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RECENT ADVANCES
IN DOPING ANALYSIS
(9)

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A. Gotzmann
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Is there a place for electroanalysis in the antidoping laboratory?

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INTRODUCTION

Analytical methods involving the use of electrodes and bioelectrodes for the detection of pharmaceuticals and their metabolites in biological fluids can be divided into three main classes:

1. combined chromatographic-electrochemical techniques, in which the electrochemical sensor or biosensor, assembled into a flow-through cell, constitutes the sensing element of the chromatographic detection unit;
2. stand-alone electrochemical or bioelectrochemical cells, where the detection unit is employed for batch measurements on a pre-purified fraction of the biological fluid (urine) to be assayed;
3. electrochemical immunosensors, where the immunological interaction between the sensor and the sample gives rise to a detectable change of a defined electrochemical parameter.

While the amounts of studies carried out on methods belonging to class 3 is still too limited to draw an even preliminary picture of the real potentiality of the relevant methods, sensors included in classes 1 and 2 have already been evaluated on real samples. More precisely, class 1 refers primarily to HPLC methods with amperometric detection, whose main advantage with respect to traditional HPLC-UV and also to GC-MS methods is given by a drastically simplified pretreatment procedure; while class 2 includes a wide variety of methods based on amperometric and voltammetric techniques.

We have evaluated the analytical performance of newly designed stand-alone amperometric and voltammetric electrodes; more specifically, we have developed and tested a newly designed amperometric bienzymatic electrode for the screening analysis of hydroxyethyl starch (HES), and a general voltammetric method for the screening analysis of corticosteroids. The former method is based on the amperometric detection of maltose (4-O- α -D-glucopyranosyl-D-glucose) produced after hydrolysis of HES: maltose is selectively detected by the combined catalytic activity of the enzymes aminoglucosidase (AG), hydrolyzing one molecule of maltose to two molecules of glucose, and glucose oxydase (GO), which in turn catalyzes the oxydation of glucose with production of hydrogen peroxide. Corticosteroids are detected by cyclic voltammetry, taking advantage of the redox reactions involving aromatic hydroxyles.

RESULTS

The bienzymatic maltose electrode, whose section is schematically represented in figure 1, was assembled by placing on the tip of the outer body of the O₂ sensing electrode (A in fig. 1) a sequence of three different membranes, in the following order: i) a teflon gas permeable membrane (B), to eliminate interferences from other electroactive substances, possibly present in the sample; ii) the bienzymatic membrane (C); 3) a dialysis membrane (letter D), to prevent any possible microbial attack of the enzymes and/or their leaking from the membrane. A rubber O-ring (E) was used to fix the three layers on the tip of the oxygen sensor.

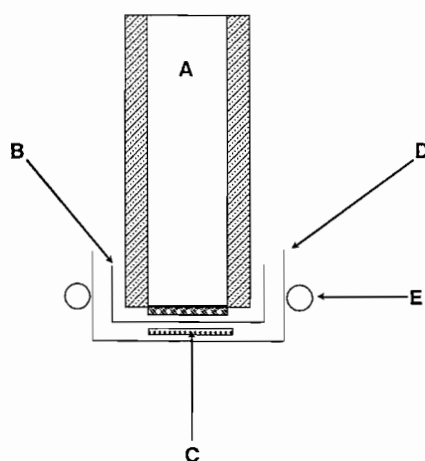


Figure 1

Amperometric measurements have been carried out in a thermostated cell, under constant

magnetic stirring, connecting the bioelectrodes to an amperometric detector (ABD, Universal Sensors Inc., New Orleans, LA, USA) and recording the current intensity values. Calibration of the bienzymatic electrode has been carried out on 2.5 ml of standard solutions of maltose (10-1000 μ M). A representative calibration curve is shown in Figure 2.

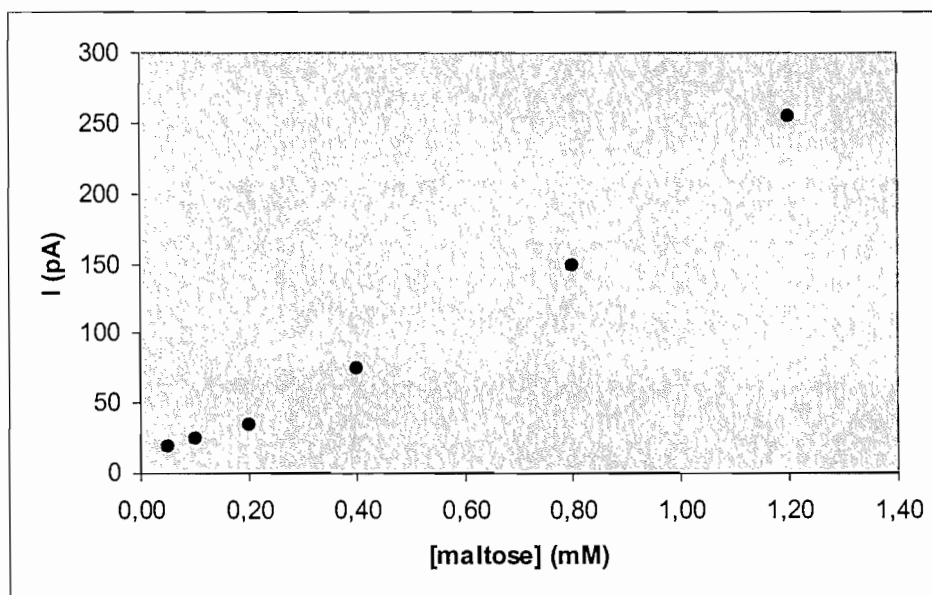
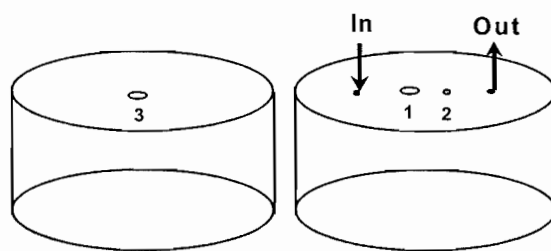


Figure 2

Voltammetric measurements have been carried out by an electrochemical analyzer (Autolab PGSTAT 10, Ecochemie), coupled to a flow-thru cell (BAS Unicell), constituted by a 3-electrode system: a) a glassy carbon working electrode (diameter: 2 mm); b) a Ag/AgCl reference electrode; c) a Pt counter electrode (Figure 3). Cyclic voltammetry assays (potential scan, triangular wave varying from -1 to -0.5 V) were carried out on standard solutions of betamethasone, showing the redox response of the $-OH$ groups. Experiments carried out in urine (both spiked and obtained by excretion studies) confirmed the possibility of performing both qualitative and quantitative analysis, without preliminary pretreatment of the sample, by characterizing the corresponding voltammograms, whose profile is not significantly affected by the matrix. Figure 4 shows a representative series of voltammetric assays, comparing the signals obtained on a 1 μ g/ml standard of betamethasone, on a positive urine and on a negative urine.



1 - Counter electrode
2 - Reference electrode
3 - Working electrode

Figure 3

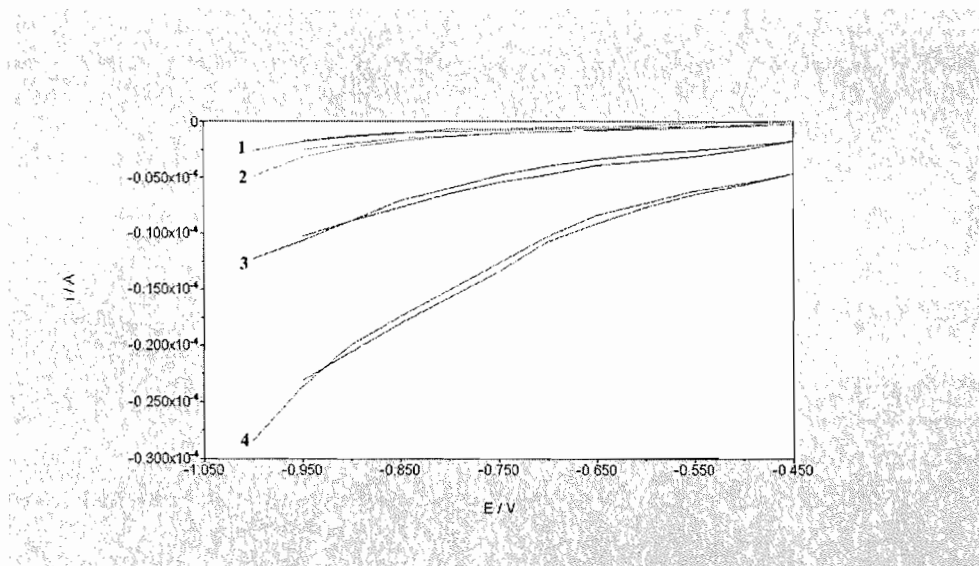


Figure 4

1: betamethasone 1 $\mu\text{g/mL}$ in CH_3OH ; 2: positive urine (5h after oral administration); 3: blank urine; 4: reagent blank

CONCLUSIONS

The recent developments in bioelectronics, micromachining and mass production of screen printed electrodes suggest that the application of electrochemical sensors and biosensors in the field of pharmaceutical and biomedical analysis will markedly grow in the near future. Electrochemical sensors and biosensors could represent a faster, simpler and more economical alternative for the preliminary screening analysis of selected classes of doping substances and methods. The use of these devices would allow to drastically reduce the pretreatment and purification of urine samples, and to avoid the chemical derivatization of the urine extracts. Table

1 summarizes the main potential advantages, including economical considerations, given by the use of electrodes and bioelectrodes in the antidoping laboratory.

Table 1 - Practical advantages of electroanalysis

- Costs:
 - ≤ 4000 € for a complete amperometric system
 - ≤ 25000 € for a complete voltammetric system
 - Electrodes/consumables/maintenance:
 - counterbalanced by the reduced use of enzyme (maltase, and theoretically amylase)
 - negligible for the voltammetric system
- Time:
 - Drastically reduced pretreatment
 - Response time:
 - 1-2 min in batch analysis;
 - 30" in FIA
- Skills:
 - Basic (\approx pH measurement by a potentiometer)

Even if it is understood that, as far as the confirmation analysis is concerned, the antidoping laboratory has to rely on chromatographic-spectrometric techniques (gas chromatography with mass spectrometry detection continuing to represent the elective analytical option), electrochemical and bioelectrochemical methods could represent a faster, simpler and more economical alternative for the preliminary screening analysis of specific doping substances and methods. Currently in progress are additional experiments aimed to compare the results obtained by the electrode here presented with those obtained by reference chromatographic spectrometric techniques, as well as to realize disposable or partially disposable electrodes.

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