Amperometric Determination of Hydrogen Peroxide in Whitening Gels Using Boron-doped Diamond Electrode

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The aim of this work was to develop an electrochemical cell and a methodology based on an amperometric determination of hydrogen peroxide in whitening gel samples under a boron-doped diamond electrode using flow injection analysis. Different parameters were evaluated to obtain the best conditions of analysis: among them, the flow of electrolyte at 2.8 mL min⁻¹, the loop sampling 175 μL (28.5 cm), an analytical length of 159 μL (25 cm) and an applied potential of +0.60 V vs. Ag/AgCl(sat). The proposed method was suitable in terms of precision of results (RSD <10%); the accuracy was confirmed in the analysis of the gels through addition and recovery studies with results between 74 and 107%. The method was then applied to the analysis of tooth-whitening gel samples, acquired in different cities of the region. Regarding the results, a medium concentration value of 2.39% (w/w) was observed.

Keywords Whitening gel, hydrogen peroxide, boron-doped diamond electrode, amperometry

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Introduction

The dental darkening process is often, but not exclusively, related to eating habits. Other factors, such as natural darkening, genetic predisposition, dental trauma, metabolic disorders, and smoking are common causes of darkening. There are several methods to work around tooth darkening; among them we can highlight scraping, the use of whitening toothpastes, and the use of whitening gels either at home or at a dental clinic.

The home bleaching technique was developed and applied for the first time by Haywood and Heymann in 1989 and it consists of applying a gel based on carbamide peroxide at 10% (w/w) on a tray for up to 8 h per night (overnight). The original technique has undergone a few changes, and one of them is to include gels based on hydrogen peroxide with concentrations ranging from 3 to 7.5% (w/w) as well as a gel containing carbamide peroxide with concentrations ranging from 16 to 22% (w/w).

Regardless of the technique, there are important side effects, such as the occurrence of tooth sensitivity and gum irritation, which are usually temporary. Another associated problem is the possible loss of tooth structure, since after a certain time, the pigments are not whitened any more, and the agent starts to oxidize and denature proteins in the enamel matrix.

Stains usually contain molecules with long double-bonded chains, phenyl and carbonyl groups, which commonly have colors in the visible region due to their absorption spectrum. These double bonds are oxidised by OH and ·OOH radicals, generating smaller products, which generally do not absorb radiation in the visible region and are therefore more clear or colorless, and make the teeth appearance more beautiful.

There are several methods for the determination of peroxide, as described by Mattos et al. in a review of the subject; among them we highlight volumetry, spectrophotometry, chemiluminescence, fluorometry, chromatography and electrochemistry. On two occasions, Matos et al. monitored the concentration of peroxide in rainwater using peroxidase immobilized on ion-exchange resin, with UV and amperometric detection. Among the papers involving electroanalytical techniques, the use of modified electrodes and biosensors could be highlighted. Several articles describe the use of Prussian blue film in peroxide detection.

Few reports describe the use of unmodified carbon electrodes. In this context the use of boron-doped diamond (BDD) electrodes stands out due to their ability to detect low levels of peroxide, even without surface modification. The development of BDD electrodes has been possible thanks to the development of techniques for the growth of diamond films, which are only possible with the advent of the latest technology. Chemical vapor deposition, CVD, is the most popular technique for depositing BDD films at low pressures. The doping of diamond becomes necessary for analytical applications, since pure diamond does not show relevant electrochemical properties. A BDD electrode has unique characteristics; the main and perhaps the most exploited one is its potential window of approximately 3.0 V, high electroactivity in water, and production of a low background current when compared to a glassy carbon electrode, or any other carbon electrode.

During the growth of a BDD film, hydrogen gas is used in excess in the reactor, and for this reason, the newly manufactured electrodes have hydrogen termination bonds. The change of the surface leads to considerable changes in the electrochemical

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behavior of the BDD electrode. The pretreatment of BDD electrodes is a topic that has culminated in the development of many works; this is because the results of each treatment are definitive for the success of an analytical determination. Generally, the electrochemical properties of the DDB electrodes are very sensitive to the surface termination. The electrochemical activation procedures or pretreatment are made under a controlled potential or current, for both the anodic region and for cathodic conditions. Generally drastic conditions, such as a potential of approximately -3.0 V and current densities of the order of 1 A cm⁻² are applied.

The aim of this work was the development of an electrochemical cell, adapting the plate with a BDD film, and the development of an analytical methodology for the determination of hydrogen peroxide in tooth-whitening gel samples using flow injection analysis (FIA) and amperometric detection with a BDD electrode.

Experimental

Reagents and chemicals

Solutions and samples were prepared with deionized water obtained from a Milli-Q water purification system. All reagents were purchased from Vetec (Rio de Janeiro, RJ, Brazil) unless it is stated otherwise. Monobasic and dibasic potassium phosphate (KH₂PO₄; K₂HPO₄) were used to prepare buffer solutions. Adjustment of pH, when required, was made by using an alkaline solution prepared from potassium hydroxide. Hydrogen peroxide solutions were prepared daily, before the experiments, by diluting the 30% (w/w) stock solution appropriately. Peroxidase EC 1.11.1.7-Horseradish 115 U mg⁻¹ was used to fill the reference electrode. A saturated KCl solution was used to fill the reference electrode.

As reagents, 4-aminantipyrine from Sigma-Aldrich (St. Louis, MO, USA), peroxidase EC 1.11.1.7-Horseradish 115 U mg⁻¹ from Seppim (France) and phenol from Vetec (Rio de Janeiro, RJ, Brazil) were used for the spectrophotometric determinations at 510 nm.

Samples

Eight samples of tooth whitening gels were selected; four of them are available in industrialized form and four others acquired at different compounding pharmacies. All samples were stored in a refrigerator at 5°C. For hydrogen peroxide analysis, approximately 110 mg of whitening gels were weighed and diluted with phosphate buffer solution (PBS) in a 10.00-mL volumetric flask. Then, an aliquot of 20 μL of the sample was diluted to 10.00 mL and injected into the FIA system.

Electrodes, electrochemical cell and instrumentation

Electrochemical measurements were performed with a μAutoLab Type III potentiostat (EcoChemie, Utrecht, Netherlands). Data acquisition and treatment was made with NOVA 1.8 software. Spectrophotometric measurements were made with an Analyser 850M spectrophotometer.

The FIA system consisted of an acrylic valve, a peristaltic pump (ISMATEC, Germany) and a lab-made electrochemical cell. The electrochemical flow cell (Fig. 1) was constructed in PTFE to work with up to 100 μL (internal volume) of solution. The developed cell had a copper plate as an adapter, to fix the BDD electrode (6000 – 8000 ppm; Adamant, Switzerland) to the cell and to establish electrical contact. A/uni00A0 rubber O-ring limited the exposed area of electrode, defining an exposed area of approximately 0.28 cm². The solution enters the cell upside and passes through the needle type counter electrode. The reference electrode was a miniaturized Ag/AgCl₀, electrode constructed in our laboratory and a platinum needle was used as an auxiliary electrode.

Procedures

BDD electrode activation. Cathodic activation was used for this work. A potential of -2.9 V was applied for 340 s in a cell containing 3 mol L⁻¹ H₂SO₄, once a day, before the beginning of the experiments. After the experiments the cell was cleaned with deionized water and filled with 0.5 mol L⁻¹ H₂SO₄.

The electrochemical cleaning of the electrode was performed either once a week or after a loss of sensitivity, by using a long anodic activation: A current of 0.1 A was applied for 2000 s, then the electrode was treated with cathodic activation as described previously.

Electrochemical study. A comparison between gold, platinum, glassy carbon and BDD was performed by cyclic voltammetry, to investigate the sensibility and response of BDD. The experiment was done with potentials ranging from 0.0 to 1.0 V with a scan rate of 50 mV s⁻¹ using 1 and 2 mmol L⁻¹ standard H₂O₂ solutions and phosphate buffer solution pH 7.00.

As carbamide peroxide is an adduct of urea and hydrogen peroxide, urea interference was investigated using the developed PTFE cell containing the supporting electrolyte, PBS 0.1 mol L⁻¹ (pH 7.00). A cyclic voltammogram was obtained by varying the potential from 0.0 to 1.0 V, with a scan rate of 50 mV s⁻¹. Possible interferences were verified by adding different concentrations of urea (1, 2 and 3 mmol L⁻¹) and measuring the corresponding signal at +0.60 V.

Study of flow injection analysis parameters. The influence of flow was conducted by monitoring the signals obtained by successive injections of 150 μL of peroxide 16 μmol L⁻¹ with flows of supporting electrolyte ranging from 1.35 to 2.80 mL min⁻¹.
The evaluation of sample injection loop was performed by monitoring the signals obtained by successive injections of peroxide 16 μmol L⁻¹ in phosphate buffer at a flow rate of 2.80 mL min⁻¹, analytical path of 25 cm and sampling loop volumes from 50 to 250 μL (8 to 34.5 cm).

Quantification of H₂O₂ in whitening gel samples. For analysis, approximately 110 mg of gel was weighed and diluted in a 10.00-mL volumetric flask. Then, an aliquot of 20 μL from this solution was diluted to 10.00 mL and injected into the FIA system.

The accuracy was verified using addition and recovery tests. In this way, two levels of spiked samples were prepared. After that, 10 μL of the spiked solutions was diluted to 10 mL, and injected into the FIA system. The final concentrations of peroxide in the spiked solutions were 48 and 68 μmol L⁻¹ for the low level and high levels, respectively.

Spectrophotometric determination. The procedure for the spectrophotometric determination involved the addition of phenol, 4-aminoantipyrine and peroxidase into a tube. Then the mixture was added to the sample containing hydrogen peroxide. The curve was constructed by adding peroxide standards in the range 9 to 95 μmol L⁻¹. The phenol, 4-aminoantipyrine and peroxidase concentrations were 1.27 mmol L⁻¹, 0.2 mmol L⁻¹ and 1.4 U mL⁻¹, respectively.

Results and Discussion

Electrochemical study

In order to evaluate the electrochemical behavior of peroxide in different working electrodes available in the laboratory, cyclic voltammetry experiments were performed using concentrations of 1 and 2 mmol L⁻¹ for the following working electrodes: boron doped diamond, glassy carbon, gold and platinum. The analysis of the cyclic voltammograms allowed confirmation of a signal from the oxidation of hydrogen peroxide at a potential close to +0.60 V vs. Ag/AgCl(sat) at the BDD, platinum and gold electrodes, while no significant current was observed for the glassy carbon electrode. In order to evaluate the capability of the BDD electrode compared to others, the currents in the voltammograms were corrected by the active area of electrodes. The comparison of the current density indicated a higher sensitivity of the BDD, since the magnitude of the peak current for the BDD (0.23 mA cm⁻²) was higher (0.07 mA cm⁻² for Au and 0.13 mA cm⁻² for Pt).

As it was mentioned before, tooth-whitening gels can contain carbamide peroxide. Therefore, to investigate the possible response of urea in the determination of peroxide, an exploratory study was conducted using cyclic voltammetry. The study demonstrated that there was no significant interference by urea in the determination of H₂O₂.

Optimization of the flow system

An important parameter involved in FIA systems is the flow condition, since it is related to sample dispersion and consequently to the quality and reliability of the analysis in general. Figure 2A shows the effect of flow on the amperometric response. Such a study was conducted by monitoring the signal obtained from successive injections of 150 μL of 16 μmol L⁻¹ hydrogen peroxide, with flows varying from 1.35 to 2.8 mL min⁻¹. It is possible to observe that the transient current increased with the flow up to 2.4 mL min⁻¹ after which the increments became smaller and insignificant. The bomb limitations allowed the study of flows up to 2.8 mL min⁻¹.

Another point worth mentioning is the evaluation of the measures of width at half-height of the signals. The peaks for low flows are wider than those at high flow rates, and the current signals are greater with increasing flow rate, which occurs because the dispersion is increased in low electrolyte flow. In this way, the value of maximum flow studied (2.80 mL min⁻¹) was established as the optimum condition for the analyses for the determination of peroxide.

The sensitivity of a method of flow injection analysis is closely related to the amount of injected sample; also the residence time is affected when changing the volume injected, which affects directly the analytical frequency. Dispersion also plays an important role in this case, as small volumes of sample that generate scattering regions may extend over the whole area of the sample and exert a great effect on dilution and consequently on the response of the system. It is therefore important to consider this variable in flow injection analysis systems.

Figure 2B shows the effect of the volume of the sample loop on the amperometric response. This study was conducted by monitoring the signals obtained by successive injections of 16 μmol L⁻¹ peroxide with a flow rate of 2.80 mL min⁻¹ and sample loops ranging from 50 to 250 μL (8.0 to 34.5 cm).

There was an increase in the transient signals up to a volume...
of 175 μL, after which there was no significant current gain, only a contribution to the widening of the signal; so for the analyses that followed, a volume of 175 μL (equivalent to 28.5 cm-loop) was used.

**Determination of H₂O₂ in whitening gel by flow-injection analysis**

After establishing the analytical conditions for the analyses, and evaluating the electrochemical behavior of urea, it was possible to apply the method to the determination of peroxide in tooth-whitening gel.

To quantify the levels of peroxide in whitening gel samples, the flow injection analysis was performed according to the optimized conditions described above.

Figure 3 shows hydrogen peroxide standard current signals (ranging from 9.77 to 95.9 μmol L⁻¹) and their respective RSD values and fortified samples. The detection (DL) and quantification (QL) limits calculated, 0.05, 6 = 0.3 < t distribution,0.05,6 = 2.36). The RSD values were below 6%, indicating good precision for the evaluated concentration level. Despite all the precautions taken to control the room temperature, some low values of recovery (between 74 and 77%) were noticed, which indicated a possible loss of analyte by thermal decomposition to form O₂ and H₂O. The others could be considered acceptable within the Horwitz coefficient of variance value of 22%, calculated from the quantification limit of the analytical curve. This value estimates the coefficient of variation for the concentration level studied.

**Table 1 Concentration and recovery values encountered for whitening gel samples**

<table>
<thead>
<tr>
<th>Origin</th>
<th>[H₂O₂], % (w/w)</th>
<th>RSD, %</th>
<th>Recovery, % (low level)</th>
<th>Recovery, % (high level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacy 1</td>
<td>2.87</td>
<td>2.25</td>
<td>83</td>
<td>101</td>
</tr>
<tr>
<td>Pharmacy 2</td>
<td>1.24</td>
<td>5.96</td>
<td>93</td>
<td>107</td>
</tr>
<tr>
<td>Pharmacy 3</td>
<td>1.29</td>
<td>3.66</td>
<td>81</td>
<td>79</td>
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<tr>
<td>Pharmacy 4</td>
<td>3.72</td>
<td>2.64</td>
<td>91</td>
<td>74</td>
</tr>
<tr>
<td>Commercial 1</td>
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<td>2.27</td>
<td>85</td>
<td>75</td>
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<tr>
<td>Commercial 2</td>
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<td>97</td>
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<tr>
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<td>89</td>
</tr>
<tr>
<td>Commercial 4</td>
<td>1.94</td>
<td>0.10</td>
<td>107</td>
<td>89</td>
</tr>
</tbody>
</table>

**Spectrophotometric determination**

In order to confirm the accuracy of the proposed methodology, a comparison with the existing enzymatic spectrophotometric method was performed. The enzymatic method is based on the reaction of H₂O₂ with 4-aminophenol in the presence of phenol, the peroxidase enzyme catalyzing the reaction for the formation of antipiril-quinonimine.

Table 2 shows the different hydrogen peroxide concentration values of the samples and their respective RSD values, obtained via amperometric and spectrophotometric methods. In order to compare the results, a paired t-test was applied. The obtained value showed no evidence of systematic differences between the two methods (t calculated,0.05,6 = 0.3 < t distribution,0.05,6 = 2.36). The RSD values obtained could be considered satisfactory since they are less than 10%, and with few variations among different samples. It can also be seen that there is no loss of precision when changing from the spectrophotometric method to the amperometric; however, in the amperometric method the enzyme was not used, reducing the cost of analysis.
Conclusions

The BDD electrodes used in this work proved excellent in determining hydrogen peroxide both at stationary conditions and associated with flow injection analysis. The electrode activation procedure was capable of conferring high reproducibility and sensitivity, since concentrations as low as 1.03 μmol L⁻¹ could be detected without requiring surface modification with metals or enzymes. The flow cell, developed to incorporate the plate with the boron doped diamond film, showed good response, without causing turbulence in the system. The materials used to build it proved to be convenient and cheap; another point is that materials such as steel, polyether ether ketone (PEEK), and others could also be used, depending on the application. The work also demonstrated a simple and rapid method for determining low concentrations of hydrogen peroxide.

The method was evaluated with respect to the voltammetric behavior of H₂O₂ under different electrodes, showing that results from the BDD electrode are superior to those obtained from other traditional electrodes described in the literature. The possible interference of urea was discarded, and other parameters were investigated, obtaining the best analytical conditions. Regarding the amperometric determination, after the linear working range was established, addition and recovery tests were performed to confirm the accuracy of the proposed methodology. The analysis system was further evaluated with respect to the repeatability of injections, and compared with the already established spectrophotometric assay. In this context, statistical tests that confirmed the validity of the proposed method were performed. The new method presented as a benefit, the complete elimination of enzyme use for whitening gels, reducing the cost of analysis considerably, since the price of enzymes is the limiting factor involved in the analysis. With respect to the obtained peroxide levels, it could be observed that the concentrations were below those stated on the information labels, but further studies are necessary to investigate whether there is a change in concentration over time, since it is a product that remains for hours in the oral cavity of the patient. However, the results were consistent with those obtained by the spectrophotometric method.

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