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New trends on modified electrodes: applications to drug analysis*

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Abstract: It is shown that activity in the area of chemically modified electrodes continues at a high level. This topic is increasing in popularity with a large research field on the incorporation of electroactive groups into a variety of conductive films. These developments are also directed towards catalyzing bioselective and biospecific reactions. Significant developments in the field of ion-selective electrodes have occurred and some exciting advances in all areas of these sensors are reported, albeit briefly.

Keywords: *Modified electrodes; ion-selective electrodes; differential pulse voltammetry; polymer electrodes; drug oxidations.*

Introduction

Progress has been achieved in electrochemistry due to the development of modified electrodes. A great deal of information has been accumulated on adsorbed layers of molecules and ions and also on techniques that enable the electrode to be built with properties designed by the experimentalist [1–5]. The field of electrocatalysis has also been developed and usefully explored [6]. It can be noted that the most well studied immobilized chemicals, enzymes or drugs are those which present good electrochemical reactivity. The principal routes to immobilization of substances can be classified as film deposition, chemisorption and covalent bonding.

Today the synthesis and application of chemical micro-structures onto electrode surfaces enhance the efficiency of a number of processes, including microelectronics and semiconductors. Intensive activity is under way to produce new types of electrodes which could lead to the production of a highly specific type of material designed for the analysis of individual pharmaceutical compounds.

Discussion

Several different lines of attack have been developed. One of them is the covalent binding to metal oxide electrode surfaces (silanization); another is the attachment to

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carbon electrodes (oxide-free carbon, carbon pastes, chemisorption on carbon and others); a third is the polymerization of films onto the electrode. The electroactive groups may be a part of the polymer or they may be inserted into the polymer by electrostatic effect, e.g. by an ion-exchange process.

Polymer-coated electrodes

The problem with polymer-coated electrodes is that the polymer films contain electrochemically or chemically reactive centres. In terms of volume, the concentration of electroactive sites in the polymer film is very high taking into account the polymer thickness.

Different ways of coating the electrodes with polymer films have been described [1, 3]. The class of organosilane derivatives has been described by Murray's group [2]. Other polymers are attached to the electrode with no complete details or explanation. An elegantly simple method of coating has been described in early 1975 by Oyama and Anson [3], who coated a polyelectrolyte onto the electrode, the thickness being between 50 and 5000 Å.

Miller *et al.* [7] exposed the electrode to a dilute solution of polymer for a defined period during which time an adsorbed polymer film formed on the electrode surface. In biologically active systems, these electrodes very often give sluggish response in terms of electrochemistry, because the redox centre is inaccessible or due to adsorption problems.

Voltammetric methods of analysis have proved extremely useful over the last few decades [8] and the number of applications has grown very rapidly. The use of solid modified electrodes as alternative working electrodes for voltammetric determinations of drugs has proved successful. Due to the oxide layer formation on gold and platinum and fairly high residual currents on glassy carbon, together with their irreproducible nature, their use has been rather limited. The area of chemically modified electrodes has matured to a large extent. Research in the author's laboratories on modified electrodes has led to other workers adopting similar lines thus making the topic and their applications to pharmaceuticals very attractive.

Doped organic polymers have also been a subject of interest for several years. At first, their structure was described by doped semiconductor ring band models. Now it is logical to admit that modifications occur in the molecular orbitals of the polymers during charge transfer processes, which lead to the formation of defects localized in the electronic structure [9, 10]. Polypyrrole synthesized or grown electrochemically has been studied extensively by Genies and co-workers [11] and polyhalopyrroles by Audebert and Bidan gave excellent results when applied to oxidation reactions of pharmaceutical interest [12].

Electrodes coated with Nafion (perfluorinated sulphonated ion-exchange polymer) are the subject of considerable current interest and have been thoroughly investigated. Nafion has hydrophobic character where the teflon fluoro-carbon dominates, and is also hydrophilic in character because ionic sulphonate groups are clustered with polar solvent molecules [1, 3]. Takeuchi and Osteryoung [13] were the first to propose square-wave voltammetry for the preparation and study of polymer-modified electrodes. Modified electrodes have been characterized by many spectral and electrochemical techniques which have been found to be well-suited for detecting small areas of coating and for precise measurements of peak potentials [2, 5].

Carbon-based electrodes

In another way, carbon-based electrodes can operate according to various mechanisms. It is not always obvious how to classify the electrodes, so that a preliminary survey of the main types of carbon-based electrode is necessary.

A key feature is the versatility of the carbon substrate and the possible selectivity of the electrode [14]. According to Midgley *et al.* [15] it is possible to summarize according to the following classification: (a) nature of carbon-substrate electrodes; (b) pH response of graphite electrode; (c) development of the Růžička electrode; and (d) non-commercial carbon-substrate ion selective electrodes.

These coated polymeric electrodes suggest several modes of reaction of the electroactive species, according to A. Bard *et al.* [16]: (a) reaction at the polymeric film-solution interface *via* electron conductivity of the film; (b) membrane diffusion in the film to the electrode-film interface; (c) electrode transfer mediation by the film; and (d) diffusion through pore and channels to react at the electrode-film interface.

Cyclic voltammetry

Cyclic voltammetry is a technique used currently in the author's laboratories to study different polymeric film electrodes. The behaviour of the carbon-paste and glassy-carbon electrodes have been investigated and compared with the results obtained with new laboratory-made types of modified electrode based on colloidal graphite or carbon black dispersed in a suitable polymer. The behaviour of the hexacyanoferrate (II/III) couple has been reported as a typical model of depolarizer. It has been shown that electrode responses were markedly influenced by the nature of the substrate. The performance of each electrode has been evaluated with regard to the shape of the voltammetric curves obtained during the investigation of these drugs.

The hexacyanoferrate (II/III) system represents the couple generally used as a model depolarizer for testing solid electrodes [17]. Since the electrode pretreatment crucially affects the kinetics of the hexacyanoferrate couple [18], care has been taken to prepare the most satisfactory surface in a reproducible manner [19].

In Fig. 1 are illustrated typical cyclic voltammograms of hexacyanoferrate (II) in 0.1 M KCl. As expected, the reversibility of the reaction (defined in terms of peak potential separation, ΔE_a) is greatly influenced by the nature of the electrode material.

Using the graphite-spray electrode (Fig. 1a) the hexacyanoferrate (II/II) couple behaves almost the same as the glassy-carbon electrode (Fig. 1b), but with the difference that the reversibility of the voltammogram is somewhat improved. The carbon–polyethylene and carbon-paste electrodes exhibit fairly large peak potential separations, currents are low and electrochemical activation by cycling between -0.7 and $+1.3$ V versus S.C.E. (saturated calomel electrode) has no marked effects upon their respective response. In Figs 2–4, typical applications are illustrated.

It can be concluded that each electrode exhibits a performance closely related to the substrate. In respect to the shape of the voltammograms, it is actually difficult to predict the most satisfactory electrode for studying certain pharmacological compounds. It seems reasonable, therefore, before initiating any investigations, to test a large number of commercially available or laboratory electrodes in order to obtain the best results.

Oxidation reactions

In the author's work, oxidation reactions are easier at the graphite-spray electrode [18]. Carbon paste is well adapted for studying organic drugs, even at very low

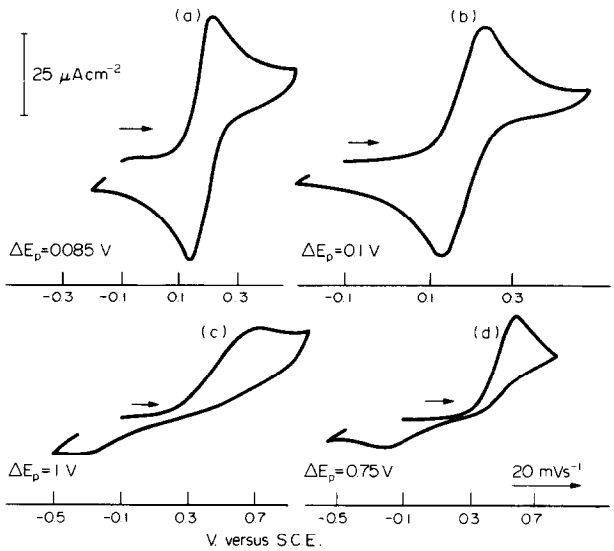

Figure 1
Cyclic voltammetry of the hexacyanoferrate (II/III) couple. Hexacyanoferrate (II), 5×10^{-4}M; KCl, 0.1 M; cyclic voltammetry: 20 mV s^{-1}. (a) Graphite-spray electrode; (b) glassy-carbon electrode; (c) carbon–polyethylene electrode; (d) carbon-paste electrode. S.C.E. = saturated calomel electrode.

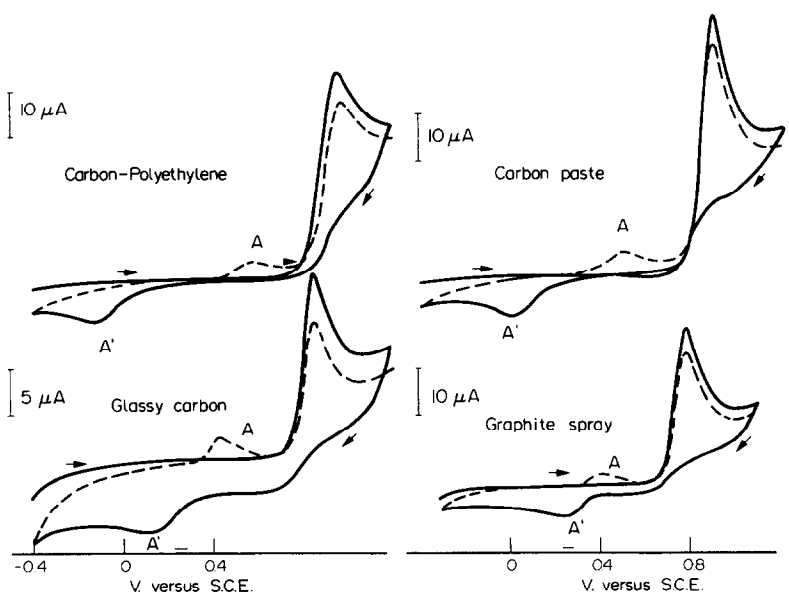

Figure 2
Cyclic voltammetry of phenacetin. Phenacetin, 5×10^{-4}M; phosphate buffer pH 6.5 with 10% v/v CH_3OH; cyclic voltammetry: 20 mV s^{-1}. The dotted line represents a second scan on the same surface.

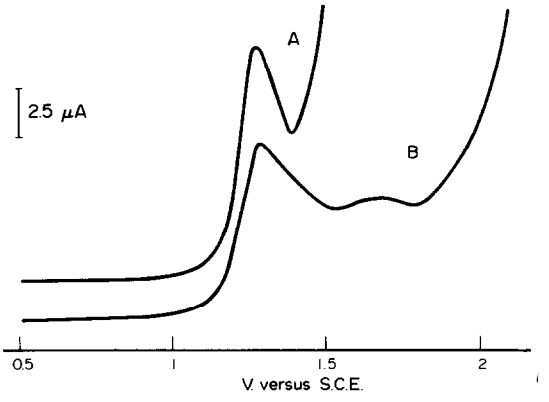

Figure 3
The analysis of codeine by differential pulse voltammetry. Codeine, 5×10^{-4}M; H_2SO_4, 0.1 M. Differential pulse voltammetry: 10 mV s^{-1}; pulse amplitude, 25 mV; pulse repetition rate, t, 0.5 s. (a) Carbon-paste electrode; (b) carbon–polyethylene electrode.

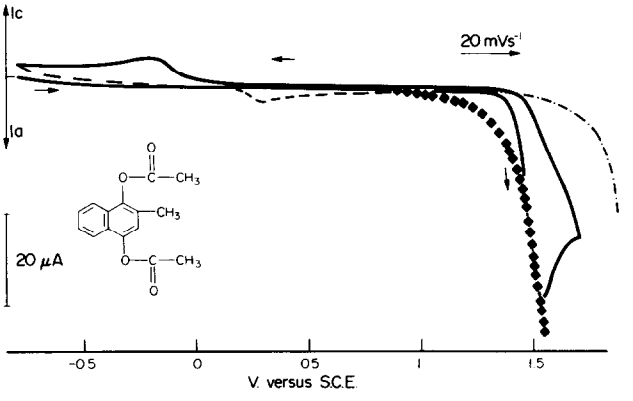

Figure 4
Cyclic voltammetry of menadiol diacetate. Menadiol diacetate, 5×10^{-4}M; H_2SO_4, 0.1 M with 30% v/v acetonitrile. Cyclic voltammetry; 20 mV s^{-1} . . . . , Carbon-paste electrode; ———, carbon–polyethylene electrode; - - - -, second scan; - · - · -, solvent discharge.

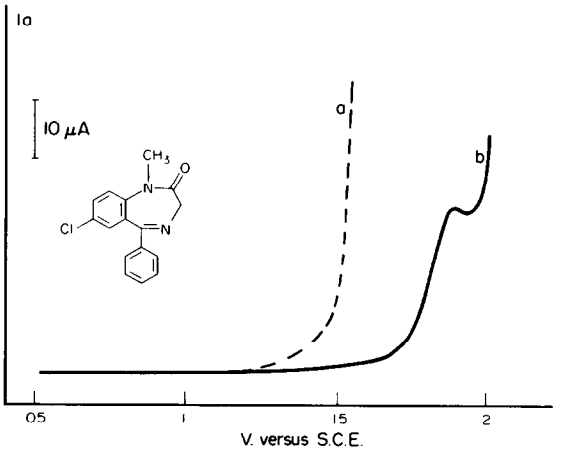

Figure 5
Differential pulse voltammetry of diazepam. Diazepam, 5×10^{-4}M; H_2SO_4, 0.1 M. Differential pulse voltammetry: 10 mV s^{-1}; pulse amplitude, 25 mV; t, 0.5 s. (a) Carbon-paste electrode; (b) carbon–polyethylene electrode.

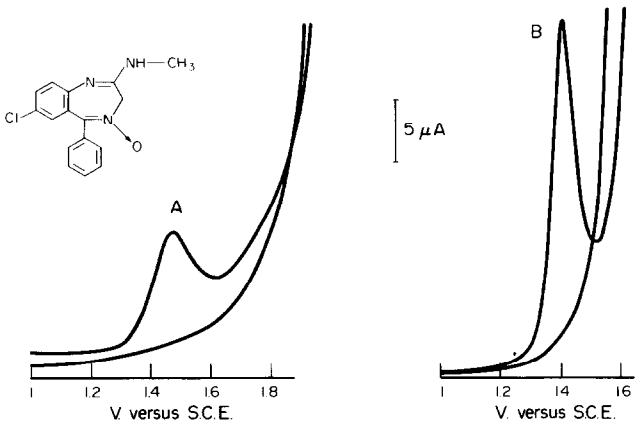

Figure 6
Differential pulse voltammetry of chlordiazepoxide. Chlordiazepoxide, 5×10^{-4}M; H_2SO_4, 0.1 M. Differential pulse voltammetry: 10 mV s^{-1}; pulse amplitude, 25 mV; t, 0.5 s. (A) Carbon–polyethylene electrode; (B) carbon-paste electrode.

concentrations. However, the reversibility in some cases is not as good as that observed with the glassy-carbon or graphite-spray electrodes (Figs 2–6). The glassy-carbon substrate must often be electrolytically activated in order to increase performance and the surface must be handled with care. The carbon–polyethylene electrode, which has been reported earlier [19], has found several applications. The kinetics of the reactions are low, but background currents are low and potential ranges very large, permitting the investigation of drugs not oxidizable at the other solid electrodes.

Another type of modification has been also proposed by the author for studying the electrochemical oxidation behaviour of cysteine, lipoic acid and disulfiram [20]. This is based on modifying the carbon-paste electrode, by incorporating cobalt (II) phthalocyanine, which offers interesting properties due to the electrocatalytic capabilities of the electrode. Using these types of electrode, different molecules have been quantitatively determined at concentrations as low as 2×10^{-7}M.

Modification of the electrode surface can confer specificity in various areas, such as electrocatalysis, photochemistry and electroanalysis. Baldwin and coworkers have shown that the addition of cobalt phthalocyanine to the carbon paste has a beneficial effect, resulting in an increase in sensitivity in high-performance liquid chromatographic studies using electrochemical detectors [21].

The electrochemical oxidation of cysteine and cystine is difficult to accomplish using solid state electrodes, since the two molecules are electroinactive at the carbon electrode. Nevertheless, with the carbon-paste electrode, a judicious selection of the supporting electrolyte did permit the author to show that these two derivatives are electroactive. In sulphuric acid, due to the large accessible anodic potentials, cysteine is oxidized at very positive potentials, close to the oxidation of the solvent. However, the study of these derivatives, and especially of cysteine, is difficult to achieve due to the poor resolution of the anodic peak. The oxidation of cysteine at the surface of the carbon paste corresponds to a slow electrochemical reaction, as well as to a spreading of the anodic peak. Modification of the electrode by incorporating into the paste a redox

mediator, such as a metalloporphyrin, improves the performance of the carbon-paste electrode. The catalytic activity of metallic phthalocyanines with respect to biochemical systems is well-known. One can interpret the electroanalytical process as being an oxidation on the surface of the electrode of the cobalt phthalocyanine (II) to cobalt phthalocyanine (III), followed by an homogeneous oxidation in a solution of thiol according to a redox process which regenerates the cobalt (II) of the organometallic complex. A study of the response of the electrode as a function of the percentage of metalloporphyrin ranging from 2–10% w/w indicated an optimum concentration of 2% w/w of redox mediator.

The behaviour of other sulphur compounds such as thiourea, methylthiouracil, thiamazole and 6-mercaptopurine was also investigated using the same redox mediator [22].

Table 1
Electrochemical characteristics of thiols and disulphide derivatives at the surface of carbon-paste and modified carbon-paste electrodes

Compound	Electrode potential E_p (V)	Current (μA) $I_{p/c}$	Limit of detection	Linear range
Carbon-paste electrode				
Cysteine	+1.380	12×10^{-2}	—	—
Cystine	+1.430	25×10^{-2}	5×10^{-6}M	8×10^{-6}–4×10^{-4}M
Lipoic acid	+0.825	12×10^{-2}	2×10^{-7}M	4×10^{-7}–1×10^{-3}M
Disulfiram	+0.880	15×10^{-2}	2×10^{-7}M	2×10^{-7}–6×10^{-5}M
Modified carbon-paste electrode				
Cysteine	+0.710	29×10^{-2}	2×10^{-6}M	4×10^{-6}–6×10^{-5}M
Cystine	+1.520	21×10^{-2}	—	—
Lipoic acid	+0.805	11×10^{-2}	2×10^{-6}M	2×10^{-6}–1×10^{-3}M
Disulfiram	+0.775	12×10^{-2}	2×10^{-6}M	4×10^{-6}–1×10^{-4}M

The analysis of benzodiazepines

A specific sensor for the determination of certain neuroleptics (dibenzodiazepines) has been developed by coating a graphite spectroscopic rod with a film of polyvinyl chloride (PVC) previously dissolved in cyclohexanone. The solvent mixture dioctylphthalate–nitrobenzene is used as plasticizer. Inside the film, dibenzodiazepine–tetraphenylborate is incorporated as an ion-pair.

The Nernstian response of the electrode was found to be between 10^{-2} and 10^{-5}M [23]. Interferences were studied. Organic substances containing the pyrrolidine, piperazine or piperidine group present a certain degree of interference. Other types of neuroleptics of the benzodiazepine family do not interfere. Methods for the accurate assay of dibenzodiazepines in tablets and injections have been described [23]. Figures 5 and 6 give typical applications. A potentiometric titration procedure using tetraphenylborate is proposed and various determinations can be carried out on pharmaceuticals at low levels of concentration [24].

For drugs, liquid and polyvinyl chloride membrane electrodes which are sensitive and selective for atropine are described. They are based on the inclusion of an atropine–reineckate ion-pair complex in the PVC matrix [25].

Conclusions

The introduction of ion-selective electrodes to clinical medicine has been relatively slow despite their advantages. Direct evaluations of physiologically active ionized acid are possible using these probes, while such measurements are often impossible by other techniques [26, 29, 30]. Sometimes they are subject to interference and are delicate. Interest is now being shown in the development of smaller automatic analysers designed to be portable [27]. A major review has been made on the subject of ion-selective electrodes [28].

The area of modified electrodes is an interesting and fascinating field of research and offers practical benefits. Amperometric biosensors, also commonly referred to as enzyme electrodes, constitute a rapidly growing field of interest to analytical chemists and also to biologists. Already about one hundred biosensors are known and several have been commercialized. Amperometric microbial sensors consisting of immobilized cells coupled to a gaseous electrode are capable of an increased oxygen uptake and assimilation of organic substances by the microbial cells [31, 32]. In this respect, the low cost of the biocatalyst and the high functional stability is interesting [31, 33].

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