Analytical Methods

Rapid and selective determination of hydrogen peroxide residues in milk by batch injection analysis with amperometric detection

Rodrigo A.B. Silva, Rodrigo H.O. Montes, Eduardo M. Richter, Rodrigo A.A. Munoz *

Instituto de Química, Universidade Federal de Uberlândia, Av. Jodo Naves de Ávila, 2121Uberlândia, MG, Brazil

ARTICLE INFO

Article history:
Received 30 August 2011
Received in revised form 30 October 2011
Accepted 3 January 2012
Available online 9 January 2012

Keywords:
Batch-injection analysis
Hydrogen peroxide
Milk
Prussian-blue
Low-potential detection

ABSTRACT

We report a highly rapid, precise, selective and sensitive analytical method for the determination of hydrogen peroxide in milk using a batch-injection analysis (BIA) with amperometric detection at a Prussian-blue bulk modified graphite-composite electrode. An electronic micropipette injected 100 μL aliquots of 10-fold diluted samples (high and low-fat milk) directly onto the modified electrode immersed in the BIA cell. The analytical features of our proposed method include low RSD between injections (0.76%, n = 9), low detection limit (10 μmol L⁻¹), elevated analytical frequency (up to 80 h⁻¹) and satisfactory recovery values for spiked samples. A fresh and highly reproductive electrode surface can be easily obtained by simple mechanical polishing (RSD = 1.6%, n = 5). The storage stability of the PB-modified graphite-composite surpassed 1 year keeping equivalent performance as initially presented. The association of BIA with an improved amperometric detector provides great promise for routine monitoring of hydrogen peroxide in milk and other beverages.

© 2012 Elsevier Ltd. Open access under the Elsevier OA license.

1. Introduction

Hydrogen peroxide (H₂O₂) is widely used in food industry for sterilization of equipment related to mixing, transporting, bottling and packing. During sterilization, H₂O₂ may become incorporated into the surface of bottles and packages and thereafter an additional process is required to decompose or remove the residual H₂O₂ (Hsu, Chang, & Kuo, 2008). Additionally, H₂O₂ has been widely used for preservation of raw milk due to its bactericidal properties (Haddadin, Ibrahim, & Robinson, 1996). However, excess of H₂O₂ can bring deleterious effects on the nutritional value of milk such as the degradation of folic acid, which is an essential vitamin to human body (Tahe & Lashmaiah, 1992). Moreover, the ingestion of H₂O₂ at high levels can cause severe gastrointestinal problems. The addition of H₂O₂ in milk at any concentration is not allowed in Brazil in such a way that the product containing H₂O₂ is considered adulterated (Brasil, 2002). In September 2007 Brazilian producers adulterated pure milk sold to manufacturing companies and end users by adding H₂O₂ due to its low-cost in order to increase the shelf-life of the product (Paixao & Bertotti, 2009). Nevertheless, the presence of adulterants (including H₂O₂) in Brazilian ultra-high temperature (UHT) processed milks from different regions of the country was recently reported (Souza et al., 2011).

There are a few analytical methods for determining H₂O₂ in milk. A highly sensitive fluorimetric method was described for the determination of H₂O₂ in milk (Abbas, Luo, Zou, & Tang, 2010). A limitation of this method for routine applications is its prior sample preparation step, which involved a 5-min reaction before measurements. Electrochemical biosensors were also presented for milk analysis (Alpat, Alpat, Dursun, & Telefoncu, 2010; Campusano, Pedrero, & Pingarron, 2005; Liang & Mu, 2008). Such analytical systems are often expensive and presents short lifetime limited to special conditions of preservation of the enzyme incorporated into the biosensor. An enzyme-free electrochemical sensor was reported for the determination of H₂O₂ based on a Prussian-blue modified electrode coated with a Nafion polymer layer (Ping et al., 2010). Due to the high selectivity provided by Prussian-blue (PB)-modified electrodes towards H₂O₂ detection, such electrochemical sensors have been denominated as 'artificial peroxidase' (Karyakin & Karyakina, 1999; Lu, Cagan, Munoz, Tangkuaram, & Wang, 2006; Munoz, Lu, Cagan, & Wang, 2007). Nevertheless, the Nafion coating was necessary in order to eliminate interferences from the sample matrix which can affect the electrode response and consequently disturbs the method accuracy (Ping et al., 2010). High-performance analytical methods are mandatory in routine laboratories of food analysis. Batch injection analysis (BIA) is a promising technique to attend such demands due to its improvements in versatility, reproducibility, analytical frequency, portability and sample size. Its combination with electrochemical detectors provides additional advantages of electrochemical sensors such as selectivity, sensitivity and fast response to the
development of analytical methods (Quintino & Angnes, 2004). An electronic micropipette injects precise sample plugs (at a programmable speed) directly onto the working electrode surface, which is immersed in a large-volume blank solution, and a fast electrochemical response proportional to the analyte concentration is registered.

In this work, we report a novel application of BIA with amperometric detection for the highly rapid, selective and sensitive method for the determination of H$_2$O$_2$ in high and low-fat milk samples. A PB-modified graphite-composite electrode provided fast and reproducible amperometric responses to H$_2$O$_2$ in 10-fold diluted samples.

2. Experimental section

2.1. Chemicals

Solutions were prepared with deionized water (Direct-Q3, Millipore, Bedford, MA, USA) with a resistivity no less than 18.2 MΩ-cm. Araldite® epoxy adhesive from Brascola (Joinville, Brazil), cyclohexanone and hydrogen peroxide from Vetec (Rio de Janeiro, Brazil), graphite (Ø: 1–2 μm) from Sigma–Aldrich (Milwaukee, WI, USA), iron(III) chloride, potassium monohydrogen phosphate, potassium dihydrogen-phosphate, and potassium ferricyanide from Proquímicos (Rio de Janeiro, Brazil) were of analytical grade and used without any further purification. Phosphate buffer solution (pH = 7.2, 0.05 mol L$^{-1}$ K$_2$HPO$_4$/KH$_2$PO$_4$) containing 0.1 mol L$^{-1}$ KCl was used as supporting electrolyte. Stock solutions of hydrogen peroxide were freshly prepared just before experiments.

2.2. Apparatus

All electrochemical measurements were performed using a μ-Autolab Type III (Eco Chemie, Utrecht, Netherlands) controlled by GPES4.9.007 software (General Purpose Electrochemical System). Injections of standard solutions or samples were conducted using an Eppendorf electronic micropipette (Multipette® stream), which permits injections from 10 to 1000 μL (using a 1 mL Combittip®) at a programmable dispensing rate (from 28 to 330 μL s$^{-1}$).

The working, counter, and reference electrodes were respectively, Prussian-blue (PB)-modified graphite-composite electrode, platinum wire, and Ag/AgCl/saturated KCl.

Amperometric measurements were performed using a homemade electrochemical batch-injection cell adapted from a previous report (Tormin, Gimenes, Richter, & Munoz, 2011). Fig. 1 illustrates a schematic diagram of the batch-injection cell that consists of a 180 mL glass cylinder (internal diameter = 7 cm) and two polyethylene covers, which were firmly fitted on the top and bottom of the cylinder. On the top, the polyethylene cover contained three holes for the counter and reference electrodes and the micropipette tip. The distance between the electronic micropipette tip (external diameter = 6.6 mm) and the center of the working electrode (positioned oppositely to the micropipette tip) was adjusted around 2 mm distant in a wall-jet configuration.

2.3. Preparation of the PB-modified graphite composite

The modification of graphite powder with PB particles used in sensors for H$_2$O$_2$, was accomplished adding 2.0 g graphite under stirring (10 min) to an equimolar mixture (40 mL) of iron(III) chloride and potassium ferricyanide (0.1 mol L$^{-1}$) containing 10 mmol L$^{-1}$ HCl. Thus, the graphite was filtered and kept at 100 °C for 1.5 h to the activation of PB particles adsorbed on graphite (Moscone, D’Ottavi, Compagnone, & Palleschi, 2011).

Fig. 1. Schematic diagram of the batch injection cell containing the three-electrode system.

PB-modified graphite was added to the pure graphite in the mass proportion of 30/70 of PB-modified graphite/pure graphite (Silva, Montes, Munoz, & Richter, 2011). This PB-modified graphite was mixed with Araldite® epoxy adhesive and cyclohexanone and kept under stirring during 24 h in order to obtain a homogeneous graphite-composite fluid (Silva, Rabelo, Bottecchia, Munoz, & Richter, 2010). The fluid was inserted into a polyamide tube (Ø = 7.2 mm) at which a copper wire was previously set (electrical contact). The time of cure was 24 h at room temperature. After that, the electrode was polished with 400 and 1200 grit sand paper in the presence of water. Before the amperometric measurements of H$_2$O$_2$, the composite electrode was submitted to a cyclic voltammetry experiment in the range of −0.1 and +0.35 V in supporting electrolyte at 20 mV s$^{-1}$ for 30 cycles.

3. Results and discussion

In a preliminary study, the composition of the PB-modified graphite-composite electrode was investigated based on the amperometric response of H$_2$O$_2$ and studies of electrical resistance (Silva et al., 2011). It was found that a composite containing 30% (wt.) of PB-modified graphite and 70% (wt.) of pure graphite provided the highest sensitivity for H$_2$O$_2$ determination. Composites containing higher amounts of PB-modified graphite (>30%) showed high ohmic drop effects, because PB particles presented lower conductivity than graphite particles (Silva et al., 2011). In this work, the working electrode was produced with 30% (wt.) of PB-modified graphite (70% wt. of pure graphite).

In order to obtain the highest selectivity, the amperometric recordings were carried out under the application of 0.0 V. The
low-potential detection of H$_2$O$_2$ eliminated interferences from electroactive substances which may be found in milk samples. The selection of phosphate buffer (pH 7.2) and 0.1 mol L$^{-1}$ KCl as electrolyte was related to the best performance of the PB-modified working electrode (Karyakin, Karyakina, & Gorton, 1999).

After selecting the composition of the working electrode and electrolyte, the PB-modified graphite-composite electrode was inserted into the BIA cell for fast and precise amperometric recordings. BIA parameters such as speed of the programmable pipette and injection volume were evaluated. A dispensing rate of 100 µL s$^{-1}$ provided the highest current response and then this parameter was kept constant. Fig. 2 shows the variation of current response of the injected volume of 100 µmol L$^{-1}$ H$_2$O$_2$ standard solutions and the respective variation of analytical frequency.

The current peak increased significantly with increasing injection volume, from 25 to 100 µL, and continued to increase slightly from 100 to 200 µL. As expected, the analytical frequency (number of sample injections per hour) decreased with higher injected volumes almost linearly. Therefore, the optimal injection volume for the BIA system was 100 µL, which provided high analytical frequency (~80 h$^{-1}$) keeping a high amperometric signal for H$_2$O$_2$.

Fig. 3 presents amperometric responses recorded at 0.0 V for injections of 100 µL (in duplicate) of solutions containing increasing and decreasing concentrations of H$_2$O$_2$ (a–f: 100–600 µmol L$^{-1}$) and the respective calibration curves.

The calibration curves (inset of Fig. 3) were found to be linear ($R = 0.999$) with similar slope values (−34.1 and −34.7 µA mM$^{-1}$ for increasing and decreasing concentrations of H$_2$O$_2$, respectively), which confirmed the absence of memory effect. The amperometric response of the modified electrode was stable and linear over a wide concentration range (0.1–4.0 mmol L$^{-1}$) in the BIA system. A repeatability experiment was obtained from successive injections of 100 µmol L$^{-1}$ H$_2$O$_2$ and the relative standard deviation (RSD) value was 0.85% ($n = 10$). The detection limit under optimized conditions was estimated in 10 µmol L$^{-1}$ (with a signal-to-noise ratio of S/N = 3).

The proposed BIA-amperometric method was applied for milk analysis. The effect of sample dilution on the amperometric detection of H$_2$O$_2$ was evaluated. Low and high-fat milk samples were diluted in electrolyte before injection at different volumetric ratios (50, 10, 5 and 2-fold dilution). If samples were 2 or 5-fold diluted, low recovery values (<70%) were obtained for samples spiked with 0.88 and 2.35 mmol L$^{-1}$ H$_2$O$_2$. Probably, this effect was observed mainly because of the high levels of potassium chloride ions (~1 g L$^{-1}$) in milk samples, which would affect the ion exchange equilibrium on the surface of PB (Karyakin et al., 1999). So, the sample matrix clearly affected the amperometric recordings, thus samples were 10-fold diluted before BIA injections.

Souza et al. (2011) reported the presence of H$_2$O$_2$ in more than 60% of the analyzed Brazilian UHT milk samples from the main producer areas of the country. The identification of H$_2$O$_2$ involved a qualitative colorimetric assay based on the oxidation of guaiacol (colorless) by H$_2$O$_2$ catalyzed by peroxidase (typical protein present in UHT-processed milk). This colorimetric method is in accordance with the Brazilian official protocol for milk analysis (Brasil, 2006). In this way, six Brazilian UHT milk samples processed in industrial plants located in regions selected in the work of Souza et al. (2011) were analyzed (four samples from the Southeast region and two samples from the Mid-west region). Hydrogen peroxide was not detected in all samples using the proposed BIA-amperometric method. In order to evaluate the accuracy of the proposed BIA method for milk analysis, all samples were spiked with 300 and 800 mg L$^{-1}$ H$_2$O$_2$ (8.8 and 23.5 mmol L$^{-1}$) and analyzed after a 10-fold dilution using a calibration curve from 0.34 to 3.40 mmol L$^{-1}$ H$_2$O$_2$. Table 1 presents the respective recovery values. Recovery values from 85% to 107% for the analysis of low and high-fat milk samples were obtained, which can be considered acceptable for such a complex sample.

Fig. 4 depicts repeatability data obtained from successive injections ($n = 9$) of a 10-fold diluted sample spiked with 300 mg L$^{-1}$ H$_2$O$_2$ (final concentration of H$_2$O$_2$ was 30 mg L$^{-1}$). These results indicated that there was no interference of sample matrix on continuous amperometric measurements. The RSD value was 0.76%.

**Table 1**

<table>
<thead>
<tr>
<th>Samples Added (mg L$^{-1}$)</th>
<th>Recovery (%)</th>
<th>Added (mg L$^{-1}$)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A 300</td>
<td>96 ± 2</td>
<td>800</td>
<td>88 ± 2</td>
</tr>
<tr>
<td>2A 300</td>
<td>92 ± 2</td>
<td>800</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>3A 300</td>
<td>101 ± 2</td>
<td>800</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>4A 300</td>
<td>107 ± 4</td>
<td>800</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>5A 300</td>
<td>91 ± 3</td>
<td>800</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>6A 300</td>
<td>85 ± 4</td>
<td>800</td>
<td>86 ± 4</td>
</tr>
</tbody>
</table>

*Low-fat milk.

*High-fat milk.
which was similar to repeatability RSD value obtained in standard solutions (0.85%).

The continuous amperometric monitoring by PB-modified electrodes can be affected not only by sample matrix but also by losses of electrocatalyst. Previous report has demonstrated that PB-modified electrodes obtained by electrodeposition underwent such an operational instability, which limited the sensor to 3 h in flow-injection-analysis systems (Karyakin & Karyakina, 1999). Polymetric coatings become necessary to overcome such a drawback and even to eliminate interferences from sample matrix on electrochemical response (Ping et al., 2010). The proposed PB-modified graphite-composite electrode was highly stable as Figs. 3 and 4 have shown and did not require any additional coating. A simple mechanical polishing provided a fresh electrode surface with elevated reproducibility of the amperometric response (RSD = 1.6%, n = 5). Moreover, the storage stability of the PB-modified graphite-composite surpassed 1 year keeping equivalent performance as initially presented. The modified electrode which presented an initial slope value of $-34 \mu A L mmol^{-1} (R = 0.999)$ (calibration curve presented in Fig. 3) was re-used 1 year later to perform a calibration curve for H$_2$O$_2$ under the same experimental conditions and the new slope value was $-30 \mu A L mmol^{-1} (R = 0.999)$. These features were reached because the composite acted as a Prussian blue reservoir. This performance was similar to PB bulk modified screen-printed electrodes (Ricci et al., 2003).

The proposed amperometric method is highly advantageous over the Brazilian official protocol based on a qualitative colorimetric assay (Brasil, 2006) because it provided the quantitative determination of H$_2$O$_2$ in milk with improved selectivity, sensitivity and accuracy derived from the use of the PB-modified electrode. The BIA system provided fast and precise determinations which results in higher analytical frequency compared with the official protocol. Amperometric measurements require commercially-available portable potentiostats and BIA system can also be easily adapted for on-site analysis (Silva, Gimenes, Tormin, Munoz, & Richter, 2011). The PB-modified graphite-composite electrode presents high storage stability and can be re-used after electrode polishing. Therefore, the proposed analytical method is cost-effective and can be used for routine and on-site (if required) analysis.

4. Conclusions

We have demonstrated the application of BIA with amperometric detection for the highly selective and sensitive determination of H$_2$O$_2$ in milk using a PB-modified graphite-composite electrode. Low and high-fat milk samples only required a 10-fold dilution in electrolyte before analysis. The proposed method is highly precise (RSD = 0.76%, n = 9), accurate (confirmed by recovery tests), and presents elevated analytical frequency (80 h$^{-1}$) employing a 100 µL sample aliquot. A fresh and reproductive electrode surface can be easily obtained by simple mechanical polishing and the storage stability of the PB-modified graphite-composite surpassed 1 year. The proposed BIA-amperometric method is promising for routine monitoring of hydrogen peroxide in milk and other beverages and can be easily applied for on-site analysis.

Acknowledgements

The authors are grateful to CNPq (478081/2010-3 and 305227/2010-6), FAPEMIG (CEX-APQ-01856-10) and CAPES for financial support. R.A.B. da Silva thanks CNPq for the doctoral scholarship Granted (141972/2009-2).

References


