

Automated determination of glucose in soluble coffee using Prussian Blue–glucose oxidase–Nafion® modified electrode

Ivanildo Luiz de Mattos*, Madalena Carneiro da Cunha Areias

Departamento de Química Fundamental - DQF, Universidade Federal de Pernambuco, Cidade Universitária, 50740-540 Recife, PE, Brazil

Received 8 July 2004; received in revised form 20 January 2005; accepted 24 January 2005

Available online 24 February 2005

Abstract

A highly selective, fast and stable biosensor for determination of glucose in soluble coffee has been developed. The biosensor electrode consist of a thin film of ferric hexacyanoferrate (Prussian Blue or PB) electrodeposited on the glassy carbon electrode (GCE) (to provide a catalytic surface for the detection of hydrogen peroxide) *glucose oxidase* immobilized on top of the electrode and a Nafion® polymer layer. The stability of the PB film and the biosensor was evaluated by injecting standard-solution (50 μ M H_2O_2 and 0.5 mM glucose) during 4 h in a flow-injection system with the electrodes polarized at -50 mV versus Ag/AgCl. The system is able to handle about 60 samples per hour and is very stable and suitable for industrial control. Determination of glucose in the range 2.5 and 15% (w/v) in phosphate buffer with precision (r.s.d. < 1.5%) has been achieved and is in agreement with the conventional procedures. Linear calibration in the range of 0.15 and 2.50 mM with detection limits of ca. 0.03 mM has been obtained. The morphology of the enzyme *glucose oxidase* on the modified electrode has been analyzed by scanning electron microscopy (SEM) measurements.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Modified electrode; Prussian Blue; Biosensor; Soluble coffee; Glucose

1. Introduction

The glucose content is a very important parameter in food industry for evaluating the quality of commercial instant coffee [1]. It indicates the degree of maturity [2] and can assess the commercial value of the product [2,3]. Among glucose determinations, are widely used several chromatographic procedures (anion-exchange chromatography [4], capillary zone electrophoresis [5], paper chromatography [6] and liquid chromatography [3,6]) normally with electrochemical detection. Alternatively, the use of Fehling's reagent [6] and enzyme electrode [7] can be used but when a fast determination is needed, procedures involving flow systems are preferable [8]. For industrial control, the use of automated methods is desirable and the flow injection analysis (FIA) is worthwhile [9–11]. For that purpose, the combination of the FIA system with biosensors became attractive due to its

versatility, simplicity and suitability for large-scale analyses [12–15].

The uses of biosensors are increasing in several applications [12–21], and the reason for that is the fact that it presents high easy fabrication procedure, low cost and portability [12–21]. One of the main factors contributed for biosensor development was the use of the electrochemical catalysts (mediators) such as ferrocene [14,22], ferrocene derivatives [14,22], meldola blue [14,22], methylene green [23], bienzyme systems [24,25] or transition metal hexacyanoferrates [26–29]. This last one attracted widespread attention due to its characteristics to form well-defined electroactive films on electrodes [28,29]. One example is the PB, $Fe_4[Fe(CN)_6] \cdot nH_2O$ – a classical mixed-valence compound which present the reduced form (Prussian White) with catalytic activity for the reduction of hydrogen peroxide even in the presence of molecular oxygen [27–31]. This mixed valence compound seems to be suitable mediator for the development of biosensors based on oxidases for analytical applications [32–38].

* Corresponding author. Tel.: +55 8121268440; fax: +55 8121268442.
E-mail address: ilmattos@ufpe.br (I.L. de Mattos).

The improvement of the procedure used for enzymatic immobilization has resulted in the development of biosensors more robust, selective and with great operational stability [12–15,21,36]. Among this strategies, the use of polymeric membrane Nafion[®] has became very attractive due to its negative charge, since foreign species such as ascorbic acid, paracetamol, uric acid, etc. are readily repelled [33–35,37,38]. The number of potential foreign species is then restricted by molecular size, permeation and/or (bio)chemical reaction. As consequence analytical sensitivity and system versatility are obtained [33–35,37,38].

The main objective of the present work was to develop a simple, fast, and low-cost automated procedure for glucose determination in instant soluble coffee using PB/Gox–Nafion[®] modified electrode.

2. Experimental

2.1. Reagents, standards and samples

All solutions used were prepared with analytical-grade chemicals such as hydrogen peroxide, potassium hexacyanoferrate, ferric chloride, ammonium hydroxide, potassium chloride, hydrochloric acid, glucose, arabinose, galactose, mannose, fructose, etc.

Solution of Nafion[®] (5%, w/v, in 90% alcohol solution), ethanol and *glucose oxidase* (type VII-S with activity 50,000 U mg^{−1} from *Aspergillus niger*) were obtained from Aldrich and Sigma, respectively.

The solutions used for deposition and activation of the PB film onto the glassy carbon electrode were prepared by mixing: 2.0 mM of K₃Fe(CN)₆, 2.0 mM of FeCl₃, 0.1 M of KCl and 0.1 M of HCl (solution defined as reagent R₁); 0.1 M of KCl and 0.1 M of HCl (as reagent R₂) and 0.25 M of KH₂PO₄ plus 0.13 M of K₂HPO₄ (as reagent R₃).

The Nafion[®] solution was diluted to 1% (v/v) with ethanol and the pH of the solution was adjusted to 5.5 by adding a concentrated solution of NH₄OH. The enzymatic solution was prepared dissolving 1.0 mg of solid *glucose oxidase* in 1 ml of distilled/deionized water.

The stock standard solution in hydrogen peroxide was stored in refrigerator. Potassium permanganate solution (0.02 M) and sulfuric acid (25%) were used for preparation and calculation of a standard solution for hydrogen peroxide. The procedure for standardized was:

- (i) Preparation of the stock solution of approximately 1000 mg L^{−1}: 10 mL of H₂O₂ (30%) was diluted into 100-mL volumetric flask and make up to the mark with water; 30 mL of this solution was diluted with 1000 mL of water and the stock solution prepared in this manner has a concentration of around 1000 mg L^{−1} hydrogen peroxide;
- (ii) Precise assay of the standard solution for hydrogen peroxide: 50.0 mL of the stock solution (ca. 1000 mg L^{−1})

was put into a 500-mL conical flask, diluted with 200 mL of water and add of 30 mL of sulfuric acid 25%; titration with potassium permanganate solution 0.02 M until the color changes to pink;

- (iii) Calculation of the precise content of the hydrogen concentration: consumption of potassium permanganate (mL) × 34.02 = content of hydrogen peroxide, in mg L^{−1}. The stock solution precisely determined according to the above procedure [39] can be used to prepare further working concentrations by dilution with water. The stock solution and any further working solutions can be used for one day when stored in a refrigerator.

Glucose solution (1 M) was left for 24 h (ambient temperature) for mutarotational equilibrium.

Working standards of H₂O₂ (50 μM) and glucose (within 0.10 and 2.50 mM) were freshly prepared by dilution of the stock standard solutions with reagent R₃.

Instant coffee samples were provided by local stores. Before introduction into the flow-injection system, 2.0 mg of the sample was diluted (200 or 500-fold, w/v) with water and this solution was diluted (50-fold, v/v) with reagent R₃ before injection.

2.2. Instruments and apparatus

Measurements were carried out employing an electrochemical system from Autolab (PGSTAT30, Germany) connected with a PC computer. The electrochemical cell was composed of three electrodes (all from Bioanalytical Systems Inc., BAS, USA): working (glass carbon with inner diameter and length as 3 mm and 7.5 cm), reference (Ag/AgCl; 3 M NaCl with length 7.5 cm) and counter (platinum wire with 6-cm length and gold connector).

Experiments with flow injection analysis (FIA) were realized using a programmable system from Ismatec (Zurich, Switzerland) composed of two peristaltic pumps (fix and variable), injection valves, tubing (0.7 mm, i.d., wall thickness < 0.2 mm), “wall-jet” electrochemical cell (Fig. 1) and other accessories.

2.3. Procedure

The electrochemical cell was filled with a 1.0 M Na₂SO₄ solution, and the glassy carbon electrode was submitted to 250 cycles (from 200 to 900 mV versus Ag/AgCl) with scan rate = 1 Vs^{−1}. After that, the working electrode was manually polished with alumina (Al₂O₃, 1 and 0.3 μm, Nuclear/Brazil) until the formation of a mirror surface and then left in sonication for 5 min (in a 1:1 mixture of water/alcohol) to eliminate micro particles of alumina adsorbed on the electrode surface. Unless mentioned, the scan rate involved in cyclic voltammetry in all experiments was fixed as 50 mV s^{−1}.

The Prussian Blue films were deposited onto the carbon electrode using the procedure developed by Mattos et al. [31], with slight modification. Shortly the process consisted of:

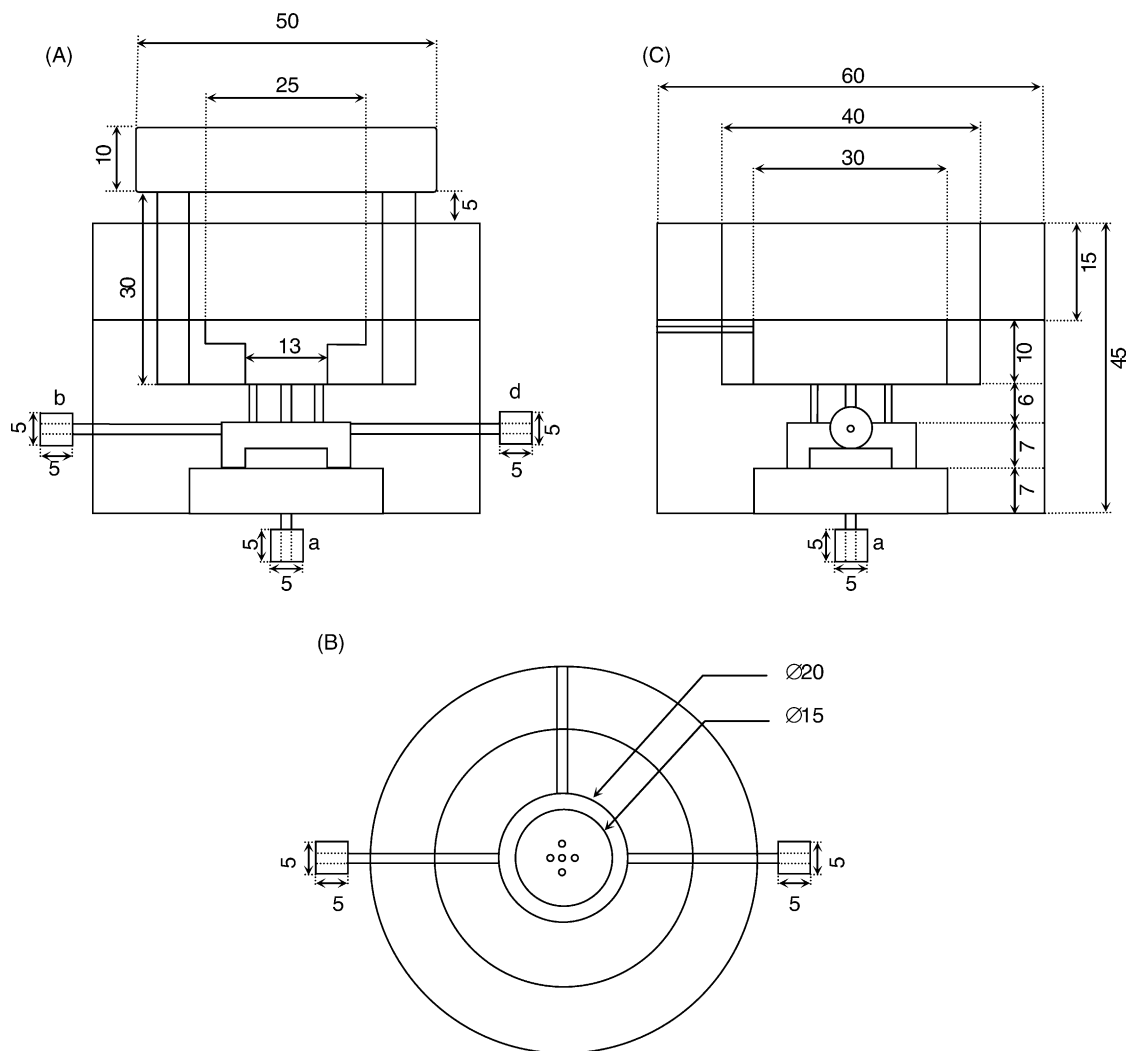


Fig. 1. Amperometric cell. (A) Frontal view, (B) top view of the open cell, (C) lateral view; (a and b) inlet and outlet of the carrier stream, (c and d) counter and reference electrodes. The working electrode is placed perpendicular to inlet, i.e. wall-jet configuration.

- (i) Production of the film (in reagent R_1): fixation of the potential in 400 mV versus Ag/AgCl during 60 s. Afterwards, the system was left in standby condition for 30 s and started the scan rate (one cycle) between 350 and -50 mV versus Ag/AgCl (Fig. 2a);
- (ii) Activation of the film (in reagent R_2): variation of the potential (50 cycles) between 350 and -50 mV versus Ag/AgCl (Fig. 2b);
- (iii) Drying of the film – after washing with water, the modified electrode was left for 1h under controlled temperature (100°C).

In the end of the third step, the material obtained was glassy carbon electrode modified with PB. Before preparation of the biosensor, the film was evaluated (concerning operational stability) by a flow injection system with successive injections ($100\ \mu\text{L}$) of hydrogen peroxide standard solution ($50\ \mu\text{M}$ diluted in reagent R_3).

The preparation of the biosensor consisted of:

- (i) Conditioning the modified electrode (in reagent R_3) – the dried PB modified electrode was submitted to potential = -50 mV versus Ag/AgCl for 600 s; after that, it was started the scan rate (25 cycles) between 350 and -50 mV versus Ag/AgCl;
- (ii) Enzymatic deposition: two drops (within 5 and $10\ \mu\text{L}$) of the enzymatic solution was deposited onto the modified electrode (the enzyme layer was maintained at ambient temperature for drying);
- (iii) Covering with polymeric membrane – over the enzyme layer was deposited two drops ($5\text{--}10\ \mu\text{L}$) of the Nafion[®] solution (diluted and neutralized to pH 5.5). The morphology of the *glucose oxidase* immobilized onto the modified PB glassy carbon electrode is showed in Fig. 3.

The drying step was accomplished by leaving the electrode at room temperature for few hours. Thereafter, the biosensor was placed in contact (around 15 min) with reagent R_3 to achieve equilibrium of the layers. The FIA system (initially

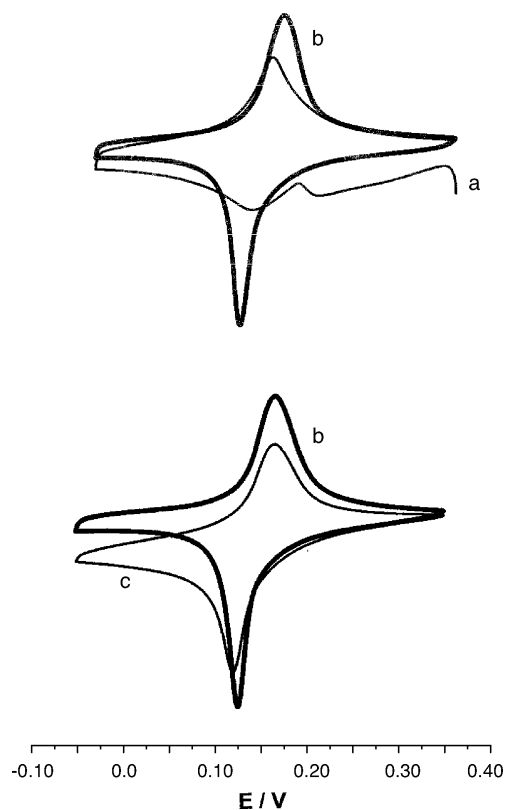


Fig. 2. Cyclic voltammograms of the PB modified glassy carbon electrode. (a) electrochemical deposition (in R_1), (b) activation of the film (in R_2), (c) reduction of hydrogen peroxide (1.0 mM in R_2). Scan rate = 50 mVs⁻¹. The potential scan was initiated at 350 mV vs. Ag/AgCl.

used for evaluation of the PB film) was employed to verify the performance of the biosensor; in this case, a standard glucose solution (0.6 mM in R_3) was used.

2.4. The flow-injection system

The sample was injected into the FIA system by a 100 μ L sampling loop and pushed by its carrier stream (0.25 M KH₂PO₄ + 0.13 M K₂HPO₄ – reagent R_3) at 0.8 mL min⁻¹. This flowed through a 25 cm coiled reactor

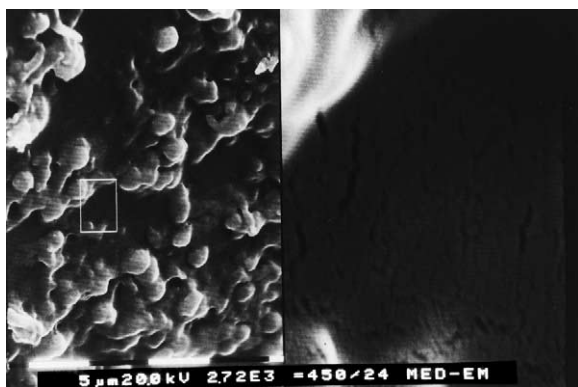


Fig. 3. Scanning micrograph of the PB film in presence of glucose oxidase immobilized in Nafion[®].

towards the detector with fixed applied potential at working electrode = -50 mV versus Ag/AgCl. The passage of the sample through the cell (Fig. 1) produced a transient signal with a peak current proportional to the glucose content in the sample. Precision was expressed as relative standard deviation calculated after successive measurements.

3. Results and discussion

The steps realized for the film preparation were of great importance to improve the stability of the films. In fact, the performance of the amperometric sensor increased by about 25% compared with the one without these steps; moreover, it is important to explain that any trace of fat or alumina adsorbed onto surface electrode before the deposition step decreases or eliminated the possibility to create a stable film of Prussian Blue.

Another aspect of great importance [31], refers to the pH value of the solutions employed for deposition and activation steps; note that alkaline medium cause the destruction of the PB film because it diminish the Fe(CN)₆⁴⁻ concentration necessary to form, together with Fe³⁺ ions, Fe₄[Fe(CN)₆] $\cdot$ nH₂O: Fe(CN)₆⁴⁻ + 2OH⁻ → Fe(OH)₂ + 6CN⁻; 2Fe(OH)₂ + H₂O + 1/2 O₂ → 2Fe(OH)₃.

3.1. Electrode optimization and electrochemical activation

Fig. 2 shows the behavior of the cyclic voltammograms of PB film onto the glassy carbon electrode after the deposition (a) and activation of the film (b). The time for deposition was fixed as 60 s, because above this value (verified until 180 s), there was an increment of the capacitive current and the peak potential (ΔE_p) values were higher than 90 mV versus Ag/AgCl. Also, it is possible to note that after activation step, the film presented a great improvement in electroactivity: the cathodic and anodic current peaks increased to ca. 180 and 50% (Table 1). The PB modified electrode presented fast response (ΔE_p = 50 mV), good definitions of the reduced and oxidized forms and value of E^0 = 142 mV very similar to that found in the literature [26,27]; these peaks were attributed to the reversible redox interconversion of Prussian Blue and Prussian White or the oxidized and reduced forms, respectively.

Also, Fig. 2 shows that in the presence of hydrogen peroxide (c) there was a variation of the PB film electroactivity, demonstrating its capacity for H₂O₂ reduction (see Table 1). As presented earlier, the reduced form of PB has a catalytic effect for electroreduction of hydrogen peroxide even in the presence of molecular oxygen [26,27]. Hence, since the product of the biosensor based on oxidase is hydrogen peroxide, the PB film can be interpreted as an “artificial” peroxidase [27,30]. Consequently, the applicability of the amperometric sensor and/or biosensor can be improved.

Table 1
Electrochemical parameters of the cyclic voltammograms of the modified glassy carbon electrode

Condition	E_{pc} (mV)	I_{pc} (μ A)	E_{pa} (mV)	I_{pa} (μ A)
Deposition	123	91.5	147.4	126
Activation	110	256	161	189
H ₂ O ₂ (1.0 mM)	112	287.6	162	113.6

3.2. Performance of the biosensor in FIA system

The long-term stability of the system and biosensor was evaluated by injecting standard solution (50 μ M H₂O₂ in R₃) during working periods (Fig. 4); no significant baseline drift was noted, which confirms the stability of the Prussian Blue film. Slight variation in the height signal, after 30 min, was due to instability of the hydrogen peroxide solution; in fact, after changing for new prepared solution, the signal was recovered. Finally, note that the profile of the first signal (1, Fig. 4) is very regular and confirm the good performance of the system; this test was done by changing the speed of the recorder paper.

After being dimensioned, the flow-injection analysis was applied to glucose analysis. Before start the registration of the standard-solution signal, it is recommended to allowed the system to run (without injection) to get the hydrodynamic equilibrium. It is easily verified by baseline stabilization. Calibration equation was attained by least-squares regression: $y = 1.12693 + 25.13441x$, where y is the cathodic current in nA and x is the concentration of glucose in mM. For $n = 5$ (0.15; 0.30; 0.60; 1.20 and 2.50 mM), the typical correlation coefficient was 0.99898. Using the proposed system about 60 samples can be run per hour. The sampling rate can be improved by reducing the available mean time for glucose reaction. This aspect is particularly attractive for analyses where sensitivity and selectivity are not critical. Considering the low cost of the proposed system, it is suitable for large-scale analysis and/or quality control. For a typical sample with glucose contents of 5.74% (w/v), the relative standard deviation of eleven results was estimated

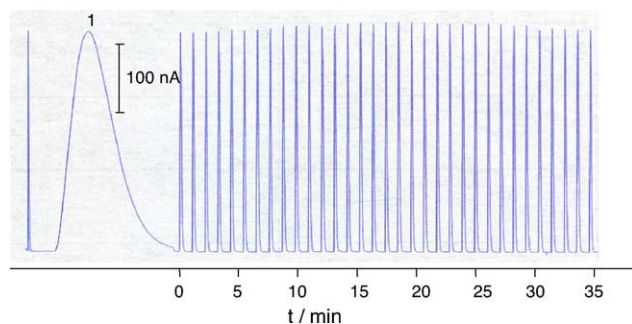


Fig. 4. Performance of the biosensor. Injection of 100 μ L of standard solution (50 μ M H₂O₂ in R₃). (1) The first signal of the standard solution, recorded in higher speed (than the others signals, in the sequence) to show the profile of the FIA-signal. Analytical frequency = 60 samples h⁻¹. Applied potential = -50 mV vs. Ag/AgCl.

Table 2
Concentration of glucose in percent (w/v) as determined in instant coffee samples by the proposed and reference methods [40]

Sample ^a	Glucose percent (w/v)	
	Present method ^b	Reference method ^b
1	14.97 $\pm$ 0.22	15.13 $\pm$ 0.16
2	7.13 $\pm$ 0.09	7.21 $\pm$ 0.10
3	5.17 $\pm$ 0.06	5.19 $\pm$ 0.07
4	5.74 $\pm$ 0.07	5.53 $\pm$ 0.07
5	2.37 $\pm$ 0.03	2.51 $\pm$ 0.03

^a Samples 2–5, dilution: 1/250 (v/v); sample 1, dilution: 1/500.

^b Analysis realized in duplicate.

as 1.5%. The accuracy was assessed by running five already analyzed samples [40], and only slight differences in results were found by different methods (Table 2).

Pure soluble coffee is characterized by low free sugar [3–7]. The concentration of sugars such as arabinose, galactose, mannose or fructose related to glucose concentration is, in the maximum, in the proportion of 5:1 [40]. So the concentration of glucose was fixed as 0.6 mM and the foreign species (separately) were fixed as 3.0 mM. The effect of these species on the glucose signal was not verified. Moreover, the use of applied potential $E = -50$ mV versus Ag/AgCl (low electrode potential – fixed to minimize or eliminate the discharge of interfering species [33]) associated with polymeric membrane based on Nafion[®] (negatively charged polyelectrolyte [33]) also contributed to diminish drastically eventual effect of foreign species [14,37].

3.3. Scanning electron micrograph (SEM)

The surface of the PB–glucose oxidase and Nafion[®] modified electrode was examined using scanning electron microscopy (SEM). On the left side of the Fig. 3, it is possible to note that the enzyme is well-fixed by Nafion[®]; this result corroborate with the long-term stability of the biosensor shown in Fig. 4. In fact, after 4 h working period, small variations in current was observed (<5%, not shown). The small square in Fig. 3 shows a region between enzymes and was selected to show the polymeric membrane covering PB film (right side).

4. Conclusion

Simplicity of the set-up for its implementation, high selectivity and stability, and a high sampling rate make the system PB/Gox–Nafion[®] modified electrode an efficient tool for performing automated analysis in a simple, fast and low-cost way. This is an attractive method for the routine measurements of glucose present in instant coffee. Replacing the recorder for a computer to access the results directly will improve the versatility of the proposal. Moreover, the proposed method can be well-adjusted for other kind of samples which may be considered as an alternative (or complementary) to

the chromatographic analysis usually required for industrial quality control.

Concerning the biosensors, there are (in principle) no limits of using other oxidases for PB/Nafion glassy carbon, Au, Pt, screen-printed electrodes, etc. for different applications. Considering, for example, a complex matrix such as product/fermentation, industrial waste, etc., the integration in the analytical system of biosensors with non-chromatographic continuous separation techniques such as pervaporation and/or concentric tubing approach make interesting choices for improve various preliminary operations of the analytical process. It represents an efficient tool to perform automated analysis improving analytical sensitivity and system versatility. Experiments are in progress.

Acknowledgements

I.L.M. would like to thank Dr Thomas Laurell from Lund University (Sweden) for scanning electron microscopy (SEM) measurements, Fapesp, CNPq and Facepe for financial support and to the Chemistry Department (Unesp/Bauru-SP) for laboratorial facilities in some stages of the work. The authors thank Dr Rajendra Mohan Srivastava and Dr Walter Mendes Azevedo for critical comments.

References

- [1] P. Jobi, *The Coffee Produced Throughout the World*, Le Hane, France, 1982.
- [2] W.J. Rogers, S. Michaux, M. Bastin, P. Bucheli, *Plant Sci.* 149 (1999) 115.
- [3] M.B. Blanc, G.E. Davis, J.M. Parchet, R. Viani, *J. Agric. Food Chem.* 37 (1989) 926.
- [4] J. Prodolliet, E. Bugner, M. Feinberg, *J. A.O.A.C. Int.* 79 (6) (1996) 1400.
- [5] Q. Hu, T.S. Zhou, L. Zhang, Y.Z. Fang, *Analyst* 126 (3) (2001) 298.
- [6] L.C. Trugo, in: R.J., R. Macrae (Eds.), *Coffee Chemistry*, vol. 1, Elsevier Applied Science, Barking, 1985, p. 83.
- [7] R. Matsukura, L.M. Aleixo, O.E.S. Godinho, G. Oliveira Neto, *Anal. Chim. Acta* 280 (1993) 49.
- [8] J.K. Foreman, P.B. Stockwell, *Automatic Chemical Analysis*, Chichester, Horwood, 1975.
- [9] J. Ruzicka, E.H. Hansen, *Flow Injection Analysis—Chemical Analysis: A Series on Analytical Chemistry and Its Applications*, John Wiley & Sons, New York, 1988.
- [10] P.B. Stockwell, *J. Autom. Chem.* 12 (1990) 95.
- [11] M. Trojanowicz, *Flow Injection Analysis: Instrumentation and Applications*, Book News, Inc, Portland, 2000.
- [12] L. Gorton, E. Csöregi, E. Dominguez, J. Emnéus, G. Jönsson-Pettersson, G. Marko-Varga, B. Persson, *Anal. Chim. Acta* 250 (1991) 203.
- [13] R.D. Schmid (Ed.), *Flow Injection Analysis (FIA) Based on Enzymes or Antibodies*, VCH, New York, 1991.
- [14] J. Wang, *Analytical Electrochemistry*, VCH, New York, 1994.
- [15] J.M. Calatayud, *Flow Injection Analysis of Pharmaceuticals: Automation in the Laboratory*, Taylor & Francis, London, 1996.
- [16] A. Pizzariello, M. Stredanský, S. Stredanská, S. Miertus, *Talanta* 54 (2001) 763.
- [17] L. Rasooly, A. Rasooly, *Int. J. Food Microb.* 49 (1999) 119.
- [18] K.R. Rogers, *Trends Biotech.* 19 (2001) 198.
- [19] I.E. Tothill, *Comp. Electr. Agric.* 30 (2001) 205.
- [20] S. Zhang, G. Wright, Y. Yang, *Bios. Bioelectr.* 15 (2000) 273.
- [21] A.O. Scott, *Biosensors for food analysis*, C.H.I.P.S., New York, 2000.
- [22] J. Kulys, W. Schuhmann, H.L. Schmidt, *Anal. Lett.* 25 (1992) 1011.
- [23] J. Kulys, L. Wang, A. Maksimoviene, *Anal. Chim. Acta* 274 (1993) 53.
- [24] U. Spohn, D. Narasiah, L. Gorton, *Electroanalysis* 8 (1996) 507.
- [25] B. Serra, A.J. Reviejo, C. Parrado, J.M. Pingarron, *Biosens. Bioelectron.* 14 (1999) 505.
- [26] K. Itaya, N. Shoji, I. Uchida, *J. Am. Chem. Soc.* 106 (1984) 3423.
- [27] A.A. Karyakin, *Electroanalysis* 13 (2001) 813.
- [28] A. Dostal, B. Meyer, F. Scholz, U. Schroder, A.M. Bond, F. Marken, S.J. Shaw, *J. Phys. Chem.* 99 (1995) 2096.
- [29] I.L. de Mattos, L. Gorton, *Quim. Nova* 24 (2001) 200.
- [30] A.A. Karyakin, E.E. Karyakina, L. Gorton, *Electrochem. Commun.* 1 (1999) 78.
- [31] I.L. de Mattos, L. Gorton, T. Ruzgas, A.A. Karyakin, *Anal. Sci.* 16 (2000) 795.
- [32] D.W. Pan, J.H. Chen, L.H. Nie, W.Y. Tao, S.Z. Yao, *Electrochim. Acta* 49 (5) (2004) 795.
- [33] I.L. de Mattos, L. Gorton, A. Malinauskas, A.A. Karyakin, *Talanta* 16 (2000) 795.
- [34] A.A. Karyakin, E.E. Karyakina, L. Gorton, *Anal. Chem.* 72 (2000) 1720.
- [35] R. Garjonyte, Y. Yigzaw, R. Meskys, A. Malinauskas, L. Gorton, *Sens. Actuat. B79* (2001) 33.
- [36] J.P. Li, T.Z. Peng, *Electroanalysis* 15 (12) (2003) 1031.
- [37] I.L. de Mattos, L.V. Lukachova, L. Gorton, T. Laurell, A.A. Karyakin, *Talanta* 54 (2001) 963.
- [38] I.L. de Mattos, L. Gorton, T. Ruzgas, *Bios Bioelectr.* 18 (2003) 193.
- [39] <http://pb.merck.de>.
- [40] S. Williams (Ed.), *Official Methods of Analysis of the Association of Official Analytical, 14th ed.*, Chemists, Association of Official Analytical Chemists, Washington, DC, 1984.