanate	In aqueous solution		45

Table 4
ADSORPTIVE STRIPPING VOLTAMMETRY OF SELECTED ORGANIC COMPOUNDS

Molecule	Indicator electrode	Supporting electrolyte	Limit of detection (*M*)
Dopamine[46]	Pt	EtOH	5.10^{-8}
Bilirubin[46]	Static Hg drop	CH_3COONa	5.10^{-10}
Heme[46]	HMDE	60% EtOH/H_2O	10^{-9}
Riboflavin[46]	Static Hg drop	10^{-3} *M* NaOH	$2.5.10^{-11}$
Chlorpromazine and other phenothiazines[46]	Impregnated graphite, carbon paste	Phosphate buffer	5.10^{-9}
Adriamycin[46]	Carbon paste	Acetate buffer	10^{-8}
Codeine, cocaine, and papaverine[46]	Static Hg drop	NaOH	10^{-8}
Diazepam and nitrazepam[46]	Static Hg drop	Acetate buffer	5.10^{-9}
Cimetidine[46]	Static Hg drop	0.1 *M* HCl	4.10^{-9}
Digoxin and digitoxin[46]	Static Hg drop	5×10^{-3} *M* NaOH	2.10^{-10}
Progesterone and testosterone[46]	Static Hg drop	5×10^{-3} *M* NaOH	2.10^{-10}
Nitro-containing pesticides[46]	Static Hg drop	BR buffer	5.10^{-10}
Thiourea[46]	Static Hg drop	0.1 *M* $NaClO_4$	2.10^{-11}

C. On-Line Electrochemical Detection

The aforementioned recent advances in the development of pulse polarographic and stripping voltammetric techniques have endowed the resulting analytical methods utilizing these electrochemical "end-steps" with a similar and, in some cases, better sensitivity for organic and organometallic molecules, as compared with rival methods employing gas chromatographic or spectrofluorimetry. In terms of selectivity, these voltammetric techniques operating in quiescent solution or in on-line situations rarely show adequate resolution for application in environmental trace analysis, where there are many naturally occurring molecules and structurally related metabolites in the matrix that can interfer. It has therefore been found necessary to incorporate sample pretreatment and chromatographic procedures such as HPLC for organics and organometallics and ion chromatography for inorganic molecular ions prior to on-line electrochemical detection.

The most widely used electrochemical detectors employed in liquid chromatography and in ion chromatography are those based on direct current hydrodynamic chronoamperometry, where the current arising from the oxidation or reduction of a molecule of interest is measured as a function of time at a fixed electrode whose potential is held constant in the flowing stream of aqueous eluent from the column. Glassy carbon is a particularly popular indicator electrode material with a potential range of -0.80 to $+1.20$ V (vs. SCE) in pH 4.5 aqueous buffer. Graphite paste (range -1.60 to $+1.10$V), mercury (range -2.00 to $+0.40$ V), and platinum (-0.50 to $+$ 1.20 V) are also used. A wide variety of detectors have been invented, with the thin-layer and wall-jet designs being the most commercially acceptable. Table 5 gives the characteristics of some of the hydrodynamic electrode systems and Table 6 compares their analytical parameters. The recent review by Stulik and Pacakova[47] should be referred to for further information on the theory and design of electrochemical detectors for use in HPLC.

A brief comparison with other HPLC detectors clearly shows the main advantages of electrochemical detectors, namely, the high sensitivity and a broad linear dynamic range. It should also be noted that for mercury electrodes used for reduction processes, the sensitivities for the appropriate molecules using electrochemical detection are inferior to UV spectroscopic detection. In addition, in those cases where the chromatographic system is unable to separate two compounds of related structure, a certain measure of selectivity in the electrochemical detector can be called into play. This has been illustrated in the selective determination of the growth-promoting hormones, dienestrol and diethylstilbestrol,[48] and the carbamate pesticides, barban and captafol.[49] When the oxidation or reduction potentials of two molecules are just separated by 50 to 100 mV, the use of DC hydrodynamic electrochemical detection may not give the required selectivity and can be improved upon by the use of the differential pulse mode of operation. In some cases, the use of the differential pulse mode may also give improved sensitivity, especially for molecules with high potentials of oxidation or reduction, e.g., organometallic cations.[50]

In recent years, a major notable advance in such electrochemical detectors has been the introduction of dual-electrode detection systems[51] which have been demonstrated to improve both the selectivity and sensitivity of certain determinations. Other advances have come about through the application of pre- and postcolumn derivitization reactions,[52] and future development is seen in the field of scanning ED systems to further aid in the identification of unknown peaks.

The application ranges for amenable organic and organometallic molecules are given in Table 7 (for electrooxidizable molecules) and Table 8 (for electroreducible molecules).

Table 5
CHARACTERISTICS OF SOME HYDRODYNAMIC ELECTRODE SYSTEMS

Electrode	Equation of limiting current	Note
Spherical	$i_{lim} = 4\pi r_0 nFDC + knr_0^2 D^{2/3} C f^{1/2}$	Turbulent flow; constant k must be determined empirically
Planar	$i_{lim} = 0.68\ nFD^{2/3} Cbl^{1/2} u^{1/2} \nu^{-1/6}$	Laminar flow
Tubular	$i_{lim} = 2.01\ nF\pi D^{2/3} l^{2/3} u^{1/3} r^{2/3} C$	k = 0.33 for laminar flow; k = 1 for turbulent flow
Conical	$i_{lim} = 0.77\ nFACD^{2/3} u^{1/2} \nu^{-1/6} l^{1/2}$	Laminar flow
Wall-jet	$i_{lim} = knFACD^{2/3} \nu^{-1/6} (u/L)^{1/2}$	Turbulent flow; k is an empirical constant
	$i_{lim} = 1.60\ knFCD^{2/3} \nu^{-5/12} V^{3/4} a^{-1/2} R^{3/4}$	

Note: Symbols are (k) constant; (u) linear flow rate of the solution; (r_o) radius of a spherical electrode; (l) length of electrode; (b) thickness of electrode; (r) radius of tubular electrode; (L) characteristic dimension of electrode; (f) frequency of solution stirring; (V) volume flow rate of the solution; (a) jet diameter; (R) disc electrode radius; the other symbols have their usual meaning.

Table 6
COMPARISON OF SELECTED ANALYTICAL PARAMETERS FOR ELECTROCHEMICAL DETECTORS

Detector	Residual current (μA)	Detection limit (ng)	Linear dynamic range/ng	Calibration curve: Slope (μA/μg)	Calibration curve: (μA/μg) Correlation coefficient (R)	Cell volume (μℓ)
Polarographic HMDE	—	15[a]	100—30,000	0.993	1.000	8
Tubular Pt	3—10	0.3[b]	0.3—2500	1.3	0.998	2.3
Thin-layer (C paste)	0.1—0.2	0.5—1.0[b]	1—5000	1.17	0.997	0.65
Thin-layer (glassy C)	2.4—5.6	0.5—1.0[b]	1—5000	2.6	0.999	0.65
Wall-jet (C paste)	0.18	0.03—0.1[b]	0.03-3000	2.25	0.958	0.35
Wall-jet (glassy C)	10	0.3[b]	0.3-3000	2.3	0.998	0.35
Metrohm EA 1096 (wall-jet, glassy C)	—	0.4[a]	10—8000	6.34	0.999	1.3

[a] Nitrobenzene.
[b] Adrenaline.

III. VOLTAMMETRIC BEHAVIOR AND DETERMINATION OF THE MOLECULES

A. Nitrogen-Containing Molecules

1. Nitro-Containing Molecules

The polarographic behavior of the nitro group has been extensively studied for many compounds and these are documented in standard texts.[63,64]

Aliphatic nitro compounds are reduced in a $4e^-$ process in acidic solution:

$$R\text{–}CH_2NO_2 \xrightarrow{4e^-,4H^+} R\text{–}CH_2NHOH + H_2O$$

Table 7
ELECTROOXIDIZABLE CANDIDATES FOR HPLC-ED

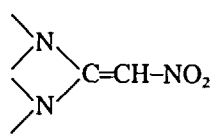
Molecular classification	Examples
Aromatic alcohols	Naturally occurring phenols (tyrosine metabolites, phenylpropionic acids, phenylpyruvic acids, benzoic acids, cinnamic acids, mandelic acids)
	Halogenated phenols (chlorinated phenols such as mono-, di-, tri-, tetra-, and penta-substituted molecules)
	Hydroxybiphenyls (polychlorinated biphenyl metabolites)
	Tocopherols
	Estrogens
	Flavones
	Antioxidants (butyl-substituted phenols and quinones)
Aromatic amines	Anilines (mono-, di-, tri-, tetra-, and penta-chlorinated anilines)
	Amino-substituted polycyclic aromatics (anthracenes, benzidines, naphthalenes, fluorenes, chrysenes)
Indoles	Tryptophan, 5-hydroxytryptophan, serotonin, 5-HIAA
Phenothiazines	Chlorpromazine
Mercaptans	RSH
Others	Vitamin A, ascorbic acid, purines, carotenes

Table 8
ELECTROREDUCIBLE CANDIDATES FOR HPLC-ED

Molecular classification	Matrix	Chromatographic separation mode	Indicator electrode	Electrochemical detector mode (using thin-layer cell)	Ref.
Benzodiazepines	Aqueous solution, plasma	Reversed phase	Glassy C, DME	DC, DPP	53
Benzoylperoxide	Aqueous solution	Reversed phase	Au/Hg	DC	54
Cystine	Aqueous solution	Ion exchange	Au/Hg	DC	55
Diacetol	Feed	Reversed phase	DME	DPP	56
Diketones, naphthoquinone	Aqueous solution	Reversed phase	DME	Square wave	57
Nitrosamines	Aqueous solution	Reversed phase	DME	NPP, DPP	57, 59
Dinitrobenzenesulfonyl-amino acids	Aqueous solution	Adsorption	Pyrolytic graphite	DC	58
Organomercury	—	Reversed phase	Au/Hg	DPP	60
Organometallics	Wastewater	Reversed phase	Au/Hg	DPP	50
Parathion	Aqueous solution, wastewater	Reversed phase	DME, Au/Hg	DC	55
Pesticides	Plants	Normal phase	DME	DC	61
Vitamin K_3, B_2, B_{12}	Aqueous solution	Reversed phase	Carbon paste	DC	62

but do not give rise to a wave in alkaline media because of the inability of the *aci*-nitro anions to reduce. These compounds are commonly encountered as explosives and polarographic methods for their analysis have been reviewed by Hetman.[65]

Ranitidine is, like cimetidine, a powerful inhibitor of gastric acid secretion and is used in the treatment of gastric and duodenal ulcers. The polarographic behavior of the electroreducible group



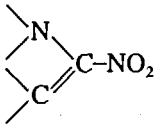
in ranitidine has been studied by Delgando Zamarreno et al.,[66] who have recommended determination by DPP in the range $2.5.10^{-7}$ to $2.05.10^{-5}$ *M*.

The reduction of aromatic nitro compounds occurs by the mechanism as shown for nitrobenzene:

$$C_6H_5NO_2 \xrightarrow[-H_2O]{4e^-,4H^+} C_6H_5NHOH \overset{H^+}{\rightleftharpoons} C_6H_5\overset{+}{N}HOH_2 \xrightarrow{2e^-,2H^+} C_6H_5\overset{+}{N}H_3 + H_2O$$

Depending on the nature of the substituents on the aromatic ring, the two electrochemical steps can occur simultaneously, thus giving rise to a single $6e^-$ process or in two separate stages. As the reduction of the $-NO_2$ group consumes at least $4e^-$ per molecule and occurs at low negative potentials, polarographic methods for the determination of compounds containing this group have been used extensively in trace analytical methods. In particular, the DPP technique can be used to determine concentrations of aromatic nitro molecules down to 1×10^{-8} *M* in pure solution, and the peaks obtained (usually at pH <4 and >10) are well resolved, with peak widths at half-height of the order of 60 to 70 mV. Aromatic nitro molecules can also be strongly adsorbed at the surface of the mercury drop. This is indicated by a "peaked" wave form in NPP where the limiting current does not obey the Cottrell Equation 8.

Because of these strong adsorptive properties, many aromatic nitro molecules can be determined directly in complex matrices, such as in body fluids without prior separation of naturally occurring interfereces, e.g., proteins, which adsorb less strongly to the mercury surface. This behavior can be used to determine concentrations of imidazoles such as metronidazole which contains the functional group



down to 50 ng $m\ell^{-1}$ directly in serum.

Direct polarographic methods for nitro molecules in wastewater and effluents have been developed, for example, nitrobenzene in industrial wastes has been determined by distillation after separating nitrophenols. The supporting electrolyte consisted of aqueous ethanol-containing hydrochloric acid.[8] Nitrochlorobenzenes in water down to 5.10^{-8} *M* (5 ng $m\ell^{-1}$) have been determined following extraction with activated charcoal and polarography of the eluted acetone solution in a pyridinium hydrochloride-supporting electrolyte.[8]

The polarographic determination of dinitro compounds commonly used as agrochemicals has been reported. Rowe and Smyth[67] have investigated the polarographic behavior of several dinitrophenols and their esters in a variety of supporting electrolytes (Table 9). Southwick et al.[68] have studied the polarographic behavior of several dinitroaniline herbicides and attempted to correlate their mechanism of reduction to their relative toxicity in the environment. These compounds give rise to a single $8e^-$ wave in acidic solution (pH <3), which then splits into two $-NO_2 \rightarrow -NHOH$ $4e^-$ waves on increase of pH.

Cimbura and Gupta[69] have determined some dinitrophenol pesticides in biological materials. The method involved the extraction of acidified samples with chloroform, followed by a back extraction into 0.1*N* sodium hydroxide solution. The aqueous phase was then taken, the pH adjusted to 7.8, the solution centrifuged, and polarography carried out on the clear supernatant between -0.20 and -1.20 V. Recoveries of dinitrophenol pesticides from blood were stated to be on the order of 75%. Dinitro-*o*-cresol (DNOC) can be determined

Table 9
HALF-WAVE POTENTIALS OF SOME DINITROPHENOL PESTICIDES IN VARIOUS SUPPORTING ELECTROLYTES

Compound	Supporting electrolyte: 0.1 *N* Nitric acid[a]	0.1 *N* Acetic acid-sodium acetate solution[a]	0.1*N* Ammonia-ammonium chloride solution[a]	BR buffer pH 9.0[b]
Dinitrophenols				
2,4-DNP	−0.06, −0.57, −0.96	−0.50, −0.88	−0.78, −1.04	
DNOC	−0.06, −0.42, −0.92	−0.45, −0.84	−0.74, −1.02	−0.50, −0.76
Dimethyl-1-*p*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate (DNBP)	−0.08, −0.40	−0.32, −0.76	−0.75, −1.04	−0.51, −0.75
Dinocap	−0.12, −0.31	−0.39, −0.67	−0.52, −0.78, −1.02	
Dinoterb				−0.50, −0.77
Medinoterb				−0.625, −0.78
Dinobuton				−0.44, −0.61
Dinitrophenol esters				
DNBP acetate	−0.08, −0.34	−0.41, −0.74	−0.69, −0.98	
Dinoterb acetate				−0.30, −0.85
Medinoterb acetate				−0.49, −0.705, −0.85

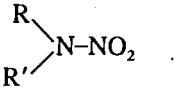
[a] $E_{1/2}$ values in DC polarography (vs. Ag-AgCl)
[b] Ep values in DPP (vs. SCE).

directly in blood and blood coagula at concentrations between 50 and 250 μg $m\ell^{-1}$ by this method. Filimonova and Gorbunova[70] described a method for the determination of *N,N*-diethyl-2,6-dinitro-4-trifluoro-methylaniline in water and vegetables. The method involved extraction of 1 ℓ water or 100 g of vegetable with hexane, purifying the extract on alumina (for tomatoes) or a cation-exchange resin (for cabbage) and carrying out the determination in 0.05 *N*-tetraethylammonium hydroxide (TEAOH) — 60% methanol. This method was used to determine the molecule in these matrices between 0.01 and 0.1 μg $m\ell^{-1}$.

Aromatic nitro compounds are usually metabolized in vivo via hydroxylamine intermediates to the corresponding amines. The application of polarographic methods to the determination of these metabolites is usually hindered, however, by the ill-defined nature of many hydroxylamine reductions (especially in DPP) and by the inability of amines to reduce at the DME. Hydroxylamines do, however, give rise to well-defined oxidation waves at the DME, as has been reported by Iversen and Lund,[20] for some aryl hydroxylamines in 0.1 *N*-potassium hydroxide — 2% sodium sulfite solution. Beckett et al.[21] have been able to resolve a mixture of primary and secondary hydroxylamine and oxime metabolites of an aliphatic amine, using DPP based on their respective oxidation processes at the DME. Sternson[71] has used the oxidation waves obtained for some aryl hydroxylamines at a carbon paste electrode in order to determine these compounds in body fluids in the concentration range 1×10^{-4} to 2.5×10^{-6} *M* in the presence of naturally occurring amines, amides, and NADPH.

Because of the usefulness of the $-NO_2$ group reduction in trace polarographic analysis, there are many instances in the literature where nitration derivitizations have been carried out on phenyl-containing compounds. The use of this derivitization procedure and its applicability to body fluid analysis is adequately illustrated by the studies of Brooks et al.[72] on the nitration of some phenyl-containing anticonvulsive drugs. In addition, there are reagents containing one or two nitro groups, which can be used for the formation of electrochemically reducible derivatives from nonreducible compounds such as alcohols, amines, amino-acids, and carboxylic acids. These reagents are used in precolumn chromatographic techniques, with the excess of reagent being separated on the particular column at the same time as the trace molecules, for example, for alcohols and amines the reagent 3,5-dinitrobenzoyl chloride is used to produce an electrochemically reducible derivative. For carboxylic acids, 4-nitrobenzyl bromide can be used.

HPLC-ED has been successfully applied in the last few years to the trace analysis of explosives and polynuclear aromatic hydrocarbons, both containing the $-NO_2$ group, in environmental samples. Maskarinec et al.[73] have utilized the reduction of nitrocompounds at a mercury-gold indicator electrode held at -1.00 V (vs. Ag/AgCl) for the determination of explosives in water samples down to detection limits of 1 ng $m\ell^{-1}$. Nitramines (e.g., TAX, SEX, HMX, and RDX) containing the functional group



nitrotoluenes (TNT, 2,6 DNT, and 2,4 DNT), and nitroaliphatics (nitrate esters such as NG and PETN containing the functional group $R-O-NO_2$) are amenable to electrochemical detection following Porapak resin adsorption of the munitions components from aqueous samples, desorption with acetone, and subsequent HPLC. Although these molecules cannot be electrochemically oxidized, nitrite can be released from them postcolumn, on line by photolysis of the HPLC effluent, followed by electrochemical detection at ca. $+1.00$ V at a glassy carbon electrode, i.e., the technique HPLC-hν-ED.[74] These workers have found detection limits of 25 ppb for RDX and TNT and 200 ppb for NG. In addition, the effect of inorganic and organic anionic interference was evaluated and it was found that the

following anions (I^-, IO_3^-, IO_4^-, HCO_3^-, NO_3^-, HSO_3^-, S^{2-}, $C_6H_5COO^-$, CNS^-, CN^-, $H_2PO_4^-$, HPO_4^-, CrO_4^{2-}, $Cr_2O_7^{2-}$) were electroactive using the technique FIA-ED, with a dual glassy carbon electrode detection system.

Recent investigations of emissions from diesel engines have focused on mutagenic and carcinogenic nitro-substituted polynuclear aromatic hydrocarbons (nitro-PAHs). Of the nitro-PAHs which have been tentatively identified in extracts of diesel-exhaust particulates, several are potent mutagens in Ames' *Salmonella* bioassay, a short-term test designed to detect chemicals which may be potential carcinogens. These compounds include 1-nitropyrene (1-NP), 2,7 dinitrofluorene (2,7-DNF), and 2- and 3-nitro-9-fluorene (2- and 3-NFO). Other mutagenic nitro-PAHs in diesel exhausts, including 2-nitrofluorene (2-NF) and 4-nitrobiphenyl (4-NB), are known to be carcinogenic in laboratory mammals. Numerous other nitro-PAHs have not been tested for mutagenicity or carcinogenicity because of the unavailability of reference compounds. Rappaport et al.[75] and Jin et al.[76] have used HPLC-ED in the reductive mode (–0.60 V vs. Ag/AgCl) to measure such nitro-PAHs at pg-ng levels and to identify 1-NP in extracts of diesel exhaust particulates by comparing the retention times and the hydrodynamic voltammogram of a reference 1-NP compound. Preliminary results indicated that some reducible species which might interfere with the analytical method (aldehydes and anhydrides) are not measured at the electrode potential used for nitro-PAHs, while others (ketones and quinones) can probably be differentiated from nitro-PAHs by their hydrodynamic voltammograms.

AdSV at the sMDE has given extremely low detection limits for the nitro-containing molecules, nitrazepam,[77] and certain nitro-pesticides,[78] i.e., 5×10^{-9} and 5×10^{-10} *M*, respectively.

2. N-Oxides

N-Oxides are usually encountered as metabolites of *N*-containing exogenous molecules, for example, in molecules containing the azomethine or azo groups or at nitrogen atoms in pyridine, piperazine, pyrimidine, or piperidine ring systems. They have also been postulates as being intermediates in dealkylation reactions. As *N*-oxides can be thermally labile in GLC determination, polarography can present an important alternative method for their trace analysis.

The polarographic reduction of heterocyclic amine *N*-oxides has been studied by Kubota et al.[19] in conjunction with electron spin resonance (ESR) spectroscopy and relationships between half-wave potential ($E_{1/2}$) values and various physical parameters observed, e.g., Hammett function, electron spin density, and energy of lowest vacant molecular orbital. These compounds, i.e., pyridine, quinoline *N*-oxides, etc. reduce at relatively negative potentials (-2.30 and -1.80 V, respectively) in solution of pH $<$ pK_a value:

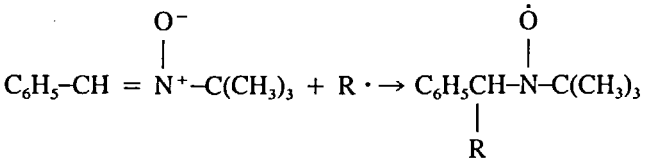
$$\overset{+}{\text{RN}}\text{–}\overline{\text{O}} \overset{\text{H}^+}{\rightleftarrows} \overset{+}{\text{RN}}\text{–OH} \xrightarrow{2e^-,2H^+} \overset{+}{\text{RNH}} + H_2O$$

In compounds containing an *N*-oxide function directly associated with an existing electroactive center, two processes are usually observed, corresponding to the separate reductions in the *N*-oxide group and that of the existing electroactive center. This is illustrated in the context of chlordiazepoxide, where the =N → O and $\diagdown$C=N– groups in the $\diagdown$C=N$\diagup$ moiety reduce at different potentials.[79] With trimethoprim, DPP has been used to differentiate between the parent compound (which exhibits a reduction wave due to the pyrimidine group in the molecule) and the two *N*-oxide metabolites formed by oxidation of the separate *N* atoms in the pyrimidine ring.[80]

When the *N*-oxidation occurs at a site that is conjugated to an existing electroactive center, then the two reduction processes can occur simultaneously. With the acetyl and benzoyl derivatives of pyridine *N*-oxide, for example, Laviron et al.[81] have shown that the $\rangle C{=}O$ and *N*-oxide reductions occur at the same potential. Similar behavior is also exhibited by a benzhydrylpiperazine derivative of pharmaceutical importance.[13]

When the *N*-oxidation occurs in a molecule that is not electroactive, then polarography often offers a specific method for the determination of the metabolic product in the presence of the parent compound; for example, chlorpromazine *N*-oxide can be determined in the presence of chlorpromazine (which is not directly reducible at the DME). In this example, however, the *N*-oxide metabolite reduces at the same potential as the *S*-oxide and *N*-oxide, *S*-oxide products. These compounds must, therefore, be separated prior to analysis.[24]

A recent paper by Stronks et al.[82] has used electrochemical detection at a pyrolytic graphite indicator electrode at +0.70 V (vs. Ag/AgCl) coupled with ESR spectroscopy to identify an homologous series of spin adduct aminoxyls (nitroxides) following their separation by HPLC, i.e.,



3. N-Nitroso- and C-Nitroso-Containing Molecules

Recent attention to *N*-nitrosamines is due to the widely held belief that they exhibit carcinogenic properties harmful to man. They can be formed in vivo by the action of nitrite on secondary or tertiary amines and are mostly encountered in food and alcoholic beverages.

The polarographic behavior of a wide range of *N*-nitrosamines has been studied by Smyth et al.[18] The polarographic determination of some carcinogenic nitrosamines has been investigated by several authors.[83-87] Heath and Jarvis[83] have described a method for the determination of dimethylnitrosamine (DMN) in animal tissues. The method involved homogenization with water, filtration, distillation from alkaline solution, and determination in 0.01 *M* sulfosalicylic acid. The minimum amount that could be determined by this method was 1 μg, with an accuracy of ±0.05 μg. Chang and Harrington have determined DMN in rat serum down to 0.2 μg $m\ell^{-1}$, using a DPP procedure.[85] Hasebe and Osteryoung[86] investigated the use of tetraalkylammonium salts in order to decrease the background current in DPP and have quoted a detection limit of 8×10^{-8} *M* for the determination of nitrosoproline in pure solution. Walters et al.[87] have investigated the separation of some *N*-nitrosamines on activated charcoal and quoted reduction potentials for 16 members of this group of compounds in 0.2 *N* hydrochloric acid vs. a mercury pool. Derivative polarographic peak potentials of the *N*-nitroso derivatives ranged from −0.69 V for *N*-methylaniline to −0.94 V for dimethylamine.

The use of polarographic methods of analysis for the identification and determination of *N*-nitrosamines in alcoholic beverages has been subject to criticism. It can be shown that a DPP polarographic method designed for the determination of these compounds in spirits can give rise to a peak which can be assigned to either nitrosamines or furfural. Gas chromatography-mass spectrometry (GLC-MS) is required for the identification of nitrosamines and can determine 1 to 10 ppb of *N*-nitroso-3-hydroxypyrrolidine in cured meat products. Pyrazines also show similar polarographic behavior as *N*-nitrosamines and these compounds should, therefore, be separated prior to analysis. Smyth et al.[88] have used DPP to determine the concentration of *N*-nitrosodiethanolamine in synthetic cutting oils, following separation

Table 10
E_p VALUES OF SOME 1,4-BENZODIAZEPINES IN BR BUFFER pH 4.0 (E_p/V VS. SCE)

Compound	Polarographic reduction peaks (using DPP)	Voltammetric oxidation peaks (using DC mode at glassy carbon)
Chlordiazepoxide	−0.37 −0.73, −1.17	+1.30
Bromazepam	−0.40, −1.12	—
Nitrazepam	−0.16, −0.78	Not oxidized
Clonazepam	−0.15, −0.73	—
Flunitrazepam	−0.16, −0.73	Not oxidized
Oxazepam	−0.76	+1.33
Lorazepam	−0.74	+1.38
Diazepam	−0.74	Not oxidized
Prazepam	−0.73	Not oxidized
Flurazepam	−0.72	+1.05
Potassium chlorazepate	−0.73	+1.49
Medazepam	−0.82	+0.88, +1.10

on Sephadex LH-20 column, and reported much lower levels of interference than when using the high performance liquid chromatography-thermal energy analysis (HPLC-TEA) procedure. Recently, Samuelsson and Osteryoung[89] have coupled HPLC with reductive electrochemical detection to selectively determine the highly polar nonvolatile *N*-nitrosamines, *N*-nitrosoproline, and *N*-nitrosodiethanolamine down to detection limits of the order of 10^{-7} *M*. They have further improved the selectivity of the link-up of HPLC and electrochemical detection by resort to rapid scan square wave voltammetric detection which results in three-dimensional chromatovoltammograms.[90]

In general, *C*-nitroso compounds give rise to even better-defined peaks than *N*-nitroso compounds. The peaks obtained for *p*-nitrosophenol, *N,N'*-diethyl-*p*-nitrosaniline, and 2-nitroso-1-naphthol in BR buffer, pH 10, have been shown to be particularly amenable to trace analysis, as their DPP peaks have peak widths at half-height of the order of 50 mV.[18] Owing to the high sensitivity offered by the *C*-nitroso reduction, it is possible to use nitrosation procedures for the determination of phenolic compounds of biological importance, e.g., resorcinol and flavones.

4. Molecules Containing the Azomethine Group

During the last 15 years there have been many instances where polarographic methods of analysis have been used for the determination of compounds containing the azomethine linkage. This has been particularly true for the 1,4-benzodiazepines, a series of drug substances that are widely prescribed as antianziety agents and sedatives and which can have additional electroreducible groups to the azomethine or $\diagdown$C=N$\diagup$ group, possessed by all members of this series of compounds, for example, chlordiazepoxide (Librium) contains three reducible functional groups, viz., $\diagdown$C=N–, =N → O, and –N=C$\diagup$, all of which reduce at different potentials in acidic media and give rise to well-defined waves of analytical importance. Hackman et al.[91] have used DPP for the determination of this compound and its electroactive metabolites in body fluids.

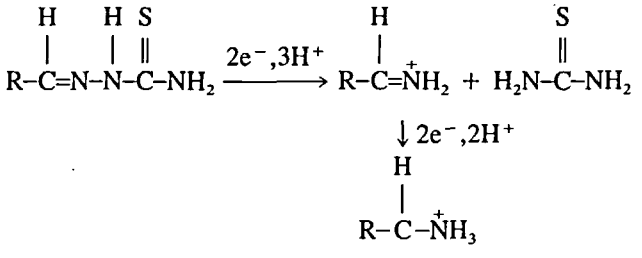
The analytical chemistry of these compounds has been fully dealt with in other reviews[8,92,93] and these should be consulted for further reference. The electrochemical reduction and oxidation[94] behavior of 12 important members of this series is summarized in Table 10.

Smyth et al. have recommended the identification of 12 therapeutically important 1,4-

benzodiazepines in a mixture based on consideration of their physicochemical, electrochemical, complexometric, and hydrolytic properties.[95] This scheme, which was devised for use in forensic situations, should provide information complementary to that obtained using GLC and TLC procedures.

Smyth and Ivaska[94] have recently investigated the voltammetric oxidation of 16 1,4-benzodiazepines and their metabolites at the glassy carbon electrode in the pH range 2 to 12. Several different mechanisms of oxidation are apparent, such as (1) *N*-oxidation on the $-(CH_2)_2N(C_2H_5)_2$ group of fluarzepam and the $=C\diagup\!\!\diagdown^{NHCH_3}$ group of chlordiazepoxide, (2) formation of radical cations on the 1-*N* atom with subsequent coupling reactions for molecules like oxazepam, and (3) lack of electroactivity in those molecules where the electron density at the 1-*N* atom is reduced through delocalization by $-NO_2$ or C=O groups and/or there is steric shielding of this 1-*N* atom by a substituent group such as $-CH_3$ or $-CH_2CH_2OH$, e.g., flunitrazepam. For trace identification and determination of these molecules, HPLC separation followed by electrochemical detection if necessary, with an electrochemical detector offering enhanced selectivity by operating potential control over a UV detector.

When the azomethine group is covalently linked to another nitrogen atom to form the $\diagup\!\!\diagdown N-N=C\diagup\!\!\diagdown$ moiety, the electrode process usually involves simultaneous saturation of the azomethine moiety and reductive splitting of the N–N bond. This occurs for several compounds of pharmaceutical interest, for example, for thioacetazone, where the mechanism of reduction involves



A similar mechanism has been shown to occur for some benzhydrylpiperazine derivatives of pharmaceutical importance[13,14] and for the antibacterial drug nitrofurantoin.[15] DPP has been used to determine the concentration of one of these benzhydrylpiperazine derivatives, SC-13504, in the plasma of baboons following therapeutic administration, and a relationship has been obtained between the plasma levels of SC-13504 and its anticonvulsant activity in this animal species.[96] Smyth and co-workers[96,97] have also made comparisons between the plasma levels of SC-13504 measured by DPP and those measured using radiochemical and GLC methods of analysis.

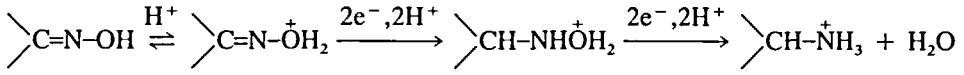
Both of these sets of results show good correlation with the DPP method. In the radiochemical/DPP experiment, DPP was unable to detect any SC-13504 in the plasma 6 hr after administration. The levels of radioactivity found in the plasma between 6 and 24 hr were, therefore, attributed to nonelectroactive metabolite(s) of SC-13504. A good correlation was obtained between the GLC and DPP results, indicating that DPP offers both a selective and an accurate method for the determination of SC-13504 in baboon plasma.

Burmicz et al.[15] described a method for the determination of nitrofurantoin in urine. This involved an extraction into nitromethane, centrifugation, evaporation of the solvent under nitrogen, and determination of the extract in BR buffer (pH 7) — 10% methanol.

Oximes are important intermediate metabolites and are interlinked with the metabolism of certain amines and hydroxylamines. This is illustrated in the case of amphetamine

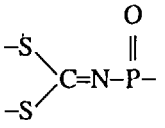
$PhCH_2CH(NH_2)CH_3$ metabolism, where phenylacetone oxime, $PhCH_2C(=NOH)CH_3$, has been isolated as a major intermediate metabolite.

Oximes are reduced in a $4e^-$ step in acidic media to form the corresponding amine. The intermediate in this process is usually the hydroxylamine derivative:



Polarography can thus offer a convenient method for studying the interrelationship between oximes and hydroxylamines in vitro and also in vivo. This is exemplified by the work of Beckett et al.[21] who have used DPP to distinguish between the primary and secondary hydroxylamine and oxime metabolites of *N*-ethyl-2-methoxy-2-(3-trifluoromethylphenyl)-ethylamine.

Smyth and Osteryoung[98] have investigated the polarographic behavior of agrochemicals, cytrolane, cyolone, chlordimeform, and drazoxolon, all of which contain exocyclic azomethine groups in the configuration



and recommended optimum conditions for their determination by DPP.

5. Azo-Containing Molecules

Compounds containing the azo group, –N=N–, are commonly used as dyestuffs and food additives, and hence can be encountered in both the aqueous environment and in body fluids. The azobenzene-hydrazobenzene system gives rise to a $2e^-$ reversible process. In solution in a strong acid, hydrazobenzene can be further reduced to aniline in an irreversible process at more negative potentials. Forence[99] has investigated in detail the mechanism of the polarographic reduction of a variety of substituted azobenzenes over a wide range of pH. He found that substitution of a nitro group stabilized the hydrazo intermediate formed initially, resulting in processes consuming $2e^-$ over a wide pH range. Substitution of hydroxyl groups in the *m* position also resulted in a $2e^-$ process over a wide pH range, e.g., as for 3,3′-dihydroxyazobenzene. However, substitution of hydroxyl groups in the *o* and *p* positions gave rise to $4e^-$ processes in acidic media, which fell off to values approaching $2e^-$ in neutral and alkaline media. The phenylazonaphthol dyes 1-phenylazo-2-naphthol and 1-(2-hydroxyphenylazo)-2-naphthol, in particular, gave rise to $4e^-$ processes in the pH range 2 to 10. The reversible reduction to the hydrazo intermediate and irreversible reduction to the resulting amines were confirmed by cyclic voltammetry. As with aromatic nitro compounds, electrochemical studies of this nature may be important in assessing the toxicity of a new compound.[100]

These compounds give rise to well-defined waves for analytical purposes and differentiation is possible between them and their corresponding diazo analogs; for example, azo- and diazoaminobenzenes can be determined in the presence of one another in 0.1 *M* lithium chloride solution, pH 7.8, containing methanol and gelatin.[100] Supporting electrolytes of acidic pH are recommended for their determination, because at these pH values reduction to the amine is more likely to occur. In addition, a decrease in the height of the peak can occur in alkaline media owing to repulsion of deprotonated hydroxyl groups by the negatively charged mercury surface.

The effect of negatively charged sulfonate groups can also reduce the analytical usefulness of polarographic waves resulting from the azo groups in such dye molecules. This has been shown by Smyth and Hassenzadeh[101] in a polarographic study of some nitrogen-containing organic compounds under aquarium conditions. This study included three textile dyes containing more than one azo group, viz., Direct Red 24, Direct Blue 84, and Acid Red 73. The dye Acid Red 73, which is structurally similar to *p*-bisazobenzene, is relatively unhindered by charged sulfonate groups and gave rise to the best-defined and most sensitive DPP peaks. It is also worthwhile noting that this was the only dye that contained the potentially dangerous 1-phenylazo-2-naphthol group and was found to be the most toxic to daphnae and plant life. Smyth and Hassenzadeh[101] used DPP to monitor the absorption of these and other organic compounds (e.g., aromatic nitro compounds) under simulated aquatic conditions. No electroactive metabolites were found to be excreted back into the body of the aquarium.

As is the case with determinations of –N=N– compounds in body fluids, investigations of this nature are hindered by the affinity that these compounds have for proteinaceous matter. The binding of azo dyes to protein has been investigated electrochemically by Malik and Ahmad,[17] and although the strength of binding affects the quantitative determination of these compounds in biological media, this property can be utilized for the release of other less strongly protein-bound compounds prior to analysis. For example, the dye methyl orange has been used in polarographic methodology to release chlorpromazine and its metabolites from their protein-bound sites prior to polarographic analysis.[24]

6. Heterocyclic Nitrogen-Containing Molecules

The polarographic behavior of heterocyclic nitrogen compounds has been reviewed by Zuman and Perrin.[64] Compounds containing only one nitrogen atom in an aromatic ring system, for example, pyridine and quinoline, generally only produce catalytic hydrogen waves in aqueous solutions of pH less than the pK_a value. The pesticide diquat gives well-defined waves in 0.1 *M* hydrochloric acid at −0.56 and −1.06 V (vs. SCE) owing to two-one-electron reductions. Diquat and the related molecule paraquat can be differentiated in mixtures based on their reduction potentials in 0.08 *M* hydrochloric acid.[102] In this medium, diquat gives rise to a wave at −0.62 V, whereas paraquat is reduced at −0.71 V. Nangniot[102] has further described a method for the determination of these compounds in residues following extraction with sulfuric acid-octan-2-ol, clean-up and separation on Celite 545 and Zeo-Karb 225 columns, and determination in the above-mentioned medium. Substituted pyridine compounds, such as those containing a carboxyl group, also give rise to well-defined waves for analytical purposes. The reduction of pyridine monocarboylic acids has been studied by Bhatti and Brown[103] at a mercury cathode and involves formation of the corresponding alcohol:

$$RCO_2H \rightleftharpoons RCO_2^- + H^+$$

$$RCO_2H + H^+ + e^- \rightleftharpoons R\dot{C}(OH)_{2\ ads}$$

$$R\dot{C}(OH)_{2\ ads} + e^- \xrightarrow[}{\text{slow}} R\bar{C}(OH)_2 \xrightarrow[\text{fast}]{H^+} RCH(OH)_2$$

Polarographic methods for the determination of the pesticide, picloram, have been reported by several authors. Gilbert and Mann[104] have described a pulse polarographic method for the determination of picloram in natural waters. This method could determine the molecule down to 0.02 μg $m\ell^{-1}$ and the authors noted the possible interference that could be caused

by the presence of various metal ions in the water system. Filimonova and Gorbunova[105] have determined picloram in water, soil, and dried maize. The method (for dried maize) involved extraction with acetone – 0.01 *N* potassium hydroxide solution followed by a liquid chromatographic separation on aluminum oxide. Picloram gave rise to a wave at −1.10 V (vs. SCE) in 0.1 *N* hydrochloric or sulfuric acid and the method could determine picloram down to 0.2 μg $m\ell^{-1}$ in water, 0.7 μg $m\ell^{-1}$ in soil, and 0.25 μg $m\ell^{-1}$ in dried maize.

Pyridines substituted by $-CO_2H$ in the 3- or 4-positions, however, undergo a different mechanism involving saturation of the azomethine group in the pyridine nucleus. The reversibility of this process permits the low-level determination of such species using AC polarography; for example, Vallon et al.[106] have determined isoniazid and isonicotinic acid using AC polarography down to 0.2 and 0.03 μg $m\ell^{-1}$, respectively. Polarographic methods can also be applied to the determination of isonicotinic acid and of 2-hydroxynicotinic acid and its *N*-1-riboside metabolite in blood and urine.[107]

Other heterocyclic compounds containing one nitrogen atom in their ring system, for example, piperidines, indoles, and phenothiazines, are not reduced at the DME, although they can exhibit oxidation waves at solid carbon electrodes corresponding to electrochemical processes involving *N*-oxidation.

Heterocyclic aromatics containing two nitrogen atoms within an aromatic ring system can be reduced at the DME, e.g., pyrimidines and pyrazines. The reduction process of pyrimidines involves saturation of an azomethine group followed by formation of tetrahydropyrimidine.

The effect of substituents on this process has been investigated by Malik et al.[108] DPP has been used to determine both the pyrimidine-containing drug trimethoprim in the presence of its two *N*-oxide metabolites in body fluids[80] and the pesticide complex nicarbazin (consisting of equimolar amounts of 4,4′-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine) in chicken tissue.[109] Imidazoles, on the other hand, are not reduced at the DME, although DPP has been applied to the determination of NO_2-substituted imidazoles. An indirect method can also be applied to the determination of piperazine in animal feeds.[110] The method involved dissolving 0.1 to 1 mg of material in 5 $m\ell$ of McIlvaine buffer, pH 5, and 1 $m\ell$ of 37% aqueous formaldehyde. After 20 min the solution was diluted to 25 $m\ell$ with water and the wave at −0.98 V (presumably due to an oxidized nitrogen derivative) measured.

Heterocyclic compounds containing three nitrogen atoms are also reducible at the DME. The polarographic behavior of several 1,3,5-triazine herbicides has been investigated by Thompson.[111] He found that 4-amino-6-alkylamino-1,3,5-triazines were polarographically active in acidic solution, giving rise to a single wave at about −1.0 V vs. SCE. In solutions of pH 1, the limiting current was found to be linear with concentration up to 2×10^{-4} *M*. The limit of detection was 5×10^{-6} *M* with DC polarography and 2×10^{-7} *M* with single-sweep polarography. The polarographic behavior of prometryne, i.e., 2-methylthio-4,6-*bis*(isopropylamino)-1,3,5-triazine, was similar in all respects to that of triazines unsubstituted at the 2-position. The 2-chloro- and 2-bromo-4,6-*bis*(alkylamino)-1,3,5-triazines were found to be polarographically active at pH values between 1.5 and 5.5, each giving rise to two waves. Detection limits were 3×10^{-6} *M* with DC and 2×10^{-7} *M* with single-sweep polarography. 2-Azido-4-ethylamino-6-*tert*butylamino-1,3,5-triazine was active at all pH values, whereas the 2-methoxy-4,6-*bis*(alkylamino)-1,3,5-triazines were polarographically inactive at all pH values.

McKone et al.[112] compared gas chromatographic, polarographic, and spectrophotometric methods for the determination of terbutryne, ametryne, and atrazine in pond and canal water. The polarographic method involved extraction with dichloromethane, taking the residue up in 0.01 *N* sulfuric acid-50% methanol and running the linear sweep voltammogram from −0.8 V to the decay of the supporting electrolyte. In this medium, atrazine gave rise to a

wave at − 1.05 V (vs. mercury anode) and could be determined down to 0.01 μg $m\ell^{-1}$, whereas terbutryne and ametryne were both reduced at −1.45 V and could be determined down to 0.005 μg $m\ell^{-1}$. The polarographic method matched the GLC procedure in terms of time of analysis and ease of calculation of the results, but suffered a little in terms of sensitivity and selectivity.

Hernandez Mendez et al.[113] have carried out electroanalytical studies of the pesticide Menazon and its hydrolysis products. Menazon is *S*-[(4,6-diamino-1,3,5-triazin-2-yl)methyl],*O*,*O'*-dimethyl-phosphorodithioate, i.e., a combination of a thiophosphate and a triazine and will hydrolyze to 4,6-diamino-1,3,5-triazin-2-methyl-mercaptan and the corresponding thiophosphate. Menazon itself produces two diffusion-controlled cathodic waves at pH 4.78, due to fission of the carbon-sulfur bond joining the triazine and the thiophosphate, followed by saturation of an azomethine group in the triazine. The second peak at −0.8 V (vs. Ag/AgCl) can be used for DPP determination down to 5.5×10^{-7} *M*. The hydrolysis products obtained in 0.1 *M* sodium hydroxide give rise to four anodic waves, and when the pH is adjusted to 4.3, to two cathodic waves and one anodic wave. DPP can determine the hydrolysis products down to 0.17×10^{-6} *M* and thus is both a selective and sensitive technique for studying the hydrolysis of this pesticide in both in vitro and in vivo situations.

The vitamin riboflavin, which contains four nitrogen atoms in a tricyclic heterocyclic system, can undergo controlled adsorptive accumulation on the sMDE[114] and can thus be determined at the nanomolar concentration level. After 30 min preconcentration, a detection limit of 2.5×10^{-11} *M* has been found.[114]

7. Amines and Amides

Amines are not reducible at the DME, but can be oxidized at a variety of solid electrodes. The anodic oxidation of amines has been reviewed by Parker[115] and, in general, the process involves either formation of a double bond between the carbon and nitrogen atoms (for primary aliphatic amines) or dimerization (for aromatic amines).

Primary amines have thus been studied little by polarographic methods of analysis. In the determination of paracetamol (acetaminophen), presumably, using the oxidation of its phenolic group at a glassy carbon electrode (GCE), Shearer et al.[116] found that its major degradation product, *p*-aminophenol, could be determined in the presence of the parent compound in formulations, no doubt due to the operation of a different oxidation mechanism, i.e., formation of the quinoneimine.

Other primary amines of environmental significance that can be selectively determined by HPLC-ED using the oxidative mode can be grouped as follows:

1. Aromatic primary amines such as 1- and 2-aminoanthracenes contained in petroleum substitutes as potent mutagens. Judicious choice of the potential of the electrochemical detector can selectively determine them in the presence of other polycyclic aromatic hydrocarbons and polycyclic aromatic nitrogen heterocycles which are oxidized at more positive potentials.[117] Benzidine and 3,3'-dichlorobenzidine have been determined by HPLC-ED at sub-ppb levels in a 50-μℓ injection and with a 50 times better sensitivity than using UV detection.[118] Also included in this category are 2- and 4-amino substituted biphenyls, 1- and 2-substituted naphthylamines, 2,7-diaminofluorene and 6-aminochrysene.
2. Chlorinated anilines such as 2-, 3-, and 4-chloroaniline, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dichloroaniline, and 2,3,4-, 2,4,5-, 2,4,6-trichloroaniline, 2,3,4,5-, and 2,3,5,6-tetrachloroanilines and pentachloroaniline. Detection limits are of the order of 10 to 50 pg.

Purnell and Warwick have described such an HPLC-ED method for the simultaneous

determination of 4,4′-methylenebis(2-chloroaniline) (MOCA) and 2-chloroaniline (OCA) in factory atmospheres.[119] Tenax-GC was employed as the adsorbent in the sampling device to collect amine vapors and a filter used to collect particular amines. The electrochemical detector was found more selective and sensitive than the UV detector and MOCA and OCA could be determined down to 20 and 10 pg injected, respectively.

Determination of hydrazine, $NH_2{\cdot}NH_2$, and 1,1-dimethylhydrazine for use in industrial hygiene monitoring has been achieved by HPLC-ED after derivitization with salicylaldehyde.[120] Less than 5 ng of the resulting derivatives could be detected using electrooxidation of the phenolic group. The detection limits for hydrazine and 1,1-dimethylhydrazine solutions were estimated to be 0.025 and 0.20 ppm, respectively.

Costa Garcia et al.[121] have used HPLC-ED to monitor the anticancer drug, Methotrexate (4-amino-N^{10}-methylpteroyl-glutamic acid, MTX), which contains an amino-substituted nitrogen heterocyclic system, in the serum of patients undergoing treatment with this drug. The mobile phase was phosphoric acid-ammonia buffer (0.025 *M*) with 25% methanol, and the potential of the glassy carbon electrode was held at +1.10 V (vs. Ag/ACl).

Secondary amines are susceptible to nitrosation procedures, although these methods have generally found application only in formulation analysis. A recent paper, however, has dealt with the trace level determination of the herbicide glyphosate in natural waters.[122] The method involved cleanup of the water sample, and the preconcentration of the molecule on an ion-exchange column followed its nitrosation directly in the eluate. The limit of detection for the DPP determination of glyphosate was quoted as 35 ng $m\ell^{-1}$.

Tertiary amines are susceptible to *N*-oxidation procedures, and these have mostly been applied to formulation analysis. The use of *N*-oxidation derivitization in trace analysis may decrease the selectivity of a polarographic assay if *N*-oxidation is also shown to occur on metabolism. Schwartz and David[123] have recently published an interesting paper on the HPLC determination of opium alkaloids, such as heroin and cocaine, using electrochemical detection at +1.20 V (vs. Ag/Ag Cl). The probable mechanism is *N*-oxidation of the aliphatic tertiary amine groups contained in these molecules. The quoted detection limits are 0.3 ng (morphine), 1 ng (heroin), and 2 ng (cocaine).

Wang et al.[124] have investigated selective voltammetric detection based on adsorptive preconcentration for flow injection analysis (FIA). They have found that the technique is highly selective, compared with conventional amperometric detection, due to combining the intimate contact between the surface-bound species and the electrode surface with the medium exchange procedure. The increased selectivity thus obtained is demonstrable by the determination of the tertiary amine, chlorpromazine, in a 10^2-fold excess of nonadsorbable solution species with similar redox potentials. Enhanced sensitivity, as examined by differential pulse quantitation, is obtained as a result of the preconcentration step. At a flow rate of 0.3 $m\ell$ min^{-1}, injection rates of 24 samples hr^{-1} and detection limits of a few nanograms are obtainable. Reproducible quantitation of chlorpromazine in urine was found possible with no sample treatment. Belal et al.[125] have used oxidative amperometric detection at a carbon fiber array electrode to determine three phenothiazines (perphenazine, triflupromazine hydrochloride, and fluphenazine dihydrochloride) by FIA down to fractions of a nanogram.

Quarternary ammonium moieties that are conjugated to an aromatic ring system can be reduced at the DME in accordance with:

$$R\text{–}\overset{+}{N}H_3 + 2e^- + H^+ \rightarrow R\text{–}H + NH_3\uparrow$$

This last mechanism has been shown to be responsible for the first portion of the first wave exhibited by tetracycline hydrochloride in acidic media[126] and has also been postulated as contributing to the electrochemical behavior of the pesticide picloram.[105] The analysis of this compound has been dealt with in the earlier section on heterocyclic nitrogen-containing

molecules. Quarternary ammonium salts are also strongly adsorbed at the DME and can thus produce peaks on application of DPP. These salts are also used in polarographic analysis to extend the usable potential range on the cathodic side to −2.50 V (vs. SCE) and to decrease the background current in DPP.[86]

The amide linkage, –CO–NH–, is generally polarographically inactive unless it is incorporated into a ring system such as for the barbiturate class of drugs. These compounds give rise to anodic waves both at the DME and at solid electrodes.[127] In the former instance, the process was shown to involve mercury salt formation, but this behavior has not been recommended for the determination of these compounds at the trace level.[128]

8. Isocyanates

The properties of isocyanates that form polymers are widely exploited in industry, particularly in the manufacture of upholstery products, paint, varnishes, printing inks, and adhesives. Organic isocyanates are, however, respiratory irritants and sensitizers and, consequently, a large number of industrial personnel are potentially at risk to occupational isocyanate exposure. Various HPLC methods have been published in recent years for the measurement of isocyanate vapors at trace levels in air. Of these, those methods with UV detection are the more established, but recently fluorescence and electrochemical detection methods have also been reported. In all of these methods the isocyanates are collected in 10 mℓ of a suitable derivitizing solution contained in a bubbler or impinger in order to stabilize them prior to HPLC separation. 1-(2-Methoxyphenyl)piperazine has been used by Warwick et al.[129] to derivitize free monomeric aromatic and aliphatic isocyanates after their collection from 101 air samples. Using a 10-μℓ injection volume, a column packing of Hypersil ODS (150 × 4.5 mm i.d.), a mobile phase of acetate buffer (pH 6) — 4% acetonitrile and electrochemical detection at +0.80 V (vs. Ag/AgCl), a detection limit of 0.2 μg m^{-3} for the determination of phenyl isocyanate, toluene diisocyanate, hexamethylene diisocyanate, and 4,4′-diisocyanate-diphenylmethane in air was calculated.

Meyer et al.[130] have used *p*-aminophenol to derivitize toluene diisocyanate during a collection step from air prior to HPLC-ED in the oxidative mode at a Kel F graphite composite electrode. Whereas the recovery of toluene diisocyanate from the air samples was found comparable to that reported by other methods, i.e., 75 to 80%, the chromatographic step was characterized by a detection limit of 94 pg of toluene diisocyanate injected, a linear working range to beyond 12 ng of toluene diisocyanate injected, and a relative standard deviation of <2%.

B. Sulfur-Containing Molecules

1. Disulfides and Dithiocarbamates

The disulfide linkage exists in many endogenous molecules and is directly reducible at the dropping mercury electrode (DME) in accordance with the overall reaction

$$R–S–S–R + 2e^- + 2H^+ \rightarrow 2R–SH$$

Those naturally occurring molecules which have been determined using polarographic methods of analysis include the cofactors oxidized glutathione[131] and lipoic acid[132] and the hormone vasopressin. Those exogenous molecules which contain this linkage and that have been studied most by polarographic methods of analysis are the thiuram disulfides. Thiram, i.e., tetramethylthiuram disulfide, $(CH_3)_2NC(=S)SSC(=S)N(CH_3)_2$, is an active fungicidal agent. Brand and Fleet[133] have studied the effect of concentration of the limiting current values of the two DC polarographic waves exhibited by thiram. They have shown that whereas the height of the first wave, i_1, is linearly dependent on concentration, that of the second wave, i_2, is independent of concentration in the range from 10^{-3} to 10^{-4} *M*. Wave i_1 was shown

to be diffusion controlled, whereas i_2 was attributed to the reduction of the molecule while in an adsorbed state. The total wave height, i.e., $i_1 + i_2$, was found to correspond to $2e^-$ process, and the mechanism can be attributed to the addition of mercury across the disulfide linkage followed by a $2e^-$ reduction to give $(CH_3)_2NC(=S)SH$. Brand and Fleet[133] have also investigated the application of cathodic stripping voltammetry (CSV) to the determination of thiram in aqueous solution. They reported that the best results were obtained by using a mercury-plated platinum electrode in a solution of thiram containing an excess of ascorbic acid. This had the effect of chemically reducing the disulfide moiety in thiram to form free –SH groups, which could then be determined using CSV. Preelectrolysis was carried out at −0.10 V vs. a saturated calomel electrode in order to remove the interference caused by the oxidation of "unreacted" ascorbic acid and or residual Cl^- ions in the buffer system. By using this method they were able to determine thiram down to 10^{-8} *M* in pure solution. This method had an increased sensitivity over the DC, linear sweep voltammetric (LSV) or AC techniques, which have limits of detection for this molecule of 6×10^{-6}, 3.5×10^{-6}, and 1×10^{-6} *M*, respectively. It is also likely to be more sensitive than the spectrophotometric or infrared attenuated total reflection spectroscopic methods commonly employed for the determination of this compound in environmental situations.

Disulfiram, tetraethylthiuram disulfide, is employed both as an antioxidant in rubber and as a drug to treat alcoholism. This compound has been studied extensively using DC, AC polarography, LSV, normal pulse polarography (NPP), differential pulse polarography (DPP), and cyclic voltammetry (CV). Its mechanism of reduction is the same as for tetramethylthiuram disulfide. Prue et al.[134] studied the NPP behavior of tetraethylthiuram disulfide, both at the DME and at a rotating graphite electrode (RGE). The wave obtained at −1.24 V (vs. SCE) at the RGE (−0.50 V at the DME) showed a linear dependence on concentration between 1.2×10^{-4} and 2×10^{-6} *M* and has found application in formulation analysis. DPP can be used to determine concentrations of this molecule down to 5×10^{-7} *M*.[135]

The metabolic fate of tetramethyl- and tetraethyl-thiuram disulfide has been shown to involve the steps shown below. Electrochemical methods, therefore, offer a convenient method for the determination of these molecules in the presence of their main metabolic products, as the dimethyl- or diethyl-dithiocarbamate metabolites will give rise to anodic waves at the

$$CS_2\uparrow \xleftarrow{\text{pH 5}} (CH_3)_2\,NC(=S)\,SSC(=S)\,N(CH_3)_2 \xrightarrow{2GSH} 2(CH_3)_2\,NC(=S)\,SH + GSSG$$

(where GSH is reduced glutathione)

$$(C_2H_5)_2\,NC(=S)\,SSC(=S)\,N(C_2H_5)_2 \rightarrow (C_2H_5)_2\,NC(=S)\,SH + (C_2H_5)_2\,NH + CS_2\uparrow$$

DME (see below in this section), whereas carbon disulfide can be determined either directly using oscillopolarography[136] or indirectly following complexation with Cu(II) ions.[137] This latter procedure can also be used for the indirect determination of tetraethylthiuram disulfide in blood following the liberation of carbon disulfide by heating in the presence of 50% sulfuric acid.

The polarographic behavior of the dithiocarbamate anion has been studied extensively by several authors. Stricks and Chakravarti[138] have showed that the anodic process observed for these compounds involved

$$R_2NC(=S) \cdot S^- + Hg^0 \rightleftharpoons R_2NC(=S) \cdot S \cdot Hg + e^-$$

$$2\ R_2NC(=S) \cdot S \cdot Hg \rightarrow [R_2NC(=S)\ S]_2\ Hg + Hg^0 \qquad \text{(UE17)}$$

The adsorption processes that accompanied this reaction were thought to be due to the orientation of the adsorbed species at the surface of the mercury. Halls et al.[139] have studied this process in greater detail and have also reported a method to determine mono- in the presence of dialkyl-substituted dithiocarbamates. This method was based on the fact that the monosubstituted compound is relatively stable in solutions of pH between 3.5 and 5.0, whereas the disubstituted compound breaks down to give carbon disulfide. They also showed that the addition of a surface-active agent, such as gelatin, improved the wave shape for analytical purposes. Canterford and Buchanan[140] have applied DPP to the determination of sodium diethyldithiocarbamate (NaDTC) and have quoted a detection limit of 2×10^{-6} *M*. Brand and Fleet[141] reported a detection limit for NaDTC of 10^{-6} *M* using both AC and LSV techniques. The AC polarography of NaDTC showed two waves in the concentration range 10^{-3} to 10^{-4} *M* at -0.4 and -0.65 V, respectively. The first wave disappeared in solutions of concentration less than 10^{-4} *M*. These authors also reported a CSV method of analysis for this compound at a mercury-plated platinum electrode. The curve relating peak current to concentration proved to be linear in the range 10^{-6} to 10^{-7} *M* and the use of a mercury-plated platinum electrode was preferred to the hanging mercury drop electrode (HMDE), because it was not affected by the rate of stirring or by spurious vibrations.

The polarographic behavior of the ethylene 1,2 *bis*dithiocarbamate (EDC) anion $^-SC(=S)NH(CH_2)_2NHC(=S)S^-$ has been studied by Brand and Fleet[142] using DC, AC, and CSV techniques. This compound exhibited four waves in DC polarography and three waves in AC polarography. CSV at a mercury-plated platinum electrode yielded three peaks, at -0.4 V (corresponding to the EDC anion), -0.7 V (corresponding to ethylenethiuram monosulfide), and -1.05 V (due to sulfide ions). These workers obtained a linear relationship between peak current at (-0.4 V) and concentration in the range from 10^{-6} to 10^{-7} *M* following preelectrolysis for 2 min at 0.00 V (vs. SCE) and then scanning at 100 mV sec^{-1}. Halls et al.[143] have also studied the polarographic behavior of this molecule and have shown that the DC polarographic wave shape could be improved by the addition of ethanol or dimethylformamide (DMF). The addition of dimethyl sulfoxide (DMSO), acetonitrile (AN), or gelatin had little or no effect on the process.

Nangniot[144] has applied DC polarography and LSV to the determination of three metal-containing dithiocarbamate pesticides, viz., ziram (zinc dimethyldithiocarbamate), zineb (zinc ethylenebisdithiocarbamate), and ferbam (iron [III] dimethyldithiocarbamate). In a 0.5 *M* ammonia — 0.5 *M* ammonium chloride supporting electrolyte solution ziram gave rise to an anodic wave at -0.41 V as a result of the oxidation of dimethyldithiocarbamic acid, whereas in 0.2 *M* sodium hydroxide solution it gave rise to a cathodic wave at -1.42 V due to the reduction of Zn(II) ions. Nangniot reported that in residue determinations the oxidation wave due to dimethyldithiocarbamic acid was affected by interferences in the matrix and suggested that residue analyses be carried out in the latter supporting electrolyte. In this medium (0.2 *M* sodium hydroxide solution), ziram could be determined down to 5 $\mu g\ m\ell^{-1}$ using LSV. Nangniot also reported detection limits of 1.0 and 0.2 $\mu g\ m\ell^{-1}$ for zineb and ferbam in 0.1 *M* sodium hydroxide solution and 0.5 *N* potassium chloride solution — 0.5 *N* sodium citrate solution — 50% acetone, respectively.

An indirect pulse polarographic method has been employed for the determination of zineb on tobacco leaves.[145] The zineb was degraded by the addition of hydrochloric acid-nitric acid, the mixture diluted with water and polarography carried out in 0.5 *M* sodium perchlorate solution, pH 2. The wave used for analytical purposes was caused by the reduction of Zn(II) ions. Halls et al.,[146] on the other hand, have suggested a method for the determination of

metal-containing dithiocarbamates based on liberation of the metal from the complex and subsequent determination of the anodic wave given by the dithiocarbamate anion. This method could be used to determine concentrations of zineb down to 0.3 μg $m\ell^{-1}$ wet mass in vegetable tissue.

Perhaps the most sensitive voltammetric method available for the analysis of these compounds is based on anodic stripping, either of the complex or of the metal ions liberated following destruction of the complex. This is illustrated by the work of Sauberlich et al.,[147] who have been able to determine ferbam down to 0.5 ng $m\ell^{-1}$ in pure solution.

2. Compounds Containing Carbon-Sulfur Linkages

a. Those Incapable of Tautomerization to Thiols

Compounds containing the C=S bond (other than the dithiocarbamates) are generally reduced at the DME. With the heterocyclic compound 4-mercaptocinnoline, Lund[148] showed that the initial reductive step involved saturation of the C=S double bond. This was followed by both protonation and slow chemical steps, which resulted in the elimination of hydrogen sulfide. A similar process can be shown to occur for 4-mercaptoquinazoline, 2,7-dimercaptopurine, and 4-thiouracil.

b. Those Capable of Tautomerization to Thiols

Compounds in this category can be oxidized at the DME; they generally give rise to waves resulting from the anodic depolarization of mercury. As a result, these compounds can be determined either by conventional polarographic methods or by CSV. If the mechanism also involves formation of a reducible group, e.g., $\rangle C{=}N-$, the reduction waves can also be used for analytical purposes.

3. Thiobarbiturates and Thioureas

The DC polarographic behavior of thiobarbiturates has been extensively studied by Smyth et al.[23,149,150] These compounds give rise to both anodic and cathodic waves at the DME, with the mechanisms of oxidation/reduction being greatly dependent on the pH and the nature of substitution in the molecule. The cathodic waves obtained in 0.05 *M* borate buffer, pH 9.3, were recommended for analytical purposes, because they were found to be operative over a wider range of concentrations than the corresponding oxidation processes and the height of the waves could be measured with greater accuracy. Smyth et al.[128] have reported that DPP can determine concentrations of thiobarbiturates down to 1×10^{-7} *M* in Britton-Robinson (BR) buffer, pH 8.0.

The polarographic behavior of thiourea (TU) has been investigated by several authors and it is generally agreed that this compound is ozidized as follows:

$$Hg \rightleftharpoons Hg^{2+} + 2e^-$$

$$Hg^{2+} + j[TU] \rightleftharpoons Hg[TU]_j^{2+}$$

where j = 2, 3, or 4. Smyth and Osteryoung[151] have also investigated the polarographic behavior of phenylthiourea (PTU) and α-naphthylthiourea (ANTU) and have shown that DPP can to determine concentrations of TU and PTU down to 1×10^{-7} *M* and of ANTU down to 2×10^{-7} *M* in 1 *M* sodium hydroxide solution as supporting electolyte. In addition, DPP is able to differentiate between TU, PTU, or ANTU and benzyl(iso)thiourea (BITU) in this supporting electrolyte. As the mechanism for TU, PTU, and ANTU involves the formation of an insoluble mercury complex, these compounds can also be determined by CSV. Although CSV can determine concentrations of TU, PTU, and ANTU down to 1 ng $m\ell^{-1}$, it was unable to differentiate between them in a mixture.

Thiourea can also be determined voltammetrically following complexation with Cu(II) ions or by liberation of the sulfur atom and subsequent determination as hydrogen sulfide. The latter procedure can be used for the determination of other sulfur-containing pesticides, for example, rogor, diazinon, and phenkapton, and utilizes reduction of the pesticide by aluminum in solution in hydrochloric acid (in the presence of nickel). The hydrogen sulfide evolved was then determined by monitoring the decrease in Pb(II) concentration in the lead acetate trapping solution. This method can determine down to 0.25 $\mu g\ m\ell^{-1}$ of TU in pure solution. A polarographic method of analysis has also been applied to the determination of ethylenethioureas in food.[152] The method involved extraction with chloroform-ethanol (9 + 1), cleanup on an alumina column, separation by paper chromatography, and nitrosation to give a polarographically active moiety. The percentage recovery was found to be about 70% and the method was used to determine concentrations of 1,3-ethylenethiourea between 0.05 and 2 $\mu g\ m\ell^{-1}$ in potatoes, tomatoes, meat, and liver.

4. Thioamides

The polarographic determination of ethionamide in in vivo samples has been reported by several authors.[153,154] Okuda[153] used DC polarography to show that whereas ethionamide exhibited both anodic and cathodic waves in spiked rabbit urine samples, these were replaced by two new cathodic waves in in vivo samples corresponding to amide and carboxylic acid metabolites as shown:

$$RC(=S)\ NH_2 \rightarrow RC(=O)\ NH_2 + RC(=O)\ OH$$

Ethionamide has also been shown to undergo further metabolic processes, for example, sulfoxidation, *N*-methylation, and dealkylation, which have been monitored by using AC polarography.[154] In this example a single polarographic scan showed good resolution between the parent compound and its seven metabolites.

Davidson and Smyth[32] have investigated the polarographic behavior of several thioamides of pharmaceutical importance. These compounds give rise to anodic waves at the DME according to the following mechanism:

$$RC(=S)\ NH_2 + Hg + H_2O \rightarrow RC(=O)\ NH_2 + HgS + 2H^+ + 2e^-$$

They have also described a differential-pulse cathodic stripping voltammetric (DPCSV) method of analysis which is specific for the parent thioamide and that can be employed directly in plasma and urine down to $2 \times 10^{-8}\ M$. Naturally occurring substances such as glutathione, thiamine, methionine, cystine, cysteine, and hydrogen sulfide, and anionic species such as Cl^-, were shown not to interfere. The results of this investigation were shown to be accurate, with a precision of 2.9% at the $5 \times 10^{-7}\ M$ level, and the method was relatively simple and rapid to perform.

5. Thiols

Thiols also produce anodic waves at the DME owing to the formation of insoluble mercury complexes. This process has been investigated by Peter and Rosset[155] who have suggested that at $pH < pK_a$ the mechanism involves

$$Hg + 2RSH \rightleftharpoons Hg(RS)_2\downarrow + 2e^- + 2H^+$$

and at $pH > pK_a$

$$Hg + 2RS^- \rightleftharpoons Hg(RS)_2\downarrow + 2e^-$$

These workers also reported a detection limit of 10^{-7} *M* for thiols using pulse polarography in which a negative pulse was applied. In effect, the analytical method was based on the reduction of the thiolate produced at the surface of the mercury during the lifetime of the drop. Much lower limits of detection may be achieved, however, using a CSV method of analysis. This is illustrated by 2-mercaptopyridine 1-oxide, which can be determined down to 8×10^{-10} *M* in pure solution[40] and cysteine, with a detection limit of 10^{-9} *M* in 0.1 *M* $HC10_4$ or 0.1 *M* H_2SO_4.[35]

An interesting paper appeared recently[156] on the CSV determination of trace amounts of penicillins which could be of considerable value in trace penicillin determination in industrial environments and in pharmaceutical formulations. After conversion to the corresponding penicilloic acid by alkaline hydrolysis, accumulation is effected by a copper complex of penicillamine, which has a free thiol group. This complex is subsequently reduced at about −0.40 V in the stripping step. After accumulation for 10 min 2×10^{-10} *M* penicillin can be detected. D-penicillamine (*d*-2-amino-3-mercapto-3-methyl-butanic acid), used for the treatment of Morbus Wilson, metal intoxication, cystinuria, and rheumatoid arthritis, can be determined in plasma and urine following separation on a cation exchange resin and oxidative amperometric detection of the SH group at a gold indicator electrode.[157] The method has been automated and separations from plasma and urine take 7 and 9 min, respectively. The detection limits are 0.05 μg $m\ell^{-1}$ in plasma and 0.20 μg $m\ell^{-1}$ in urine, with a coefficient of variation of 2.9% (n = 10). Drummer et al.[158] have improved on this method by use of a high-efficiency C_{18} reversed-phase column and have been able to determine *N*-acetylcysteine, as well as penicillamine, with a detection limit of 25 ng $m\ell^{-1}$, using a 500-μℓ blood sample. Halbert and Baldwin[159] have determined cysteine and glutathione in plasma or blood by HPLC-ED using a chemically modified carbon paste electrode incorporating colbalt phthalocyanine with detection limits of less than 4 pmol. The electrocatalytic activity of these chemically modified electrodes permitted optimum response at a potential of +0.75 V (vs. Ag/AgCl), several hundred millivolts lower than that required at conventional carbon electrodes.

Thiols (and disulfides) can be simultaneously determined by using HPLC and thin-layer dual-mercury amalgam electrodes in the series mode.[160] After HPLC separation, disulfides were first converted to the corresponding thiols at an upstream electrode at −1.00 V (vs. Ag/AgCl). Both thiols and disulfides were then detected as thiols downstream at +0.15 V via their catalytic oxidation at a mercury surface. The specificity of this reaction aids a low level of interference. It has been applied to the determination of oxidized and reduced glutathione in human blood (1.1 m*M* GSH and 1.8 μ*M* GSSG) after a simple dilution and citrus leaf homogenate after filtration and ion exchange processes. This analytical method is much more rapid than enzymatic methods for specific GSH and GSSG levels.

6. *Thioethers*

Kok et al.[161] have employed postcolumn oxidation of thioethers such as $(CH_3)_2NCH_2-(C_4H_2O)-CH_2S-CH_2CH_2NHC(=CHNO_2)NCH_3$ by bromine, which is generated electrochemically in the column eluent. Detection was performed by amperometric measurement of the excess of bromine downstream at a platinum electrode after reaction with eluting thioethers. For the determination of ampicillin in plasma and urine a superior sensitivity and selectivity compared with the usual UV detection was shown. Plasma concentrations of 0.2 to 10 μg $m\ell^{-1}$ with a precision of ±6% could be obtained. Ranitidine could be determined in plasma after sample cleanup and concentration by liquid-liquid extraction with a limit of detection of 2 ng $m\ell^{-1}$.

7. *Thionitrites*

Takeuchi et al.[162] have studied the electrochemical behavior of *N*-acetyl penicillamine

thionitrite, $CH_3C(=O)NHCH(COOH)C(CH_3)_2SNO$, proposed as an intracellular intermediate in organic nitrate-induced mammalian vascodilation. They have found a reversible polarographic anodic wave at pH <6.0, rather similar to that given by penicillamine, and due to $RSNO + Hg + H_2O \rightleftharpoons RSHg + HONO + H^+ + e^-$. An irreversible reduction wave was observed on both carbon and mercury indicator electrodes due to $(RSNO)H^+ + e^- \rightarrow RSH + NO$. Linear calibration in the range 1 to 200 $\mu g \ell^{-1}$ was found using square wave voltammetry.

8. Oxides of Sulfur

S-Oxides can be encountered as metabolic products of some *S*-containing exogenous compounds and give rise to well-defined polarographic waves when the group is conjugated to an aromatic ring system. Polarographic methods exist for the determination of the *S*-oxide metabolites of ethionamide,[154] chlorpromazine,[24] and fluphenazine. In the instance of ethionamide *S*-oxide, AC polarography was used to determine the metabolite in the presence of the parent compound, which is itself electroactive.

With the phenothiazines, however, the parent compounds are not reducible at the DME and thus polarography can offer a specific method for the determination of their metabolic products. Beckett et al.[24] have shown that the *S*-oxide, *N*-oxide, and the *N*-oxide-*S*-oxide metabolites of chlorpromazine all reduce at the same potential (−1.07 V vs SCE in BR buffer, pH 5.0) and must, therefore, be separated prior to analysis. The method chosen involved a selective solvent extraction procedure shown in Beckett et al.[24] and the LSV method of analysis was found to determine concentrations of the metabolites down to 5×10^{-8} *M*. Although the phenothiazine nucleus is not directly reducible at the DME, it is susceptible to bromination, nitration, and oxidation procedures, which can be applied to the determination of the parent compound in formulations and body fluids.

9. Sulfones and Sulfonamides ($R\text{-}SO_2\text{-}R'$)

Sulfones are generally reduced only at negative potentials in solutions containing a tetraalkylammonium (R_4N^+) salt as the supporting electrolyte. With phenothiazine, the *S*-oxide and sulfone derivatives can be resolved polarographically as the sulfone is not reduced in acidic solution.

Sulfonamides are also reduced in aprotic media containing a tetraalkylammonium salt as the supporting electrolyte.[163] With arylsulfonamides, the mechanism involves cleavage of the S-N bond:

$$ArSO_2NHR' + 2e^- + 2H^+ \rightarrow ArSO_2H + R'NH_2$$

Sulfonamides containing a strongly electron-withdrawing group in the phenyl substituent can cleave, however, at the C-S bond:

$$p\text{–}XC_6H_4SO_2NH_2 + 2e^- + H^+ \rightarrow p\text{–}XC_6H_5 + \bar{S}O_2NH_2$$

The waves obtained for sulfonamides in aprotic media are usually well defined and have found application in formulation analysis. Fogg and Ahmed[164] have described an indirect method for their trace determination based on diazotization and coupling with 1-naphthol to form the corresponding azo derivatives. This procedure utilizes DPP in the determination step and can determine down to 5×10^{-8} *M* sulfanilamide, sulfathiazole, and sulfacetamide and 1.2×10^{-7} *M* of sulfaguanidine in pure solution.

10. Sulfonates

Sulfonate compounds can only be reduced in aprotic media and the waves obtained are

not generally recommended for trace analytical purposes. They also give rise to tensammetric waves in aqueous solutions (caused by desorption of the species at the mercury surface, thus affecting a change in the double-layer structure) which are manifested as sharp incisions in AC polarography and which can be used for quantitative purposes down to 10^{-6} *M*.

Sulfonate compounds can also be determined indirectly by their complexation with dyes such as methylene blue[165] or by nitration if they contain a phenyl moiety.[166] This procedure involves the nitration of microgram amounts of the alkylbenzenesulfonate (ABS) of interest with fuming nitric acid (0.1 mℓ) for 15 min at 30°C. The excess of nitric acid is blown off with nitrogen and the residue dissolved in 5 mℓ of BR buffer, pH 12.0. All of the sulfonates nitrated under these conditions showed only one peak, suggesting mononitration. The reduction potential of the straight-chain ABS molecules was found to occur in the region −0.66 to −0.67 V, branched-chain sulfonates between −0.72 and −0.74 V, and phenyl-containing alkanesulfonates between −0.73 and −0.74 V. A DPP scan of a mixture of the nitro derivatives of a straight-chain ABS (sodium 4-pentadecylbenzenesulfonate) and of a branched-chain ABS (sodium 4-[1-hexyl]heptylbenzenesulfonate) showed a degree of resolution between the two compounds. Alkanesulfonates with a phenyl ring, however, gave reduction peaks at the same value as branched-chain ABS molecules and differentiation between the two was not possible. Sulfonates without a phenyl ring were found not to give nitro derivatives. Calibration graphs were found to be linear in the region 5 to 50 μg. The coefficient of variation at the lower end was 8.25% and at the upper end 7.90%. This method is potentially applicable to for the trace determination of these surfactants in effluents.

A polarographic method has also been described for the determination of the anionic surfactants sodium dodecylsulfate and sodium dodecylbenzenesulfonate in surface water.[167] DPP has also been used for the determination of the lignin sulfonic acids in natural waters.[168]

C. Oxygen-Containing Molecules

1. Aldehydes and Ketones

Aldehydes and ketones can either be reduced or oxidized at the DME or can serve to influence the reduction of a $\rangle C{=}C\langle$ double bond to which the carbonyl function is conjugated.

Aliphatic aldehydes are reduced according to

$$RCH(OH)_2 \underset{}{\overset{-H_2O}{\rightleftharpoons}} RCHO \overset{+1e^- + 1H^+}{\rightleftharpoons} \dot{R}\dot{C}HOH$$

$$\xrightarrow{+1e^- + 1H^+} R\bar{C}HOH \xrightarrow{+H_2O} RCH_2OH + OH^-$$

and the process is governed by the rate at which dehydration to the free aldehyde can take place. There are direct polarographic methods for the determination of the simple aliphatic aldehydes, such as formaldehyde, acetaldehyde, and butyraldehyde, in wastewaters with limits of detection in the low μg $m\ell^{-1}$ region.

The use of derivitization of the carbonyl group has been exploited by Afghan et al.[169] in a systematic study of aldehydes and ketones in various supporting electrolytes, using twin-cell potential sweep voltammetry. The formation of the semicarbazone was found to be most satisfactory. The formation of the semicarbazone is optimized at pH 4 to 6 to ensure that a significant fraction of the carbonyl compound is protonated and, at the same time, to ensure that the concentration of the protonated amine remains low. This ensures optimum reaction to form the semicarbazone. Excess of the electroinactive semicarbazide is added to ensure complete reaction: using a citrate buffer with EDTA added to complex interfering heavy

metals, a limit of detection of 0.25 ng $m\ell^{-1}$ was claimed for the determination of some carbonyl compounds present in natural waters and industrial effluents without any separation or preconcentration of the sample. The method was, however, unable to differentiate between many aliphatic and aromatic aldehydes and ketones. A recent paper has improved upon the selectivity of this method by the use of reversed-phase HPLC and UV detection at 360 nm. Vigh et al.[170] have achieved resolution and identification of the 2,4-dinitrophenyl hydrazone derivatives of a range of aldehydes and ketones.

The reversibility exhibited by aliphatic carbonyl compounds on the first electron-consuming step results in an enhancement of the waves obtained for those compounds during AC polarography. This has been utilized by Fleet et al.[171] for the determination of mesityl oxide in the presence of diacetone alcohol, using a fast-scan, phase-sensitive AC technique.

Aromatic carbonyl compounds are irreversibly reduced at the DME in either one- or two-electron processes.[64] Well-defined waves of analytical importance have been obtained for several compounds of biological significance. Polarography can be recommended for the determination of furfural in alcoholic beverages. Compounds related to benzophenone are commonly encountered in the analytical chemistry of the 1,4-benzodiazepines,[92] and in the case of bromazepam, de Silva et al.[172] have used DPP in order to determine urinary benzophenone metabolites in the presence of metabolites that retain the basic seven-membered ring structure. Meshkova et al.[173] utilized the adsorption properties of some aromatic aldehydes and ketones for their determination at a stationary mercury drop. This procedure involved accumulation of the adsorbed species at the electrode surface (at -0.30 V vs. SCE in 0.1 *M* lithium chloride solution-30% ethanol) and subsequent measurement of the stripping peak. The peak obtained at -1.29 V for anthraldehyde was used to determine this compound down to 10^{-7} *M* in pure solution.

In devising a polarographic method of analysis for carbonyl compounds of biological significance, it is a general rule that the waves obtained for these compounds will lie in a more analytically usable range of potential with an increasing degree of conjugation in the molecule. It is important, however, to identify the nature of the electrode reaction in compounds where the $\rangle C{=}O$ group is conjugated to a π-bond system, as there are instances where the carbonyl function is reduced, e.g., haloperidol; those in which the adjacent $\rangle C{=}C\langle$ bond is reduced, e.g., 3-keto steroids with 4,5-unsaturation; those in which both functions are reduced, e.g., cinnamaldehyde; and compounds where a mixture of products is obtained, e.g., mesityl oxide.[171] With the tetracycline antibiotics, part of the complicated electrochemical reduction of this molecule species is thought to be caused by reduction of the keto group in ring A of the molecule.[126] The polarographic behavior of these drugs was further investigated by Smyth et al.[174] in relation to their complexation properties with metal ions. Significant changes were observed in the presence of Cu(I) and Fe(III) ions and these observations were interpreted as being due to the binding of these cations to ring A in the molecule. No changes in polarographic behavior were observed in the presence of divalent ions, eg., Ca(II) and Mg(II), which are known to complex with the diketone system in rings B and C of the molecule. This has been taken as further evidence to support the conclusion that the polarographic behavior of tetracycline is the result of processes occurring in ring A of the molecule.

The combination of photoelectrochemical detection (PED) with HPLC and FIA has recently been investigated by La Course et al.[175] for the determination of a series of carbonyl compounds. They have designed a flow-through, thin-layer amperometric cell modified to irradiate the working electrode surface and so take advantage of new and/or altered electrochemical properties of photogenerated excited states, intermediates, or products. PED has been found responsive to alkyl and aryl ketones and aldehydes, i.e., to compounds that

undergo n-π^* excitation as opposed to π-π^* excitation. Response is linear over 3 to 4 orders of magnitude and minimum detection limits for conjugated carbonyl derivatives are in the range 2 to 10 ng, comparable to UV detection.

The keto-enol equilibrium plays an important role in redox systems in the body and there are many published methods for the determination of compounds such as ascorbic acid in body fluids and food products. In general, the quinone-hydroquinone system undergoes a quasi-reversible process at the DME,[64] but it is usually the oxidation process that is monitored for analytical purposes. The DPP behavior of ascorbic acid has been studied by Lindquist and Farroha[176] and the determination of ascorbic acid in food products has been dealt with by several authors. Owen and Smyth[177] have reported that the LSV method of analysis for this molecule in various food matrices showed a large degree of tolerance towards suspended matter. Kajita et al.[178] measured the levels of ascorbic acid and dehydroascorbic acid in mixtures. The determination of the latter molecule was accomplished by condensation with *o*-phenylenediamine. Ascorbic acid can also be determined at a carbon-paste electrode[179] and Thrivikraman et al.,[180] among others, have used a combination of HPLC with electrochemical detection at such an electrode in order to determine ascorbic acid in brain tissue.

2. Phenolic Compounds

Phenols are not reducible at the DME, but do exhibit anodic waves at a variety of solid electrodes.[181] Of particular analytical significance is the work of Kissinger and co-workers, who have been able to determine subnanogram amounts of biologically important compounds (both pharmaceutical products and neurochemically active molecules such as DA, NE, HVA, DOPAC, 5-HIAA, 5-HT, etc.) in body fluids following HPLC separation and electrochemical detection at electrodes such as the semimicro carbon-paste electrode. These studies and applications have been reviewed in the literature.[182,183] Goto et al.[184] have designed a dual electrochemical detector having two working electrodes (anode and cathode) suitable for HPLC using microcolumns for the selective detection and determination of catecholamines on the basis of their electrochemical reversibility. Catecholamines have been determined in human urine injected directly into a micro high performance liquid chromatograph with an alumina preconcentration microcolumn. Oxidation waves obtained for other phenolic compounds at solid electrodes have been used for their determination, for example, the oxidation of the hydroxyl group in vitamin E has been used in formulation analysis.[185]

Until the aforementioned developments in electrochemical detection for use with HPLC, most polarographic methods for the determination of phenolic compounds were based on prior derivitization procedures. In particular, the use of nitration has found many applications in the determination of these compounds in biological fluids. For the determination of phenol in water, the method involves nitrating a concentrated diethyl ether extract of water at 90°C for 1 hr by using a mixture of 13 *M* nitric acid and 18 *M* sulfuric acid. The derivative was then taken up in a BR buffer and determined by use of either superimposed AC or derivative-pulse polarography. The latter technique was found to be the most sensitive and the method can be applied to the determination of a wide range of phenol concentrations. In this instance a nitrosating nitration procedure was employed, although other phenolic compounds containing a vicinal –OH group were found to interfere with the analysis. Pasciak and Gajewska[186] have shown that in the presence of ethanol, *p*-nitrosophenolic compounds may be formed (owing to the action of ethyl nitrite). In such derivitization procedures it is necessary, therefore, to identify the product(s) of the reaction and to separate compounds of related structure which may interfere with the analysis.

Armentrout et al.[187] have recently selectively detected individual phenolic compounds, including the more toxic halogenated ones, at low ppb levels using HPLC with a polymeric cation exchange resin column, acidic acetonitrile/water eluent, and an electrochemical elector containing a unique carbon-black/polyethylene tubular anode. The detection limits (0.2 pbb

for phenol to 0.7 ppb for pentachlorophenol) compare more than favorably with those obtained by temperature-programmed GLC and electron capture detection of heptafluorobutryl derivatives of phenols and HPLC-UV detection of nitrophenols, solvent extracted by CH_2Cl_2 from water samples.

Water/ethanol extracts from plant material are used extensively to analyze a wide range of secondary plant metabolites. These extracts can be used as such or some subfraction can be prepared for analysis with a suitable technique such as HPLC. Such plant extracts are very complex and the chromatograms contain numerous peaks with appreciable peak overlap. Nagels et al.[188] have found single electrode electrochemical detection two to five times more selective than UV detection and that dual electrode parallel electrochemical detection can reduce detection limits by a factor up to 5 for phenolics with high oxidation potentials. Kafil et al.[189] have used HPLC with a scanning coulostatic electrochemical detector to separate, help identify, and determine a mixture of ten phenolic acids at the sub-nmol level.

HPLC-ED has also been recently used for the determination of new drug molecules containing phenolic groups at trace levels in body fluids, i.e., potential antianginal and antihypertensive drugs, 4-(2-di-*n*-propylaminoethyl)-7-hydroxy-2-(3H)-indolone and *N*-[2′-hydroxy-5′-(*N*,*N*-di-*n*-propylaminoethylphenyl)] methane sulfonamide,[190] and analgesics, ciramadol, and dezocine.[191]

Preconcentration and quantitation of the cancer chemotherapy drug, doxorubicin, have been achieved by a flow injection approach utilizing adsorption of the drug onto a carbon paste electrode, medium exchange, and differential pulse voltammetry on the adsorbing surface.[192] The oxidation peak at +0.57 V (vs. Ag/AgCl), which no doubt corresponds to oxidation of the aromatic hydroxy group(s) in the fused ring system, was used for quantitative purposes. Linear response was obtained for concentrations from 10^{-6} *M* to the detection limit of 10^{-9} *M*, and it was possible to determine the drug in urine by direct injection of the sample with no preliminary steps. The sensitivity, linearity, and selectivity of the FIA approach were markedly improved relative to a batch method in which the adsorption step was carried out in a stationary sample solution.

3. Organic Acids, Alcohols, and Sugars

The polarographic behavior of organic acids has been well documented in standard texts. In general, most carboxylic acids are reduced at relatively negative potentials (from −1.50 to −1.80 V vs. SCE). The main exceptions to this rule are those compounds which contain two carboxylic groups joined by ethylenic linkage, for example, maleic and fumaric acids and the pyridine carboxylic acids. There are relatively few instances, therefore, in which polarographic methods have been applied to the determination of biologically important compounds containing this linkage, unless these compounds also contain another electroactive center, which gives rise to waves more suited to trace analysis. In addition, derivatization procedures can be employed, for example, in the determination of mandelic acid in urine (based on formation of benzaldehyde by a back-distillation procedure) and 2,4-dichlorophenoxyacetic acid in blood and urine (following nitration). In the latter instance, interfering substances in the chloroform extract must be removed by thin-layer chromatography (TLC) prior to the method being applied in toxicological situations.

Alcohols are not directly amenable to the polarographic analysis unless they contain another functional group that is electroactive; for example, 2,3-dimercaptopropanol (known more commonly as British Antilewisite, BAL) can be determined in blood serum by a method based on monitoring the oxidation of the –SH groups in the molecule. Other polarographic methods have also been described for some biologically important alcohols.[193]

Sugars give rise to kinetic waves at relatively negative potentials (from −1.50 to −1.70 V), but the i_{lim} values of these waves are generally too small to be considered suitable for trace analysis. They are amenable, however, to nitration procedures, and as such have been

shown to interfere with the nitration of other compounds when present as impurities in the nitration mixture, e.g., 4-hydroxybenzoic acid.

4. Carbamates

The reduction of carbamates at negative potentials can be investigated by polarography after derivitization via nitration or nitrosation, and recently Anderson and Chesney[194] have applied their little-investigated oxidation reactions to a reverse phase liquid chromatographic method with thin layer Kel-F-graphite electrochemical detection operated in the constant potential amperometric mode at +1.10 V (vs. Ag/AgCl). Calibration curves were linear over at least three orders of magnitude with relative standard derivations of 1 to 2%. Detection limits of 2 to 7 ng $m\ell^{-1}$ were obtained and the method compared favorably with GLC, with electron capture detection following hydrolysis to the corresponding phenols or amines and reaction with halogen-rich reagents. The electrochemical detector, in this case, was found more sensitive than both the UV detector operated at 190 to 210 mm, and the fluorescence detector in which dansyl derivatives were formed prior to injection or postcolumn derivitization carried out with *o*-phthalaldehyde.

Anderson et al.[195] have further investigated the application of HPLC-ED for the determination of trace concentrations of carbamates in river water by the use of microarray electrochemical flow detectors operating at high applied potentials. These Kelgraf micorarray detectors effectively discriminated between oxidation reactions limited by the rate of mass transport and reactions (including solvent oxidation) limited by the rate of electron transfer or other surface processes and thus can afford improved detection limits at high applied potentials. Sub-ng detection limits were obtained for cabamates, Aminocarb, Carbendazim, and Desmedipham which have the general structure RNHC(=O)OR′.

D. Hydrocarbons (Containing Carbon-Carbon Multiple and Carbon-Halogen Bonds)

The carbon-carbon double bond is generally very difficult to reduce (polynuclear hydrocarbons are reduced between −2.00 and −2.50 V [vs. SCE] in 0.175 *M* tetrabutylammonium iodide [TBAI] — 75% dioxan and hence there have been relatively few examples in which voltammetric methods have been applied to the determination of compounds containing this bond). Relationships do exist, however, between $E_{1/2}$ values and various physical parameters, for example, energy of the lowest vacant molecular orbital, and studies of this nature may aid in an understanding of the carcinogenicity of several members of this series.

Compounds containing a C=C bond conjugated to a carbonyl moiety are reduced at more positive potentials. In the instance of 3-keto steroids with unsaturation between C4 and C5, e.g., cortisone and testosterone, well-defined DPP peaks are obtained for the reduction of the C=C double bond at −1.77 and −1.84 V (vs. SCE), respectively, in 0.1 *M* TEAP — 50% methanol. Adsorptive stripping voltammetry has been used by Wang et al.[196] to obtain detection limits of 1.6×10^{-10} *M* for testosterone, 2×10^{-10} *M* for progesterone, and 3.3×10^{-10} *M* for methyltestosterone, with 15 min preconcentration at the sMDE. Norethisterone (17-α-ethynyl-17 β-hydroxyoestr-4-en-3-one) is a pharmaceutical compound used in oral contraceptives. It has been assayed rapidly in tablets by DPP without the need for prior extraction. The procedure involves dissolution in 40% methanolic solutions in the presence of 0.2 *M* TMAB, degassing with nitrogen for 30 to 40 min, followed by polarography at the DME.[197] Both peak potential and peak current varied as a function of pH. One peak was obtained for norethisterone in the pH ranges 2.9 to 6.1 and 9.0 to 11.6, while two peaks were obtained between pH 7.1 and 8.1. A linear peak current-concentration relationship was found in the range from 7×10^{-6} to 7×10^{-4} *M*.

The cardiovascular drugs digoxin and digitoxin also contain the basic steroidal nucleus and the reduction occurs at the C=C bond in the five-membered ring structure containing a

conjugated $\rangle C{=}O$ group. Digoxin and digitoxin give rise to waves at −2.28 and −2.32 V (vs. SCE), respectively, in propan-2-ol −0.01 *M* tetrabutylammonium bromide (TBAB). The limit of detection for these compounds using DPP was stated to be 2.5×10^{-6} *M*.[198] Wang et al.[199] have improved upon this sensitivity by adsorptive stripping voltammetry and achieved detection limits of 2.3×10^{-10} *M* for digoxin, 8×10^{-10} *M* for digitoxin, and 7.5×10^{-10} *M* for digitoxigenin. These results were based on 15-min accumulation at the static mercury drop.

The reduction of coumarins has been shown to involve saturation of the C=C double bond conjugated to the $\rangle C{=}O$ moiety. Reduction of the C=C bond has also been shown to occur in the cephalosporin nucleus and polarographic methods of analysis for these compounds have been reported.[200] For griseofulvin, the reduction process involves saturation of both the C=C and the $\rangle C{=}O$ bonds. Kenyherez and Kissinger[201] have studied the electrochemical behavior of the growth-promoting agent, diethylstilbestrol pOH–$C_6H_4C(C_2H_5){=}C(C_2H_5)C_6H_4$ p-OH and found that, on application of cyclic voltammetry, the molecule would oxidize in a $2e^-$ process at + 0.45 V (vs. SCE) and rereduce at −0.09 V (vs. SCE). They used HPLC-ED to determine diethylstilbestrol at ppb levels in animal tissues.

The carbon-carbon triple bond is not directly reducible at the DME. With the artificial steroid lynestrenol, however, heating with a 60% sulfuric acid-methanol mixture converted the $-C{\equiv}CH$ group into a polarographically active moiety.[11]

Halogen-containing hydrocarbons are reduced according to

$$RX + 2e^- + H^+ \rightarrow RH + X^-$$

(where R = aryl) and their potentials of reduction are affected markedly by the nature and number of substituents to the aromatic ring. Although organochlorine compounds are best determined using GLC with electron-capture or coulometric detection, there are several examples in the literature in which voltammetric methods have found particular application.

Kemula and Kreminska[202] have combined column chromatography and polarography to separate quantitatively the *pp'*- and *op'*-isomers of DDT. They used a column packed with powdered rubber swollen with heptane. The DDT isomers were eluted by using a solution of 0.05 *M* tetraethylammonium iodide (TEAI) — 87% DMF. The eluate was collected from the column and the isomers determined at a reduction potential of −1.20 V. By use of this separation procedure, *pp'*-DDT was eluted before *op'* DDT. Davidek and Janicek[203] tried to determine DDT in biological material using a direct method, but found that interferences in the matrix caused a deformation of the DDT peak. In order to overcome this problem they developed an indirect technique. DDT was nitrated at 90 to 95°C for 10 min using a mixture of concentrated sulfuric acid and fuming nitric acid. On cooling, the solution was diluted with water, followed by methanol, to give a water-methanol (50 + 50) mixture. In this strongly acidic medium, the tetranitro derivative was reduced in a single wave with a half-wave potential of −0.13 V (vs. SCE). The limit of detection of the method was stated to be 0.4 μg $m\ell^{-1}$.

The γ-isomer of hexachlorocyclohexane (also known as benzene hexachloride, γ-BHC, and lindane) is employed as an insecticide and is reduced according to

$$C_6H_6Cl_6 + 6e^- \rightarrow C_6H_6 + 6Cl^-$$

in solutions of pH > 6. γ-BHC can be determined in aerosols, in water samples down to 0.5 μg ℓ^{-1}, and in soil. This last method would involve extraction of the soil with hexane,

filtering through sodium sulfate, concentration of the extract, and separation on silica gel. This method can then determine γ-BHC in the presence of heptachlor and heptachlor epoxide and the polarographic analysis should be carried out in 0.05 *M* TMAB containing either 10% (for γ-BHC or heptachlor) or 40% (for heptachlor epoxide) ethanol.

Supin and Budnikov[204] have investigated the adsorption properties of γ-BHC at a slowly DME. By careful choice of accumulation time (7 sec) and applied potential (−0.40 V vs. SCE) they were able to concentrate γ-BHC at the surface of the mercury, followed by measurement of the stripping peak obtained using fast-scan pulsed oscillographic polarography. This method was found to be 60 times more sensitive than the DC procedure and could be used to determine down to 0.06 μg $m\ell^{-1}$ in pure solution. Supin and Budnikov[204] have also applied this technique to the determination of mucochloric acid and quoted a detection limit of 0.2 μg $m\ell^{-1}$.

Berck[205] has developed an indirect method for the determination of the grain fumigant chlorpicrin. This involved liberation of Cl^- ions by heating the substance in 60% propanol-3.5% monoethanolamine for 4 hr at 60°C. The mixture (8.5 mℓ) was then cooled, 1 mℓ of 8 *N* nitric acid and 0.5 mℓ of 1% gelatin added, and the Cl^- released determined by anodic polarography at +0.22 V (vs. SCE). He was also able to determine chlorpicrin in the presence of other fumigants, for example, methyl bromide, ethylene dibromide, acrylonitrile, and carbon tetrachloride, and the method was successfully applied to the determination of this compound in air samples taken from fumigated grain, flour, and soil.

Colas et al.[206] have used a silica gel column in order to separate a mixture of the two isomers of endosulfan, followed by polarography in a water-acetone (50 + 50) mixture containing lithium chloride as the supporting electrolyte. This compound has also been detected in the stomach contents of poisoned chickens using polarographic and GLC techniques. The presence of parathion was also detected in this investigation.

Recently, Farwell et al.[207] have reported on the use of interrupted-sweep voltammetric analysis for the identification of polychlorinated insecticides and other polychlorinated aromatics. The apparatus consisted of a three-electrode, potentiostatically controlled circuit with a logic-controlled interrupted linear sweep mechanism. By using DMSO — 0.1 *M* tetraethylammonium bromide (TEAB) as the supporting electrolyte, voltammograms for many polychlorinated aromatic compounds have been obtained.

This method suffers from the fact that one requires at least 9 μg of relatively pure compound in order to obtain a positive identification. It is, therefore, of little use in the analysis of pesticide residues in the environment, but could find more application in the identification of isomeric products found during the synthesis of polychlorinated agrochemicals. Hexachlorophane has been determined in plasma using phase-sensitive AC polarography.[208] The method was able to determine down to 100 ng $m\ell^{-1}$ in this body fluid, although proteins were shown to interfere with the analysis.

Chatten et al.[209] have recently investigated the reduction mechanism of antiherpes compounds. (E)-5-(2-bromovinyl) 2′-deoxyuridine (BVDU) and 5-iodo-2′-deoxyuridine(idoxuridine), and found that the former compound is reduced at −1.80 V (vs. SCE) in a $4e^-$ process involving loss of −Br and reduction of the vinyl group. The latter compound is reduced in pH 7 buffer at −0.97 V (vs. SCE) due to loss of the iodo group.

E. Organophosphorus Compounds

With the large-scale development of organophosphorus insecticides in the early 1950s, methods of analysis were based either on colorimetric techniques or on the determination of total phosphorus. These methods are inherently nonspecific, however, and were slowly superseded by the development of GLC procedures employing flame photometric or thermionic detection. As many organophosphorus insecticides are unstable under the conditions required for GLC procedures, increasing importance has been placed on the development of "cold" methods of analysis.

Nangniot[210] has studied the polarographic behavior of a wide range of organophosphorus pesticides. Although phosphoric acid esters cannot be reduced at the DME, compounds containing either the $\rangle P(=S)-$ or $\rangle P-S-$ moieties in their molecular structures can produce sharp adsorption peaks when using a fast-scan LSV technique. This method can be used to determine concentrations of the organophosphorus compounds at the micromolar level.

Thiophosphates also produce anodic waves at the DME. This has been demonstrated by some dialkyl (or alkylamine) thiophosphates and dialkyldithiophosphates. Supin and Budnikov[204] used a CSV method of analysis for the determination of the insecticides phthalophos and benzophosphate in apples. This procedure involved extracting 200 g of apples with acetone, evaporation of the extract, and treatment of the final residue with alkali. Under these conditions, the insecticides are hydrolyzed to dimethyl- and diethyl-thiophosphate, respectively. These compounds can then form insoluble salts with Hg and can be determined by CSV using a scan rate of 1 V s^{-1}. The method could be used to determine down to 0.2 $\mu g\ kg^{-1}$ of each insecticide in apples.

Davidek and Seifert[211] have developed an enzymopolarographic method for the determination of the organophosphorus pesticide intration. The method is based on the inhibition of anticholinesterase activity by the organophosphorus moiety (a reaction which parallels the in vivo biological activity of these compounds). Unreacted enzyme is then incubated with β-naphthol acetate and the β-naphthol liberated is measured by polarography, following nitrosation. The method has been applied to the analysis of intration in lettuce, cabbage, cherries, and tomatoes. Naturally occurring enzymes, which would be capable of hydrolyzing β-naphthyl acetate, are removed by precipitation with ethanol and subsequent centrifugation. No interference was observed in the presence of carotenes, xanthophyll, chlorophyll, or anthocyanidines and the method was found to be relatively simple and rapid to perform.

The selectivity of polarographic methods towards $-NO_2$-containing compounds can be illustrated with reference to the pesticide parathion $(C_2H_5O)_2P(=S)OC_6H_4\ pNO_2$. This compound is metabolized in vivo to give the highly toxic substances paraoxon, $(C_2H_5O)_2P(=O)OC_6H_4pNO_2$, and *p*-nitrophenol. Although *p*-nitrophenol can be determined in the presence of the other two compounds on the basis of its peak potential in BR buffer, pH 3, it is more difficult to differentiate between parathion and paraoxon. These compounds exhibit similar DC and DPP behavior in simple buffered systems, although it has been reported that only parathion gives rise to a sharp adsorption peak in LSV, presumably, due to the presence of the P=S moiety in its molecular structure.[210] This adsorption process is also manifested to a certain degree in DPP (in aqueous solution containing R_4N^+ salts as the supporting electrolyte), but the differences observed between parathion and paraoxon are only useful for qualitative purposes. With parathion, the polarographic method is also unable to differentiate between this compound and its structurally related analogs methyl parathion and *O*-ethyl-*O*-(*p*-nitrophenyl) phenylphosphonothioate (EPN). Compounds of related structure should, therefore, be separated prior to analysis, as has been described for the polarographic determination of parathion and methylparathion in lettuce following a liquid-chromatographic separation.[61]

Clark et al.[212] have developed a method for the determination of ethyl- and methylparathion residues in vegetable materials and in surface water samples using reversed-phase HPLC with series UV detection and ED (vs. Ag/AgCl) at −0.85 V. Sample preparation techniques were developed which avoided usual preliminary column fractionations and which allowed the parathions to be recovered with average of 95% at concentrations less than 50 ng g^{-1} for plant material. Relative standard deviations of 5% were obtained using five different plant examples. Concentrations less than 10 ng $m\ell^{-1}$ were readily measured in

water samples using a column concentration procedure. The selectivity of electrochemical detection made it unnecessary to chromatographically resolve the electroactive plant components from the pesticides and allowed rapid analysis. Series detection proved useful in distinguishing various components in the samples from pesticides, in distinguishing various pesticides, and in comparing the characteristics of the two detectors. The pesticides alachlor, atrazine, carbaryl, carbofuran, chloropyrifos, diazinon dyfonate, and lannate did not give ED under the conditions of the experiment.

Recently, Whittaker and Osteryoung[213] have investigated the polarographic behavior of the aromatic NO_2-containing rodenticide Vacor and its main metabolite *p*-nitroaniline. These compounds were found to reduce at similar potentials in a range of supporting electrolytes and must, therefore, be separated by TLC prior to analysis. Osteryoung et al.[214] have used this procedure in order to determine *p*-nitroaniline in the liver of an accidental poisoning case. The method involved the homogenization of 5 g of tissue with 25 mℓ of water, salting out 12-mℓ aliquots with 4 g of sodium chloride, and extracting with 2 mℓ of tetrahydrofuran (THF).

F. Organometallics

The electrochemical behavior and resulting analytical applications of organometallic compounds of Hg, Sn, Pb, and Ge have been reviewed by Fleet and Fouzder.[8] The pertinent analytical applications will be summarized in this text.

1. Organomercurial Species

Fleet and Jee[215] determined Hg(II) olefin addition compounds by AC polarography in the solvent CH_3OH–H_2O (9 + 1) with 0.1 *M* NaOH or $NaNO_3$ as the supporting electrolyte. The method has been proposed for the analysis of a wide range of olefins and could be used for the analysis of mixtures in view of the variation in reaction rates. The sample of olefin (0.1 to 2 meq) dissolved in CH_3OH was placed in a 10-mℓ volumetric flask. An excess of methanolic mercury (II) acetate solution was added so that the final concentration would be 2 to 3 $\times$ $.10^{-4}$ *M*. After suitable time had elapsed for the formation of the organomercury compound, 1 mℓ of 1 *M* NaOH was added as supporting electrolyte and the solution made up to 10 mℓ with methanol. This solution was dearated and subjected to AC polarography. Olefins such as allylacetone, vinyl acetate, *N*-vinyl carbazole, vinyl *n*-butyl ether, etc. will react to form derivatives in 2 min at room temperature and give AC summit potentials in the range -0.41 to -0.55 V (vs. SCE) and half-peak widths in the range 80 to 129 mV.

Heaton and Laitinen[216] have shown a linear dependence of DPP current on concentration for the reduction of the environmentally significant entity CH_3Hg^+ in the range 10^{-4} to 10^{-7} *M*. CH_3Hg^+ is believed to reduce according to the following mechanism:[8]

$$RHg^+ \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} RHg\cdot \xrightarrow{+e^-} RH + Hg$$

$$2\ RHg\cdot \rightarrow (RHg)_2 \rightarrow R_2Hg + Hg$$

MacCrehan et al.[217] have combined HPLC with reductive ED to separate organomercurials CH_3Hg^+, $C_2H_5Hg^+$, $PhHg^+$ as their zero-charged 2-mercaptoethanol complexes on a Spherosorb ODS 5-μm column, 4.6 $\times$ 250 mm, with a mobile phase of methanol-water (40 + 60) containing 0.06 *M* NH_4OAc, pH 5.5, and 5 $\times$ 10^{-4} *M* 2-mercaptoethanol, flow rate 1.0 mℓ min^{-1}, and a detector potential set at -0.90 V. Linear calibration curves can be obtained over a wide concentration range and the detection limits are in the low nanogram range.

2. Organotin Species

The pesticide fentin, which contains the triphenyltin species PH_3Sn^+, will reduce in a reversible $1e^-$ process to give the radical $Ph_3Sn\cdot$ which can adsorb on the mercury surface. Further reduction and protonation to give Ph_3SnH or dimerization to $Ph_3SnSnPh_3$ can then take place. Booth and Fleet[218] have recommended that the first adsorption peak, observed on the application of DPP and corresponding to the formation of the organotin-free radical, be used for trace analytical purposes. In addition, they have shown that fentin residues can be determined in the region 10^{-7} to 10^{-8} *M* by ASV using the adsorption of the species $Ph_3Sn\cdot$ as the accumulation step. While there was some slight loss of radical during this step due to dimerization or deactivation, the method was found quite precise. It was found necessary to control the length of this accumulation step so that less than a monolayer of the free radical was deposited on the electrode surface. When more than a monolayer was deposited, two distinct stripping peaks were obtained corresponding to the removal of the bulk and surface monolayer. Using this procedure, levels of fentin in potatoes were determined following extraction of the macerated sample with acetonitrile and direct measurement in the aqueous organic extract.[218] The resulting method was approximately 100 times more sensitive than conventional spectrophotometric procedures.

Polarographic determination of dialkyltin compounds can be carried out over the range 10^{-4} to 10^{-9} *M* by DPP using acetate buffer (pH 7) containing 80% (v/v) ethanol as supporting electrolyte and utilizing the first reduction peak due to $Bu_2Sn^{2+} \underset{-e^-}{\overset{+e^+}{\rightleftharpoons}} Bu_2Sn^{+}\cdot$ Using the same solution conditions, monoorganotin compounds could be determined down to 5×10^{-7} *M*.[8] Polarographic analysis of mixtures of organotin species could also be carried out in that the monoorganotin compound $PhSnCl_3$ (which reduced in a $3e^-$ process to yield $PhSn\cdot + Cl^-$) could be determined in the presence of di- and triphenyltin compounds.[8]

3. Organolead Species

In acetate buffer (pH 7.0)-supporting electrolyte containing 50% ethanol, Ph_3Pb^+-containing compounds, showed two well-defined DPP peaks which were diffusion controlled and linearly dependent on concentration over a wide range (e.g., 2×10^{-4} to 5×10^{-7} *M* for the first peak corresponding to $Ph_3Pb^+ \overset{+e^-}{\rightarrow} Ph_3Pb\cdot_{ads}$). A similar medium could be used for analysis of dialkyllead compounds over a similar concentration range (p 284).[8] An ASV method has been recommended for the determination of organolead in petroleum[219] — a solution of bromine in chloroform has been used to decompose R_4Pb followed by extraction of Pb(II) into 0.1 *M* HNO_3. In this method accumulation was carried out at -0.80 V (vs. SCE) for 1.5 min and Pb(II) determined at -0.39 V (vs. SCE). A limit of detection of 8 parts Pb per 10^{13} with a coefficient variation of 7% was claimed.

MacCrehan et al.[217] have used reversed-phase HPLC-reductive ED to detect 0.1 ng (corresponding to 2×10^{-8} *M* or 5 ppb) of the species $(CH_3)_3Pb^+$, with linear calibration in the range 10^{-3} to 10^{-7} *M*. These authors stated that some form of sample pretreatment and concentration would be necessary for the analysis of "real" water samples.

4. Organoarsenic Species

The vast majority of analytical methods described in the literature do not differentiate between either the oxidation states or the number of organic substituents on the As atom without a prior separation or extraction method. Watson[8] has investigated the polarographic behavior of a wide variety of organoarsenic compounds (arsonic acids, phenylarsenoxide, diphenylarsinic acid, diphenylarsine oxide, triphenylarsine oxide, etc.) and exploited the selectivity of the DC technique for these compounds. Jan et al.[220] have exploited the application of DPP at the HMDE for the simultaneous determination of selected organoarsenic

compounds at the 10^{-8} to 10^{-9} *M* concentration level. It was found difficult to determine As(III) by this technique when interfering organoarsenic molecules were present in the mixture.

5. Other Organomettallics

The determination of the metal-containing dithiocarbamates, for example, ziram, ferbam, and zineb, has been dealt with in a previous section. Bond and Wallace[221] have recently used HPLC-ED in the oxidation mode at a variety of indicator electrodes for the determination of dithiocarbamate complexes of Cu, Ni, Co, Cr(VI), and Cr(III). Limits of detection substantially less than 1 ng have been obtained. For simultaneous determination of all five species, external formation of complexes prior to injection on the column is essential.

G. Selected Inorganic Molecules

The polarographic behavior of inorganic molecules in which the central metal atom exists in a high oxidation state, e.g., MoO_4^{2-}, CrO_4^{2-}, WO_4^{2-}, and VO_3^-, have been extensively studied in the literature.[222,223] Jan et al.[224] have used the DPP technique at the HMDE to achieve limits of detection of 2.7×10^{-6} *M* for CrO_4^{2-}, 7.7×10^{-9} *M* for WO_4^{2-}, and 1.4×10^{-5} *M* for VO_3^-. The method was selective enough to allow VO_3^-, CrO_4^{2-}, and WO_4^{2-} or VO_3^-, MoO_4^{2-}, and WO_4^{2-} to be determined simultaneously at the 10^{-5} *M* concentration level.

An increasing number of references can now be observed in the literature referring to the detection and determination of selected inorganic molecules using ion chromatography or HPLC combined with electrochemical detection, for example, $S_2O_3^{2-}$ in human urine and plasma,[225] aqueous polythionates and thiosulfate,[226] nonoxidizable anions,[227] CN^- and other anions,[228] and pertechnetate.[229]

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