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Simultaneous and Accurate Real-Time Monitoring of Glucose and Ethanol in Alcoholic Drinks, Must, and Biomass by a Dual-Amperometric Biosensor

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ABSTRACT: In this work the optimization and application of a dual-amperometric biosensor for simultaneous monitoring of glucose and ethanol content, as quality markers in drinks and alcoholic fermentation media, are described. The biosensor is based on glucose oxidase (GOD) and alcohol oxidase (AOD) immobilized by co-cross-linking with bovine serum albumin (BSA) and glutaraldehyde (GLU) both onto a dual gold electrode, modified with a permselective overoxidized polypyrrole film (PPYox). Response, rejection of interferents, and stability of the dual biosensor were optimized in terms of PPYox thickness, BSA, and enzyme loading. The biosensor was integrated in a flow injection system coupled with an at-line microdialysis fiber as a sampling tool. Flow rates inside and outside the fiber were optimized in terms of linear responses (0.01−1 and 0.01−1.5 M) and sensitivities (27.6 ± 0.4 and 31.0 ± 0.6 $\mu A \cdot M^{-1} \cdot cm^{-2}$) for glucose and ethanol. Excellent anti-interference characteristics, the total absence of "cross-talk", and good response stability under operational conditions allowed application of the dual biosensor in accurate real-time monitoring (at least 15 samples/h) of alcoholic drinks, white grape must, and woody biomass.

KEYWORDS: *dual-amperometric biosensor, glucose, ethanol, microdialysis, alcoholic drink, must, biomass*

INTRODUCTION

The alcoholic fermentation process represents a fundamental step in the production of various biotechnological foodstuffs, such as wine and other beverages. The fermentation performance is very important to the requirements of drinks manufacturers as it allows them to respond to consumers increasing food sensitivity. For this reason, determination and monitoring of particular substances or products, like quality markers, during fermentation, are important as they provide information of good fermentation performance and give the possibility to modify almost in real time the process when there are abnormalities. In particular, in wine production, glucose and ethanol can be considered quality markers and continuous monitoring of these variables during fermentation is a prerequisite for effective process control. In fact, glucose and ethanol are not only the carbon source for the fermentation activity of yeasts and the main product of alcoholic fermentation, respectively, but they also have important effects on yeast growth.[1]

Basically, in clinical and industrial laboratories, ethanol and glucose are determined by classical methods, such as colorimetry,[2] spectrophotometry,[3,4] chemiluminescence,[5,6] and enzymatic methods,[7,8] which are poorly selective and time consuming. Chromatographic methods[9−13] are very selective and reliable, but they require extensive sample pretreatment and expensive and cumbersome equipment. On the other hand, enzyme amperometric biosensors are very attractive alternatives in food-quality monitoring, agricultural, and biotechnological applications because they can provide specific, rapid, and repetitive assays by miniaturizable and low-cost transducers.[14−16] To operate in complex matrices, biosensors should fulfill several requirements: they must be robust and easy to calibrate, should possess a wide linear range, and must be fouling free and interference free. In the case of multianalyte biosensing devices other requirements, such as the absence of cross-over effects, should be respected.[17,18]

Glucose and ethanol amperometric biosensors are usually based on oxidase or NAD-dependent dehydrogenase (GDH and ADH).[19−21]

Use of NAD-dependent dehydrogenase as a biological component is rather complicated by the copresence of NAD coenzyme whose electrode regeneration is necessary for the action of those devices;[20] on the other hand, use of oxidase in the presence of oxygen produces H_2O_2 whose detection with amperometric transducers can be subjected to interferences or 'cross-talk' effects in the case of adjacent sensors, due to interelectrode diffusion of the biocatalytically generated hydrogen peroxide.[17,22] A number of attempts have been made in order to face these problems, but the above requirements have not been completely met.[23−38]

Due to their characteristics, biosensors are particularly suitable for online monitoring analysis, in particular, coupled with flow injection analysis (FIA) techniques.[29−31] It has been also demonstrated that biosensors can be used in FIA systems for multicomponent analyses in real matrices.[32−34]

Dynamic sampling by microdialysis[35,36] is a method which is based on passive diffusion of analytes across a semipermeable membrane in the presence of a concentration gradient so that analyte recovery is a mass transport-controlled process. It can be conveniently coupled to a variety of analytical techniques, such as mass spectrometry, voltammetry, and chromatography,[37] and a number of applications described the coupling of microdialysis samplers with a biosensor for monitoring of real

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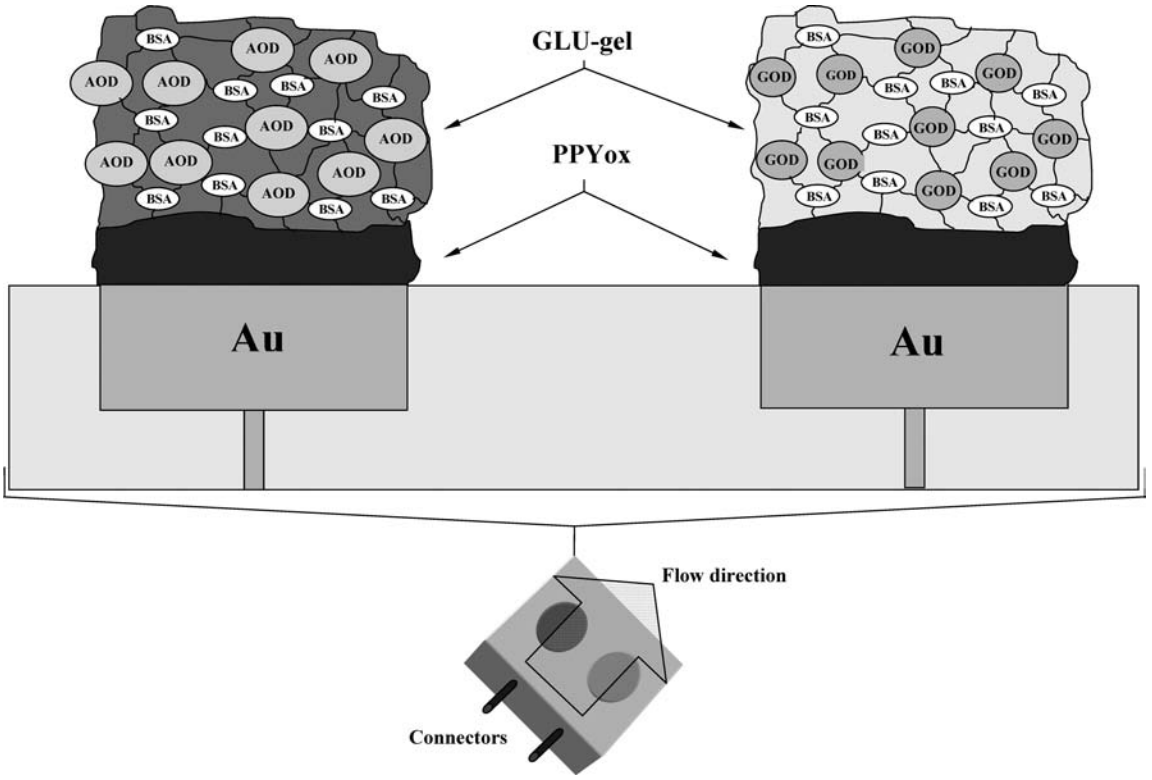
Scheme 1. Schematic View of a Typical Dual Biosensor for Simultaneous Monitoring of Glucose and Ethanol[a]



[a]GLU, glutaraldehyde; BSA, bovine serum albumin; AOD, alcohol oxidase; GOD, glucose oxidase; PPYox, overoxidized polypyrrole; Au, gold electrode.

samples.[26,29,38,39] Dynamic microdialysis allows an effective and a reliable sampling dilution step and a very efficient removal of fouling macroscopic matters (e.g., high molecular weight species such as proteins, peptides, triglycerides, etc.). Use of biosensors coupled with microdialysis samplers in FIA can be considered a very useful approach for determination of analytes in real matrices.

In a recent work[40] the interference introduced by ethanol in the analysis of real samples performed by a first-generation biosensor have been efficiently overcome by use of a gold electrode and a permselective overoxidized polypyrrole film. This approach has been exploited[41] in the attempt to develop a dual-amperometric biosensor for glucose and ethanol monitoring.

In the present work the dual-biosensor performances, such as sensitivity, selectivity, and stability, have been optimized in terms of BSA and enzyme loading, overoxidized polypyrrole film thickness, and flow rate inside/outside the at-line microdialysis fiber sampler. A total absence of cross-talk effects between the GOD- and the AOD-modified electrodes has been demonstrated, and the device also showed a high sensitivity of response toward both analytes, a good stability in operational condition, and suppression of the electroactive interferences present in real matrices. Biosensor performances have been tested by simultaneous determination of glucose and ethanol content in commercial alcoholic drinks, white grape must, and biomass.

MATERIALS AND METHODS

Chemicals. Alcohol oxidase (EC 1.1.3.13., from *Pichia pastoris*, 33 U mg^{-1} protein in a 60% saccharose solution with 0.1 M phosphate buffer at pH 8), glucose oxidase (EC 1.1.3.4., type II from *Aspergillus niger*, 15 U mg^{-1} protein), glutaraldehyde (grade II, 25% aqueous solution), and bovine serum albumin were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA) and used as received.

Pyrrole (Aldrich, Steinheim, Germany) was purified by vacuum distillation at 40 °C and stored in the dark under a nitrogen atmosphere at −20 °C.

Ethanol and all other chemicals of analytical-reagent grade were purchased from Baker (Mollinckrodt Backer B.V. Deventer, Holland) and used without further purification.

Real Samples. Commercial liqueurs, Campari (25% vol.), Herbal liqueur (30% vol.), and Mandarin liqueur (30% vol.) were purchased in a local supermarket. White grape must and woody biomass samples were collected from local factories, filtered on cellulose acetate (0.2 μm, Phenex-RC), and stored at −20 °C until analysis.

Apparatus. Electrochemical measurements were carried out with an Autolab PGSTAT 12 and GPES software, version 4.9 (Eco Chemie B.V., Utrecht, Holland).

The flow injection apparatus consisted of a Minipuls 3 peristaltic pump (Gilson, Villiers Le Bel, France), a six-way low-pressure injection valve (Rheodyne model 5020, Cotati, CA, USA), and a conventional thin-layer electrochemical cell (EG&G Princeton Applied Research, Princeton NJ, USA) with a dual Au working electrode (3 mm diameter, 1 mm electrode gap) and a thin-layer flow cell gasket of 255 μm thickness. All potentials were referred to a Ag/AgCl (3 M Cl^-) reference electrode. A pH 7.4 phosphate buffer solution (PB) was used as the carrier. Spectra/por hollow fibers (regenerate cellulose, 150 μm i.d., 168 μm o.d.) having a molecular weight cutoff of 9000 Da were purchased from Spectrum Medical Industries (Los Angeles, CA). The microdialysis fiber sampler was previously described.[38]

Electrosynthesized Polymer-Modified Electrodes. Gold electrodes were polished with alumina (0.3 and 1 μm), washed, sonicated in purified water, and electrochemically pretreated by potential cycling between −0.2 and +1.5 V versus Ag/AgCl in 0.5 M sulfuric acid until a steady state voltammogram was obtained. Polypyrrole (PPY) films were electrochemically grown, on pretreated gold electrodes, at +0.7 V

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versus Ag/AgCl in a 10 mM KCl solution containing 0.4 M pyrrole. The deposition charge was 537 mC·cm^{-2} corresponding to a film thickness of about 1.2 μm.[42] Au/PPY electrodes were overoxidized at +0.7 V versus Ag/AgCl in PB at pH 7.4 for at least 5 h until a steady state background current was obtained.

Biosensor Preparation. GOD and AOD dual biosensor (Scheme 1) was prepared as follows: 200 μL of a PB solution at pH 7.4 containing 13 mg of BSA and 69 U of GOD was mixed with 20 μL of 2.5% glutaraldehyde solution (25% GLU solution diluted 1:10 with PB). AOD solution was prepared by mixing 200 μL of a PB solution at pH 7.4 containing 24 mg of BSA and 11.6 U of AOD with 20 μL of 2.5% glutaraldehyde solution. A 3 μL amount of GOD and AOD final solutions were carefully pipetted onto the surface of the Au/PPYox-modified electrodes avoiding any mixing of the two solutions. Cross-linking reaction took place, leaving the electrodes for 60 min at a temperature of 25 °C. Afterward, the dual biosensor was washed to remove any weakly bound and/or adsorbed enzyme, and the device was stored in PB at 4 °C when not in use.

RESULTS AND DISCUSSION

Optimization of GOD and AOD Dual Biosensor. *Effect of PPYox Thickness.* PPYox as a permselective film in the development of amperometric biosensors represents a very interesting anti-interferent mean toward the most common electroactive interferents. The film thickness is the most important parameter that can affect the permselectivity and anti-interferent characteristics of the biosensor. Thickness was optimized by measuring the absolute bias (expressed as glucose concentration) of the most common interferents present in food matrices and biological fluids at their typical concentrations: 0.58 mM uric acid, 1.1 mM sodium metabisulfite, 0.1 mM ascorbic acid, 0.2 mM paracetamol, 0.08 mM cysteine, 0.48 mM gallic acid. The alcohol biosensor based on a 0.67 μm PPYox film and tested in flow injection conditions at 0.5 mL·min^{-1} gave biases in the range 3.2−22 μM, but gallic acid, which is the most representative polyphenol compound present in alcoholic drinks and biomasses, gave an unacceptable bias of 898 ± 6.9 μM. As expected, by increasing the film thickness the anti-interferent capabilities improved, and at 1 μm the bias produced by gallic acid was 673.0 ± 3.5 μM, while at 1.2 μm thickness it was 172.0 ± 0.6 μM; the overall bias relevant to all the interferents tested was below 200 μM. High film thicknesses gave long response time, low repeatability, and loss of mechanical stability of the biosensor with a consequent loss of sensitivity. Similar results were obtained at the glucose biosensor.

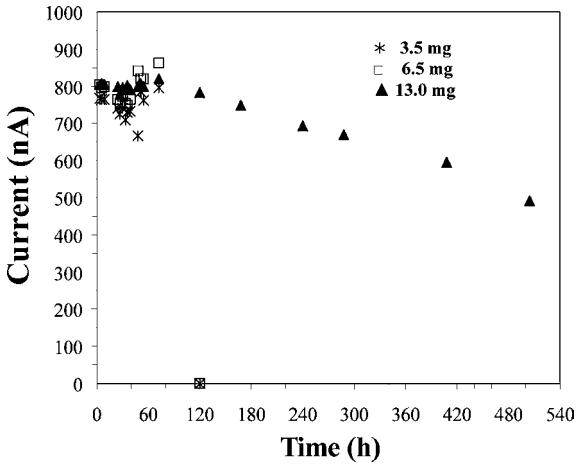

Figure 1. Operational stability of Au/PPYox/GOD-glutaraldehyde-BSA biosensors prepared with different BSA amounts measured by injections of glucose. Data are means of 3 repetitive injections of a 0.5 mM glucose standard solution. Flow rate, 0.5 mL·min^{-1}. Injection loop, 110 μL.

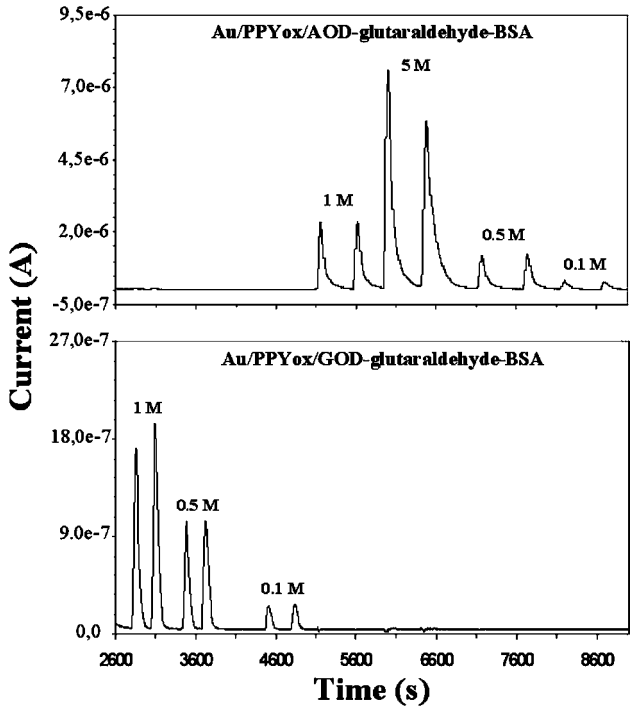

Figure 2. Flow responses obtained by duplicate injections of ethanol and glucose at different concentrations with a dual GOD- and AOD-based biosensor coupled with an at-line microdialysis fiber sampler. Flow rate (inside/outside the fiber), 0.2 mL·min^{-1}. Injection loop, 110 μL.

Effect of BSA Concentration. Use of BSA in the immobilization of enzymes by co-cross-linking prevents possible biocomponent denaturation due to the involvement of amino acids present in the active site. On the other hand, co-cross-linking efficiency as the mechanical stability of the enzyme entrapping gel also depends on the number of functional lysine groups of either BSA or enzyme molecules. Therefore, BSA concentration has been optimized in terms of biosensor sensitivity and operational stability. For this purpose, tests using BSA in the range 2.4−32 mg were carried out keeping the temperature (25 °C), concentrations of enzymes, and amount of glutaraldehyde (2.5%) unchanged. In particular, at BSA concentrations above 40 mg, co-cross-linking occurred rapidly (less than 5 s) without any possibility of spotting the solution onto the PPYox film-modified electrode.

Glucose biosensors with 24 mg of BSA showed a lower sensitivity value (2122 ± 142 μA·M^{-1}·cm^{-2}) with respect to biosensors with lower BSA amount (3.5−13 mg), which exhibited sensitivities in the range of 21 079−22 777 μA·M^{-1}·cm^{-2}.

Furthermore, BSA loading consistently affects the glucose biosensor stability. BSA concentrations above 24 mg gave mechanically nonflexible gels with a consequent deterioration of the PPYox anti-interferent film and glucose biosensor itself, while concentrations lower than 2.4 mg gave an inefficient co-cross-linking that implied a consequent gel instability and a rapid loss of biosensor sensitivity.

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Table 1. Comparison of Enzyme-Based Systems for Simultaneous Monitoring of Glucose and Ethanol

biosensor configuration (operational mode)	sensitivity glucose ethanol ($\mu A \cdot M^{-1} \cdot cm^{-2}$)	linearity/LOD glucose ethanol (mM)	stability	real sample/ sampling rate (samples/h)	ref
Au/PPYox/AOD-glutaraldehyde-BSA and Au/PPYox/GOD-glutaraldehyde-BSA dual bisensor (FIA with and without a microdialysis fiber sampler)	27.6 ± 0.4 31.0 ± 0.6 22777 ± 848[a] 29001 ± 283[a]	10−1000/5 10−1500/5 0.05−1/0.005[a] 0.05−1/0.005[a]	sensitivity retained: 100% and 90% (glucose and ethanol) after continuous analysis of 400 untreated biomass real samples injected in a 3 day period of time	white grape must, woody biomass, and diluted liqueurs and Campari/15	present work
graphite-Teflon-GOD-HRP-ferrocene and graphite-Teflon-AOD-HRP-ferrocene composite electrodes in parallel (FIA)	8629 ± 28[b] 1897 ± 28[c]	0.01−0.8/0.0019[b] 0.1−4.0/0.019[c]	15 days, but a daily renewal of the electrode surface before use was necessary	diluted red wine and sweet sherry/15[d]	49
controlled-pore glass/AOD and GOD reactors (sequential injection analysis with filtration and dialysis units)	−[e]	0.028−4.17/−[a] 0.0033−0.65/−[a]	−	beer fermentation broth/50	26
graphite/PEGDGE/PVI_{13}dmeOs/GDH/PQQ and graphite/PEGDGE/PVI_{13}dmeOs/ADH/PQQ biosensors (FIA)	0.087 0.22	0.02−0.8 0.0025−0.25	sensitivity retained: 80% after 1 month of storage at 4 °C; 60% after 20 h; and 90% after 100 h of continuous operation for glucose and ethanol, respectively	diluted wines	28

[a]Obtained without the microdialysis sampler. [b]Data from ref 48. [c]Data from ref 25. [d]Value estimated from the reference. [e]− = not reported.

In Figure 1 stability data obtained under operational conditions for a GOD biosensor at BSA loading in the range 3.5−13 mg are shown. Biosensors with 3.5 and 6.5 mg of BSA were stable up to 3 days, while after 5 days the response was negligible owing to detachment of the enzyme membrane during the FIA. The device with 13 mg of BSA showed a good stability of response, retaining 65% of the initial signal after 3 weeks of use in operational conditions. A similar trend has been obtained for the AOD biosensor with the best results in terms of sensitivity and stability using 24 mg of BSA. An optimized BAS amount higher than that of GOD can be explained considering a different number of lysine groups present in the AOD involved in the co-cross-linking process.

Effect of the Enzyme Concentration. In the development of biosensors enzyme loading strictly affects the sensitivity of response; therefore, several dual biosensors prepared by different amounts of enzymes cross-linked onto PPYox-modified Au electrodes were investigated, maintaining the optimized BSA amount, glutaraldehyde, and volume of cross-linking solution (3 μL) constant.

AOD and GOD concentrations in the range 29−222 and 90−345 $U \cdot mL^{-1}$ were used in the cross-linking solution, respectively. The AOD biosensor showed an increase of sensitivity by increasing the enzyme loading up to 58 $U \cdot mL^{-1}$ (29 001 ± 283 $\mu A \cdot M^{-1} \cdot cm^{-2}$), while at higher enzyme concentrations the sensitivity decreased strongly (16 552 ± 280 and 3678 ± 270 $\mu A \cdot M^{-1} \cdot cm^{-2}$ at 110 and 222 $U \cdot mL^{-1}$, respectively). This behavior suggests[43] a change in the rate-determining step, i.e., substrate diffusion limitation, occurring in the cross-linked matrix, whose chemical and mechanical characteristics depend on the cross-linking degree and hence on the enzyme concentration−number of lysine groups.[44]

On the contrary, the GOD biosensor displayed an enzyme loading dependent sensitivity with an increase up to 22 777 ± 848 $\mu A \cdot M^{-1} \cdot cm^{-2}$ at an enzyme concentration of 345 $U \cdot mL^{-1}$. This trend is expected for amperometric biosensors in which the rate of enzyme catalysis is comparable or slower than the diffusion of enzyme substrate inside the immobilization layer.[45−47]

Optimization of the Dual-Amperometric Biosensor in FIA with a Microdialysis Fiber Sampler. As described in the previous sections, the optimized GOD- and AOD-based biosensors were fabricated onto a gold dual working electrode, giving a single biosensor device (Au/PPYox/GOD-glutaraldehyde-BSA and Au/PPYox/AOD-glutaraldehyde-BSA, see Scheme 1) for simultaneous monitoring of glucose and ethanol. Analyses carried out in a FIA system operating at 0.5 $mL \cdot min^{-1}$ allowed determination of performance parameters. Sensitivities of 22 777 ± 843 and 29 001 ± 283 $\mu A \cdot M^{-1} \cdot cm^{-2}$ for glucose and ethanol, respectively, linearity in the range 0.05−1 mM (r^2 = 0.999), and detection limits of 5 μM were obtained. In complex real matrices the concentration of the analytes is out of the linear range of the biosensor response; therefore, use of a microdialysis fiber sampler can allow both at-line and online analyses without any pretreatment step of dilution and/or filtration, which are very useful for monitoring in the food-processing industry.

Recently, different applications have been described concerning the coupling of microdialysis sampling with biosensors.[26,29,38,39] In fact, electrode fouling caused by high

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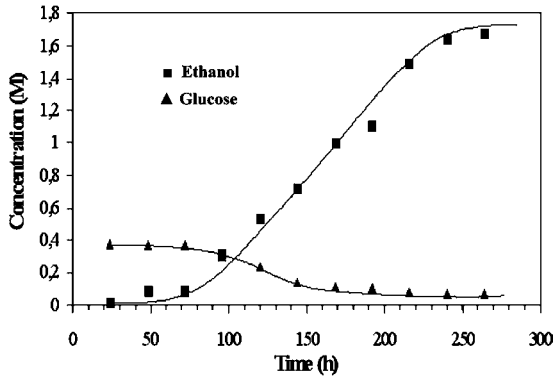

Figure 3. Ethanol and glucose concentration profiles of a typical alcoholic fermentation of a white grape must monitored by the dual GOD- and AOD-based biosensor coupled with an at-line microdialysis fiber sampler.

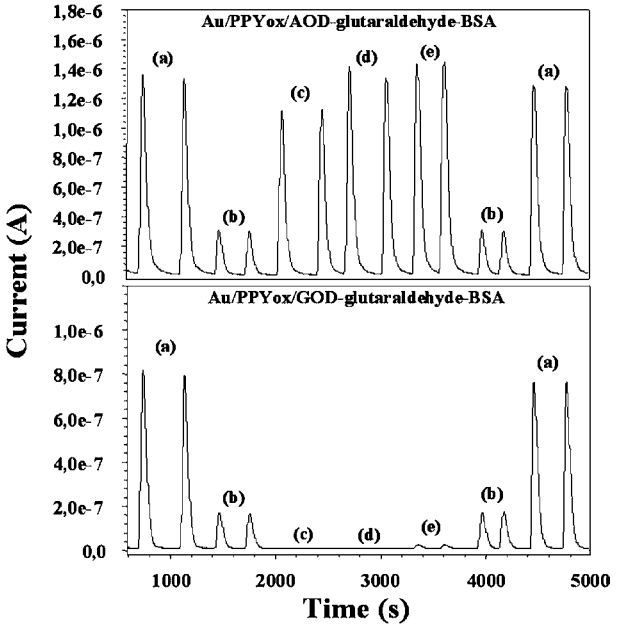

Figure 4. Flow injection analyses of standard solutions and alcoholic drinks diluted 1:10 carried out at the dual GOD- and AOD-based biosensor coupled with an at-line microdialysis fiber sampler: (a) standard mix solution of ethanol and glucose 0.5 M each; (b) standard mix solution of ethanol and glucose 0.1 M each; (c) Campari; (d) mandarin liqueur; (e) herbal liqueur. Flow rate (inside/outside the fiber), 0.2 mL·min^{-1}. Injection loop, 110 μL.

molecular weight components (e.g., proteins) normally occurring in biofluids and tissues and the low K'_M value of the immobilized enzymes, which can be inadequate for practical applications, are problems that can be coped with the use of a microdialysis sampler. Recovery of a microdialysis sampler is defined as the ratio between the concentration of the sample and the dialysate. This value depends on a number of parameters, e.g., the active area involved in the microdialysis process and the flow rate in the sample and in the dialysate. For example, a decrease in the flow rate usually increases the recovery but at the same time increases the analysis time.

The microdialysis sampler is placed in a FIA system,[38] and the fiber is contacted by a limited sample plug (typically around 100 μL) injected in a carrier stream; the higher the sample velocity, the lower the sampling time and then recovery. Moreover, another important parameter that should be considered in order to optimize the flow rates inside and outside the fiber is the sample throughput. In present case, a flow rate of 0.2 mL/min inside and outside the fiber guarantees a sufficient sensitivity (27.6 ± 0.4 and 31.0 ± 0.6 μA·M^{-1}·cm^{-2} for glucose and ethanol, respectively) and a sampling rate at least of 15 samples/h. These operational conditions allowed a linear range of response up to 1.0 (r^2 = 0.998) and 1.5 M (r^2 = 0.996) for glucose and ethanol, respectively, with detection limits of 5 mM.

The particular fabrication approach avoids any cross-talk between the two biosensors integrated in the same transducer, as can be noticed in Figure 2, where injections of glucose or ethanol standard solutions in the concentration range 0.1−5 M showed no responses on the other modified electrode. The dual biosensor integrated in a FIA system with an at-line microdialysis sampler can be used for analyses of real samples because the concentrations normally found in alcoholic drinks, musts, and biomasses are in the linear range of the biosensor response. A problem that often influences the reliability of a biosensor for analysis of real samples is the stability under operational condition, which can be affected by the deterioration of the permselective film or leaking of the enzyme during analysis. In this study the biosensor stability under operational flow conditions was assessed by analysis of 400 untreated biomass real samples injected in a 3 days period of time, during which the dual biosensor was biased continuously at the working potential; at the end of the explored period the retained sensitivities of response to glucose and ethanol were 100% and 90%, respectively.

Performances of the proposed first-generation dual-amperometric biosensor are better than those (see Table 1) of other systems for simultaneous glucose and ethanol monitoring, based on more complex detection schemes, such as reactors[26] or mediators with[49] and without[28] HRP. In particular, the dual biosensor possesses higher sensitivities of response, a higher operational stability, and excellent anti-interferent properties, which can allow an accurate monitoring of complex matrices even at low concentrations of substrates.

Application to Real Samples. The optimized dual GOD- and AOD-based biosensor in combination with an at-line microdialysis fiber sampler was successfully used to measure glucose and ethanol concentrations in real samples, even during biological processes, such as alcoholic fermentation of musts, allowing a real-time monitoring of the process. Figure 3 shows ethanol and glucose concentration profiles during the fermentation process of a white grape must. The slight increase of ethanol and decrease of glucose during the first 48 h can be probably ascribed to irreversible yeasts inhibition by SO_2 and a consequent selection of SO_2-resistant yeasts. In the following 3 days the activity of *Saccharomyces cerevisae* is demonstrated by the glucose total consumption (after about 300 h). At the end of the fermentation process, which also involved other sugars, the alcoholic grade had a final value of 11%.

In Figure 4 the use of the dual-biosensor device coupled with a microdialysis sampler for simultaneous determination of glucose and ethanol in alcoholic drinks is displayed. Low glucose values were found in real samples, since saccharose is typically used as a sweetener in those drinks. The alcoholic contents determined by the dual biosensor were not significantly different (according to a *t* test at 95% confidence level) from those reported on the labels of the drinks. The good response reproducibility of glucose and ethanol standard

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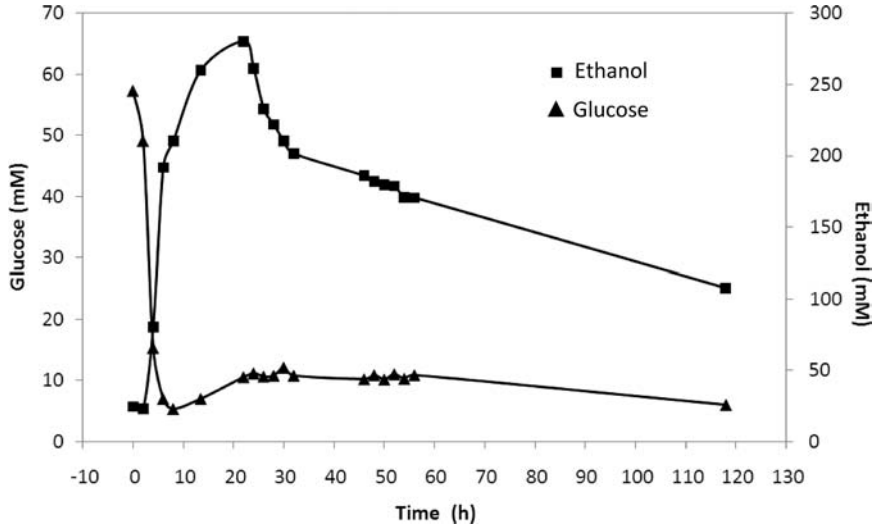

Figure 5. Ethanol and glucose concentration profiles in a woody biomass monitored by the dual GOD- and AOD-based biosensor coupled with an at-line microdialysis fiber sampler.

solutions, obtained before and after injections of real samples, proved the absence of any fouling effect and/or enzyme denaturation and then system reliability.

The potential of the dual biosensor was also tested by analysis of woody biomass whole samples, which were only filtered before injection. Figure 5 shows typical glucose and ethanol profiles of a woody biomass with an initial glucose level of 10 $g \cdot L^{-1}$, spiked with increasing concentrations of glucose (4 $g \cdot L^{-1}$ every 2 h) until a final value of 26 $g \cdot L^{-1}$. The biosensor detected a decrease of the glucose with an increase of ethanol up to the 22nd hour, with an expected delay of 2 h. Monitoring of microbial growth (data not reported) showed a plateau (maximum rate) starting from the 24th hour, confirming that microrganisms consumed ethanol as a carbon source when the glucose was depleted. The results obtained by the dual biosensor compared well (paired *t* test at 95% confidence level) with those of standard reference methods based on colorimetric enzymatic assays.

In this study a dual-amperometric biosensor based on AOD and GOD entrapped by co-cross-linking onto a dual gold electrode modified with a permselective overoxidized polypyrrole film was developed and optimized for the first time. The dual-amperometric biosensor that is cross-talk and interference free was easily integrated in a conventional FIA system coupled with an at-line microdialysis fiber-based sampler. The biosensor showed in flow conditions very wide linear ranges of response and a high operational stability that allowed accurate simultaneous analyses of glucose and ethanol in real complex matrices with no pretreatment, even at low concentrations of substrates, with a good sample throughput. Results compared well with those obtained by standard reference methods based on colorimetric enzymatic assays; then the proposed device represents an advantageous alternative to the official methods for the fast and accurate monitoring of both drinks and bioprocesses.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

GOD, glucose oxidase; AOD, alcohol oxidase; BSA, bovine serum albumin; GLU, glutaraldehyde; PPY, polypyrrole; PPYox, overoxidized polypyrrole; NAD, nicotinamide−adenine dinucleotide; GDH, glucose dehydrogenase; ADH, alcohol dehydrogenase; FIA, flow injection analysis; PB, phosphate buffer

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