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Amperometric multidetection with composite enzyme electrodes

A. Guzman-Vázquez de Prada, N. Peña, C. Parrado, A.J. Reviejo, J.M. Pingarrón*

Department of Analytical Chemistry, Faculty of Chemistry, Universidad Complutense de Madrid, E-28040 Madrid, Spain

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Abstract

The use of composite biosensors for multianalyte detection strategies is discussed. Graphite–Teflon rigid composite biosensors offer the possibility of coimmobilization of several enzymes by simple physical inclusion in the bulk of the electrode matrix with no covalent linkages. A novel trienzyme graphite–Teflon–glucose oxidase (GOD)–alcohol oxidase (AOD)–peroxidase (HRP)–ferrocene bisosensor yielded amperometric steady-state currents similar to those obtained with graphite–Teflon–GOD–HRP–ferrocene and graphite–Teflon–AOD–HRP–ferrocene electrodes for the same concentration of glucose and ethanol, respectively. The performance of the trienzyme biosensor for multianalyte detection was evaluated with the simultaneous determination of glucose and ethanol after separation by HPLC, in samples of sweet wine. The simultaneous analysis of several analytes in the same sample should imply that, with an adequate dilution, the concentration levels of the analytes can be included within the ranges of linearity of the corresponding calibration plots. The use of two composite biosensors in a parallel configuration, so that different analytes can be simultaneously detected with no need of chromatographic separation, is also discussed. The usefulness of this approach was evaluated by the simultaneous analysis of glucose and ethanol in sweet wine, and of glucose and lactic acid in red wine.

Keywords: Multianalyte detection; Composite biosensors; Amperometric detection

1. Introduction

Some important analytical problems need the monitoring of several (bio)chemical parameters to be solved in a complete way. This is specially interesting in food analysis in which a lot of the samples contain a complex mixture of analytes of interest. Traditionally, chromatography has been used for the determination of several analytes of interest in one single analysis. However, some limitation occurs concerning detection, because it can be performed only for compounds belonging to the same family or exhibiting similar characteristics with respect to the detection principle. Moreover, it is not always possible to chromatographically resolve analytes of different chemical characteristics in real samples due to the absence of adequate columns or derivatizing substances. Probably, the multidetection devices of a higher importance at the time are electronic noses or tongues [1]. Most of these systems are based on chemical sensors, but they are only semi-selectives and exhibit a low signal-to-noise ratio produced by the sensors noise and drift.

Enzyme biosensors constitute an interesting alternative to carry out multidetection, because they allow the analysis of samples containing analytes unable to be simultaneously detected at a conventional detector, by incorporation of different enzymes or coupling of several enzyme reactions, provided that each biological recognition element gives an individual response [2].

Multianalyte systems based on enzyme bioelectrodes have been used for the determination of analytes of interest in fermentation processes (glucose, lactic acid, ethanol) [3,4], as well as for the determination of glucose and L-lactate in tomato juice [5], galactose and glucose in dairy products [6], malic and lactic acids in wine [7] and fructose and glucose in honey [8].

The possibility of coinmobilization of several enzymes by simple physical inclusion in the bulk of graphite–Teflon composite matrices has been extensively demonstrated by our group in the last years [9–14]. Advantages as the easy renewability by polishing, the absence of covalent linkages and membranes on the electrode surface, and the bulk incorporation of other components such as mediators of cofactors

* Corresponding author. Tel.: +34-913-944-315; fax: +34-913-944-329.
E-mail address: pingarro@quim.ucm.es (J.M. Pingarrón).

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allow the fabrication of robust and versatile electrochemical biosensors.

These advantages have been profited in this work for the construction of a multienzyme graphite–Teflon biosensor in which glucose oxidase (GOD), alcohol oxidase (AOD), horseradish peroxidase (HRP), as well as the mediator ferrocene were coimmobilized into the graphite–Teflon electrode matrix. The performance of this novel trienzyme biosensor both for the single analysis of the substrates corresponding to each enzyme, and for multianalyte detection in liquid chromatography is evaluated in this article. The analytical usefulness of the graphite–Teflon–GOD–AOD–HRP–ferrocene electrode was demonstrated with the simultaneous determination of glucose and ethanol in sweet sherry wine. Moreover, the possibility of using two different composite enzyme electrodes in a parallel configuration connected to a multichannel detector, for the simultaneous analysis of glucose and ethanol in sweet wine, and of glucose and lactic acid in red wine, with no need of chromatographic separation, is also discussed.

2. Experimental

2.1. Apparatus, electrodes and electrochemical cells

Batch amperometric measurements in stirred solutions were performed on a BAS LC-4C potentiostat connected to a Limeys LG512B recorded, except those corresponding to dual electrochemical detection with two biosensors in a parallel configuration. These measurements were carried out on a BAS Epsilon amperometric detector connected to a computer equipped with the BAS chromatograph 2.0.01 package.

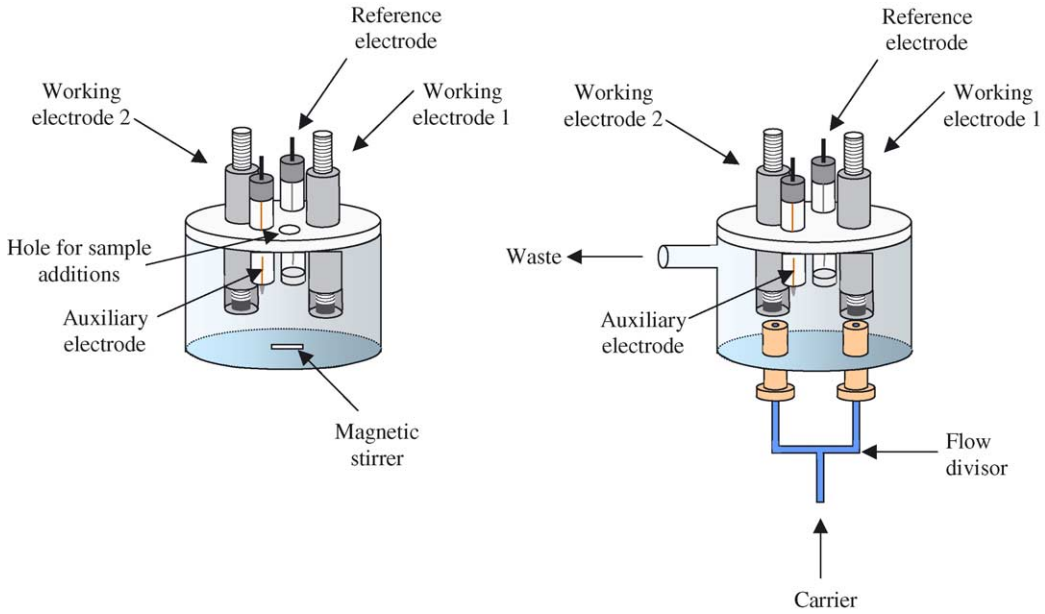
The flow-injection arrangement consisted of a WIZ peristaltic pump and a Rheodyne model 7725i injection valve with variable injection volumes. Chromatographic separations were carried out using a JASCO PU-980 HPLC pump and a Rheodyne model 7725i injection valve. A Phenomenex 125 mm × 4 mm i.d. Hypersil ODS column with a particle size of 5 μm was used. In both cases, the electrode potentials for amperometric measurements were controlled by means of an EG&G PAR model 400 detector using a computer equipped with the Biocrom 200 3.0 software for data adquisition. A BAS RE-1 Ag/AgCl/KCl (3 M) reference electrode and a Pt wire auxiliary electrode were used in all cases.

A large volume (50 ml) home made metacrylate wall-jet cell [15] was used for HPLC measurements. Specially designed cells for the use of several working electrodes were employed for dual amperometric detection (Scheme 1).

Graphite–Teflon–glucose oxidase–horseradish peroxidase–ferrocene, graphite–Teflon–alcohol oxidase–horseradish peroxidase–ferrocene and graphite–Teflon–lactate oxidase–horseradish peroxidase–ferrocene electrodes were prepared in the form of cylindrical pellets as described previously [9–11]. A similar procedure was followed for the construction of the graphite–Teflon–glucose oxidase–alcohol oxidase–horseradish peroxidase–ferrocene electrode. In this case, graphite, 2700 U HRP, 2724 U GOD and 286 U AOD were thoroughly mixed by mechanic stirring in a 0.40 ml suspension of a 0.05 mol l^{-1} phosphate buffer solution of pH 7.4 at 4 °C.

2.2. Reagents and solutions

HRP (EC 1.11.1.7, type II, activity 180 U mg^{-1} of solid, Sigma), GOD (EC 1.1.3.4, activity 181.600 units per g of solid, Sigma), AOD (EC 1.1.3.13, activity 1430 U ml^{-1},



Scheme 1. Batch and flow-injection cells used for dual amperometric detection.

Sigma), LOD (EC 1.1.3.2, activity 35 U mg^{-1} of solid, Sigma), ferrocene (Merck), graphite (ultra-F purity, Carbone of America) and Teflon powder (Aldrich) were used to prepare the composite enzyme electrodes.

Other reagents were: D(+)-glucose (Panreac), methanol (99.98% (w/w), Scharlau), ethanol (96% (w/w), Scharlau), L-lactic acid (Sigma). All chemicals were of analytical reagent grade and the water used was obtained from a Millipore Milli-Q purification system.

Stock 0.10 mol l^{-1} solutions of glucose, methanol, ethanol and lactic acid were prepared in 0.05 mol l^{-1} phosphate buffer of pH 7.4. More dilute standards were prepared by suitable dilution with the same buffer solution.

2.3. Samples

Commercial red wine (El Sotillo, 12 vol.%) and sweet sherry (Valdespino, 15 vol.%).

2.4. Procedures

Amperometric measurements in stirred solutions were performed at room temperature by applying the desired potential and allowing the steady-state current to be reached. Amperometric flow-injection measurements were carried out by using a 0.05 mol l^{-1} phosphate buffer solution of pH 7.4 as the carrier, whereas a 0.01 mol l^{-1} phosphate buffer of the same pH was employed as mobile phase in HPLC experiments.

2.4.1. Determination of glucose and ethanol in sweet sherry by HPLC with amperometric detection at the trienzyme electrode

This determination required only a 5 μl sample dilution in 5.0 ml of mobile phase as sample treatment. Fifty microlitres of this solution were injected into the chromatographic column. A flow rate of 1.0 ml min^{-1} and a detection potential of 0.00 V were used. The determination of the analytes was carried out by interpolation of the peak areas in the corresponding calibration plots.

2.4.1.1. Determination of glucose and ethanol in sweet sherry with dual amperometric detection using bienzyme composite electrodes in parallel. As for the chromatographic determination, simple dilutions of 100 μl of sample in 5.0 ml of phosphate buffer solution, in the batch mode, and of 5 μl in 5.0 ml of carrier solution, in the FI mode, were needed as sample treatment. In the first case, the determination was carried out by applying the standard additions method. This implied that after the obtention of the amperometric signals corresponding to the addition of a 50 μl aliquot of the diluted sample solution, with each of the two biosensors used, successive 25 μl additions of 0.1 mol l^{-1} ethanol and 0.1 mol l^{-1} glucose were carried out and the amperometric responses recorded. The determination by FI with amperometric detection was performed by interpolation of the peak currents obtained after the injection of the diluted sample into previously constructed calibration plots for the analytes.

2.4.1.2. Determination of glucose and lactic acid in red wine with dual amperometric detection using bienzyme composite electrodes in parallel. No dilution step was needed for this sample when batch amperometry was used. Twenty-five microlitres of sample were added to the electrochemical cell and the amperometric responses for each analyte obtained. Then, the standard additions method was applied, which involved additions of 25 μl aliquots of 0.01 mol l^{-1} glucose and lactic acid stock solutions. The FI mode required a dilution of 50 μl of the sample in 5.0 ml of the carried solution. The standard additions method was also used by injecting growing concentrations of lactic acid and glucose together with 50 μl of the diluted sample.

3. Results and discussion

In a former article we reported on the construction and performance of a bienzyme graphite–70% Teflon–peroxidase–glucose oxidase–ferrocene electrode for the determination of glucose in must and wine samples [9]. Neither ethanol nor methanol affected the response of the analyte even for a glucose-to-alcohol ratio of 1:10. More recently, we reported also on graphite–Teflon–alcohol oxidase–peroxidase–ferrocene electrodes for the reliable monitoring of alcohols in food and beverages [11]. Also, the additions of glucose showed no effect on the responses obtained with this biosensor. Therefore, it can be expected that the responses of a trienzyme GOD–AOD–HRP–ferrocene composite biosensor for glucose or ethanol would correspond to the hydrogen peroxide generated in the reaction of each substrate with the corresponding enzyme. In both cases, the amperometric signal employed for monitoring the enzyme reactions corresponded to the electrochemical reduction of ferricinium. Some experiments were made to verify that the coimmobilization of enzymes, that are not involved in the reaction chain produced after a substrate addition, did not affect the stability and the analytical characteristics of the biosensor. The working pH used (ca. 0.05 mol l^{-1} phosphate buffer solution of pH 7.4) was the same employed with each of the bienzyme electrodes mentioned above. Also, the loadings of the enzymes and the mediator were those optimized previously for the bienzyme biosensors.

The graphite–Teflon–GOD–AOD–HRP–ferrocene electrodes yielded amperometric steady-state currents similar to those obtained with the graphite–Teflon–GOD–HRP–ferrocene and graphite–Teflon–AOD–HRP–ferrocene electrodes for the same concentration of glucose and ethanol, respectively. Moreover, the currents measured remained constant over the whole working day. Concerning the useful lifetime,

the behaviour of the trienzyme electrode was the same than that observed for the bienzyme biosensors for each analyte [9,11]. Therefore, the useful lifetime of the trienzyme biosensor was limited by the stability of AOD in approximately 15 days with no need of enzyme stabilizers in the composite matrix. Furthermore, it was necessary to polish the electrode surface daily before using it for the determination of alcohols.

Calibration plots at $E_{app} = 0.00\,V$ for glucose and ethanol were obtained with the trienzyme biosensor with linear ranges of (0.1–9.0) $\times 10^{-4}\,mol\,l^{-1}$ ($r = 0.9986$) and (0.2–20) $\times 10^{-4}\,mol\,l^{-1}$ ($r = 0.9984$), respectively. The slopes and intercepts of this plots were (6.01 ± 0.05) $\times 10^{2}\,\mu A\,mol^{-1}\,l$ and (1 ± 4) $\times 10^{-3}\,\mu A$, respectively, for glucose, and (6.1 ± 0.1) $\times 10^{2}\,\mu A\,mol^{-1}\,l$ and (11 ± 7) $\times 10^{-3}\,\mu A$ for ethanol. These analytical characteristics are very similar to those found with the GOD–HRP–ferrocene electrode for glucose (linear range 1.0×10^{-5} to $8.0 \times 10^{-4}\,mol\,l^{-1}$, slope 610 ± 2 $\mu A\,mol^{-1}\,l$), and with the AOD–HRP–ferrocene biosensor for ethanol (linear range 2.0×10^{-5} to $2.0 \times 10^{-3}\,mol\,l^{-1}$, slope (5.51 ± 0.03) $10^{2}\,\mu A\,mol^{-1}\,l$).

3.1. HPLC with amperometric detection at the trienzyme composite electrode

The amperometric detection at the trienzyme biosensor in HPLC was evaluated for three analytes: glucose, ethanol and methanol. A Hypersil reversed-phase column was used since no modifiers are needed with this column for an adequate separation of the alcohols [11]. A $0.01\,mol\,l^{-1}$ phosphate buffer solution of pH 7.4 was used as the mobile phase. The applied potential was 0.00 V and the injection volume was 50 μl.

The effect of the mobile phase flow rate was checked by injecting in the chromatographic system a solution containing $1.0 \times 10^{-3}\,mol\,l^{-1}$ glucose, $5.0 \times 10^{-4}\,mol\,l^{-1}$ methanol and $5.0 \times 10^{-3}\,mol\,l^{-1}$ ethanol. R_s values lower than 1.5 for glucose and methanol were obtained in the whole flow rate range studied (1.0–0.1 ml min^{-1}). Therefore, the selection criterion chosen was the lower relative error in the peak area measurement. A $\varepsilon_r = -2\%$ was obtained for a flow rate of 0.5 ml min^{-1}, which can be considered as acceptable for further applications. Although a $\varepsilon_r = -1\%$ was found at 0.2 ml min^{-1}, this value would imply an unacceptable increase of the analysis time. Fig. 1 shows a chromatogram obtained using flow rate of 0.5 ml min^{-1}.

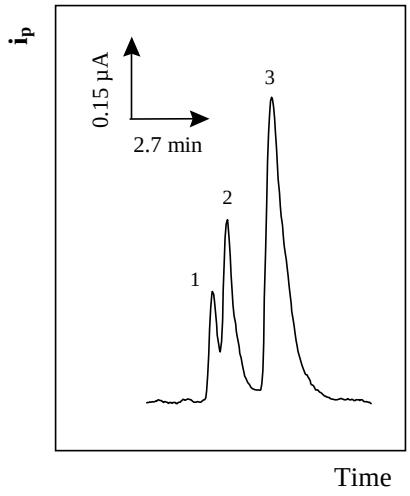


Fig. 1. Chromatogram obtained at a tryenzime graphite–Teflon–GOD–AOD–HRP–ferrocene electrode for the injection of 50 μl of a solution of $1.0 \times 10^{-3}\,mol\,l^{-1}$ glucose (1); $5.0 \times 10^{-4}\,mol\,l^{-1}$ methanol (2); and $5.0 \times 10^{-3}\,mol\,l^{-1}$ ethanol (3). Mobile phase, $0.01\,mol\,l^{-1}$ phosphate buffer of pH 7.4: flow rate, 0.5 ml min^{-1}; $E_{app} = 0.00$ V.

Table 1 summarizes the average values of retention times, capacity factors, peak heights and peak areas for five repetitive injections, as well as the corresponding confidence intervals, for a significance level of 0.05, and the relative standard deviations (R.S.D.). As can be observed, the repeatability of these parameters was fairly good, thus indicating a reliable inmobilization of the enzymes being unaffected by the hydrodynamic conditions.

The analytical characteristics of the peak area vs. concentration plots for the three analytes are shown in Table 2. The limits of detection were calculated according to the $3s_b/m$ criterion, where m is the slope of the linear range of the corresponding calibration plot, and s_b was estimated as the standard deviation ($n = 10$) of $1.0 \times 10^{-4}\,mol\,l^{-1}$ glucose, $5.0 \times 10^{-5}\,mol\,l^{-1}$ methanol and $1.0 \times 10^{-4}\,mol\,l^{-1}$ ethanol. These limits are sufficient for the determination of these analytes in real samples. The obtained results permit to conclude that the trienzyme biosensor is suitable to be used for a multianalyte determination when it is employed as amperometric detector in a HPLC system.

3.2. Application to the analysis of real samples

One of the main practical problems in the use of multianalyte biosensors for the analysis of real samples is that

Table 1
Retention times (t_R), capacity factors (k'), peak heights (i_p) and peak areas (A_p) obtained for five successive injections of 50 μl of a solution containing $1.0 \times 10^{-3}\,mol\,l^{-1}$ glucose, $5.0 \times 10^{-4}\,mol\,l^{-1}$ methanol, and $5.0 \times 10^{-3}\,mol\,l^{-1}$ ethanol

Analyte	t_R (min)	k'	i_p (μA)	R.S.D. (%)	A_p (μA s^{-1})	R.S.D. (%)
Glucose	2.38 ± 0.05	0.0	0.143 ± 0.001	1.0	214 ± 3	1.1
Methanol	2.9	0.22	0.24 ± 0.03	1.3	500 ± 20	2.6
Ethanol	4.5	0.89	0.496 ± 0.005	0.8	1900 ± 90	3.8

Mobile phase, $0.01\,mol\,l^{-1}$ phosphate buffer of pH 7.4; flow rate, 0.5 ml min^{-1}; $E_{app} = 0.00$ V.

Table 2
Analytical characteristics of the calibration graphs obtained for glucose, methanol and ethanol by HPLC with amperometric detection at a graphite–Teflon–GOD–AOD–HRP–ferrocene composite electrode

Analyte	Linear range ($mol\,l^{-1}$)	intercept (μA)	Slope ($\mu A\,min\,mol\,l^{-1}$)	r	Limit of detection ($mol\,l^{-1}$)
Glucose	$(1.0–32) \times 10^{-4}$	16 ± 7	$(174 \pm 5) \times 10^3$	0.9984	4.0×10^{-5}
Methanol	$(0.5–8.0) \times 10^{-4}$	25 ± 10	$(750 \pm 30) \times 10^3$	0.9984	4.0×10^{-5}
Ethanol	$(1.0–32) \times 10^{-4}$	12 ± 10	$(392 \pm 6) \times 10^3$	0.9995	5.0×10^{-5}

$E_{app} = 0.00$ V.

the different analyte concentration levels generally do not coincide to allow the determination to be carried out from a sole aliquot of the sample solution. The applicability of the trienzyme biosensor as multianalyte detector in HPLC was evaluated by analysing samples of sweet wine, which is characterized by its high alcoholic content and sugar concentration [16]. This makes possible that both the ethanol and glucose concentration were included within the linear range of the corresponding calibration plot for each of them, provided that an adequate dilution of the sample was carried out (see Section 2). This dilution step in the buffer used as the mobile phase was indeed the only sample treatment needed prior to the injection of an aliquot in the chromatographic system.

The determination of methanol in this sample (which may be present in a small amount as a product of pectines degradation) was not possible because its content was below the detection limit of the method. Taking this into account, and with the aim of shortening the time of analysis, we decided to increase the mobile phase flow rate to $1.0\,ml\,min^{-1}$, at which the peaks of glucose and ethanol are well resolved. At this flow rate, linear relationships between peak area and concentration were found for glucose and ethanol over the ranges $(1.0 \pm 32) \times 10^{-4}\,mol\,l^{-1}$ ($r = 0.9985$; slope $(117 \pm 6) \times 10^3\,\mu A\,min\,mol^{-1}\,l$; intercept $= 24 \pm 9\,\mu A$), and $(2.0–128) \times 10^{-4}\,mol\,l^{-1}$ ($r = 0.9987$; slope $(131 \pm 3) \times 10^3\,\mu A\,min\,mol^{-1}\,l$; intercept $= 25 \pm 17\,\mu A$), respectively. The limits of detection, calculated from the standard deviation ($n = 10$) of $1.0 \times 10^{-4}\,mol\,l^{-1}$ glucose, and $2.0 \times 10^{-4}\,mol\,l^{-1}$ ethanol were $7.0 \times 10^{-5}\,mol\,l^{-1}$ in both cases. If these analytical characteristics are compared with those obtained at $0.5\,ml\,min^{-1}$, it can be observed that the sensitivity increases as the flow rate of the mobile phase decreased, as expected for biosensing detection in flow systems.

Fig. 2 shows a chromatogram obtained after the injection of an aliquot of the sweet sherry wine (15°). The results obtained for five analysis yielded glucose and ethanol contents of 85 ± 3 and $120 \pm 8\,g\,l^{-1}$, respectively. These results were compared with those obtained by applying commercial enzymatic kits using spectrophotometric detection supplied by Boehringer. The glucose concentration in the sweet wine using the corresponding test kit was $88 \pm 5\,g\,l^{-1}$, while the ethanol concentration found with its enzymatic kit was $120 \pm 8\,g\,l^{-1}$. Obviously, the application of the Student's *t*-test demonstrated there were no significant differences between the values obtained by HPLC with the trienzyme electrode and with the reference methods at a 0.05 significance level.

Although these results show fairly well the usefulness of the graphite–Teflon–GOD–AOD–HRP–ferrocene as a detector in HPLC, it must be pointed out that the simultaneous analysis of several analytes in the same sample should imply that, with an adequate dilution, the concentration levels of such analytes could be included within the ranges of linearity of their corresponding calibration plots.

3.3. Simultaneous detection using bienzyme composite electrodes in parallel

The use of sensor arrays for multicomponent analysis in real samples is, with no doubt, one of the growing areas in modern analytical chemistry [1]. In this context, we have employed several composite electrochemical biosensors in a parallel configuration immersed in the same working cell, and using only one reference electrode and one counter electrode, both in the batch and flow-injection modes. The electrochemical cells reported in Section 2 were designed for this purpose, and they can be referred as a first order sensor array. The usefulness of this approach was evaluated by the simultaneous analysis of glucose and ethanol in sweet wine and of glucose and lactic acid in red wine.

3.3.1. Determination of glucose and ethanol in sweet wine (sherry)

This sample and the analytes determined (the same than those analysed above) were chosen with the aim of evaluating the behaviour of this kind of electrode array as an

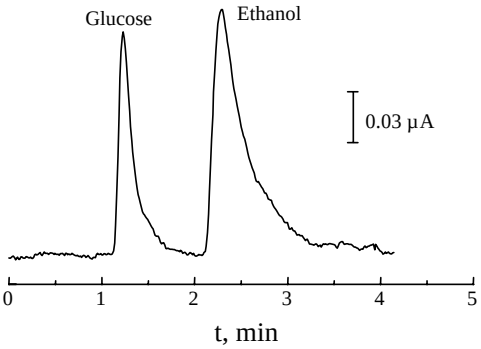


Fig. 2. Chromatogram obtained at a tryenzime graphite–Teflon–GOD–AOD–HRP–ferrocene electrode for the injection of 50 μl of a sweet sherry wine. Conditions as in Fig. 1.

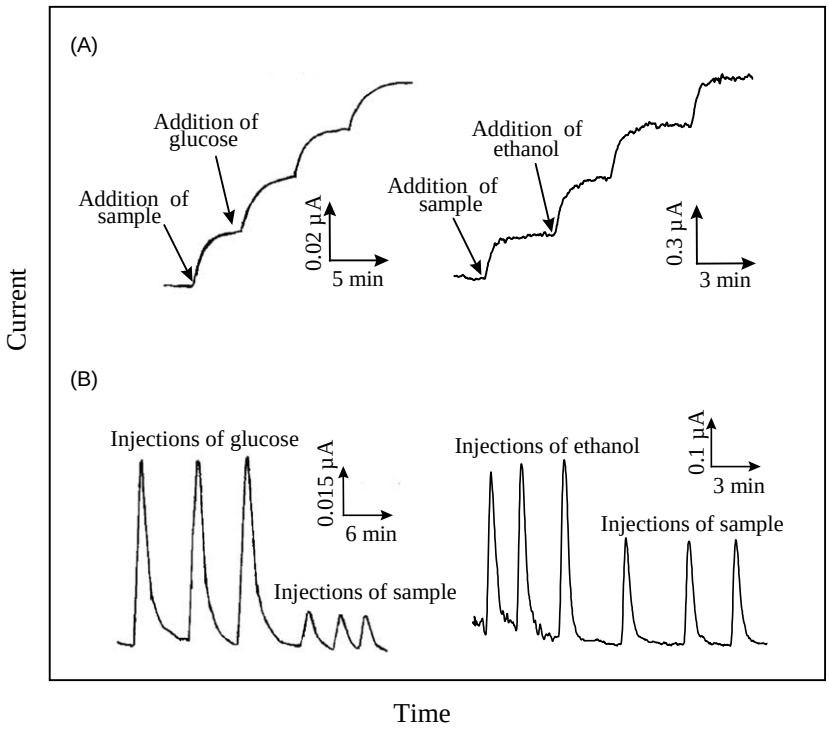


Fig. 3. Amperometric responses obtained in the batch mode (A); and in the flow-injection mode (B) for a sweet wine sample, and additions of glucose and ethanol standard solutions using graphite–Teflon–GOD–HRP–ferrocene and graphite–Teflon–AOD–HRP–ferrocene electrodes in a parallel configuration. Phosphate buffer solution (0.05 mol l^{-1}) of pH 7.4 as background and carrier solutions: $E_{app} = 0.00$ V; flow rate = 1.0 ml min^{-1}.

alternative to the use of chromatographic columns. The determination of glucose and ethanol was carried out using the previously reported graphite–Teflon–GOD–HRP–ferrocene and graphite–Teflon–AOD–HRP–ferrocene bioelectrodes [9,11], both in the batch and flow-injection modes.

The determination of the analytes by batch amperometry in stirred solutions was carried out by applying the standard additions method. Thus, after the addition of the sample solution aliquot, three 25 μl additions of a 0.1 mol l^{-1} glucose standard solution and of a 0.1 mol l^{-1} ethanol standard solution were performed (Fig. 3A). Concerning the determination using the flow-injection methodology, interpolation of the signals obtained after the sample injection into the corresponding calibration plot of standards was employed. This methodology required a flow splitting to allow the sample to reach the two electrodes. Fig. 3B shows some of the fiagrams obtained for successive injections of standards and sample solution.

Table 3 summarizes the results obtained by both methodologies, which are compared with those reported above by HPLC and using the trienzyme biosensor as amperometric detector. Application of the Student's *t*-test demonstrated that the *t* experimental values were in all cases lower than the tabulated *t* (2.306), indicating the absence of significant differences between the methods.

3.3.2. Determination of glucose and lactic acid in red wine

Since the graphite–Teflon composite matrix exhibits excellent characteristics for the construction of multienzyme biosensors, we decided to incorporate a fourth enzyme, lactate oxidase (LOD), to the graphite–Teflon–GOD–AOD–HRP–ferrocene for its application to the analysis of wines.

The constructed graphite–Teflon–GOD–AOD–HRP–ferrocene composite electrode permitted the determination of each analyte separately, with similar analytical characteristics to those found with the bienzyme electrodes prepared with each one of the oxidase enzymes which compose the multienzyme biosensor [9–11]. However, when this electrode was used as a detector in the HPLC system, it was verified the impossibility of separation of the peaks for glucose and lactic acid with the Hypersil column employed.

Besides the applications of the array cell described in the case (a), this cell design is very useful for this kind of situations in which the separation of the analytes with a determined chromatographic column is not possible. Thus, the simultaneous determination of glucose and lactic acid in red wine was carried out using the multidetection cell. The

Table 3
Results obtained for the analysis of glucose and ethanol in sweet wine (sherry) using graphite–Teflon–GOD–HRP–ferrocene and graphite–Teflon–AOD–HRP–ferrocene electrodes in a parallel configuration

	Batch	t_{exp}	FIA	t_{exp}	HPLC[a]
Glucose (g l^{-1})	88 ± 5	1.15	87 ± 5	0.77	85 ± 3
Ethanol (g l^{-1})	120 ± 8	0.0	121 ± 9	0.1	120 ± 8

[a] Using the graphite–Teflon–GOD–AOD–HRP–ferrocene electrode as amperometric detector.

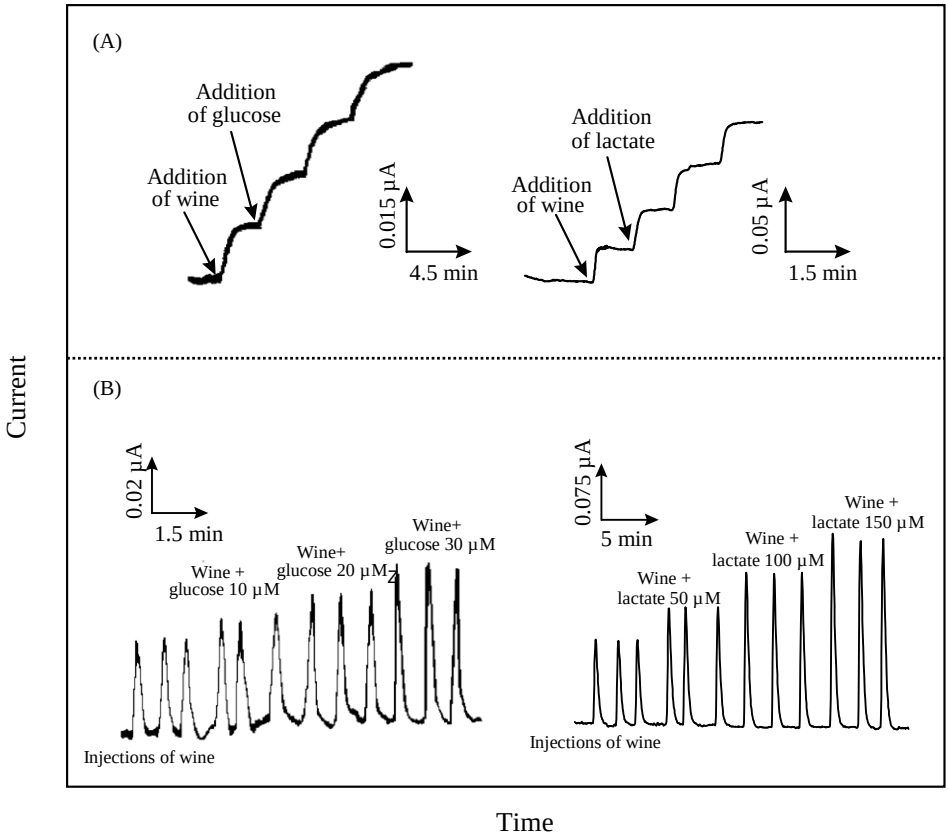


Fig. 4. Amperometric responses obtained in the batch mode (A); and in the flow-injection mode (B) for a red wine sample, and additions of glucose and lactate standard solutions using graphite–Teflon–GOD–HRP–ferrocene and graphite–Teflon–LOD–HRP–ferrocene electrodes in a parallel configuration. Conditions as in Fig. 3.

Table 4
Results obtained for the determination of glucose and lactic acid in red wine using graphite–Teflon–GOD–HRP–ferrocene and graphite–Teflon–LOD–HRP–ferrocene electrodes in a parallel configuration

	Batch	t_{exp}	FIA	t_{exp}	Reference method[a]
Glucose ($g\,l^{-1}$)	0.13 ± 0.06	0.26	0.12 ± 0.05	0.59	0.14 ± 0.03
Lactic acid ($g\,l^{-1}$)	0.9 ± 0.8	0.0	1.1 ± 0.4	0.27	0.9 ± 0.7

[a] Results obtained with the graphite–Teflon–GOD–HRP–ferrocene electrode for glucose, and with the graphite–Teflon–LOD–HRP–ferrocene electrode for lactic acid.

above mentioned graphite–Teflon–GOD–HRP–ferrocene biosensor was employed for the determination of glucose, whereas a graphite–Teflon–LOD–HRP–ferrocene composite electrode previously developed in our laboratory [10] was used for the determination of lactic acid.

Fig. 4A and B shows some amperograms in stirred solutions and fiagrams obtained for glucose and lactic acid. In both cases the applied potential was 0.00 V and the standard additions method was used for quantification. The red wine sample was added undiluted in the batch procedure while a 50 µl aliquot of diluted sample in the carrier solution was injected in the FI methodology. The results obtained are summarized in Table 4, and they were compared with those achieved with the specific graphite–Teflon bienzyme biosensors of each substrate (GOD–HRP for glucose and LOD–HRP for lactic acid). As can be observed, the results obtained with the biosensors in a parallel configuration agree fairly well with those obtained using the bienzyme electrodes separately, since experimental t values were in all cases lower than the tabulated t (2.306).

4. Conclusions

The results obtained demonstrate that biosensors based on graphite–Teflon composite matrices are very appropriate to bulk incorporation of several biological recognition elements with an extremely simple fabrication procedure. This allows multiple possibilities for these biosensors to be used for multidetection, either using multienzyme electrodes as amperometric detectors in liquid chromatographic systems, or using array cells for the simultaneous

determination of several analytes both in the batch and flow-injection modes. This later approach constitutes an advantageous alternative to a lot of methods of analysis which require a much more complex and costly instrumentation, allowing also a simplification of the analytical methodology.

References

[1] H. Ulmer, J. Miltrovics, U. Weinar, W. Göpel, Sens. Actuators B: Chem. 65 (2000) 79.
[2] P.I. O'Connell, G.G. Guilbault, Anal. Lett. 34 (2001) 1063.
[3] M.A. Kumar, M.S. Thakur, A. Senthuran, V. Senthuran, N.G. Karanth, R. Hatti-Kaul, B. Mattiasson, World J. Microbiol. Bioctehnol. 17 (2001) 23.
[4] H. Lidon, T. Buttler, H. Jeppsson, G. Marko-Varga, J. Vola, L. Gorton, Chromatographia 47 (1998) 501.
[5] F. Palmisano, R. Rizzi, D. Centonze, P.G. Zambonin, Biosens. Bioelectron. 15 (2000) 531.
[6] S. Mannino, M.S. Cosio, S. Buratti, Italian J. Food Sci. 11 (1999) 57.
[7] J. Katrlik, A. Dizzariello, V. Martihuba, J. Suorc, M. Stred-ansky, S. Miertus, Anal. Chim. Acta 379 (1999) 193.
[8] R. Antiochia, G. Palleschi, Anal. Lett. 30 (1997) 683.
[9] M.A. Cerro del, G. Cayuela, A.J. Reviejo, J.M. Pingarrón, J. Wang, Electroanalysis 9 (1997) 1113.
[10] B. Serra, A.J. Reviejo, C. Parrado, J.M. Pingarrón, Biosens. Bioelectron. 14 (1999) 505.
[11] A. Guzman-Vázquez de Prada, N. Peña, M.L. Mena, A.J. Reviejo, J.M. Pingarrón, Biosens. Bioelectron., in press.
[12] G. Cayuela, N. Peña, A.J. Reviejo, J.M. Pingarrón, Analyst 123 (1998) 371.
[13] N. Peña, G. Ruíz, A.J. Reviejo, J.M. Pingarrón, Anal. Chem. 73 (2001) 1190.
[14] D. Domínguez, B. Serra, A.J. Reviejo, J.M. Pingarrón, Anal. Biochem. 298 (2001) 275.
[15] J. Wang, B. Freiha, Anal. Chem. 57 (1985) 1776.
[16] Belitz and Grosch, Análisis de Alinatos, 1992 second ed., Acribia.