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ELECTROCHEMICAL BEHAVIOUR OF ALKALOIDS: DETECTION AND INTERACTION WITH DNA AND PROTEINS

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In memory of Professor František Šantavý

Abstract – Electroanalysis can provide valuable information on the molecular interactions, biological activity and pharmacological potential of alkaloids. This article summarizes current knowledge on the electrochemical properties of a broad spectrum of alkaloids using polarography and voltammetry. Alkaloid interactions with DNA and proteins using electrochemical techniques are also described. More than 100 references published in the last 70 years are reviewed.

CONTENT

1. INTRODUCTION

Alkaloids are one of the most abundant and diverse groups of secondary metabolites found mainly in higher plants. They are defined as physiologically active basic compounds in which at least one nitrogen atom forms part of a cyclic system. Due to their structure, biosynthesis and pharmacological effects, they can be divided into several groups. Most play an important role in biomedical research. The total number of structurally known natural substances was estimated to be about 50,000 in 2008. Of these, ~21,000 alkaloids were identified.[1] Analytical methods used for the determination of alkaloids include: thin layer chromatography,[2,3] high performance liquid chromatography,[4,5] gas chromatography,[3,6] capillary electrophoresis[7,8] usually on-line combined with mass spectrometry,[3,9] diode-array detectors,[5] Fourier transform infrared spectroscopy,[10] fluorescence spectroscopy,[11] nuclear magnetic resonance,[12] and also electrochemical detectors.[13,14] Electrochemical methods, especially with mercury electrodes (polarography) are one of the oldest and a major pioneer in the polarographic determination of alkaloids was František Šantavý (Figure 1).[15] Today, electrochemistry is an indispensable experimental tool for investigating redox and adsorption properties, molecular interactions and the analysis of alkaloids.

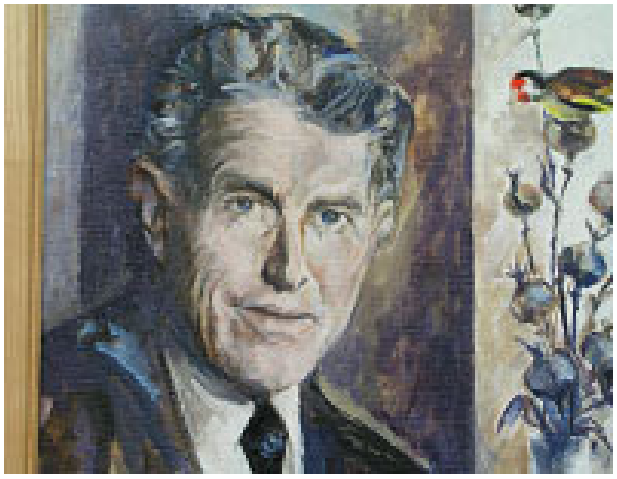

Figure 1. František Šantavý (*23.4.1915–†27.3.1983), Professor of Biochemistry, Palacký University in Olomouc (former Czechoslovakia). His scientific interests covered the isolation and chemical characterization of alkaloids, especially colchicines and benzylisoquinolines. This greatly respected person is captured on an oil-on-canvas by painter Oldřich Šimáček.

The aim of this review is *(a)* to summarize knowledge on electrochemical properties of alkaloids, *(b)* to describe the use of electrochemistry in the study of interactions of alkaloids with DNA and proteins, and *(c)* to present an overview of electroanalytical approaches to alkaloid analysis. The review does not intend to give a complete overview of the topics considered.

2. POLAROGRAPHY OF ALKALOIDS

Polarographic methods, especially d.c. polarography, were used to study the following alkaloids; atropine, hyoscyamine,[16] arecoline,[17] berberine, hydrastine, hydrastinine and emetine,[18,19] cocaine,[16] quinine, chelerythrine and sanguinarine,[20] erythrophleine,[21] cotarnine,[22] colchicine,[15,16,23] lobeline,[24] morphine and its derivatives,[25] opium, narceine and meconic acid,[26] nicotine, pilocarpine,[16] papaverine, piperine,[27] piperidine,[28] protopine and cryptopine,[29] santonin and sinomenine,[30,31] and sparteine.[16] Protopine was also investigated by oscillographic polarography.[29] In the 1940s and 1950s, polarography was one of the most sensitive analytical methods with a detection limit 10^{-3}-10^{-5} mol L^{-1}. In the 60s, classic dropping mercury electrode was replaced by hanging mercury drop electrode and from this emerged d.c. polarography by pulse voltammetric methods. Differential pulse polarography and d.c. polarography were used for the study of codeine, thebaine and dionin.[32] This comparative study was based on the dissolution of the alkaloids in concentrated nitric acid and subsequent reduction of the nitro derivatives. Colchicine and reserpine were studied using a.c. polarography.[32] This study is a comparison of aprotic organic and aqueous electrolytes for the determination of both alkaloids.

These are two electrochemical approaches for the analysis of alkaloids at mercury electrode. The first is non-direct reduction of alkaloids based on the formation of the catalytic reduction wave(s). Analytical applications of catalytic currents are typical for alkaloids that contain any directly reducible functional group(s). Electrocatalytically active alkaloids include morphine and its derivatives,[25] atropine, hyoscyamine, hyoscine, homatropine, cocaine, nicotine, sparteine, pilocarpine,[16] and hydrastine and emetine[19] and others. Electrocatalytic determination of berberine in the presence of H_2O_2 has been described in medicinal plants.[33] The second approach is based on 'direct' reduction of alkaloids on the mercury surface. For example, electrochemical multi-step reduction of chelerythrine and sanguinarine was investigated polarographically under both acidic and alkaline conditions.[20] Chelerythrine and sanguinarine, hydrastinine, cotarnine, berberine and many other alkaloids are also reducible.[18]

In polarographic studies of alkaloids, buffers or solutions of strong acids and hydroxides were used. In the case of unbuffered media, hydrochloric acid and its lithium salt and lithium hydroxide were used for polarographic analysis of several isoquinoline alkaloids.[19] Lithium chloride was applied for analysis of morphine and its derivatives,[25] and ammonium chloride was used for polarography of arecoline.[17] Sulphuric acid and its lithium salt were applied in the study of the polarographic behaviour of erythrofleine[21] and santonine.[30,34] Sodium hydroxide and other inorganic salts found application in the polarographic reduction of colchicine,[15,23] narceine and meconic acid.[26] Britton-Robinson buffer is one of the most commonly used buffers in polarographic studies on alkaloids. This has been used for the determination of berberine, hydrastine, hydrastinine,[18] chelerythrine, sanguinarine,[20] cotarnine,[22] lobeline,[24] morphine and its derivatives,[25] narceine and meconic acid,[26] piperine,[27] protopine and

cryptopine,[29] santonine,[30] sinomenine,[31] and colchicine.[23] Borate buffer was used for the investigation of cocaine, atropine, hyoscyamine, nicotine, pilocarpine, sparteine and colchicine,[16] and phosphate-citrate buffer was used in the study of colchicines.[15,23]

3. VOLTAMMETRY OF ALKALOIDS

Electrooxidation: Various types of working electrodes have been used for the investigation of the anodic reactions of alkaloids. A commonly used solid electrode is glassy carbon electrode (GCE). This is used for its good electrical and mechanical properties such as wide potential range, chemical inertness and relatively good reproducibility. Bare and modified GCE have been compared in studies of the electrochemical properties of alkaloids.[35-37] In general, modified electrodes can provide substantial benefits such as higher current response,[14,38] wide potential window, low background current, long-term signal stability, improved detection limits and reproducibility, higher sensitivity and resistance to surface contamination and passivation. There are several types of electrode modifications used for alkaloid analyses, *e.g.* determination of caffeine[39] and theophylline[40] by Nafion-graphene modified GCE and Nafion/lead-ruthenium oxide pyrochlore modified GCE.[38] GCE modified by single-walled carbon nanotubes was used for the oxidation of brucine,[41] colchicine,[42] and multi-walled carbon nanotubes were used for determination of atropine,[43] berberine,[44] thebaine,[45] caffeine,[46,47] nicotine,[48] and noscapine.[37] In addition to GCE, carbon paste and pyrolytic graphite electrodes (CPE and PGE) were applied under certain modified/unmodified conditions, *e.g.* determination of aminophilline,[49] brucine,[50] caffeine,[51] morphine,[52,53] strychnine,[50] theophylline,[49] vinblastine,[54] and vincamine.[55] Oxidation of protopine,[56] sanguinarine and dihydrosanguinarine,[57] purine alkaloids such as theobromine and caffeine, xantine and theophylline[58-60] were studied using bare PGE. For determination of nicotine, PGE modified by multi-walled carbon nanotubes was used.[61] Oxidation mechanisms of purine alkaloids such as xanthine, theophylline, theobromine and caffeine,[62-64] codeine[65] were studied by boron-doped diamond electrode (BDE). The summarization of voltammetric studies for the electroanalysis of alkaloids is presented in Table 1.

Of noble metal based electrodes, platinum and gold were applied for oxidation of brucine,[66] lysergic acid-type ergot alkaloids,[67] morphine,[68] and vinblastine.[69] Mercury electrodes, primarily used for cathodic reactions (see Sec. 2), were also applied to simultaneous determination of morphine and noscapine and investigation of their oxidation mechanisms. Adsorptive accumulation of morphine and noscapine on a hanging mercury drop electrode (HMDE), were followed by oxidation of adsorbed species by DPV.[70]

In practically all voltammetric studies of alkaloids, cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) predominate. In some cases, these electrochemical methods, are supported by impedance spectroscopy, quartz crystal microbalance

measurement and selected spectrometric approaches.

To improve the detection limits for alkaloids, preconcentrated samples are analyzed on the electrode surfaces by adsorptive stripping voltammetry. Adsorptive stripping SWV was used for determination of vincamine on Nujol-based CPE. Detection limits 6.0×10^{-9} mol L^{-1} were achieved in human serum with a recovery of 99.5 ± 1.79 % without extraction or preconcentration step.[55] Adsorptive transfer stripping CV and SWV were also used for determination of protopine, sanguinarine, its dihydroderivate[56,57] and brucine with a detection limit 6.0×10^{-8} mol L^{-1}.[66] Sensitive determination of caffeine and thebaine was carried out on a multi-walled carbon nanotubes modified GCE using adsorptive stripping CV[46] or DPV.[45] The effect of accumulation potential on the stripping peak current was studied. For example, high sensitivity was achieved with an accumulation potential of –1.0 V for caffeine. The detection limit was found to be 0.5×10^{-6} mol L^{-1} and the recoveries were in the range of 88 to 96 % for the determination of caffeine in real samples.

Electroreduction. Reduction and adsorption of brucine,[71] berberine,[72] cocaine,[73] colchicine,[74] and quinine[75] were investigated using HDME. Brucine was determined indirectly, after the reaction with nitric acid it was transformed into cacotheline. Adsorptive accumulation of cacotheline at HDME was followed by cathodic linear sweep voltammetry (LSV). A detection limit of 2.0×10^{-9} mol L^{-1} was obtained.[71]

Reduction and adsorption of berberine were investigated by a.c. polarography and SWV in supporting electrolytes of various pH. This is an irreversible process, occurring between –0.95 V and –1.35 V, depending on berberine concentration and a pH value. Canadine, a reduction product of berberine is electro-inactive and cannot be reoxidized on mercury electrode. Both berberine and canadine form very stable condensed films, even at –0.5 V on the mercury surface.[72]

As well as cathodic reaction of berberine, the reduction and adsorption of cocaine and its metabolites such as benzoylecgonine, ecgonine, and ecgonine methyl ester were investigated using SWV. Adsorptive stripping voltammetric method for determination of cocaine was developed on the basis of benzoylecgonine reduction because of its stronger adsorption on the mercury surface compared to cocaine. In phosphate buffer (pH=7) cocaine undergoes a reduction at a potential of about –1.49 V. Benzoylecgonine undergoes reduction at potential of about –1.1 V, depending on its concentration and pH of supporting electrolyte. Thus, at pH=8.5, cocaine solution gives rise to two peaks due to the reduction of unhydrolysed cocaine and benzoylecgonine. The reduction product of benzoylecgonine undergoes dimerisation and then protonation leading to final products, ecgonine and benzaldehyde.[73]

Reduction of colchicine was investigated by CV and HMDE. Under acidic conditions, colchicine undergoes irreversible reduction around –1.0 V.[74] The electrochemical behavior of quinine was investigated by CV, SWV and coulometry in the presence of different surfactants,

cetyltrimethylammonium bromide, sodium dodecylbenzene sulfonate and Tween-20. Quinine gave one well-defined reduction peak which is attributed to the reduction of quinoline moiety.[75]

Table 1. Voltammetry of alkaloids. *Abrev.:* CV – cyclic voltammetry, CSV – cathodic stripping voltammetry, DPV – differential pulse voltammetry, SWV – square wave voltammetry, a.c. impedance (a.c. – alternating current), LSV – linear sweep voltammetry, GCE – glassy carbon electrode, CPE – carbon paste electrode, SPE – screen printed electrode, HMDE – hanging mercury drop electrode, BDE – boron-doped carbon electrode, PGE – pyrolytic graphite electrode, Pt – platinum.

Alkaloids	Methods	Electrodes	Supporting electrolytes	References
Aminophylline	CV	GCE, CPE, SPE	Britton-Robinson buffer (pH=2-12), phosphate buffer (pH=7), acetate buffer (pH=4.5), $LiClO_4$ in 0.1 M EtOH	Campean et al.[49]
	CSV	HMDE	Phosphate, borate and Britton-Robinson buffer (pH=4-10)	Shubietah et al.[76]
Atropine	CV, DPV	Multi-walled carbon nanotube electrode	Tetramethyl ammonium hydroxide, sodium dodecyl benzene sulfonate	Dar et al.[43]
Brucine	CV, SWV, a.c. impedance	4-Amino-2-mercapto pyrimidine self-assembled monolayer modified gold electrode	H_2SO_4	Zhang et al.[66]
	LSV	HDME	Acetate buffer (pH=4.0)	Li et al.[71]
	CV	Single-walled carbon nanotubes modified GCE	H_2SO_4	Liu et al.[41]
	CV, chronocoulometry DPV	CPE modified with multi-walled carbon nanotubes	Britton-Robinson (pH=2-10)	Behpour et al.[50]
Berberine	CV, SWV, a.c. polarography	HMDE	Phosphate buffer (pH=7), citrate buffer (pH=4), borate buffer (pH=10)	Komorsky-Lovric[72]
	CV, DPV	GCE	Britton-Robinson, phosphate buffer (pH=3.5, 7.4, 11), NaOH, Na_2SO_4	Skopalová et al.[77]
	CV, DPV, SWV	GCE	HCl/KCl (pH=1.2, 2.0), acetate buffer (pH=3.3-5.9), phosphate buffer (pH=7-8), NH_3/NH_4Cl (pH=9.6)	Diculescu et al.[78]
	CV, DPV	GCE	Britton-Robinson buffer (pH=5.7)	Tian X. et al.[79]
	CV, DPV	Multi-walled carbon nanotube modified SPE	Phoshate buffer (pH=7)	Ovádekova et al.[44]
Caffeine	CV, SWV	1,4-benzoquinone modified CPE	HCl + NaOH (pH=1-12)	Aklilu et al.[51]
	CV, SWV	4-Amino-3-hydroxynaphthal ene sulfonic acid modified GCE	Acetate buffer (pH=5)	Amare et al.[35]
	DPV	Multi-walled carbon nanotubes/Nafion modified GCE	Britton-Robinson buffer (pH=1-12)	Zhang et al.[46]
	CV, DPV	Nafion-covered GCE	H_2SO_4, HCl, HNO_3, acetate buffer (pH=5), phosphate buffer (pH=7), KNO_3	Brunetti et al.[80]
	CV, DPV	Nafion/graphene modified GCE	H_2SO_4, HNO_3, HCl, CH_3COOH	Sun et al.[39]
	CV	GCE, BDE	Britton-Robinson buffer (pH=1.8-12)	Spataru et al.[62]
	CV	GCE, CPE, SPE	Britton-Robinson buffer (pH=2-12),	Campean et al.[49]

			phosphate buffer (pH=7), acetate buffer (pH=4.5), $LiClO_4$ in 0.1 M EtOH	
	CV, DPV, SWV	GCE	Acetate buffer (pH=4.5)	Campean et al.[81]
	CV	PGE	Acetate buffer (pH=4.7)	Hansen & Dryhurst[58]
	CV, SWV	PGE	Phosphate buffer (pH=4-11)	Goyal et al.[59]
	SWV	Multi-walled carbon nanotubes modified GCE	Phosphate buffer (pH=4-10)	Gupta et al.[47]
	CV, DPV	BDE	0.4M $HClO_4$	Švorc et al.[63]
	DPV, SWV	BDE	Acetate buffer (pH=4.5)	Lourencao et al.[64]
	CV, DPV	Nafion/multi-walled carbon nanotubes modified GCE	H_2SO_4	Yang et al.[82]
Camptothecin	CV, DPV, SWV	GCE	HCl+KCl (pH=1.2-2.1), acetate buffer (pH=3.4-5.1), phosphate buffer (pH=6.1-7), NH_3+NH_4Cl (pH=9.1)	Shah et al.[83]
Chelerytrine	CV, DPV	GCE	Britton-Robinson buffer, phosphate buffer (pH=7.5)	Hrbáč et al.[84]
Cocaine and its metabolite (benzoylecgonine)	SWV	Paraffin-impregnated graphite electrode	KNO_3 (pH=2)	Komorsky-Lovric et al.[85]
	CV	Cobalt hexacyanoferrate film modified Pt	Acetonitrile + 0.1 M $NaClO_4$	Oiye et al.[86]
	SWV	HMDE	KNO_3 (pH=9.2)	Komorsky-Lovric et al.[87]
	SWV	HMDE	Phosphate buffer (pH=7, 8.5), ammonia buffer (pH=10) and NaOH	Pavlova et al.[73]
Codein	DPV	GCE	KCl + HCl (pH=1.2-2), acetate buffer (pH=3.4-5.4), phosphate buffer (pH=6.9-8.1), $Na_2B_4O_7$ + NaOH (pH=9.3), KCl + NaOH (pH=12.1-12.8)	Garrido et al.[88]
	CV, DPV	BDE	Britton-Robinson buffer (pH=7)	Švorc[65]
Colchicine	CV, DPV, a.c. impedance	Poly(*o*-phenylenediamine)/ single-walled carbon nanotubes modified GCE	H_2SO_4 (pH=1)	Zhang et al.[42]
	CV, DPV	SPE	H_3PO_4/$HClO_4$ (pH=2.05)	Bodoki et al.[89]
	CV, DPV	Acetylene black-dihexadecyl hydrogen phosphate modified GCE	$HClO_4$	Zhang[90]
	CV	GCE, HMDE	Perchloric acid, H_3PO_4, KCl, KNO_3, sodium perchlorate, phosphate buffer	Kasim et al.[74]
Ephedrine	CV	Polypyrrole film modified GCE	Britton-Robinson buffer (pH=9.2)	Mazzotta et al.[91]
	CV, SWV	CFE	0.1 M NaOH, Britton-Robinson buffer (pH= 6-12)	Platts et al.[92]
Ergot alkaloids	CV, DPV, amperometry	GCE	Acetonitrile-0.02 M KH_2PO_4 (pH=2)	Inczeffy et al.[93]
	CV, coulometry	Pt	$LiClO_4$ + acetonitrile containing perchloric acid	Dankházi et al.[67]
Morphine	CV	GCE	Phosphate buffer (pH=7.4), Britton-Robinson buffer (pH=3-9)	Li et al.[94]
	CV, amperometry	Prussian blue/indium tin oxide electrode	KCl/HCl (pH=2.75-5.75)	Ho et al.[95]
	CV, amperometry	Ordered mesoporous carbon modified GCE	Phosphate buffer (pH=7)	Bo et al.[36]
	CV, SWV	Poly(3,4-ethylenedioxythio phene) modified Pt	Britton-Robinson buffer (pH=2-9)	Atta et al.[68]
	CV, DPV	Gold nanotubes/GCE	Phosphate-citric acid buffer (pH=5.2-7.2)	Yang et al.[96]
	DPV	GCE	KCI + HCI (pH=1.2-2), acetate buffer (pH=3.4-5.4), phosphate buffer (pH=6.9-8.1), $Na_2B_4O_7$ + NaOH (pH=9.3), KCI + NaOH (pH=12.1-12.8)	Garrido et al.[97]

	DPV	HMDE	Britton-Robinson buffer (pH=1-11)	Niazi et al.[70]
	CV, DPV, amperometry	CPE spiked with 4-hydroxy-2-(triphenyl phosphonio) phenolate and multi-walled carbon nanotubes	Phosphate buffer (pH=7.0)	Shishehbore et al.[52]
	CV, SWV, amperometry	ZnO/CNT nanocomposite, 1-methyl-3-butylimid azolium bromide modified CPE	Phosphate buffer (pH=5-9)	Afsharmanesh et al.[53]
Nicotine	CV, DPV, SWV	Pencil graphite electrode	Phosphate buffer (pH=4.8-10)	Levent et al.[98]
	CV, SWV	BDE	Britton-Robinson buffer (pH=4-8)	Suffredini et al.[99]
	CV, SVW	Poly(4-amino-3-hydroxy naphthalene sulfonic acid) modified GCE	Phosphate buffer (pH=7.5)	Geto et al.[100]
	CV	Multi-walled carbon nanotubes modified PGE	Britton-Robinson buffer	Sims et al.[61]
	CV, amperometry	Multi-walled carbon nanotube/alumina-coated silica nanocomposite modified GCE	Phosphate buffer (pH=8)	Wang et al.[101]
Navelbine (5'-noranhydro vinblastine)	CV, DPV	GCE	KCl + HCl (pH=1.2-2), acetate buffer (pH=3.4-5.4), phosphate buffer (pH=6.9-8.1), $Na_2B_4O_7$ + NaOH (pH=9.3), KCl + NaOH (pH=12.1-12.8)	Brett et al.[102]
Noscapine	CV	Multi-walled carbon nanotubes modified GCE	Britton-Robinson buffer (pH= 2-10)	Rezaei & Zare[37]
	DPV	HMDE	Britton-Robinson buffer (pH=1-11)	Niazi et al.[70]
Papaverin	LSV	GCE, PGE, Pt	H_2SO_4	Ziyatdinova et al. [103]
Protopine	CV, SWV	PGE	Britton-Robinson buffer (pH=2-10.5)	Vrublová et al.[56]
Quinine	CV, DPV, SWV, coulometry	HMDE	Britton-Robinson buffer (pH=4.5-10.4)	Dar et al.[75]
Sanguinarine	CV, DPV, SWV	GCE	HCl/KCl (pH= 1.2-2), acetate buffer (pH=3.3-5.6), phosphate buffer (pH= 6-8), NH_3/NH_4Cl (pH= 9.8)	Diculescu et al.[104]
	CV, SWV	PGE	Britton-Robinson buffer (pH=2-11.5)	Vacek et al.[57]
	CV, DPV	GCE	Britton-Robinson buffer, phosphate buffer (pH=7.5)	Hrbáč et al.[84]
Strychnine	CV, chronocoulometry DPV	CPE modified with multi-walled carbon nanotubes	Britton-Robinson buffer (pH=2-10)	Behpour et al.[50]
Topotecan	CV, DPV	Acetylene black nanoparticles modified GCE	Phosphate buffer (pH 5.7-8)	Cheng et al.[105]
Thebaine	CV, impedance spectroscopy	Multi-walled carbon nanotube modified GCE	Britton-Robinson buffer (pH= 2-10)	Rezaei & Damiri[45]
Theobromine	CV	GCE, BDE	Britton-Robinson buffer (pH=1.8-12)	Spataru et al.[62]
	CV	PGE	Acetate buffer (pH=4.7)	Hansen & Dryhurst[58]
Theophylline	SWV	Nafion/lead-ruthenium oxide pyrochlore modified GCE	Phosphate buffer (pH=3)	Zen et al.[38]
	CV, DPV, SWV	GCE	Acetate buffer (pH=4.5)	Campean et al.[81]
	CV	GCE, BDE	Britton-Robinson buffer (pH=1.8-12)	Spataru et al.[62]
Vinblastine	CV, DPV, SWV	Pt	Acetonitrile, sodium perchlorate and pyridine	Haque & Saba[69]
	CV, DPV	CPE	Acetate buffer in ethanol/water (1:1), pH 5.6	Rusling et al.[54]
Vincamine	CV, SWV	CPE	Britton-Robinson buffer (pH=2-11), acetate buffer (pH=3.8-6.2), phosphate buffer (pH=2-7.5), KCl/KNO_3	Beltagi[55]

5-Noranhydrovinblastine, vinblastine, vincristine, vindesine	CV, DPV	GCE	KCl + HCl (pH=1.2-2), acetate buffer (pH=3.4-5.4), phosphate buffer (pH=6.9-8.1), $Na_2B_4O_7$ + NaOH (pH=9.3), KCl + NaOH (pH=12.1-12.8)	Brett et al.[106]
Xanthine, hypoxanthine	CV	GCE, BDE	Britton-Robinson buffer (pH=1.8-12)	Spataru et al.[62]
	CV	PGE	McIlvaine buffer (pH=2.2-8), acetate buffer (pH=3.5-5.8), NH_4Cl+NH_4OH (pH=8.5-9)	Hansen & Dryhurst[60]

4. LIQUID SEPARATION COMBINED WITH ELECTROCHEMICAL DETECTORS

One of the disadvantages of polarographic and voltammetric techniques is fact that these techniques do not reach the selectivity of separation methods. Therefore, for the analysis of complex plant extracts application of chromatographic separation coupled with on-line detection techniques such as amperometry or coulometry is preferred.

4.1. HPLC with Electrochemical Detectors

High performance liquid chromatography (HPLC) with amperometric detection was used for determination of â-carboline alkaloids at carbon nanotubes modified GCE. The HPLC (C_{18} reversed phase) separation and determination of â-carboline alkaloids were carried out using a mobile phase consisting of 20% (v/v) methanol, 20% (v/v) acetonitrile and 0.05 mol L^{-1} monohydrogen phosphate. Detection limits were 28 × 10^{-9} mol L^{-1} (for harmalol) and 88 × 10^{-9} mol L^{-1} (for harmaline). The applicability of the method was demonstrated using food samples, beer, coffee and cheese. Recoveries ranging between 92 and 102 % for beer, 92 and 101 % for coffee, and 88 and 100 % for cheese were achieved.[14,107] HPLC with amperometric detection was also used for the simultaneous determination of four isoquinoline alkaloids including berberine, jatrorrhizine, coptisine and palmatine in Chinese medicinal plant *Coptis chinensis*. In comparison with the previous study, unmodified bare GCE was applied. The HPLC analysis was performed on C_{18}-column with the mobile phase containing phosphate buffer (pH=7) and acetonitrile (40:60, v/v). The limits of detection of the four alkaloids ranged from 0.01 to 0.03 × 10^{-6} mol L^{-1} where the detection limit of berberine was 80-times lower in comparison to UV detection.[108] HPLC-amperometric detection of caffeine, theobromine, theophylline and adenine was developed using C_{18}-column with an isocratic elution of phosphate buffer (pH=3.5) and methanol (90:10, v/v). The optimal detection potential was +1.4 V. The limits of detection were 0.4 ng for adenine, 1 ng for theophylline, and 2.5 ng/injection for caffeine and theobromine. The method was applied for the determination of the above purine alkaloids in beverages (tea, coffee and cacao).[109] Amperometric detection at carbon fibre electrode was developed for determination of narciclasine in the blood of mice. For separation, C_{18} stationary phase and mobile phase of methanol/0.025 mol L^{-1} potassium dihydrogen

phosphate (pH=5.5) was used.[110] The HPLC method for determination of homoharringtonine in plasma was also developed. The oxidation of homoharringtonine occurred at +1.0 V at GCE. The chromatographic method was performed on CN-column with acetonitrile/15 × 10^{-6} mol L^{-1} KH_2PO_4-H_3PO_4 (pH=6.8) mobile phase. This method was applicable for plasma or serum, and it has been demonstrated to be useful for study of the pharmacokinetics of homoharringtonine in patients suffering from acute non-lymphocytic leukaemia.[111] Comparison was made of amperometric and UV detection of morphine, papaverine, dihydrocodeinone, oxycodone, dihydromorphinone, heroin, apomorphine, codeine, narcotine, hydrastinine, vindesine, vincristine, vinblastine and desacetylvinblastine.[112]. Amperometric detection at GCE was more sensitive than UV detection. The separation process was based on CN-column with mobile phase composed of acetonitrile/phosphate buffer (pH=3) with 1 × 10^{-6} mol L^{-1} NaCl.

HPLC assay with coulometric electrochemical detection was developed for the determination of morphine in human, rabbit, pig and dog plasma. This includes a one-step extraction procedure with hexane/isoamyl alcohol (pH=8.9) and reversed-phase liquid chromatography on a μPorasil column. The mobile phase was composed of 5 × 10^{-6} mol L^{-1} sodium acetate buffer (pH=3.75) and acetonitrile (25:75, v/v). The limit of detection of morphine was 100 ng per one liter of plasma.[113]

4.2. Capillary Electrophoresis with Electrochemical Detectors

Nonaqueous capillary electrophoresis (CE) coupled with electrochemiluminescence and electrochemical dual detection was demonstrated for the analysis of atropine, anisodamine, and scopolamine. The method was applied for the determination of these three alkaloids in *Flos daturae* extract. A mixture of acetonitrile and 2-propanol containing 1 mol L^{-1} acetic acid, 20 × 10^{-3} mol L^{-1} sodium acetate, and 2.5 × 10^{-3} mol L^{-1} tetrabutylammonium perchlorate was used as the electrophoretic buffer. The linear concentration ranges of atropine, anisodamine, and scopolamine were 0.5–50, 5–2000, and 50–2000 × 10^{-6} mol L^{-1}, respectively.[114] As well as in the study above, nonaqueous CE with electrochemical detection was applied to the determination of nicotine. The measurements were performed using acetonitrile-based buffer. Nicotine was shown to yield well-defined voltammetric signals suitable for anodic detection. The limit of detection for nicotine was 13 × 10^{-9} mol L^{-1}.[13]

The separation and determination of theobromine, theophylline and caffeine have also been repeorted by micellar electrokinetic capillary chromatography with amperometric detection. A carbon disk electrode was used as the working electrode and phosphate buffer solution (pH=8.5) with sodium dodecyl sulphate as a medium. The method was applied successfully to determine the active ingredients of the composite theophylline tablets.[115] CE combined with electrochemiluminescence detection and/or dual performance of electrochemical/optical detectors is preferred to classical electrochemical detector.[114,116-119]

5. ALKALOID INTERACTIONS WITH DNA AND PROTEINS

5.1. Interactions with DNA

Investigation of the mechanism of interaction between the alkaloids and DNA studied by electrochemical methods is primarily based on examination of the electrochemical behavior of the alkaloid in the presence and/or absence of DNA. The mechanism of interaction can be investigated in two different ways, using interaction of DNA with alkaloids in solution followed by electrochemical analysis and/or interaction of alkaloids with DNA modified electrodes (DNA biosensors). Interactions of alkaloids with DNA were investigated on different types of working electrodes such as PGE,[57] GCE,[48,79] HMDE,[120] CPE and pencil graphite electrode,[44,121] screen printed electrode (SPE),[44,89] using different electrochemical techniques, SWV,[120] DPV,[44,48,79,121,122] and *ex situ* (adsorptive transfer) SWV.[57]

5.1.1. Interaction in the solution

Alkaloid and DNA are mixed and incubated together in aqueous medium and electrochemical signals of alkaloid and/or DNA are then measured. These electrochemical signals are compared with the signals of DNA and alkaloid itself. This experimental approach is preferred because the incubation of alkaloids with DNA adsorbed onto electrode surfaces (DNA biosensors) can lead to 'artificial' results, i.e. conformational changes of DNA involved after adsorption (vs. native DNA in solution).

Interactions of DNA with sanguinarine or dihydrosanguinarine were studied in Britton-Robinson buffer (pH=7.4) using PGE and *ex situ* SWV. Study of the interactions is based on the observation of sanguinarine and dihydrosanguinarine (main sanguinarine metabolite in mammals) oxidation peaks before and after incubation with DNA. The results showed that sanguinarine intercalates into the double stranded structure of DNA, while increased binding affinity was observed for its quaternary cation. In comparison with dihydrosanguinarine, which does not possess a strictly planar molecular structure shows no intercalative DNA binding. Electrochemical study of the interactions was confirmed and complemented with measurements using gel electrophoresis and steady-state and time-resolved fluorescence spectroscopy.[57]

The interaction of nicotine and DNA was studied by DPV at GCE in 0.05 mol L^{-1} $Na_2C_2O_4$ buffer solution (pH=4.2). The peak currents of nicotine decreased and the peak potentials shifted negatively with increasing DNA concentration. The results imply that nicotine probably binds to DNA in solution via electrostatic interaction.[48]

Berberine also interacts with DNA. The potential of the oxidation peak for berberine shifts to negative values with increasing DNA concentration, which probably indicates the electrostatic interaction of berberine cation and polyanionic DNA. The height of the peak current associated with the redox reaction of berberine decreased according to increasing concentration of DNA. This may be associated with the

formation of DNA-berberine adducts, which are not electrochemically active under these conditions. Verification of electrostatic interaction between berberine and DNA, as well as between nicotine and DNA were investigated using the effect of ionic strength.[79]

The interaction of lycorine with DNA was studied electrochemically based on the oxidation DNA signals of guanine and adenine by using DPV at CPE and pencil graphite electrode. The oxidation signals of guanine were observed after lycorine-DNA interaction in buffered solution. When DNA interacted with lycorine, a dramatic decrease in DNA peaks was observed.[121]

Interactions of codeine and morphine with DNA were also investigated. DP voltammograms of codeine showed decrease in peak current and negative shift of the anodic peak potential with increasing DNA concentration in solution. Also the oxidation signal of morphine decreased with increasing DNA concentration but a significant positive change was observed in the peak potential after interaction with DNA. It may be concluded that codeine is bound to DNA mainly in an electrostatic mode.[122]

5.1.2. DNA biosensors

The interactions of lycorine with DNA and synthetic polynucleotides poly(G) were studied electrochemically based on the oxidation signals of lycorine and DNA oxidation peaks of guanine and adenine at CPE and pencil graphite electrode by DPV. Immobilization of DNA samples on the electrode surface was carried out using a potential +0.5 V or wet-adsorption. As a result of the interaction of lycorine with DNA, the voltammetric signal of guanine and adenine greatly decreased. This phenomenon can be explained by blocking of electroactive bases, providing an electrochemical signal, during the interaction of the alkaloid with DNA.[121]

The sensing strategy for the study of nicotine-DNA interactions is based on the immobilization of DNA onto GCE surface. Observation of decrease in the peak current of nicotine and a negative shift in the oxidation potential of nicotine in the presence of increasing concentrations of the DNA was probably associated with nicotine electrostatic interaction with DNA. The effect of changing the ionic strength with NaCl was studied to confirm the electrostatic binding.[48]

Detection of interaction of colchicine with DNA on the surface of SPE was studied using SWV in 0.25 mol L^{-1} acetate buffer containing KCl. No colchicine interaction with DNA has been demonstrated. This is proof that the antimitotic effect of colchicine is not accompanied by colchicine binding to DNA, but to protein.[89]

Interaction of the berberine cation with DNA is probably mediated by electrostatic binding. The interaction was examined in Britton-Robinson buffer (pH=5.7). The Langmuir-Blodgett technique was used for immobilization of DNA on the surface of GCE. This technique has been confirmed as suitable for the immobilization of DNA on the electrode surface and DNA retains its native form as double

stranded DNA.[79] Interaction of berberine was also investigated using DNA modified multi-walled carbon nanotubes-based SPE sensors. DNA was labeled by Co-complex as the DNA redox marker. The potential anticancer effect of berberine may be due to an effect on DNA. DNA from non-cancer cells was structurally much more stable toward the action of berberine than DNA from U937 cancer cells.[44]

The SWV was used to study interaction of ellipticine with DNA.[120] The first step was adsorption of DNA on HMDE and then adsorptive transfer technique was used to establish ellipticine interaction with DNA modified-HMDE. A decrease in adenine and cytosine DNA reduction signals can be observed and this is probably associated with interaction of ellipticine with DNA. In additional experiments, the authors investigated changes in the reduction signal in DNA treated with ellipticine *in vitro* and DNA isolated from UKF-NB-3 neuroblastoma cells treated with 1×10^{-6} mol L^{-1} ellipticine.

The interaction between codeine and/or morphine with DNA was determined at DNA modified multi-walled carbon nanotubes-pencil graphite electrode using DPV. It was shown that both alkaloids were electrochemically oxidized due to the presence of phenolic and amino groups in their structures. As described previously, the interaction modes were electrostatic for codeine and intercalation for morphine.[122] Interaction of sanguinarine from adulterated mustard oil with DNA was investigated using DNA modified PANi/ClO_4-ITO electrode. Sanguinarine intercalated into DNA strands forming complexes and this resulted in a decrease in redox peak currents.[123]

5.3. Interactions with Proteins

The effectiveness of alkaloids in pharmacological applications can be influenced by interactions with serum albumin. This may be crucial for understanding its toxicity and distribution in the human organism. Interaction between nicotine and bovine serum albumin (BSA) was studied using sensor based on BSA and poly-*o*-phenylenediamine (PoPD) film modified GCE. The interaction was observed via changes in the peak currents of PoPD, which was used as electrochemical probe. The PoPD peak current decreased with increase in nicotine concentration in the presence the BSA. The decrease in PoPD peak current at BSA/PoPD/GCE was attributed to the binding of nicotine to BSA. The interaction of nicotine with BSA formed an electro-inactive complex on the electrode, which inhibited the interfacial electron transfer of PoPD.[124] Interaction of the albumin with colchicine was studied on graphite-based SPE[89] and Fe_3O_4 nanoparticles modified CPE[125] using BSA immobilization on the electrode surface or in solution. The results showed that the interaction between colchicine and BSA, leads to the formation of electro-inactive complexes, in which colchicine is less favorable for electron transfer. Due to these changes, the anodic peak of colchicine decreases and shifts to positive potentials.[125] Investigation of the interaction of BSA with papaverine is based on the same principle as was described above. After formation of electro-inactive complex of papaverine and BSA, the peak current of PoPD at GCE was decreased.[126]

6. CONCLUSION

The electrochemical study of alkaloids was pioneered by František Šantavý and his colleagues in the 1940s. These studies focused on application of polarography in the determination and redox behavior of alkaloids. The development of advanced instrumentation and introduction of novel electrode materials have opened up new application fields, such as study of electrooxidation, reactivity and adsorption for a wide range of alkaloids. In the case of sensitive analysis, electrochemical sensors have been developed, often based on modification of carbon electrodes by nanomaterials or polymeric structures. Despite the considerable number of analytical studies, the potential for application of electrochemical methods is in the study of reactivity, molecular interactions and redox mechanisms. Thus, mechanistic studies can contribute significantly to the interpretation of principles that are responsible for the biological effects of the studied alkaloids. Electrochemical methods can also be used for the electrosynthesis of alkaloids or their analogues (oxidation or reduction products), whose chemical synthesis has not been described, is time-consuming or for other reasons made more difficult. Alkaloids are a highly diverse group of substances and include thousands of individual species. Only a small percentage of this total has been electrochemically characterized. This is a motivation for the future. Systematic study on the redox transformations of different groups of alkaloids can reveal much information about their behavior in living organisms.

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