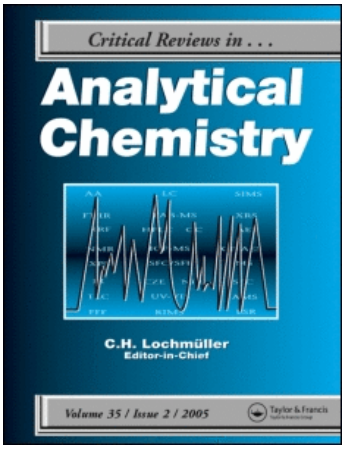

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Critical Reviews in Analytical Chemistry, 30(1):37–57 (2000)

Polarographic and Voltammetric Determination of Chemical Carcinogens

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ABSTRACT: The polarographic and voltammetric behavior of chemical carcinogens is reviewed and the possible role of electrochemistry in elucidation of their genotoxic and ecotoxic properties, mechanism of their action, metabolism, fate in the environment, etc. is briefly discussed. The use of modern electroanalytical techniques, namely, differential pulse polarography, differential pulse voltammetry, adsorptive stripping voltammetry and high-performance liquid chromatography with electrochemical detection for the determination of trace amounts of these substances is described.

KEY WORDS: differential pulse polarography (voltammetry), adsorptive stripping voltammetry, high-performance liquid chromatography with electrochemical detection, chemical carcinogens.

ABBREVIATIONS: APAHs, aminoderivatives of polycyclic aromatic hydrocarbons; **BR,** Britton-Robinson buffer; **CSV,** cathodic stripping voltammetry; **DL,** detection limit; **DNA,** desoxyribonucleic acid; **DPP,** differential pulse polarography; **DPV,** differential pulse voltammetry; **DME,** classical dropping mercury electrode; **DMF,** dimethylformamide; **DMSO,** dimethylsulfoxide; **$E_{1/2}$,** half-wave potential; **GC,** glassy carbon; **HMDE,** hanging mercury drop electrode; **HPLC-ED,** high-performance liquid chromatography with electrochemical detection; **LOD,** limit of determination; **LSV,** linear scan voltammetry; **MeOH,** methanol; **MeCN,** acetonitrile; **RDE,** rotating disk electrode; **NPAHs;** nitrated polycyclic aromatic hydrocarbons; **PAHs,** polycyclic aromatic hydrocarbons; **SCE,** saturated calomel electrode; **SMDE,** static mercury drop electrode; **TBABr,** tetrabutylammonium bromide; **TLC,** thin layer chromatography

TABLE OF CONTENTS

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I. INTRODUCTION

Cancer exacts substantial costs in treatment and preventive measures on a worldwide scale, as well as causing immeasurable human suffering. The approximately 20% cancer mortality together with the fact that environmental causes contribute to the majority of cancers emphasizes the potential benefits of environmental detection of chemical carcinogens and raises carcinogenic substances monitoring in general and working environment to the highest priority. Analytical measurement procedures should have a critical role in molecular epidemiology and exposure regulation, as well as in environmental monitoring.

In many cases, modern electroanalytical methods can be a viable alternative to more frequently used spectrometric or separation methods. Moreover, in some cases there is a relationship between polarographic and/or voltammetric behavior and genotoxic properties of organic compounds, and the knowledge of the mechanism of their electrode reactions can give us a useful clue in elucidation of the mechanism of their interaction with living cells and their fate in the environment. It is challenging that in many cases there can be drawn a link between polarographic or voltammetric behavior and genotoxic properties of organic species.[9] The knowledge of an electrode process of chemical carcinogens, mutagens, and anticancer drugs provides a useful aid for understanding of its enzymatic processes, dangerous radical reactions and its inactivation pathways in living cells. Polarography and voltammetry was applied successfully for monitoring the efficiency of the destruction of chemical carcinogens.

According to our experience, modern polarographic and voltammetric methods (e.g., DPP,[9–12] LSV,[9–11] AdSV,[9,10,11,–17] or HPLC-ED) are among those fulfilling the stringent conditions on selectivity and sensitivity essential in the determination of chemical carcinogens (see Figure 1). Sensitivity of voltammetric methods can be increased further by electrochemical or adsorptive accumulation at the surface of the working electrode by stripping methods using analyte preconcentration.[15,19–21]

The proper choice of the base electrolyte is of extreme importance. In many cases, it is necessary to use mixture of aqueous buffer with a suitable organic solvent because of the limited solubility of test substances in water. For the determination of extremely low concentrations of chemical carcinogens, 10- or 100-fold diluted buffer or other base electro-

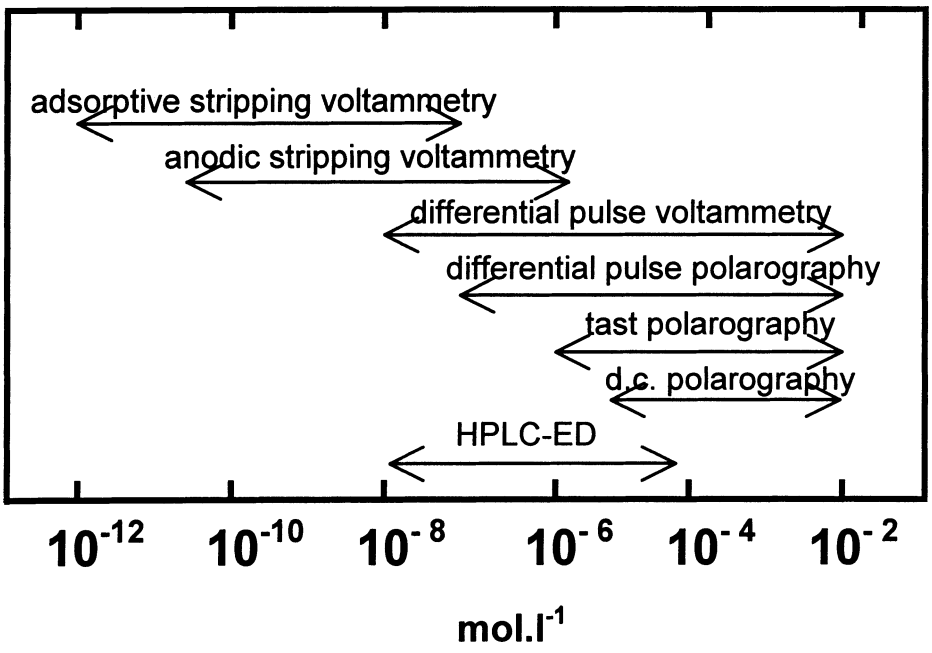


FIGURE 1. The application range of various polarographic and voltammetric techniques.

lyte used yields a smoother curve of the supporting electrolyte due to lower concentrations of trace impurities in the chemicals used for its preparation. This enables to reach much lower LOD, especially with AdSV. With this technique, it is advisable to decrease the concentration of organic solvent as low as possible, because its presence usually decreases the tendency of organic substances to adsorb on the surface of HMDE. In many cases, extremely high sensitivity of modern polarographic and voltammetric techniques can be combined with highly efficient separation by high-performance liquid chromatography.[18] The theoretical backgrounds and many practical applications of these methods (e.g., in environmental,[22] clinical,[13] and biological[23,24] sciences) are described in the above-quoted monographs and reviews. Many useful information on the mechanism of the electrode reactions of these substances can be found in an extensive treatment.[25]

Moreover, the study of polarographic or voltammetric behavior of chemical carcinogens can provide an extraordinary amount of information about their electron-transfer reactions. Both electrochemical and biological reactions are essentially heterogeneous processes occurring at the electrode-solution or enzyme-solution interface. They can occur at similar pH and temperature and in both cases it is likely that the substrate molecule has to be oriented in a rather specific fashion before the electron transfer can occur. According to our opinion, there is sufficient superficial similarity between electrochemical and biological reactions to warrant extensive study of the electrochemistry of chemical carcinogens, which should shed considerable light on the fundamentals of the biological reaction mechanisms.

It is the aim of this review to show that modern polarographic and voltammetric techniques can be successfully used for the determination of trace amounts of various chemical carcinogens. The extent of this review makes it impossible to quote all papers dealing with polarographic or voltammetric determination of chemical carcinogens. Thus, only selected examples demonstrating the

applicability of these methods for various classes of chemical carcinogens are given.

II. OVERVIEW OF CHEMICAL CARCINOGENS WITH RESPECT TO THEIR ELECTROCHEMICAL PROPERTIES

Overall evaluations of chemical carcinogens can be found in IARC (International Agency for Research on Cancer) monographs database on carcinogenic risk to humans.[26] Carcinogenic substances can be basically divided into three following groups (see Table 1). If you compare the list of carcinogenic substances with the review of electrochemically active organic functional groups (see Table 2) you can see the wide applicability of modern polarographic and voltammetric techniques in the analysis of chemical carcinogens. (In Table 1, electrochemically reducible substances amenable to polarographic and voltammetric determination mainly on mercury electrodes are printed in italics, while electrochemically oxidizable substances amenable to voltammetric determination mainly on solid or carbon paste electrodes are underscored. Where appropriate, chemical abstract numbers are given in square brackets.)

III. POLYCYCLIC AROMATIC HYDROCARBONS AND THEIR DERIVATIVES

A. Polycyclic Aromatic Hydrocarbons

Polarographic methods are not too suitable for the determination of PAHs, because these substances are reducible at very negative potentials (about –2.5 V vs. SCE) at which it is difficult to attain the appropriate selectivity and sensitivity. It can be demonstrated by the attempted polarographic determination of 3,4-benzopyrene,[27] which is too imprecise for practical purposes. Better results were obtained by anodic DPV on GCE, which was used for the determination of anthracene, benzo[*g,h,i*]-perylene, benzo[*a*]pyrene, chrysene, coronene, 9,10-diphenyl-anthracene, pyrene, perylene, and triphenylene in sulpholane medium with LOD around 5.10^{-7} *M* or in MeCN with LOD around 2.10^{-8} *M*.[28] Anodic DPV on platinum electrode was used for the analysis of three- and four-component mixture of PAHs in MeCN medium in the region of 1 to 5 ppm.[28] Polarography was used to study the correlation between carcinogenity and half-wave potentials of reduction of PAHs in DMF or DMSO[30] or of their oxidation in MeCN[31] or DMF.[32] Similarly, the relationship between carcinogenity and polarographic behavior of dibenz[*a,h*]anthracene[33] and dibenzo[*a,h*]pyrene[34] was studied and the influence of proton donors on the reduction of carcinogenic PAHs in nonaqueous media was investigated.[35]

B. Nitrated Polycyclic Aromatic Hydrocarbons

So far, mostly chromatographic methods, such as gas chromatography–mass spectrometry or HPLC were used for the determination of NPAHs.[36,37] However, these methods are characterized by high investment and running costs. Because of easy polarographic reducibility of nitro group, modern polarographic and voltammetric methods (DPP, DPV, and AdSV) were applied successfully[38] for the determination of trace amounts of 1-nitropyrene, 9-nitroanthracene, 2-nitrofluorene, and 2,7-dinitro-fluorene in the concentration range of 1.10^{-4} to 10^{-9} *M*. Practical applicability of these methods was demonstrated on the determination of trace amounts of selected chemical carcinogens in river water and on monitoring of the chemical destruction of the test substances. DPP and DPV were used for the determination of traces of 4-nitrobiphenyl[39,40] and for monitoring the efficiency of chemical destruction of this substance.[7] An interesting correlation[41] was found between half-wave potential and mutagenicity of a

TABLE 1
Selected Chemical Carcinogens

Group 1: Substances carcinogenic to humans
Arsenic [7440-38-2] and arsenic compounds, Beryllium [7440-41-7] and beryllium compounds, Cadmium [7440-43-9] and cadmium compounds, Chromium[VI] compounds, Nickel compounds, Aflatoxins, naturally occurring [1402-68-2], 4-Aminobiphenyl [92-67-1], *Azathioprine* [446-86-6], Benzene [71-43-2],Benzidine [92-87-5], N,N-Bis(2-chloroethyl)-2-naphthylamine (Chlornaphazine) [494-03-1], Bis(chloromethyl)ether [542-88-1], chloromethyl methyl ether [107-30-2], 1,4-Butanediol dimethanesulfonate [55-98-1], Chlorambucil [305-03-3], *1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Methyl-CCNU; Semustine)* [13909-09-6], *Ciclosporin* [79217-60-0], *Cyclophosphamide* [50-18-0] [6055-19-2], Diethylstilboestrol [56-53-1], Ethylene oxide [75-21-8], Melphalan [148-82-3] , *8-Methoxypsoralen* (Methoxsalen) [298-81-7], Mustard gas (Sulfur mustard) [505-60-2], 2-Naphthylamine [91-59-8], *Tamoxifen* [10540-29-1],2,3,7,8-Tetrachlorodibenzo-para-dioxin [1746-01-6], *Thiotepa* [52-24-4], Vinyl chloride [75-01-4],
Group 2A: Substances probably carcinogenic to humans
Cisplatin [15663-27-1], Acrylamide [79-06-1], *Adriamycin* [23214-92-8], *Azacitidine* [320-67-2], Benz[a]anthracene [56-55-3], Benzidine-based dyes, Benzo[a]pyrene [50-32-8] , *Bischloroethyl nitrosourea* (BCNU) [154-93-8], 1,3-Butadiene [106-99-0] , Captafol [2425-06-1],*Chloramphenicol* [56-75-7], a-Chlorinated toluenes (benzal chloride [98-87-3], *benzotrichloride* [98-07-7], *benzyl chloride* [100-44-7]) and benzoyl chloride [98-88-4], *1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea* (CCNU) [13010-47-4], para-Chloro-ortho-toluidine [95-69-2], *Chlorozotocin* [54749-90-5] , Dibenz[a,h]anthracene [53-70-3], Diethyl sulfate [64-67-5], Dimethylcarbamoyl chloride [79-44-7], 1,2-Dimethylhydrazine [540-73-8], Dimethyl sulfate [77-78-1], Epichlorohydrin [106-89-8], Ethylene dibromide [106-93-4], *N-Ethyl-N-nitrosourea* [759-73-9], *Formaldehyde* [50-00-0], *2-Amino-3-methylimidazo[4,5-f]quinoline* [76180-96-6], *5-Methoxypsoralen* [484-20-8], 4,4´-Methylene bis(2-chloroaniline) [101-14-4], Methyl methanesulfonate [66-27-3], *N-Methyl-N´-nitro-N-nitrosoguanidine* (MNNG) [70-25-7], *N-Methyl-N-nitrosourea*, Nitrogen mustard

TABLE 1 (continued)
Selected Chemical Carcinogens

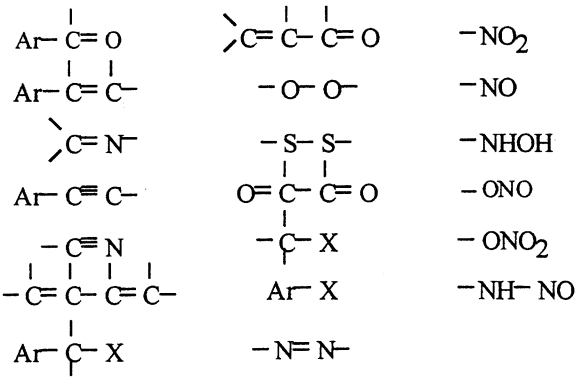
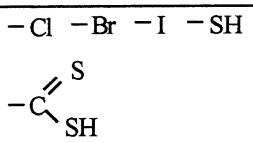
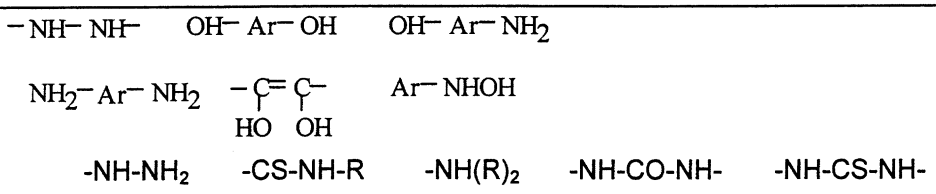
[51-75-2], *N-Nitrosodiethylamine* [55-18-5], *N-Nitrosodimethylamine*, *Phenacetin* [62-44-2],Procarbazine hydrochloride [366-70-1], Styrene-7,8-oxide [96-09-3], Tetrachloroethylene [127-18-4], Trichloroethylene [79-01-6] , 1,2,3-Trichloropropane [96-18-4], Tris(2,3-dibromopropyl) phosphate [126-72-7], Vinyl bromide [593-60-2], Vinyl fluoride [75-02-5]
Group 2B: Substances possibly carcinogenic to humans
A-a-C (2-Amino-9H-pyrido[2,3-b]indole) [26148-68-5], *Acetaldehyde* [75-07-0], Acetamide [60-35-5], Acrylonitrile [107-13-1],*2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide* [3688-53-7], *Aflatoxin M1* [6795-23-9], *para-Aminoazobenzene* [60-09-3], *ortho-Aminoazotoluene* [97-56-3], *2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole* [712-68-5], Amitrole [61-82-5] , ortho-Anisidine [90-04-0], *Auramine* [492-80-8], *Azaserine* [115-02-6], Aziridine [151-56-4], Benzo[b]fluoranthene [205-99-2]. Benzo[j]fluoranthene [205-82-3], Benzo[k]fluoranthene [207-08-9] , Benzofuran [271-89-6], Benzyl violet 4B [1694-09-3], *Bleomycins* [11056-06-7], Bromodichloromethane [75-27-4], Butylated hydroxyanisole (BHA), b-Butyrolactone [3068-88-0], Caffeic acid [331-39-5],Catechol [120-80-9], Chlordane [57-74-9], Chlordecone (Kepone) [143-50-0], Chlorendic acid [115-28-6], para-Chloroaniline [106-47-8], Chloroform [67-66-3], 1-Chloro-2-methylpropene [513-37-1], 4-Chloro-ortho-phenylenediamine [95-83-0], Chloroprene [126-99-8], Chlorothalonil [1897-45-6], *CI Acid Red 114* [6459-94-5], *CI Basic Red 9* [569-61-9], *CI Direct Blue 15* [2429-74-5], *Citrus Red No. 2* [6358-53-8], para-Cresidine [120-71-8], Cycasin [14901-08-7], *Dacarbazine* [4342-03-4], *Dantron (Chrysazin; 1,8-Dihydroxyanthraquinone)* [117-10-2], *Daunomycin* [20830-81-3], DDT [p,p'-DDT, 50-29-3], N,N'-Diacetylbenzidine [613-35-4], 2,4-Diaminoanisole [615-05-4], 4,4'-Diaminodiphenyl ether [101-80-4], 2,4-Diaminotoluene [95-80-7], *Dibenz[a,h]acridine* [226-36-8], *Dibenz[a,j]acridine* [224-42-0], *7H-Dibenzo[c,g]carbazole* [194-59-2], Dibenzo[a,e]pyrene [192-65-4], Dibenzo[a,h]pyrene [189-64-0], Dibenzo[a,i]pyrene [189-55-9], Dibenzo[a,l]pyrene [191-30-0], 1,2-Dibromo-3-

TABLE 1 (continued)

chloropropane [96-12-8], para-Dichlorobenzene [106-46-7], 3,3'-Dichlorobenzidine [91-94-1], 3,3'-Dichloro-4,4'-diaminodiphenyl ether, 1,2-Dichloroethane [107-06-2], Dichloromethane (methylene chloride) [75-09-2], 1,3-Dichloropropene [542-75-6], Dichlorvos [62-73-7] , Di(2-ethylhexyl) phthalate [117-81-7], 1,2-Diethylhydrazine [1615-80-1], Diglycidyl resorcinol ether [101-90-6], Dihydrosafrole [94-58-6], Diisopropyl sulfate [2973-10-6], 3,3'-Dimethoxybenzidine (ortho-Dianisidine) [119-90-4], *para-Dimethylaminoazobenzene* [60-11-7], *trans-2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)-vinyl]-1,3,4-oxadiazole* [25962-77-0], 2,6-Dimethylaniline (2,6-Xylidine) [87-62-7], 3,3'-Dimethylbenzidine (ortho-Tolidine) [119-93-7] , 1,1-

number of NPAH. Electrochemical cells used for HPLC-ED determination of NPAHs are of the thin-layer[42–44] or wall-jet[45] type, each with a three electrode system, i.e., working, auxiliary, and reference electrodes. Typical detectors contain a glassy carbon[42,44,45] or a gold/mercury[43] working electrode with a Ag/AgCl reference electrode, or a porous graphite working electrode[46] with a palladium-based modified H_2/H^+ reference electrode. Almost all electrochemical detectors are operated in reductive amperometric mode with the working potentials typically between –500 and –650 mV vs. reference electrode. Attempts to use electrochemical detectors in the differential pulse mode were not very successful.[43] The measurement of hydrodynamic voltammograms can help to confirm the analytes identity.[43–45] 2-nitronaphthalene, 9-nitroanthracene and 1-nitropyrene have been determined by HPLC-ED in atmospheric samples[45] with DLs of 200 to 1600 pg, some other NPAHs in ambient air particulates with DLs in the subnanogram levels,[46] and 16 NPAHs were determined in diesel exhaust[44] (2-acetamido-3-nitro-9-fluorenone, 7-nitrofluorene-1-carboxylic acid, 2,7-dinitro-9-fluorenone, 3-nitro-9-fluorene, 2,7-dinitrofluorene, 1-nitronaphthalene, 2- nitrobiphenyl, 2-nitronaphthalene, 3-nitrobiphenyl, 4-nitrobiphenyl, 2-nitrofluorene, 1,3,6-trinitropyrene, 9-nitro-anthracene, 1,3-dinitropyrene, 1-nitropyrene, 4-nitrofluoranthene). Compared with a similar system employing a conventional column,[44] sensitivities in the micro HPLC system (0.91 μL thin layer flow cell, two 500 mm × 1 mm i.d. columns, injection valve and connections of minimal internal dead volume) increased 3 to 7 times.[42] A large-area porous graphite working electrode[47] allowed not only the determination of NPAHs in reductive amperometric mode, but the high degree of electrochemical conversion to APAHs can also be used in subsequent fluorescence detection. A gold/mercury thin layer electrochemical cell,[43] operated in the differential pulse mode under gradient elution conditions, gave very poor results. Several systems[48,49] utilize post-column electrochemical reduction of NPAHs to amino derivatives followed by fluorescence detection. However, it is worth mentioning the APAHs are amenable to oxidative amperometric detection as well (see Section III.C). The extent of conversion using this technique is not very high (typically 5 to 15%), and it depends on the flow rate of the mobile phase.

TABLE 2
Electrochemically Active Organic Substances and Functional Groups

Electrochemically reducible organic substances and functional groups
aldehydes, ketones,  heterocycles (O,S,N) with double bonds, PAHs with double bonds, alkaloids, vitamins, hormones, steroids, saccharides
Substances or groups reacting with Hg and thus providing anodic waves: 
Electrochemically oxidizable organic substances and functional groups  -NH-NH_2 -CS-NH-R -$NH(R)_2$ -NH-CO-NH- -NH-CS-NH-

An attempt[50] to combine HPLC with AdSV on HMDE for the determination of 2-nitrofluorene and 2,7-dinitrofluorene has lead to relatively high DL (around 2.10^{-7} *M*).

C. Amino Derivatives of Polycyclic Aromatic Hydrocarbons

These substances are chemical carcinogens on one side and metabolites of carcinogenic NPAHs on the other side. They are amenable to anodic oxidation on suitable solid or paste electrodes. This fact was exploited for voltammetric determination of 4-aminobiphenyl,[39] 2-aminofluorene,[51] and benzidine and its derivatives[52] on GC-RDE, of 4-aminobiphenyl on CPE[53] and for HPLC-ED determination of 1-aminopyrene,[54] 3-aminofluoranthene,[55] 1-aminonaphthalene,[56] benzidine and dichlorobenzidine,[57] and benzidine derivatives in laboratory wastes,[61] in water and soil, in urine,[59] and

in waste water[60] on thin-layer,[54] carbon-fiber[61] or platinum tubular[56] detector. DPV on CPE was used for the determination of ppm to ppb amounts[62] and HPLC-ED for picomole amounts of some other carcinogenic aromatic amines. We believe that HPLC-ED can be used for biological monitoring of the human exposure to NPAHs based on the determination of corresponding metabolites (APAHs) in body fluids in a similar way as competitive ELISA.[64] Further examples can be found in a review.[65]

D. Hydroxy Derivatives of Polycyclic Aromatic Hydrocarbons

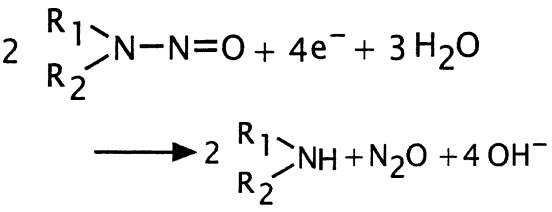
These substances, which are important metabolites of PAH and thus can be used for biological monitoring of human exposure to PAH, are amenable to anodic oxidation so that HPLC-ED can be used for their determination.[18] This can be demonstrated by HPLC-ED determination of 1-hydroxypyrene in urine on carbon-fiber detector with DL around 5.10^{-8} *M*.

IV. HETEROCYCLIC AROMATIC HYDROCARBONS AND THEIR DERIVATIVES

Heteroaromatic compounds with a π-electron deficit are characterized by low energies of the lowest occupied molecular orbital, owing to which these compounds are polarographically reducible. Moreover, their polarographic reduction can be further facilitated by protonation of the nitrogen atom, which brings about additional decrease in the electron density at sites of potential electron transfer. The addition of a next aromatic ring to acridine results in an energy increase for the lowest occupied molecular orbital. This is naturally mirrored by the $E_{1/2}$ shift to more negative values. Due to limited solubility, aqueous-methanolic solutions has to be used for the determination of acridine, benz(c)acridine and dibenz(a,h)acridine by DPV and AdSV[67] with LOD around 10^{-8} *M*. The polarographic behavior of acridine and its derivatives with fused benzene rings in DMSO[68] and DMF[69,70] has been examined in relation to their carcinogenity. Further references on polarographic behavior of heteroaromatic compounds can be found in the article.[67] 4-Nitroquinoline-1-oxide, 4-hydroxylaminoquinoline-1-oxide and 4-aminoquinoline-1-oxide are polarographically reduced in the region of nitro and oxide group[71] and possible correlation of polarographic and carcinogenic properties of these substances was also investigated.[72]

V. *N*-NITROSOCOMPOUNDS

N-nitrosocompounds are among the most commonly occurring chemical carcinogens and are present in trace amounts in a number of products of the chemical and even food industry, in various agricultural products, and also in the living and working environment. They are polarographically irreversibly reduced in alkaline medium with an exchange of two electrons according to Eq. 1 and in acidic medium with the exchange of four electrons (Eq. 2). The limiting diffusion current is thus roughly twice higher in acidic than in alkaline medium.



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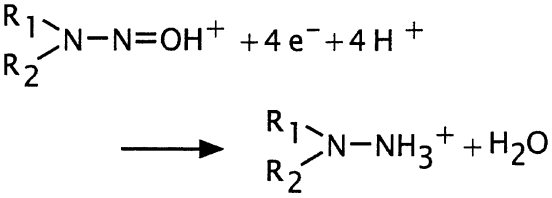
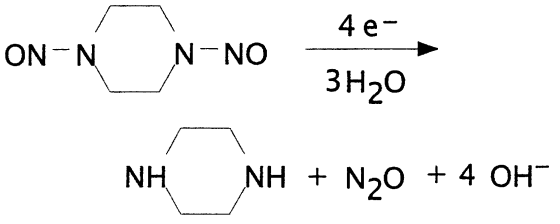


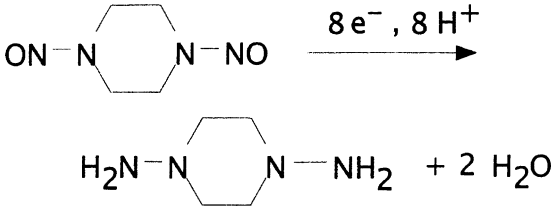
(2)

For that reason, polarographic determination is usually carried out at low pH. DCP was used for the determination of aliphatic *N*-nitrosocompounds[73–76] with LOD around 10^{-5} *M* and DPP for the determination of N-nitrosamines with LOD around 10^{-7} *M* in biological matrices,[77,78] or in drugs.[79,80] There is a danger of false-positive results when determining trace amounts of these substances in food,[81] spirits,[82] and beer.[83,84] However, the elimination of the DPP peak by UV irradiation is a reliable proof of the presence of *N*-nitrosamines. Further, DPP was used for the determination of *N*-nitrosoureas used as cytostatics.[85,86] HPLC with polarographic detection was used for both volatile and nonvolatile *N*-nitrosamines.[87,88] 4-substituted derivatives of *N*-nitroso-*N*-methylaniline (R = H, CH_3, OCH_3, Cl, CN, OH, and NO_2) were determined by DPV at HMDE, which can be used for the analysis of a mixture of the test substances either directly or after their separation by TLC.[89] False-positive results frequently occurring in environmental analysis can be eliminated by UV irradiation of the test sample leading to denitrosation of N-nitrosocompounds under investigation yielding polarographically inactive products.[89]

HPLC-ED of these substances on a C18 chemically bonded stationary phase with DC voltammetry on HMDE, anodic voltammetry on a glassy carbon fiber array detector, and indirect anodic voltammetric detection after photolytic denitrozation of the analytes yielding corresponding aromatic amines was described.[90] *N*,*N*′-dinitrosopiperazine[91] is polarographically reduced at pH 10 according



(3)



(4)

to Eq. 3, while at pH 2.4 an eight-electron reduction according to Eq. 4 occurs.

Attempts at increasing the sensitivity of the voltammetric determination of all tested *N*-nitrosocompounds via their adsorptive accumulation on the surface of HMDE failed.

VI. AZOCOMPOUNDS

A. Derivatives of *N*,*N*-Dimethyl-4-Aminoazobenzene

The easy polarographic reduction of azocompounds, whose mechanism is discussed in monograph,[25] permits the very sensitive polarographic and voltammetric determination of a number of genotoxic derivatives of *N*,*N*-dimethyl-4-aminoazobenzene (see Table 3). Polarographic behavior of derivatives with polarographically inactive substituents depends on pH because azogroup can be reduced to hydrazo or amino groups. Polarographic behavior of *N*,*N*-dimethyl-4-amino-3′nitro-azobenzene is even more complicated because of the presence of nitro group that can be reduced with the exchange of 2, 4, or 6 electrons to –NO, –NHOH, or $–NH_2$ group in dependence on the pH.[92] Similarly, the mechanism of reduction of *N*,*N*-dimethyl-4-amino-4'-aminoazobenzene is effected by the mutual interaction of azo and amino group. The selectivity of these methods enables direct determination of *N*,*N*-dimethyl-4-aminoazobenzene in blood plasma or analysis of a mixture of azobenzene, *N*,*N*-dimethyl-4-aminoazobenzene and *N*,*N*-dimethyl-4-amino-4′-aminoazo-

TABLE 3
Polarographic and Voltammetric Determination of *N,N*-dimethyl-4-Aminoazobenzene Derivatives

Substituent	Technique	Medium	LOD	Ref.
H	DPP	NH_3-NH_4Cl-MeOH (1:1) pH 9.7	1.10^{-8} M	94
	AdSV	NH_3-NH_4Cl-MeOH (1:1) pH 9.7	3.10^{-9} M	
3'-CH_3	DPP	BR-MeOH (1:9) pH 9.6	2.10^{-8} M	97
	AdSV	BR-MeOH (1:9) pH 4.6	3.10^{-9} M	
4'-NH_2	DPP	BR-MeOH (1:9) pH 9.7	2.10^{-8} M	93
	AdSV	BR-MeOH (1:9) pH 4.6	2.10^{-8} M	
4'-X	DPP	BR-MeOH (1:9) pH 6.7	5.10^{-8} M	100
(X=F,Cl,Br,I)	AdSV	BR-MeOH (1:1) pH 5.5	1.10^{-8} M	
3'-X	DPP	BR-MeOH (1:9) pH 6.1	2.10^{-7} M	101
(X=F,Cl,Br,I)	AdSV	BR-MeOH (1:9) pH 2.8	2.10^{-9} M	
4'-NO_2	DPP	BR-MeOH (9:1) pH 2.1	1.10^{-7} M	92
	AdSV	BR-MeOH (4:6) pH 8.4	2.10^{-9} M	
2´-COOH	DPP	BR pH 5.0	5.10^{-8} M	95
	AdSV	100xdiluted BR pH 4.0	1.10^{-9} M	
4´-SO_3H	DPP	BR pH 12.0	2.10^{-7} M	96
	AdSV	100xdiluted BR pH 9.0	3.10^{-10} M	

benzene by DPP.[93] Moreover, this selectivity can be increased by a preliminary separation using extraction by chloroform,[94] diethylether,[95] benzene,[96] or *n*-butyanol[96] or by TLC.[93,97] Anodic DPV at a glassy carbon RDE was proven to be useful not only to the initial derivatives of *N,N*-dimethyl-4-aminoazobenzene but also for products of their reductive splitting.[98] HPLC-ED was used for the analysis of mixtures of *N,N*-dimethyl-4-aminoazobenzene derivatives.[99]

B. Azodyes

All dyes are polarographically active and most of them can be anodically oxidized on solid or paste electrodes as well. Optimum conditions for polarographic and voltammetric determination of trace amounts of some genotoxic azodyes are summarized in Table 4.

VII. DERIVATIVES OF 1-PHENYL-3,3-DIMETHYLTRIAZENE

Derivatives of 1-phenyl-3,3-dimethyltriazene are among genotoxic substances that act via an alkylation mechanism. At the same time, they exhibit carcinostatic properties. With regard to the easy polarographic reducibility of triazene group, modern polarographic, and voltammetric techniques were used for the determination of trace amounts of these substances (see Table 5).

TABLE 4
Polarographic and Voltammetric Determination of Genotoxic Azo Dyes

Substance	Tchnique	Medium	LOD	Ref.
Congo red	DPP	BR buffer, pH 7	2.10^{-8} M	102
	DPV	BR buffer, pH 7	2.10^{-8} M	
	AdSV	BR buffer, pH 7	2.10^{-9} M	
Trypan blue	DPP	0.1M H_3PO_4	8.10^{-8} M	103
	DPV	0.1M H_3PO_4	6.10^{-8} M	
	AdSV	0.1M H_3PO_4	5.10^{-9} M	
Semitrypan blue	DPP	0.1M NaOH	8.10^{-8} M	104
	DPV	BR buffer, pH 8	5.10^{-9} M	
	AdSV	BR, buffer pH 8	8.10^{-11} M	

TABLE 5
Polarographic and Voltammetric Determination of Some Derivatives of 1-phenyl-3,3-Dimethyltriazene

Substituent	Technique	Medium	LOD	Ref.
2'-$CONH_2$	DPP/SMDE	BR-MeOH (9:1) pH 4.1	1.10^{-7} M	105
	AdSV	BR-MeOH (99:1) pH 5.1	3.10^{-9} M	
3'-$CONH_2$	DPP/DME	BR-MeOH (1:1) pH 5.9	2.10^{-7} M	106
	AdSV	BR-MeOH (99:1) pH 5.1	4.10^{-9} M	
4'-$CONH_2$	DPP/DME	BR-MeOH (1:1) pH 5.8	2.10^{-7} M	107
	AdSV	BR-MeOH (999:1) pH 4.0	4.10^{-9} M	
2'-NO_2	DPP/DME	BR-MeOH (1:1) pH 11.1	1.10^{-7} M	108
	AdSV	BR-MeOH (1:1) pH 7.2	1.10^{-8} M	
4'-C_6H_4-N=N-	DPP/DME	BR-MeOH (1:1) pH 8.0	1.10^{-7} M	109
	AdSV	BR-MeOH (999:1) pH 8.2	2.10^{-11} M	110
4'-Br	DPP/DME	BR-MeOH (9:1) pH 5.1	5.10^{-8} M	111

VIII. MYCOTOXINS

The structures of most mycotoxins (see Figure 2) suggest their polarographic reducibility and/or voltammetric oxidizability. Therefore, polarographic and voltammetric methods are frequently used for their determination (see Ref. 112). DPP determination of Aflatoxine B1, B2, G1, and G2 in food after its column chromatography on Sephadex LH 20[113] and of T2 toxine and deoxinivalenol in corn after extraction with hexane and TLC separation[114]

with LOD around 3.10^{-8} *M* are just two examples. Mytomycine B and C are reversibly reduced with the exchange of two electrons in quinone moiety and follow-up opening of the aziridine ring,[115,116] while in griseofulvin α,β-unsaturated carbonyl group is reduced.[117] HPLC-ED was used for the determination of *cis* and *trans*-zearalenone in which a phenolic ring is oxidized to quinoid system with the exchange of two electrons.[118] This method enables its determination in corn with less complicated preliminary separation than HPLC with UV spectrophotometric or fluorimetric detection.

IX. SOME OTHER CHEMICAL CARCINOGENS

A. Inorganic Substances

Arsenic can be determined[119] using DP CSV at a HMDE in the presence of pyrrolidine dithiocarbamate with LOD ~3.10^{-9} *M*. Further references on arsenic determination using DPP, ASV, or CSV can be found in the above-quoted paper.

Potentiometric-stripping analysis at MFE of beryllium in complex with Thorin,[120] AdSV at HMDE of cadmium[121] in complex with 8-hydroxyquinoline with LOD ~10^{-10} *M*, and AdSV at MFE of nickel in complex with dimethylglyoxime[122] are further examples of trace electroanalysis of inorganic carcinogens.

One of the obvious advantages of modern voltammetric techniques is the possibility of speciation, i.e., determination of different oxidation forms or different complexes of the same metal, which can be demonstrated on the determination of carcinogenic Cr(VI) and noncarcinogenic Cr(III) in the presence of diethylene-triaminepentaacetic acid using catalytic CSV at HMDE with LOD for Cr(VI) ~10^{-10} *M*.[123]

Polarographic and voltammetric methods of determination of various platinum complexes, which are classified as probably carcinogenic to humans, are described in monograph.[124]

B. Antineoplastic Agents

Most antineoplastic agents are genotoxic and thus their dosage must be carefully controlled. Modern polarographic and voltammetric methods were used successfully for monitoring plasma levels, tissue distribution, or excretion of anticancer drugs (see Ref. 124 and selected examples in Table 6). Moreover, polarography was used for modeling of metabolic processes of cancerostatic anthracyclines[6] and for correlation of half-wave potentials with cytostatic effects of some fenanthrenequinones.[125]

C. Halogen Compounds

Benzyl chloride is an effective alkylation agent whose reactivity toward cellular nucleophiles leads to high genotoxicity. C-Cl bond is rather difficult to reduce polarographically. Therefore, TBABr in DMF was used as a base electrolyte. In this medium, benzyl chloride yields one, well-developed, strongly irreversible, diffusion controlled wave with $E_{1/2} = -2.25$ V vs. SCE.[146] Because of this highly negative potential, DPP on DME gives relatively high LOD ~2.10^{-6} *M*.

D. Nitrogen Compounds

Hydrazine and its derivatives are amenable to anodic oxidation at solid electrodes, which was exploited for their HPLC-ED determination using carbon fiber voltammetric detector[147] or platinum tubular detector[148] with LD around 10^{-5} *M*, which is at least two orders better than UV spectrophotometric detection.

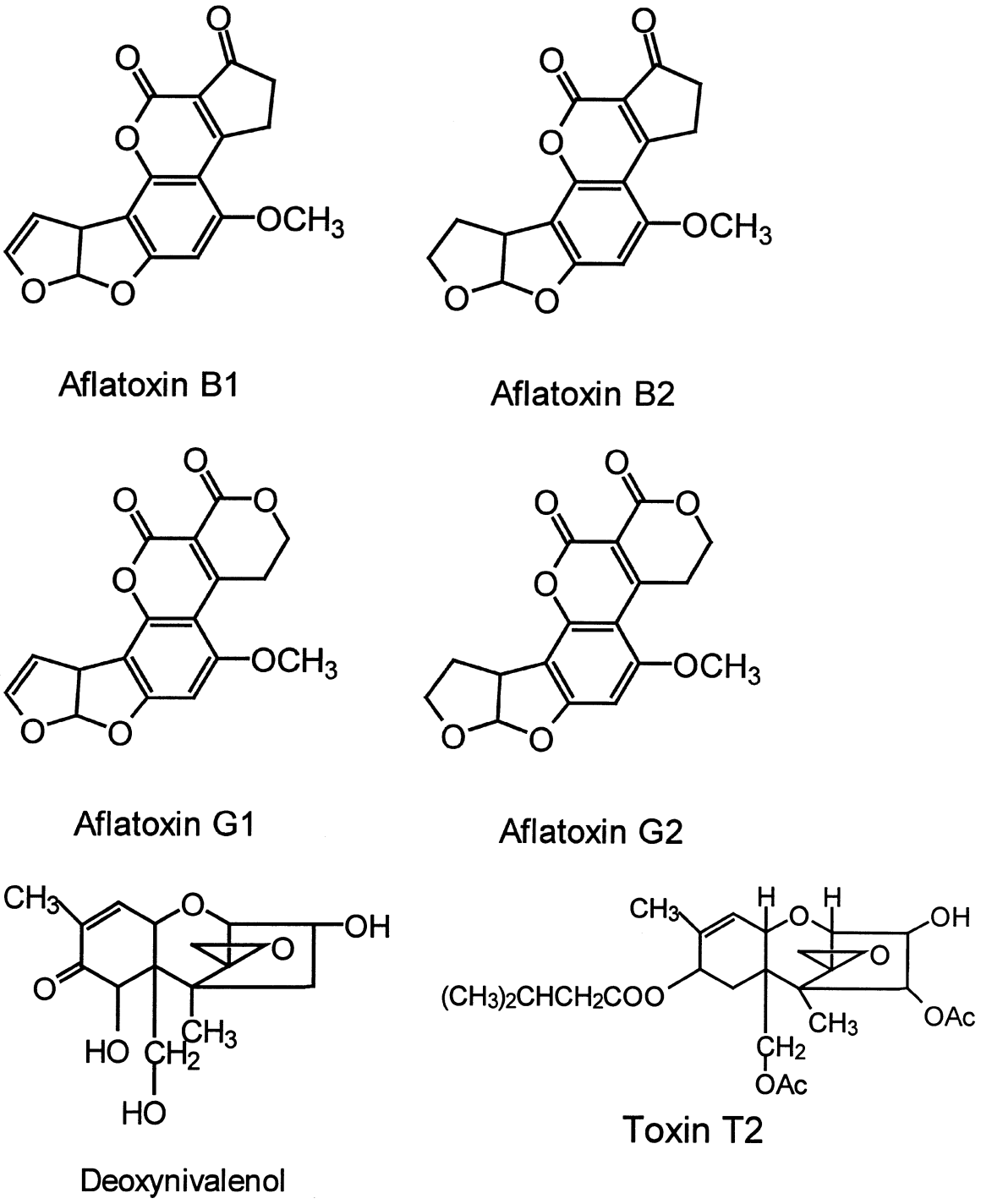


FIGURE 2. Structures of some mycotoxins.

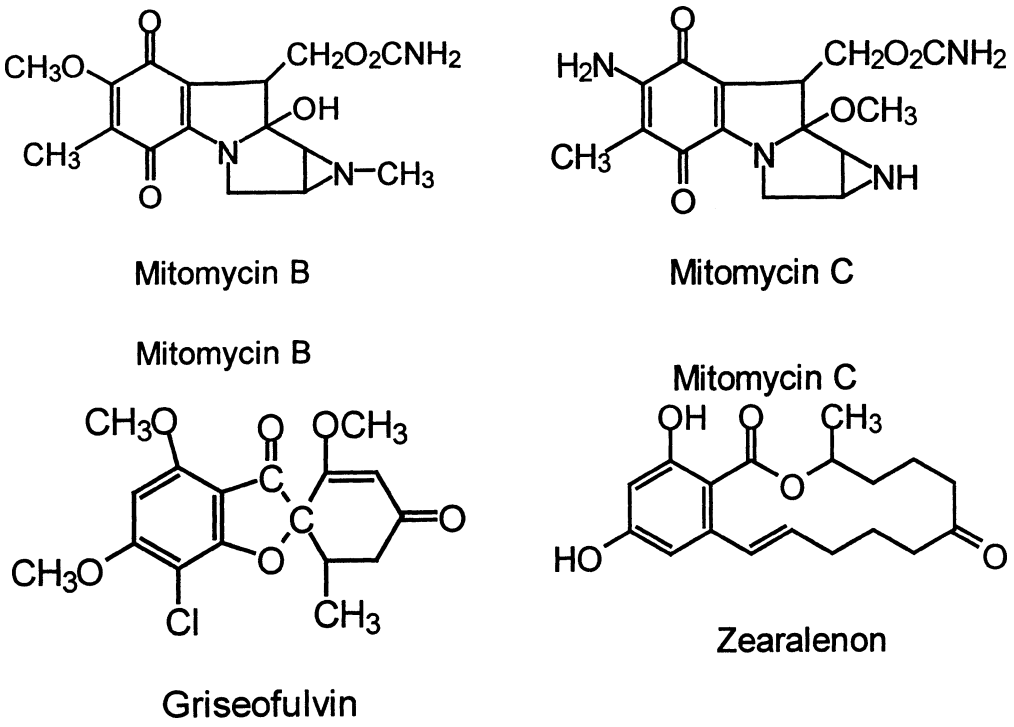


FIGURE 2 (continued).

E. Sulfur Compounds

Many sulfur-containing chemical carcinogens form insoluble compounds with mercury, which can be used for their AdSV determination at HMDE. Determination of thiourea[149] with LOD ~4.10^{-11} *M* is just one example.

X. FUTURE TRENDS

It can be expected that new polarographic and voltammetric techniques will be developed for the determination of trace amounts of formerly classified chemical carcinogens and for new classes of these dangerous substances as well. We believe that especially high sensitivity of adsorptive stripping voltammetry and increased selectivity of HPLC-ED makes these techniques a suitable alternative to so far prevalent spectrometric and separation techniques. Voltammetric immunoassays[150] represent another interesting field of development that can be possibly exploited in the analysis of chemical carcinogens. A new strategy based on DNA modified electrodes[151] for detecting nanomolar levels of carcinogenic aromatic amines[152] or hydrazines,[153] which utilizes the fact that the action of chemical carcinogens is related to their interaction with DNA, is another promising approach. Moreover, voltammetry with nucleic acid-modified electrodes can be used to detect damages to the DNA resulting form its interaction with carcinogenic substances.[154]

XI. SUMMARY

It is true that most carcinogens can be determined by other spectrometric and separation techniques, which are frequently more sensitive or more selective then polarography or voltammetry. However, these methods are usually much more expensive from the point of view of both investments and running costs. The main advantage of modern polarographic

TABLE 6
Polarographic and Voltammetric Determination of Selected Anticancer Drugs

Drug	Technique	LOD	Ref.
chlorambucil	AdSV on HMDE	3.10^{-8} M	126
melphalan	DPV on GC RDE	2.10^{-6} M	127
cyclophosphamide	DPP after nitrosation	1.10^{-5} M	128
methotrexate	NPP	1.10^{-6} M	129
methotrexate	DPP	1.10^{-6} M	130
5-fluorouracil	DPV at HMDE	1.10^{-8} M	133
5-fluorouracil	AdSV at HMDE	3.10^{-9} M	126
6-mercaptopurine	DPP at DME	1.10^{-6} M	134
Vinca alkaloids	NPV and DPV at CPE	1.10^{-6} M	135
Vinca alkaloids	AdSV	2.10^{-8} M	136
daunorubicine	AdSV at HMDE	1.10^{-9} M	137
doxorubicine	AdSV at HMDE	1.10^{-9} M	138
doxorubicine	AdSV at CPE	1.10^{-8} M	139
doxorubicine	FIA-AdSV	1.10^{-9} M	140
marcellomycine	AdSV	5.10^{-9} M	141
marcellomycine	AdSV at lipid modified GCE	5.10^{-6} M	142
mitomycine c	AdSV at HMDE	2.10^{-9} M	143
mitoxantrone	AdSV at CPE	5.10^{-11} M	144
dicarbazine	AdSV at HMDE	4.10^{-9} M	145

and voltammetric methods is their high sensitivity, wide concentration range from 10^{-3} to 10^{-10} *M*, applicability both to inorganic and organic chemical carcinogens, and last but not least the low cost of equipment, which enables building extensive networks of analytical laboratories required for large-scale monitoring. It should be stressed that they present an independent alternative to separation and spectrometric techniques that can be used for validation of results obtained by other techniques and that their relationship to other methods is complimentary rather the competitive. The low cost is especially important for economically weak central and east European countries.

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