A FAST, ROBUST ELECTROCHEMICAL METHOD FOR THE AUTOMATED DETERMINATION OF HYDROGEN PEROXIDE

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ABSTRACT

A suitable flow-injection method for the analysis of commercial solutions of hydrogen peroxide is described. A Nafion-coated glassy carbon electrode, modified by deposition of copper hexacyanoferrate (CuHCF), is employed as an amperometric sensor. The method allows the analysis of 45 samples per hour, with peroxide concentrations in the range of 1.25 - 10.0 mM, with relative standard deviations smaller than 1%.

Keywords: Nafion-coated copper hexacyanoferrate modified electrode; FIA system; Hydrogen peroxide quantification

INTRODUCTION

Hydrogen peroxide is present in several biological reactions as main product of enzymatic reactions^{1,2} and is a very important parameter for treatment and (bio) process monitoring³⁻⁴. It is also a key species in the reactions of the troposphere and in reactions such as the catalyzed and uncatalyzed aqueous phase oxidation of SO₂ and the UV-enhanced aqueous phase oxidation of organic species⁵. As an antiseptic, H₂O₂ can be compared to a preservative, since it quickly acts to kill microorganisms and has no long-term or preserving effect⁶; it has been used as an antimicrobial agent since the early 1800's, and is well known for its use as a topical skin application in 3% concentration⁷. In addition, H₂O₂ has been used in several important areas such as food^{8,9}, and environmental^{5,10}, in the industry^{11,12} and in clinical evaluations^{13,14}. In food industry, hydrogen peroxide has been used as a disinfectant in milk as early as 1904 and is approved by the FDA for packaging and surface sterilization¹⁵. For antimicrobial purposes, hydrogen peroxide is used for milk treating in cheese manufacturing, thermophile microorganism free starch production^{6,7}. It is also used as an oxidizing and reducing agent in wine, dried eggs, corn syrups and as a bleaching agent¹⁶.

The quantification determination of hydrogen peroxide has been carried out by a variety of analytical methods, based on titration^{17,18}, spectrophotometry^{19,20}, fluorimetric^{21,22}, chemiluminescent²³, spectroscopic^{24,25} and electrochemical²⁶⁻²⁸ measurements. Because of its simplicity and versatility, the latter have been more widely used^{28,29}. The use of chemically modified electrodes based on transition metals has improved their application²⁹⁻³⁶.

We have previously described glucose biosensors from copper hexacyanoferrates (CuHCF) electrodeposited on glassy carbon electrodes³⁶. Besides being effective for the detection of glucose, these systems could be employed for the amperometric determination of hydrogen peroxide.

In the present communication we describe a simple, fast and low-cost flow-injection procedure for the automated hydrogen peroxide quantification in commercial samples based on the use of Nafion-coated CuHCF deposited electrodes.

EXPERIMENTAL

Apparatus

An Autolab potentiostat-galvanostat (PGSTAT30, Germany) system connected with a computer was employed. The three-compartment electrochemical cell contained a platinum auxiliary electrode, an Ag/AgCl reference electrode and a glassy carbon electrode (diameter 3.0 mm) as working electrode.

Hydrodynamic experiments were performed with a LC4B electrochemical analyzer (Bioanalytical Systems Inc., West Lafayette, IN). Flow-injection experiments were carried out with peristaltic pump, home-made injector-commutator, home-made electrochemical cell, tygon pumping tubes and connectors. For reactors and transmission lines was used a 0.8 mm i.d. polyethylene tubing with wall thickness = 0.3 mm (Fig. 1).

Reagents, solutions and samples

Concentrated solutions of H₂O₂ (30%), Nafion perfluorinated ion-exchange (5% solution in 90% alcohol) were supplied by Aldrich. All employed reagents

were analytically pure and the solutions prepared with doubly distilled water (Millipore-Q).

The solutions used for the electrodeposition of the CuHCF films onto the glassy carbon electrode were prepared as follows: 0.1 M CuSO₄ + 0.5 M H₂SO₄ (solution, S₁); 5 mM K₃Fe(CN)₆ + 0.1 M KCl (S₂). A pH 5.5 buffer solution was prepared as following: 0.025 M 0.05 M K₂HPO₄ + 0.05 M KH₂PO₄ + 0.1 M KCl (S₃). The last solution was used for conditioning of the CuHCF film after activation, as well as carrier stream in the flow system (Fig. 1).

The samples used were acquired at local store, and consisted of aqueous solution of H₂O₂. Before being injected into flow-injection system, the samples were diluted with buffer (S₃) between 500 or 2000-fold (v/v).

Hydrogen peroxide standard solutions were prepared from a stock (0.10 M) and diluted with buffer solution S₃.

Figure 1. Flow diagram. S - sample; L - sampling loop; IC - injector; C - carrier stream; B - coiled reactor (20-cm); a, b and c - working, auxiliary and Ag/AgCl (3.0 M KCl) reference electrodes. Arrows indicate inlet from peristaltic pump. Dotted line indicates next position (sample injection).

PROCEDURE

Preparation of the CuHCF-Nafion modified electrode

Prior to any surface modification, the glassy carbon electrode was polished with alumina powder (Al₂O₃, 1 and 0.25 μm).

The polished electrode was then dipped into solution S₁ and a cathodic current of 1 mA was applied for 2 s. The system was left in standby condition

for 10 s and then scanned for one cycle between 350 and -50 mV. The deposited film was then activated by immersing the electrode in solution S_2 and applying a constant potential of 1.0 V for 200 s. After washing with water and drying, the modified electrode was conditioned in solution S_3 , by applying a potential of -50 mV for 600 s, followed by voltammetric scanning between 350 and -50 mV for 10 cycles. Finally, the electrode was coated with a polymeric film by allowing two drops of a Nafion solution, at pH 5.5, to evaporate on its surface.

The obtained Nafion-coated CuHCF-modified electrode was stable in solutions with pH values lower than 6. In alkaline media the hexacyanoferrate anions is gradually converted to the corresponding ferrous and then ferric hydroxides. Therefore this media was not considered in the present work. The electrode had a sensitivity of $2.4 \text{ mA} \cdot \text{cm}^{-2} \cdot \text{M}^{-1}$ and a detection limit of 0.10 mM, quite appropriate for the hydrogen peroxide concentrations employed by us (3-10% (v/v)).

The flow-injection system

Figure 1 depicts the flow-injection system employed in this work. The sample was injected into the system by a 10-cm (ca. 50 μL) loading loop L, and pushed by its carrier stream (solution S_3 at 1.0 mL min^{-1}) through conduct C and coil B towards the detector with a fixed potential (-200 mV vs. Ag/AgCl). The passage of the sample through the electrochemical cell and the contact with the Nafion-coated CuHCF at the tip of the glassy carbon electrode produced a transient current proportional to the hydrogen peroxide content in the sample. After defining the hydrodynamic conditions, the system was applied to analysis of hydrogen peroxide in pharmaceutical formulations (aqueous solutions). Experimental precision was expressed as the relative standard deviation estimated after eleven successive analyses of typical sample (about 6% v/v hydrogen peroxide), and the accuracy was determined by the reference method¹⁷.

RESULTS AND DISCUSSION

Figure 2 shows a typical cyclic voltammogram of the CuHCF modified electrode. One reversible peak arising from the redox process below is observed^{36,37}: $\text{Cu}_3^{2+}[\text{Fe}(\text{CN})_6]_2 + 2\text{K}^+ + 2\text{e}^- \leftrightarrow \text{K}_2^+\text{Cu}_3^{2+}[\text{Fe}(\text{CN})_6]_2$. In the presence of hydrogen peroxide, Fe^{3+} is first reduced electrochemically to Fe^{2+} which reacts chemically with the H_2O_2 resulting in the reduction of H_2O_2 to H_2O and in the regeneration of the catalyst^{30,31}.

Figure 2. Cyclic voltammogram of the copper hexacyanoferrate film in 0.1 M KCl solution with a scan rate = 100 mV s^{-1} ; start potential at 900 mV vs. Ag/AgCl.

The flow-injection analysis was done using standard solutions of hydrogen peroxide in the range of 1.25 to 10.0 mM in the buffered (pH 5.5) S_3 solutions with an applied potential of -200 mV vs. Ag/AgCl in the working electrode. Increasing the injected volume (10-100 μL) led to increased signals, as expected. However, above 100 μL the signal no longer increased and pronounced losses in linearity between peroxide concentration and response current were observed.

An optimum injected volume of 50 μL led to a very good linear correlation ($R^2 = 0.9973$) between concentration (1.25 - 10.0 mM) and response current. The influence of the flow rate on the analytical signal was investigated by varying it between 0.8 and 1.5 mL min^{-1} , keeping the injection volume equal to 50 μL . A reduction of the flow rate to values lower than 0.8 mL min^{-1} led to an increase of the recorded peak intensities, but this effect had the unacceptable cost of reducing the sampling rate to only 20-25 samples per hour. For values higher than 1.5 mL min^{-1} , in spite of the high sampling frequency (>60 samples per hour), the system stability was deteriorated. In fact, standard deviations larger than 6% for a set of 10 samples were observed. Therefore, the optimum flow rate was thus fixed at 1.0 mL min^{-1} .

Figure 3. Performance of the amperometric sensor. Injection of 50 μL of standard solutions of hydrogen peroxide in S_3 with concentrations (a) 1.25; (b) 2.5; (c) 7.5 and (d) 10.0 mM. Applied potential = -200 mV vs. Ag/AgCl.

A comparison was made between the results obtained with our method and those of a standard hydrogen peroxide determination based on reference method¹⁷. This test method determines the concentration of H_2O_2 in aqueous solutions particularly used in textile bleaching. A specimen is acidified with sulfuric acid and titrated with standardized potassium permanganate solution. The concentration of hydrogen peroxide is calculated using the volume and normality of the permanganate solution used¹⁷.

Table 1 lists the obtained values with both methods, showing a very good coincidence between them.

It is important to clarify that in cosmetic compositions containing hydrogen peroxide, it is necessary to stabilize this species and a great number of stabilizers for hydrogen peroxide are described, including, among others, certain organic compounds. However, the low interferant-to-analyte ratio and the very high sample dilution involved suggested problems with potentiality interfering species were likely to be minimal. Moreover, the use of Nafion membrane covering the modified electrode also contributes for eliminating this trouble.

The long-term stability of the modified electrode was investigated using a 2.5 mM hydrogen peroxide solution in phosphate buffer at pH 5.5 during 2-3 h. Six samples of aqueous hydrogen peroxide at pH 5 were evaluated, employing the optimal conditions described above. Figure 3 illustrates the performance of the sensor reflected in the profile of the signals. For higher concentrations (signals c and d) the reproducibility of the signals decreased slightly. However, this drawback did not result in a bad quality of the analysis. With the proposed system, about 40-50 samples could be run per hour, with standard deviations smaller than 1%.

Table 1. Comparison between hydrogen peroxide determinations carried out with a Nafion-coated CuHCF modified electrode and a reference method¹⁷.

Sample*	Hydrogen peroxide %(v/v)	
	Present method**	Reference method**
1	3.01±0.03	2.80±0.05
2	2.93±0.03	2.98±0.05
3	6.00±0.05	6.01±0.07
4	5.98±0.06	6.03±0.07
5	9.06±0.08	9.12±0.09
6	8.98±0.09	9.01±0.10

Mean of three determinations±SD

CONCLUSION

In conclusion, we have described a fast, robust and easily implemented flow-injection method for hydrogen peroxide determination based on an amperometric sensor developed from a Nafion-coated copper hexacyanoferrate-modified electrode. The modified electrode presents good performance with a minimum decrease in activity after 2 h. The Nafion coating avoids important interferences. Thus, covering the modified electrode with this polymeric membrane it is possible to increase selectivity and the applicability of the sensor to different commercial samples in the food and pharmaceutical industry, and in the environmental control.

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