

Amperometric Applications to The Assay of Some Biocompounds of Interest in Food and Clinical Analysis: An Editorial

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Editorial

Electrochemical techniques have gained increasing interest in food analysis and clinical domain [1]. Amperometry relies on applying a constant potential to a working electrode, and consequently measuring the intensity of the current generated, as a result of the electron-transfer reaction that involves an electroactive analyte [2-4].

At a constant potential value, the electron transfer occurs at elevated rate, so the process becomes controlled by mass transfer. Hence, the intensity of the diffusion current I_d depends on: the thickness of the diffusion layer δ , the diffusion coefficient of the analyte D , the number of electrons transferred n , the electrode surface A , the analyte concentration S , and the Faraday number F (96480 C mol^{-1}), following the equation [2,3].

$$I_d = nAFDS/\delta$$

The measured current intensity generated by the electrochemical reaction is directly correlated to the analyte concentration [5,6].

Kumar and Narayan have investigated a method for vitamin C determination, relying on a cobalt hexacyanoferrate modified graphite electrode. The cobalt hexacyanoferrate modified electrode presented good electrocatalytic activity towards ascorbic acid oxidation with a linear response ranging from $5.52 \times 10^{-5} \text{ M}$ to $3.23 \times 10^{-2} \text{ M}$, a detection limit of $3.33 \times 10^{-5} \text{ M}$ and stable analytical response [7].

Lowering the applied potential in the case of amperometric assays based on anodic oxidation, is possible with the use of mediators, such as ferrocene derivatives [8], or redox couples (ferrocyanide/ferricyanide) [9]. The use of ferricyanide immobilized by sol-gel technique on a screen printed electrode, allows for ascorbic acid determination at +0.3 V vs Ag/AgCl, with linearity up to 300 μM , sensitivity and detection limit of 2.85 nA/ μM and 46 nM, respectively, at a signal per noise ratio of 3 [9], whereas the use of unmodified Pt electrodes results in ascorbic acid detection at +0.6 V vs. Ag/AgCl, after separation on an anion-exclusion column [10].

O'Connell et al. [11] developed a sensor for ascorbic acid assay in foodstuffs and pharmaceutical preparations, by aniline electropolymerization on the surface of glassy carbon or screen-printed electrodes. The low applied potential of 100 mV results in selective electro-oxidation of L-ascorbic acid minimizing interferences from commonly present electroactive species such as 4-acetamidophenol (paracetamol), uric acid and citric acid [11].

Modified amperometric sensors have also been applied to the assessment of several antioxidant compounds such as butylated hydroxyanisole, whose determination has been achieved at a silver ferricyanide-based graphite-wax composite electrode. The amperograms showed that the oxidation current increased linearly with butylated hydroxyanisole concentration within the range of 7.4×10^{-6} to $8.3 \times 10^{-4} \text{ M}$. The limit of detection was $3.7 \times 10^{-6} \text{ M}$ [12].

Amperometric biosensors rely on the oxidation/reduction of an electroactive compound generated/consumed in an enzyme reaction. The biocatalyst consumes/generates an electroactive species (such as

O_2 or H_2O_2) stoichiometrically related to the substrate concentration. Glucose, ascorbic acid, lactate, cholesterol, oxalate, malate, can be determined by such biosensors relying on the corresponding oxidase as biocatalyst, and amperometric detection of either consumed molecular oxygen or generated hydrogen peroxide.

Various immobilization procedures involving electropolymerization and the use of mediators have been applied recently, aiming at optimizing the analytical parameters.

A glucose biosensor has been developed, by glucose oxidase entrapment in an electropolymerized polyaniline-polyvinylsulphonate film. Electropolymerization of aniline was performed on a Pt electrode, by applying a constant potential value, 0.75 V vs. Ag/AgCl. The prepared glucose biosensor preserved 80.6% of initial activity after 40 days when stored in phosphate buffer solution 0.1 M at 4°C [13].

In another study, an enzymatic biosensor was constructed, relying on TiO_2 nanotube arrays for transducing, and immobilization of both Prussian Blue and glucose oxidase. A very good sensitivity of the sensor was obtained, namely 248 $\text{mA M}^{-1} \text{ cm}^{-2}$, and a detection limit of 3.2 μM [14].

A novel glucose biosensor integrated in a flow injection system was developed, by coating a tin oxide-modified screen printed electrode with glucose oxidase entrapped in Nafion. The amperometric response of the biosensor was linear up to 200 mg/L with a detection limit (3σ) of 6.8 mg/L. The relative standard deviation for the repeated measurements of 100 mg/L glucose was 2.9%. All tested interferent effects from electroactive compounds (uric acid, acetaminophen, xanthine, hypoxanthine and ascorbic acid) could be hampered by employing the standard addition method. This biosensor allowed for viable determination of glucose in human blood plasma [15].

L-ascorbic acid content was analysed in fruit juices and effervescent tablets, by a biosensor with ascorbate oxidase from *Cucurbita* sp., immobilized on a screen-printed carbon electrode, using poly(ethylene glycol) diglycidyl ether as a crosslinking agent. Bovine serum albumin was also immobilized on the surface of this electrode, due to the presence of poly(ethylene glycol) diglycidyl ether. The linear range of analytical response for L-ascorbic acid corresponded to 5 - 150 $\mu\text{mol/L}$ [16].

In Figure 1, a chronoamperogram obtained by the author of this

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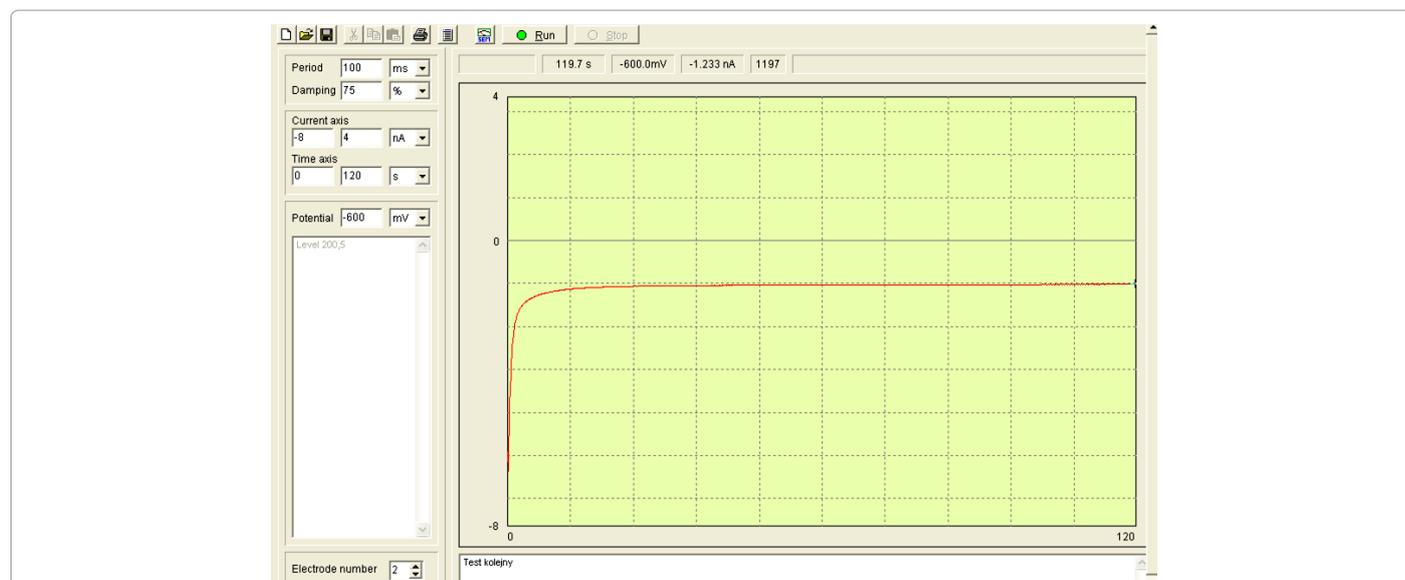


Figure 1: A chronoamperogram obtained by using an ascorbate oxidase based biosensor: 0.30 mM ascorbic acid; 100 U enzyme immobilized on a nylon membrane.

Editorial is presented, illustrating an ascorbic acid amperometric signal, applying the conditions described at [6].

Amperometric sensitive assay of cholesterol and H_2O_2 was possible with a biosensor based on a hybrid material derived from nanoscale Pt particles and graphene. Enzymes cholesterol oxidase and cholesterol esterase were used as biocatalysts [17].

The bienzyme amperometric electrode was characterized by a sensitivity respect to cholesterol ester of $2.07 \pm 0.1 \mu A/\mu M/cm^2$ and a limit of detection of $0.2 \mu M$. The assessed apparent Michaelis-Menten constant was 5 mM. The sensor's response was stable and was not affected by interferences from other common electroactive compounds [17].

A dimethylferrocene-modified linear poly(ethylenimine) hydrogel was employed to both immobilize and mediate electron transfer from lactate oxidase immobilized at a carbon anode, and an enzymatic cathode, aiming at amperometric lactate biosensing. The biosensor operated at 300 mV vs. SCE. When coupled with the bilirubin oxidase-based biocathode, the biosensor detected lactate between 0 mM and 5 mM, with a sensitivity of $45 \pm 6 \mu A cm^{-2} mM^{-1}$ [18].

The analytical characteristics (sensitivity, low detection limit, specificity) and validation by numerous applications in various sample types (foodstuffs, biological media), confirm the viability of the amperometric chemical sensors and biosensors, for the assessment of key biocompounds.

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