Electroanalytical Techniques for the Future: The Challenges of Miniaturization and of Real-Time Measurements

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Abstract
A brief survey of electrochemical methods which can be employed to respond to the challenge of measuring electroactive species in untreated solutions in real-time is made. Particular attention is given to the advantages that can be obtained from miniaturization and protection of the electrode surface against fouling with polymers, together with employment of novel instrumentation techniques. The merits of the batch injection analysis approach in addressing these questions are discussed and illustrated.

Keywords: Real-time measurements, Miniaturization, Electrode fouling, Polymer-modified electrodes, Stripping voltammetry

1. Introduction

Electroanalytical techniques have undergone many important developments in recent decades. This has stemmed from a better understanding of electrode processes and improvements in instrumentation, which have allowed faster measurements to be made under better-controlled conditions, particularly those involving pulse voltammetric techniques [1]. Such developments were first introduced for the dropping mercury electrode, with voltammetric techniques devised to sample the currents at certain points in the drop life. It was soon realized that this approach is useful at electrodes of constant area and at all solid electrodes. This led to the widespread acceptance of differential pulse voltammetry and more recently of square-wave voltammetry [2] and fast-scan cyclic voltammetry [3].

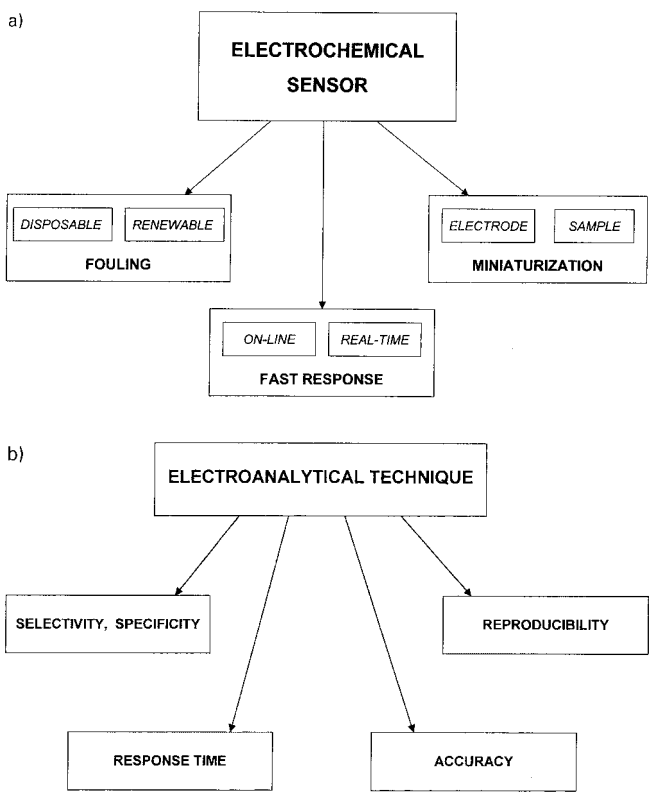

Fig. 1. Important aspects for the use of a) electrochemical sensors and b) electroanalytical techniques.

Two essential challenges remain for the use of electrochemical sensors, and which need to be addressed; these are shown in Figure 1a. One is miniaturization of the electrode and of the sample. Microelectrodes have permitted measurements to be made in media of higher resistivity than hitherto. There are also important developments in the reduction of sample volume, and even of cell size [4]. The other challenge is the direct measurement of electroactive species in as-collected samples, not just of natural waters but also of effluents, both domestic and industrial. These samples can contain large amounts of adsorbable organic matter which can rapidly block an electrode surface. Electrochemistry offers a unique opportunity for providing a diagnostic of the concentration of labile species in a liquid sample in real time, so long as the problem of irreversible adsorption can be solved.

In Figure 1b the important criteria for the use of electroanalytical techniques are illustrated, which are influenced by the aspects referred to in Figure 1a. This article will concentrate mainly on how electroanalytical techniques can respond to the challenges of miniaturization and real-time measurements. Sensors will also be described but only to the extent of how they are used with the electroanalytical techniques. The use of analytical separation techniques will not be specifically addressed, but there is the possibility of coupling it to the systems to be described in order to increase selectivity.

2. Novel Instrumentation Techniques

The advent of computer-controlled instruments has permitted many waveforms to be invented and applied, and high resolution responses to be obtained. At the same time, there have been advances in the simulation of electrode response and fitting of experimental curves to different reaction mechanism models, particularly for cyclic voltammetry [5]. The benefits of the approach are clear: instead of being restricted to one or two points on the voltammetric curve such as peak potential and the peak width at half-height, the whole curve is fitted, leading to

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improved accuracy and precision. The data obtained can be used for mechanistic or electroanalytical purposes. Previous approaches relied on transformation of the response by convolution or semi-integration [6] into a curve similar to a steady-state response, such as would be obtained at, for example, a rotating disk electrode. Analysis of such transformed responses could lead to missing certain mechanistic nuances.

With respect to the specific challenges posed by electroanalysis, square-wave voltammetry (SWV) [2] has become particularly important since it has brought a number of simplifications to experimental procedures. Amongst the advantages of SWV are: the fast effective scan rate, effective subtraction of charging currents, the fact that on the diffusion-limited current plateau the net current is zero. Of particular importance in anodic stripping voltammetry is the fact that during the square-wave scan, species in solution outside the "diffusion layer" usually do not have time to diffuse to the electrode surface. During accumulation, all oxygen in the vicinity of the electrode is reduced and during the positive-going scan only a negligible amount of oxygen has time to reach the surface again to react.

Many of the advantages described are also possible on employing cyclic voltammetry with high-resolution modern instrumentation, particularly if a scan in background electrolyte can be subtracted from the response. This is extremely important if fast scan rates are used where charging currents can be very large, even at microelectrodes [2]. Migration effects may also have to be taken into account at microelectrodes in highly resistive media [7].

An interesting approach involves the combination of a pulse waveform and cyclic voltammetry [8]. Since most modern instruments are digitally based, cyclic voltammetry is normally implemented as cyclic staircase voltammetry, with the staircase potential increment made sufficiently small so that the response is the same as that from a true ramped potential waveform, and with current sampling near the end of each step. Using pulses or a square-wave superimposed on the staircase enhances the charging current rejection. The waveform can also be viewed as a pulse or square-wave voltammetric scan with reversal of the scan direction.

3. Microvolumes

The probing of small volumes in electrochemical measurements can permit mapping of local concentrations in large volumes of solution, or permit measurements in small total volumes of solution in microcells, which can reach down to volumes less than a nanoliter [9]. A schematic of how measurements can be made is shown in Figure 2. As can be seen, there are two approaches. An example of the droplet on a flat surface approach is the fast anodic stripping of heavy metals in droplets of 5 μL volume [10]. Regarding microchamber cells, these can be manufactured by micromachining techniques such as lithography, for example to investigate the response of single living cells to hormones [9]. In both schemes shown in Figure 2 solvent evaporation during the timescale of the experiment has to be carefully avoided. Microelectrode systems (two or three electrodes) are necessary to probe these microcells, usually with submicron dimensions. An additional advantage is that measurements can generally be made without the need of adding an electrolyte.

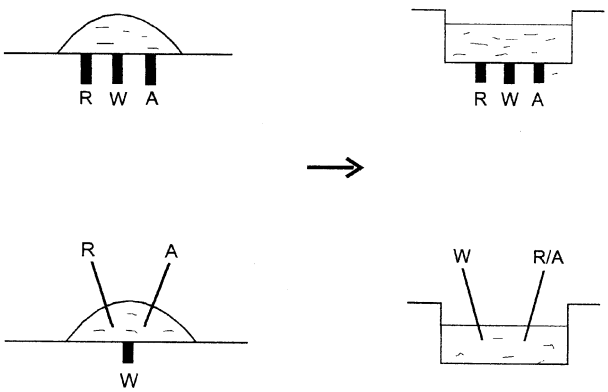

Fig. 2. Techniques for measuring microvolumes, showing the transformation from "droplet" cells to microchambers, with electrodes embedded or inserted in the cell.

Recent advances in potentiometric measurements in small volumes have been made possible with ion-selective field effect transistors (ISFETs) and chemical field effect transistors (ChemFETs) [11]. Such an approach can be extended to voltammetric measurements with microelectrode arrays [12].

An additional advantage of probing microvolumes may be that contact time between sample and detector is reduced. This will be exemplified below.

4. Surface Modification for Electrode Protection

Electrode surfaces need to be protected against non-electroactive interferents, which can be irreversibly adsorbed on the surface of the electrode during voltammetric scans. These interferents lead to a decrease in electrode response with time and preclude the possibility of analysis of untreated samples. Such protection can be achieved by using suitable electrode materials or, alternatively, suitably modified electrode materials. Much attention has been devoted to carbon electrodes and their modification, given their widespread use in electroanalytical chemistry [13]. Polymer modification is an approach which has been used with success for reducing adsorption phenomena. These polymers can either represent a new electrode surface in themselves, i.e., conducting polymers or redox polymers, or allow only the electroactive species of interest to reach the electrode substrate underneath. Thus, selectivity can be due to their porosity, excluding large molecules such as proteins, or due to ion-exchange characteristics if it is desired to make the membrane selective to charged species, or both of these. In the case of the analysis of cations by anodic stripping voltammetry at mercury thin film electrodes, amongst a number of strategies partially hydrolyzed cellulose acetate [14, 15] and perfluorosulfonated ionomers [16, 17] have found important application.

5. Batch Injection Analysis

An example of a system where the above concepts of miniaturization and electrode protection are all brought together is electrochemical batch injection analysis (BIA) [18, 19]. Two types of example will be given, first of trace metal ions at polymer-coated mercury thin-film electrodes using square-wave

anodic stripping voltammetry (SWASV) and second of hemoglobin at poly(methylene blue) modified electrodes.

The BIA cell is a modified large-volume wall-jet cell filled with inert electrolyte and containing working, auxiliary and reference (normally saturated calomel, SCE) electrodes. It exhibits many advantages of electroanalytical measurements in flow streams [20] and of flow injection analysis [21]. Samples are injected directly over the center of a disk electrode from a programmable, motorized, electronic micropipette. The concepts described in previous sections are achieved in the following way:

- interference reduction through the small injected sample volume, usually 50 μL, and protection of the electrode surface by polymer coatings, usually Nafion-based cation-exchange coatings;
- miniaturization through the small sample volume (50 μL)
- high reproducibility and sensitivity owing to the controlled flow wall-jet-type convective mass transport during injection; there are the benefits of flow systems during injection without the carrier stream in flow injection analysis;
- no necessity for added electrolyte since the sample is thin compared to the distance between working and auxiliary electrodes, these not contributing significantly to the ohmic drop.

Thus "real-time" analysis of small samples by direct injection over the electrode can be contemplated in order to give the concentration of labile species.

Such a strategy can also be combined with separation techniques such as capillary electrophoresis – capillary batch injection analysis [22].

5.1. Analysis of Trace Metal Ions in Environmental Samples

Trace metal ions, zinc, cadmium, lead and copper, can be analyzed by BIA-SWASV at polymer-modified mercury thin film electrodes [23, 24] with detection limits of the order of 5 nM. The experimental procedure is relatively simple, involving application of a polymer-film coating to a glassy carbon substrate: these films are made of Nafion [24] or, more recently, Nafion mixed with a small percentage of another sulfonated polymer such as 5% poly(vinyl sulfonic acid) [25]. A volume of 5 μL of a 0.25 wt% solution in low-weight alcohols is applied to the surface followed by 3 μL of N,N′-dimethylformamide casting solvent. The solvent is evaporated and the film cured with a warm air gun giving a film of approximately 1 μm thickness. The electrode is then introduced into the batch injection analysis cell and injection of 10 μL of a 0.1 M Hg^{2+} solution in 0.1 M KNO_3/5 mM HNO_3 is done, at an applied potential of −1.0 V (vs. SCE), to form a mercury thin film (in fact a collection of closely-spaced droplets [26]) between substrate and Nafion film. The film serves two purposes: first, it protects the mercury thin film against irreversible adsorption and secondly it helps to prevent any movement of mercury over the surface, which could be a problem after many consecutive injections. These films have been evaluated for their performance in "clean" solutions and on adding model surfactants exemplified by Triton-X-100 detergent, sodium dodecyl sulfate polyelectrolyte and a protein standard which is mainly albumin [24]. The Nafion film itself led to a signal reduction of about 15%, and the interferents to further reductions, only significant in the case of detergent. Slightly superior results were obtained on using Nafion/PVSA films rather than Nafion films.

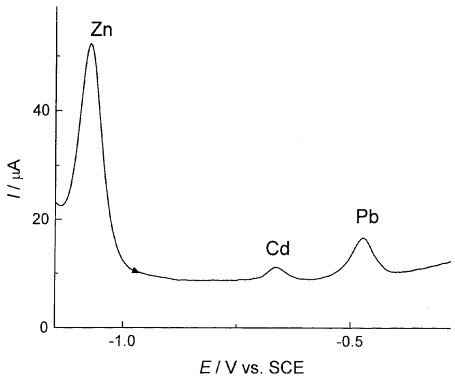

Fig. 3. Typical trace for BIA-SWASV of a mixture of heavy metal ions at a Nafion-coated MTFE. Injections of 50 μL, $E_{dep} = -1.3$ V for 30 s. SW parameters: amplitude 25 mV, frequency 100 Hz, potential increment 2 mV [27].

Figure 3 shows a typical trace from analysis of metal ions in a real environmental sample at such an electrode and Figure 4 the analysed results before and after acid digestion [27].

Additionally, the relative advantages of employing a random array of carbon microdisk electrodes as substrate for mercury microelectrodes have been investigated [28]. Although this leads to a significant enhancement of current densities, the detection limits remain about the same as with a large glassy carbon disk electrode substrate for the mercury film, owing to the increased signal noise.

It should be pointed out that although this system may not appear to be as appropriate for field analysis as systems which employ, for example, disposable screen-printed electrodes [29], it can satisfy most of the criteria necessary for such analyses.

5.2. Analysis of Hemoglobin

In this application, the reduced form of hemoglobin is oxidized at a poly(methylene blue) electrode [30]. The polymer film formed on the glassy carbon substrate is a new electrode material at which the electroactive species reacts. It had been shown previously [31] that methylene blue adsorbed on electrodes prevents adsorption of hemoglobin and enhances the electron transfer rate for hemoglobin oxidation. Thus, films of poly(methylene blue) (PMB) were grown by electropolymerization from a solution containing the monomer onto a glassy carbon

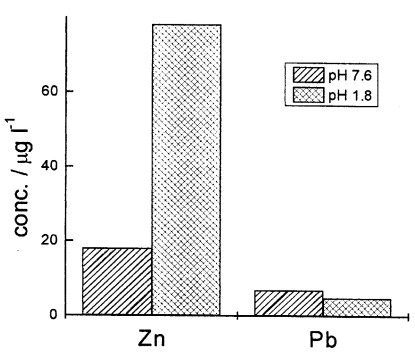

Fig. 4. Results obtained for BIA-SWASV analysis over Nafion-coated MTFE of effluent samples immediately after collection (pH 7.6) and after digestion in acid medium for 48 h (pH 1.8) [26]. Other parameters as in Figure 3 [27].

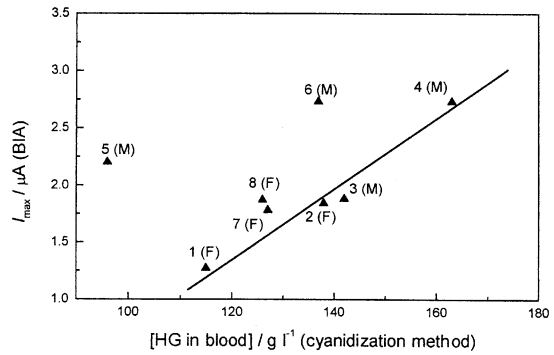

Fig. 5. Plot of maximum peak current for oxidation of hemoglobin in whole blood at PMB electrode ($E = +0.55$ V vs. SCE) by BIA (injection of 50 μL) vs. hemoglobin levels determined by the cyanidization method. Whole blood diluted 1 : 5 in pH 5.0 acetate buffer. F: female, M: male; subjects 5–8 potentially ill [32].

substrate and the electrode introduced into the batch injection analysis cell. Whole blood samples, after addition of anticoagulant, are then directly injected over the electrode held at a potential of 0.55 V (vs.SCE) to oxidize the reduced form of hemoglobin to methemoglobin. This is a rapid procedure and injection can be carried out almost immediately after taking the blood sample from the patient, requiring only 50 μL of blood. Figure 5 demonstrates some typical results [32]. Compared to the normal clinical protocol, which uses cyanidization for all forms of hemoglobin, this BIA method measures only the reduced form. This in itself could be a useful parameter for probing potentially ill patients. In fact, deviations from a linear correlation were found for patients with hematological problems, supporting this hypothesis.

6. Future Perspectives

There is great importance in meeting the challenges of real-time electrochemical measurements and in miniaturization, as exemplified in previous sections. The approaches will become increasingly important with schemes such as batch injection analysis becoming widespread as diagnostic tools. It is unlikely that the approach will substitute detailed laboratory analyses with analytical separation or hyphenated techniques. Important complementary studies regarding environmental analyses involve investigating which chemical species are assimilated by living organisms and the mechanism of uptake. Such research will show what is the real value and extent of the role of "real-time" electroanalytical diagnostic experiments.

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