Electrochemical Analysis of Opiates—An Overview

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ABSTRACT

The analysis of opiates is of vital interest in drug abuse monitoring and research. This review presents a general overview of the electrochemical methods used for detection and quantification of opiates in a variety of matrices. Emphasis has been placed on the voltammetric methods used for study and determination of morphine, codeine, and heroin. Specific issues that need to be solved and better explained as well as future trends

in the use of electrochemical methods in the examination of opiates are also discussed.

Key Words: Opiates; Voltammetry; Electroanalysis; Electrochemical detection; Review.

INTRODUCTION

Opiates were originally available from the opium poppy (*Papaver somniferum*), native in Asia Minor. The alkaloids which occur in the poppy include morphine, noscapine, codeine, papaverine, and thebaine (Fig. 1). Traditionally, only natural substances from opium and semisynthetic derivatives are termed opiates, whereas opioids are synthetic derivatives, e.g., methadone. However, in recent times the term opioid has been understood to encompass opiates.

Opiates and their derivatives are very potent analgesics. Although commonly used as therapeutic agents, some of these compounds are also frequently abused as illicit drugs. Indeed, opiates are responsible for more than 80% of all drug deaths occurring in Portugal.[1]

With the rise in the use of illegal drugs there is also an increased demand to identify illegal drug consumption. Consequently, forensic science laboratories

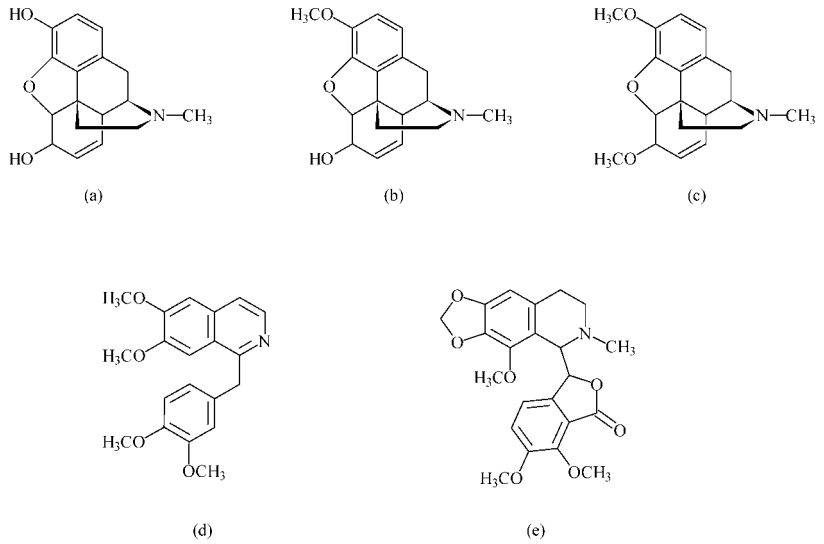

Figure 1. Molecular structures of opium alkaloids; (a) morphine, (b) codeine, (c) thebaine, (d) papaverine, and (e) noscapine.

must have specific and sensitive techniques to discriminate between the legal and illegal intake of opiates. Clinical and forensic testing of these compounds generally involve screening by immunological methods and confirmation using chromatographic techniques, usually of biological fluids, like blood and urine.

Different methods have been developed for the detection and determination of opiates and their derivatives. Many of the suggested methods are based on the use of gas chromatography combined with mass spectrometry (GC-MS) because of its sensitivity and specificity.[2,3] The necessity of sample derivatization and the cost of the technique itself restricts and constrains its applicability and use. Liquid chromatographic (LC) procedures are more often used for the determination of opiates than GC-MS methods.[4,5] The LC methods in combination with electrochemical detection or fluorescence detection are comparable in sensitivity with GC-MS methods. Electrochemical techniques are especially attractive for the determination of opiates because of their remarkable detectability, experimental simplicity, and low cost. Among electrochemical methods, oxidative detection has been used much more extensively than reductive approaches.[6]

The primary focus of this review will be the analytical use of electrochemical methods, for the study and quantification of the opiates. Since recent reviews have been published dealing with polarographic (reductive) methods for study of some alkaloids of the morphine series,[7,8] special emphasis will be given to oxidative detection.

OPIATE METABOLISM

The opiate substances most frequently detected and determined are morphine, codeine, heroin and its metabolite 6-monoacetylmorphine (Fig. 2).

In man, morphine metabolism depends largely on the route of drug administration and its rates of absorption and distribution.[9] A detailed review on the metabolism of opiates identified four major routes of metabolism of morphine and its surrogates: *N*-dealkylation, *O*-dealkylation, conjugation, and hydrolysis.[10] Morphine is converted into the 3-glucuronide (M3G) and to a lesser extent into the 6-glucuronide (M6G) and 3,6-diglucuronide (M3,6G).[3,9] More than 50% of administered morphine is eliminated as M3G.[3] The M6G has potent analgesic activity and its level in urine can reach 10% of that of M3G.[3] About 5–10% of administered morphine is converted into the 3-ether sulphate and 3–5% into normorphine.[3,10] Codeine was previously reported as a metabolite of morphine[10,11] but recently this pathway was rejected unequivocally.[12]

Codeine is generally administered orally. Depending on the dose, the mean half-time of codeine in plasma ranges from 1.6 to 2.4 hr. It is metabolized by *N*-demethylation to norcodeine (~10%), by *O*-demethylation to its active

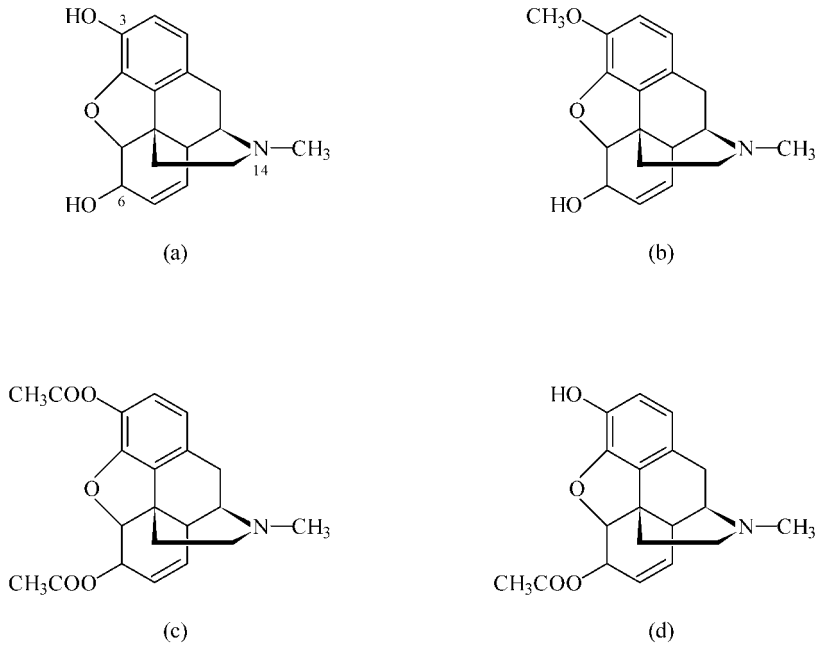

Figure 2. Molecular structures of opiates; (a) morphine, (b) codeine, (c) heroin, and (d) 6-monoacetylmorphine.

metabolite morphine, and by conjugation (major route) to the 6-glucuronide (C6G). The C6G has a similar activity to codeine itself.[13]

Heroin is usually administered by a parenteral route and intravenous injection seems to be favored by most heroin addicts. Heroin quickly disappears from the blood, its half-life being estimated to be ca. 2 min.[3,14] Heroin is deacetylated in the human body first to 6-monoacetylmorphine (6-MAM) and further to morphine. The pharmacological effects of heroin and 6-MAM are equipotent.

Figure 3 summarizes the main routes of opiate biotransformation.

ASSAYS OF OPIATES BY ELECTROCHEMICAL METHODS

Morphine

There are many papers dealing with the use of electrochemical (EC) detection for the assay of morphine. This is due to the ease with which

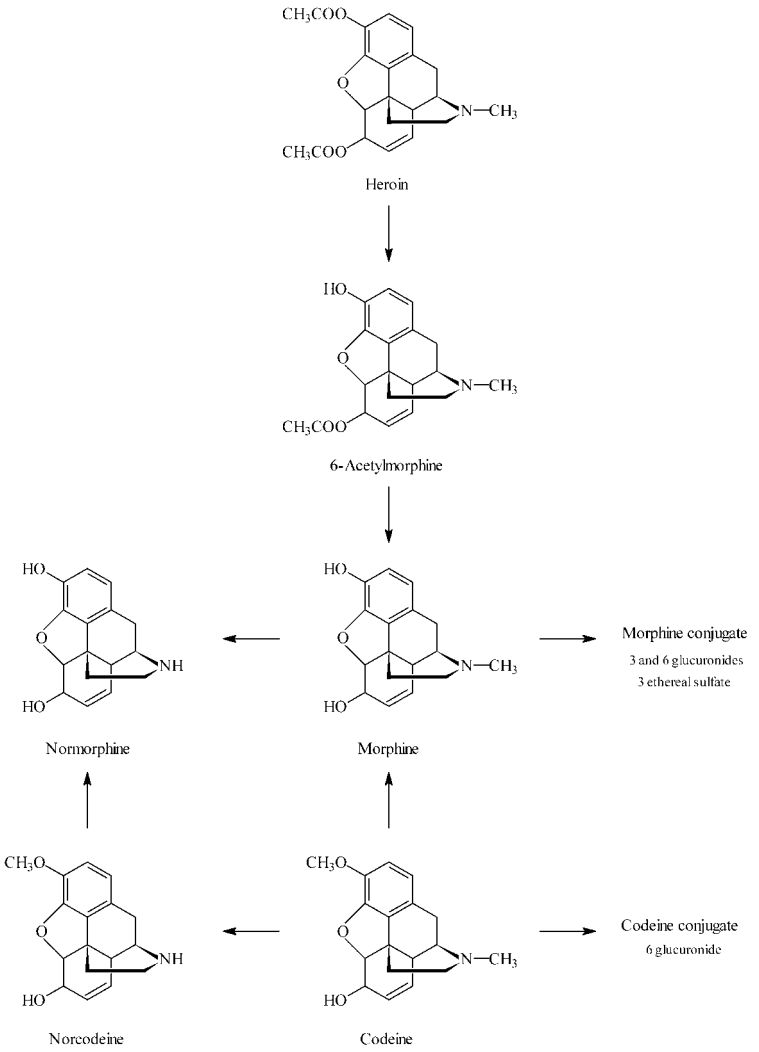

Figure 3. An overview of the pathways of opiates biotransformation.

morphine, and some of its metabolites, may be electrochemically oxidized. Although the first studies involving the oxidation of morphine occurred in the early 1960s,[15] its boom only happened at the end of the 1970s.[16,17] The study of the oxidative electroactivity of morphine and analogs showed that the phenolic group present in the structure of these compounds was the electroactive centre. The oxidative mechanism proposed involved one electron

oxidation of morphine followed by dimerization of the free radical to pseudomorphine.[16]

While morphine is easily oxidized at relatively low potentials, most of the other opiates are not oxidizable except at high potentials. This is due to the fact that these opiates contain a phenol ether or ester instead of a hydroxyl group at the 3-position of morphine (Fig. 2). Thus, compounds such as codeine, heroin, thebaine, or papaverine are not readily detectable electrochemically. This selectivity for morphine has been used to advantage for the direct determination of morphine in poppy straw extracts.[18]

Another common feature in the molecular structure of morphine and analogs is an aliphatic tertiary nitrogen atom. The ability of aliphatic amines to be electrochemically oxidized in basic solution led to the development of chromatographic methods of wide applicability.[6,19] Moreover, due to its high sensitivity and specificity, in a review of different detectors the use of HPLC with electrochemical detection was recommended for the determination of morphine in biological fluids.[20]

Notwithstanding this wide application of electrochemical methods to the determination of morphine, the first detailed study of its voltammetric behavior only appeared in the beginning of the 1990s.[21] Three distinct waves were seen. The first was attributed to the oxidation of the phenoxide ion at the 3-position, the second to further oxidation of the product formed during the first oxidative process, i.e., pseudomorphine, and the third to the two-electron oxidation of the tertiary amine group.[21] Although these results were consistent with the data already published by other workers, further investigation and evidence are needed to support the mechanism of the subsequent oxidation of pseudomorphine.

Recently, some interest has centered on the use of enzyme sensors[22] and chemically modified electrodes[23] for the determination of morphine. The use of these methods significantly improved the sensitivity (Fig. 4) and outlook for future applications to in vivo analysis.

Codeine

Unlike morphine, the electrochemical behavior of codeine raised a number of questions during the last 20 years that need to be clarified and explained.

The first reference to the voltammetric behavior of codeine on a glassy carbon electrode (GCE) appeared in the end of the 1970s and it was stated that its oxidation gives rise to three anodic waves.[17] Nevertheless, during the following years it was claimed that codeine was hardly oxidizable or even devoid of electroactivity,[24,25] which is curious in view of its structure similarity with morphine.

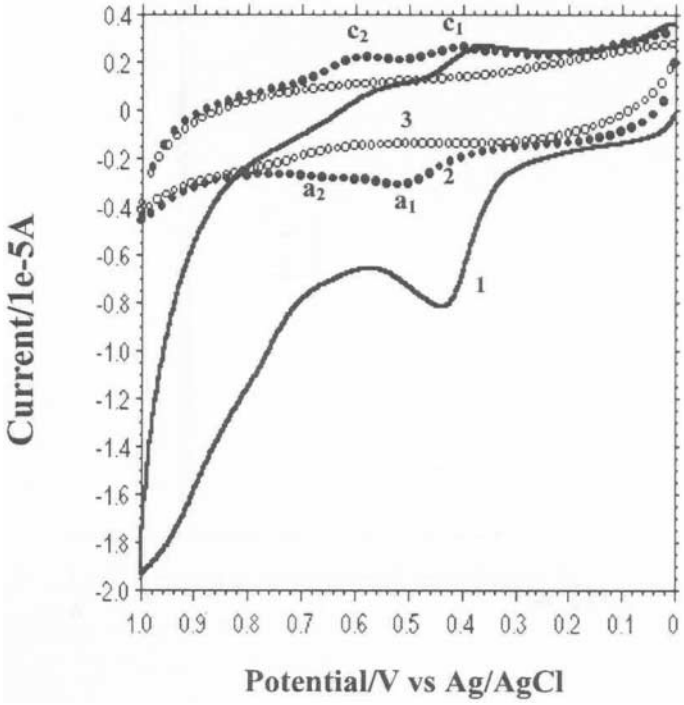

Figure 4. Cyclic voltammograms of the bare glassy carbon electrode (GCE) and the cobalt hexacyanoferrate modified electrode (CoHCF CME) in 2.0×10^{-4} M morphine. Curve 1: CoHCF CME in 2.0×10^{-4} M morphine; curve 2: CoHCF CME in blank electrolyte; curve 3: bare GCE in 2.0×10^{-4} M morphine. Electrolyte: 0.1 M phosphate-buffered saline (pH 4.5); Scan rate: 100 mV sec^{-1}. Reproduced with permission from Ref.[23].

In the middle of the 1980s a full investigation was undertaken of the anodic voltammetry of codeine at rotating disc electrodes of platinum and gold. A single wave was observed at both types of electrode and from the results obtained a reaction mechanism was proposed involving the formation of a quinonoid structure.[26]

Surprisingly, despite the lack of information on the electrochemistry of codeine, particularly using GCE, there are many reports regarding the use of electrochemical detection in HPLC assays for this compound and its metabolites.[19,27–29] Most of them use as basis for the electrochemical detection the oxidation of the tertiary nitrogen atom, yet the presence of a disubstituted catechol group in codeine structure is also suggested to be

involved in the oxidative process.[27] This last assumption seems rather unlikely considering the molecular structure of codeine and the electrochemical behavior of similar compounds such as morphine, heroin, or papaverine.[19]

As a result of the apparent difficulty of oxidizing codeine at bare electrodes, in recent years several approaches have been proposed for its quantification using different modified electrodes and a biosensor.[22,25,30,31] All these methods present the advantage of sensitivity (Fig. 5) and selectivity and are demonstrated as a good possibility to be used in routine analysis of codeine

Recently, an electroanalytical procedure based on the use of a bare GCE was proposed for the determination of codeine by square wave voltammetry and flow injection analysis.[32] The results presented therein showed that

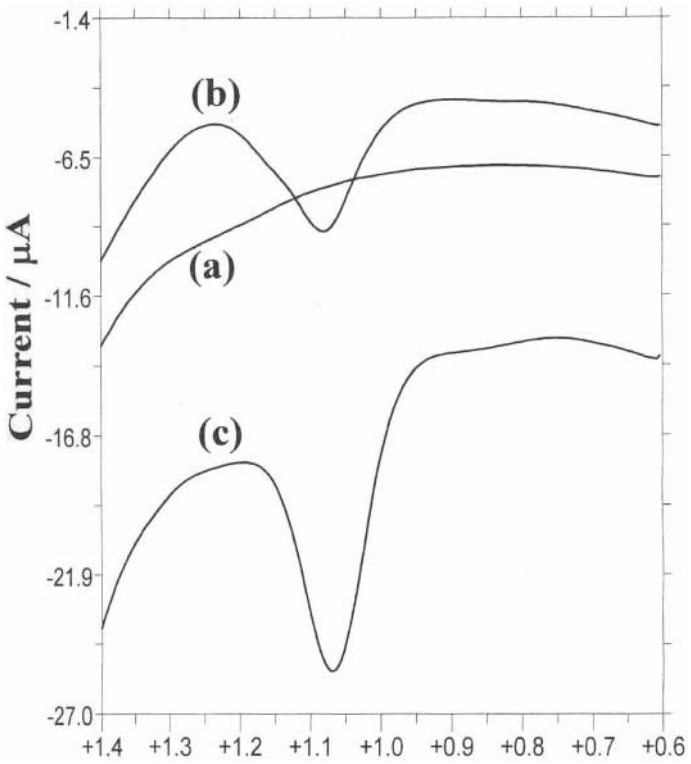

Figure 5. SW voltammograms for 10 μM codeine in 0.05 M $HClO_4$ solution at a bare GCE (a), the Nafion/GCE (b), and the Nafion/ruthenium oxide pyrochlore chemically modified electrode (c). SWV amplitude: 50 mV; SW frequency: 15 Hz; step height: 4 mV. Reproduced with permission from Ref.[30].

codeine is electroactive and that its oxidation mechanism is complex and pH dependent. Moreover, the new data found are in accordance to what was described for codeine 25 years ago and that has been ignored in most of the subsequent studies regarding codeine oxidative processes. An interesting feature of these procedures based on the use of a bare GCE is the sensitivity reached being close to that obtained using modified electrodes (Fig. 6).

Heroin and 6-Monoacetylmorphine

Contrary to the other opiates, there are very few analytical procedures for electrochemical determination of heroin and 6-monoacetylmorphine. The first reference to the electrochemical behavior of heroin and 6-MAM appeared in the middle of the 1980s and attributed its activity to the fact that these compounds contain the morphine structure.[19] The effect of pH on the electrochemical response for heroin was found to be similar to that of codeine which reflects the fact that both are nonphenolic and possess an oxidizable tertiary nitrogen group. Another important result was that 6-MAM

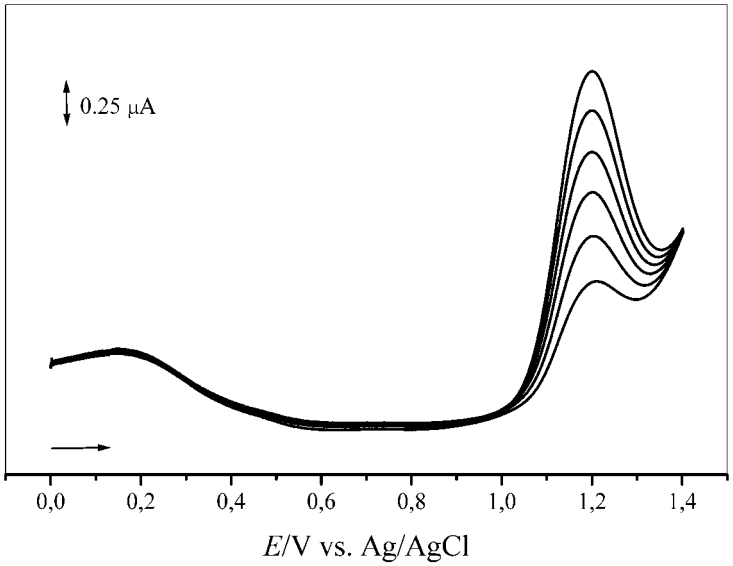

Figure 6. Successive square wave voltammograms in pH 3 0.2 mol L^{-1} buffer electrolyte of codeine: 41.4, 61.8, 82.0, 102.0, 121.8, 141.4 μmol L^{-1}: Frequency 150 Hz; pulse amplitude 50 mV. Reproduced with permission from Ref.[32].

is significantly more easy to oxidize, since it possesses two electrochemically active centres—the tertiary nitrogen and the phenolic group.

Despite the relevance of the previous results, only at the beginning of the last decade was there a detailed study of the voltammetric behavior of heroin, using a carbon paste electrode.[33] It was verified that heroin is irreversibly oxidized giving rise to a single wave whose half-peak potential is strongly influenced by pH. A mechanism for the electrochemical oxidation was postulated and consists in four steps in which an intermediate ammonium radical is converted to a quaternary Schiff base, which is then rapidly hydrolyzed to give a secondary amine and a ketone (Fig. 7). Although it is stated that this mechanism is consistent with the oxidation processes of aliphatic tertiary amines some new evidence still needs to be obtained to confirm ketone formation.

Although there is no study of the oxidative behavior of 6-monoacetylmorphine, recently new chromatographic procedures based on electrochemical detection were proposed for its determination.[34,35] As concluded previously, the electrochemical activity of 6-MAM was attributed to the presence of a phenolic group in its molecular structure, by analogy to what is observed for morphine.[35] However, it was found that the optimal detection potential is approximately 150 mV higher than that of morphine. This difference was justified due to the presence of an acetyl substituent on the 6-hydroxy group.[35] Further studies are still necessary to support and consolidate this conclusion,

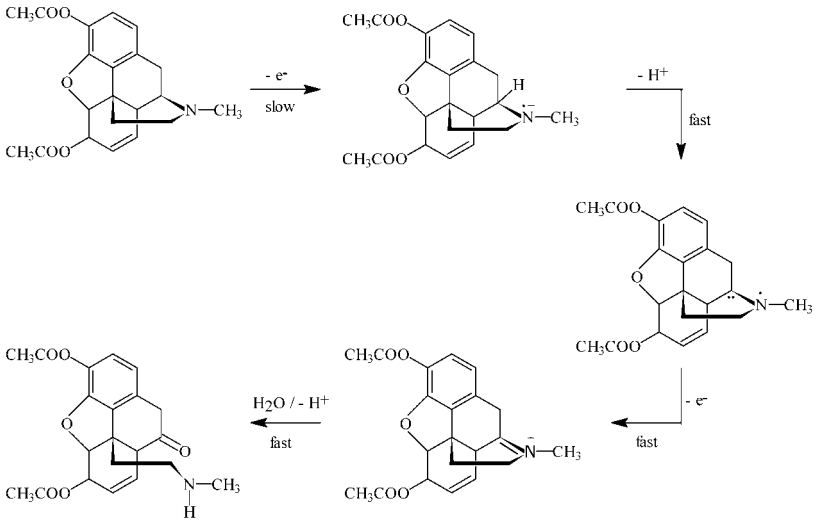

Figure 7. Proposed mechanism for the oxidation of heroin on a carbon paste electrode. Reproduced with permission from Ref.[33].

since the data available up until now for similar compounds hardly explains such differences in the oxidation potentials between morphine and 6-MAM. In fact, a much smaller difference is found comparing the half-peak potentials obtained for morphine and hydromorphone (6-keto group).[17]

An interesting and promising future application for electrochemical detection in heroin abuse would be discrimination between street (illegal) and pharmaceutical use. The presence of the heroin metabolite 6-MAM is considered to be definitive evidence of heroin use. Also, acetylcodeine is a synthesis by-product present in street heroin but not in pharmaceutical heroin. Considering that the voltammetric behavior of 6-MAM and acetylcodeine are both substantially different from pure heroin, their electrochemical detection can be used for qualitative and quantitative differentiation between street and pharmaceutical heroin.[36]

CONCLUSIONS

This review has described the electrochemical methods that have been developed and used for the determination of opiates.

Opiate abuse continues to represent significant health problems for modern society. The variety of metabolites originating from biotransformation and degradation processes have and will require special attention from analytical chemists and toxicologists in order to analyze them in biological specimens and drug seizures. The use and interest in electrochemical sensors will continue unabated, stimulated by the wide range of potential applications. An area of particular interest for electroanalytical chemists lies in in vivo monitoring. The suitability of electroanalytical techniques to miniaturization and the success obtained so far using appropriate modified electrodes suggests a great potential for use of these methods for routine analysis and in vivo studies.

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