Analytical Methods

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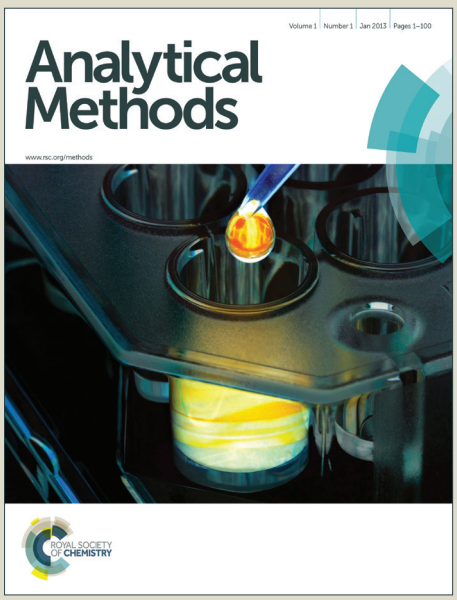

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Amperometric determination of hydrogen peroxide using a copper microelectrode

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Abstract

The cathodic reduction of hydrogen peroxide at copper microelectrodes was investigated in phosphate buffer solutions. Following the optimization of the experimental conditions, the proposed sensor presented excellent analytical properties for the amperometric detection of hydrogen peroxide at -0.2 V *vs.* Ag/AgCl (saturated KCl). The usefulness of the fabricated electrochemical sensor was confirmed by the determination of hydrogen peroxide in commercial products and values obtained by the proposed method agreed well with those found by using a standard method.

Keywords: Hydrogen peroxide, Copper, Microelectrodes.

Introduction

Fast and accurate hydrogen peroxide (H_2O_2) determination has become a relevant issue due to its importance for the food[1, 2] and pharmaceutical industries[3, 4] and environmental analysis[5]. Hydrogen peroxide takes part in many biological redox reactions that generate hydroxyl radicals, hence its quantification is an important parameter in biochemical processes control. Electrochemical methods have been employed for the determination of H_2O_2 as they can reduce costs, present good selectivity and rapid response time, achieve low detection limits and possess large dynamic concentration range. Moreover, there are advantages concerning inherent miniaturization and portability. Aiming to avoid the influence of interfering species, a large number of chemical mediators have been attached onto electrode surfaces to minimize the high overpotential required in the electrochemical reduction or oxidation of hydrogen peroxide.[6-10]

The use of copper surfaces to promote reduction/oxidation processes has been explored as copper oxide layers have a role in typical electrocatalytic processes.[11, 12] Hence, several studies have been reported in the literature on the use of copper surfaces aiming at the quantification of different species such asglucose[13], carbohydrates[14], ethanol[15-17], nitrate[18-20], nitrite[21], sufite[22], glyphosate[23, 24] and selenium(IV)[25]. The electroreduction of H_2O_2 at copper surfaces has also been examined and the process is facilitated due to an electrocatalytic process involving reduced copper oxides.[26-28] At electrode surfaces of micrometric dimensions, this electrochemical process is expected to be carried out with some advantages, such as very fast mass transport owing to radial diffusion. The steady-state regime is reached in very short time, with no need of solution stirring, and reliable information is obtained in a fast and simple way. Accordingly, in this paper we present our

attempt to use copper disc microelectrodes as electrochemical sensors for hydrogen peroxide determination.

Experimental

Chemicals, materials and samples

All chemicals were used without further purification. The reagents used were methyl viologen (Aldrich – Steihheim, Germany), hydrogen peroxide and potassium chloride (Merck – Darmstadt, Germany), sodium hydroxide, potassium phosphate and potassium permanganate (Synth – São Paulo, Brazil). The solutions were prepared with deionized water, which was processed through a Nanopure Infinity purification system ($18 M\Omega cm^{-1}$) (Barnstead, Dubuque, IA, USA). Hydrogen peroxide solutions were daily prepared from a stock solution (30% m/m) and standardized with a potassium permanganate solution in acid medium, as reported in the literature.[29] Oral antiseptic samples were acquired in a local supermarket and no preparation was required before measurements. Dental whitening samples were acquired in a specific store and were diluted in phosphate buffer under stirring for 10 min.

Electrodes and instrumentation

All measurements were performed with an Autolab PGSTAT 30 (Eco Chemie) bipotentiostat. The experiments were carried out in a three-electrode cell using an Ag/AgCl (saturated KCl) reference electrode, a platinum wire as a counter electrode and a

commercial copper macroelectrode (d = 2mm) and a copper disc microelectrode as working electrodes.

Copper disc microelectrodes were fabricated by sealing a copper microfiber (Puratronic® - Alfa Aesar) of nominal radius of 12.5 μm (approximately 2 cm length connected to a Ni/Cr wire) directly to a Pasteur pipette using Araldite epoxy resin, according to conventional procedures described in the literature.[30, 31] Before experiments the surface of the microelectrode was polished with sandpaper (no. 400) and alumina slurry (1 μm, Alfa Aesar, Massachusetts, USA). Then, the microelectrode surface was rinsed with water and sonicated during 5 minutes in distilled water. The radius of the microelectrode was determined by measuring the steady-state current in a 20 mmol L^{-1} MV^{2+} (methyl viologen) solution containing 0.1 mol L^{-1} KCl as supporting electrolyte and the value was found to be 14 μm.

Titrimetric analysis

The accuracy of the method was evaluated by comparison with results obtained from titrimetric analysis of hydrogen peroxide with potassium permanganate in acid medium.[29]

Results and discussion

Electrocatalytic reduction of hydrogen peroxide

Fig. 1 shows cyclic voltammograms recorded with a copper macroelectrode (d = 2mm) in 0.1 molL^{-1} phosphate buffer (pH = 7.0) in the absence and presence of H_2O_2. Because the influence of EC catalytic processes on the current response at microelectrodes

is less pronounced owing to the fast rate of diffusion to and from the electrode surface[32], results of experiments involving the electroreduction of H_2O_2 were preliminary performed using a copper macroelectrode. In free-H_2O_2 solutions, an anodic current starting at -0.1 V is noticed during the potential scan towards more positive values, which defines a broad anodic current peak at 0.0 V corresponding to the formation of CuO[33] and soluble Cu^{2+} species[34]. The reverse sweep shows two well-separated peaks for the reduction of Cu(II) to Cu (I) and Cu(I) to copper metal.[26, 35]A large cathodic current enhancement with a concurrent decrease of the peak corresponding to the formation of CuO were noticed when the experiment was repeated in the presence of 1.7 mmol L^{-1} H_2O_2, suggesting the chemical consumption of some reduced copper species by H_2O_2[11]. The results of this experiment confirm the advantages of using copper as an electrodic surface in the development of analytical methods to measuring hydrogen peroxide at relatively low overpotentials and with enhanced sensitivity.

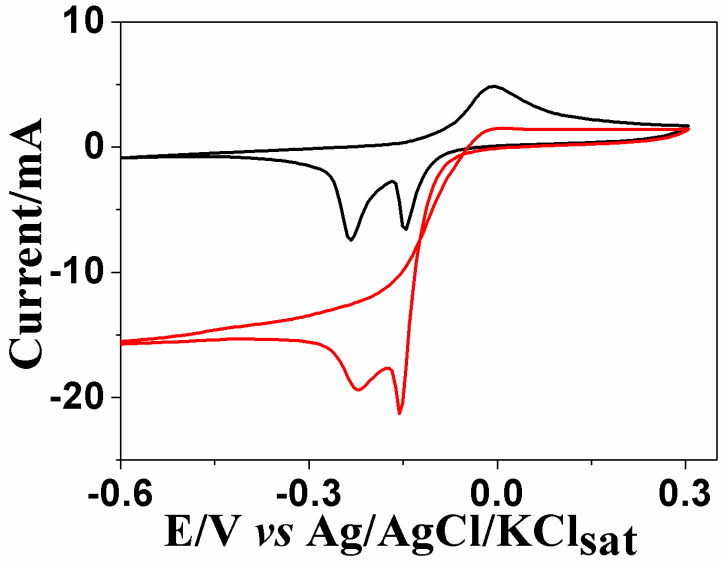

Fig. 1. Cyclic voltammograms recorded with a copper macroelectrode (d = 2 mm) in 0.1 mol L^{-1} phosphate buffer (pH = 7.0), in the absence (—) and presence (—) of 1.7 mmol L^{-1} H_2O_2, ν = 5 mV s^{-1}.

Optimization of the experimental parameters

Experimental parameters involving the electroreduction of H_2O_2were optimized using a copper microelectrode. Experiments were performed in 0.1 mol L^{-1} phosphate buffer during repetitive additions of H_2O_2 and the influence of pH and applied potential was examined. Because the mass-transport rate to a microelectrode is very fast, stirring was only required after injection of the H_2O_2 solutions to homogenize the mixture. Fig. 2A shows the current responses obtained upon addition of H_2O_2over a pH range comprising 5to 8, the potential being set at -0.2 V. A linear relationship between current and concentration was noticed at all pH values, with higher sensitivity at pH 7.0. This pH value was then selected for further experiments. The electrochemical behavior of Cu in phosphate solutions involves the formation of copper phosphate layers, in a pH dependent process. The higher catalytic activity of the Cu microelectrode towards the cathodic reduction of H_2O_2 at pH 7.0 is likely dependent on the nature ofthese compounds.[34]

The effect of the applied potential on the sensitivity was studied by varying the potential in the range -0.1 to -0.5 V (Fig. 2B). The increase in sensitivity as the potential was made more negative is clearly seen. However, as a compromise has to be reached between sensitivity and selectivity and taking into account the influence of possible interfering species present in real samples, the selected potential was -0.2 V.

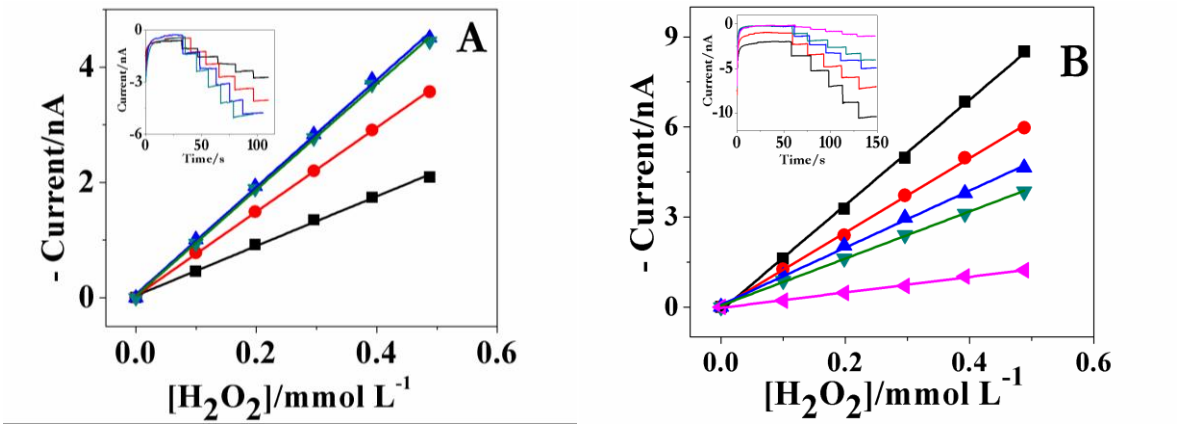

Fig. 2. Calibration curves and current responses (inset) recorded with a copper microelectrode during addition of aliquots of a 20 mmol L^{-1} H_2O_2 solution to 0.1 mol L^{-1} phosphate buffer. **(A)** Influence of pH: 5 (—), 6 (—), 7 (—), 8 (—), E = -0.2 V. **(B)** Influence of potential: -0.5 V (—),-0.4 V (—),-0.3 V (—),-0.2V (—) e -0.1 V (—), pH = 7.0.

The selected potential was located in the negative potential range, hence the influence of O_2on the H_2O_2 reduction current was also investigated. Fig. 3 shows amperometric curves obtained using a copper microelectrode in a 0.1 mol L^{-1} phosphate buffer (pH 7.0), polarized at -0.2 V containing dissolved O_2 at room temperature. The removal of dissolved O_2 was accomplished by bubbling argon gas for 10 minutes. By comparing the sensitivity values obtained in the absence and presence of O_2 one can conclude that the influence of O_2 is negligible, at least for the investigated concentration range of hydrogen peroxide.

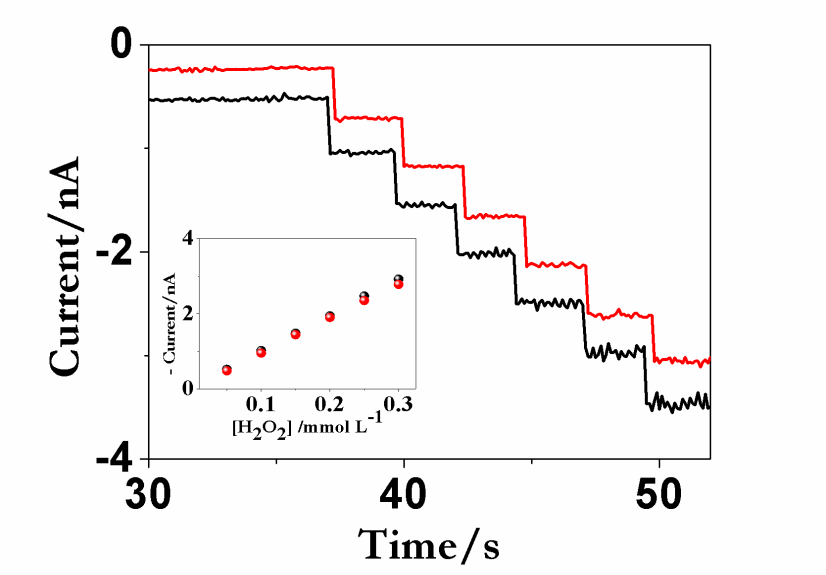

Fig. 3. Current responses monitored as a function of time with a copper microelectrode in 0.1 mol L^{-1} phosphate buffer during repetitive additions of H_2O_2 in the presence (—) and absence (—) of dissolved O_2. E = -0.2 V. Inset: Calibration plot.

The relationship between limiting current and concentration of H_2O_2 was assessed by means of a calibration plot. Fig.4 shows amperometric signals obtained during successive additions of a H_2O_2 solution. A linear relationship in the range 0.015 to 1.82mmol $L^{-1}$was obtained (I (nA) = 0.1 + 32.1 [H_2O_2] (mmol L^{-1}), with a correlation coefficient of 0.9999. The decrease in sensitivity for higher H_2O_2 concentrations is likely attributable to a kinetic limitation, as the sensor is not able to handle with such a high flux of electroactive species at the same rate. The limits of detection and quantification were estimated as 2.8 and 9.4 μmol L^{-1}, respectively. The repeatability of the method was investigated by comparing the current response to 10 successive measurements of a 0.55 mmol L^{-1} H_2O_2 solution and the relative standard deviation was 0.5%, demonstrating that the method produces an acceptable precision.

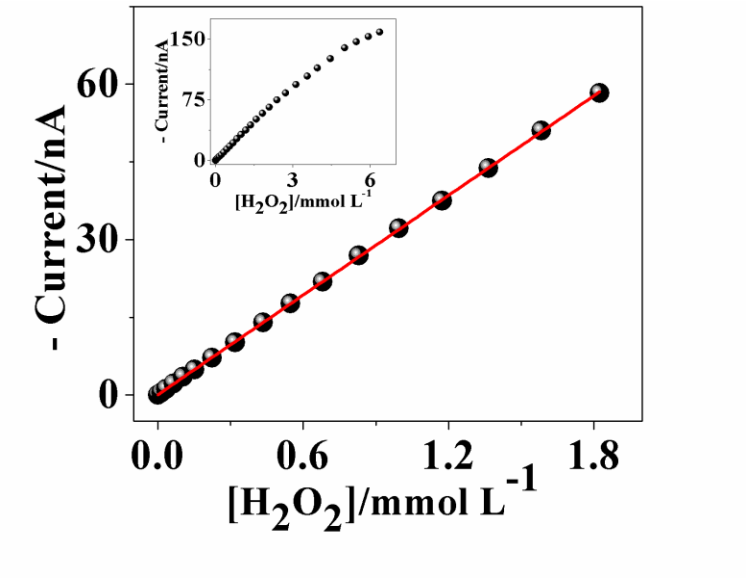

Fig. 4. Calibration curve recorded with a copper microelectrode during additions of a H_2O_2 standard solution in 0.1 mol L^{-1} phosphate buffer (pH = 7.0). The inset shows the entire analytical curve. E = -0.2 V.

Interference effect and real sample analysis

The proposed method was used for the determination of H_2O_2 concentration in oral antiseptic and dental whitening samples. In order to assess the influence of interfering species other than O_2 in the amperometric response of the copper microelectrode sensor, some compounds present in oral antiseptic samples were chosen as model substances. Accordingly, the interference effect of sorbitol, glycerin, ethanol and sodium saccharin at a concentration 10 times higher than that of H_2O_2 was studied. By analyzing the results shown in Fig. 5, it is possible to conclude that the proposed sensor does not respond to such foreign compounds, hence it can be selectively used for H_2O_2 detection in the proposed real samples. It should be emphasised that as measurements were performed at -0.2 V, the developed sensor can be used for H_2O_2 analysis in biological samples with no interference

of compounds such as ascorbic acid and uric acid, which undergo oxidation processes at positive potential values.

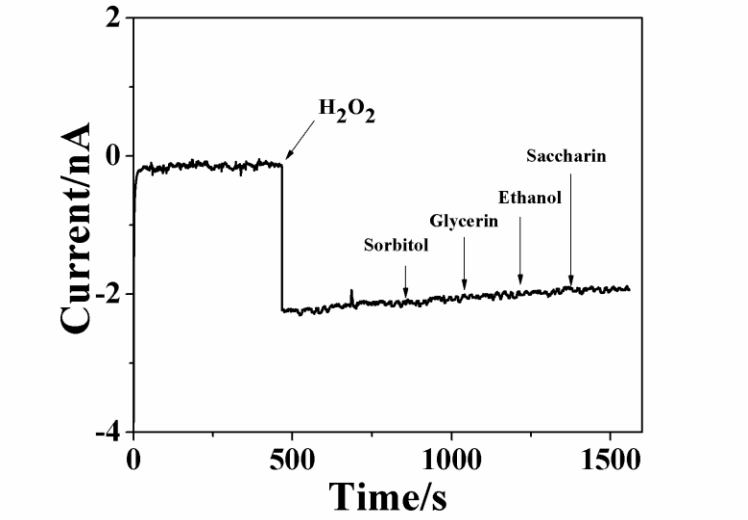

Fig. 5. Amperometric responses recorded after injection of H_2O_2 (to give a final concentration of for 0.5 mmol L^{-1}) and interfering species (to give a final concentration of 5.0 mmol L^{-1}) in an experiment performed with a copper disc microelectrode at E = -0.2 V in 0.1 mol L^{-1} phosphate buffer (pH 7.0).

The proposed microelectrode sensor was examined for its ability to determine hydrogen peroxide concentration in oral antiseptic and dental whitening commercial products. Samples were diluted using 0.1 mol L^{-1} phosphate buffer and the standard addition method was followed for the analysis in order to exclude the influence of matrix. Fig. 6 presents results on the successive additions of a standard H_2O_2 solution to the electrochemical cell containing a commercial sample diluted in 0.1 mol L^{-1} phosphate buffer (pH 7.0). The copper microelectrode was polarized at -0.2 V and the current response was continuously measured during the experiment. The limiting current value

increased with the addition of the H_2O_2 standard solution, according to the linear equation I (nA) = 1.0 +7.7 [H_2O_2] (mmol L^{-1}) (R^2 = 0.9990).

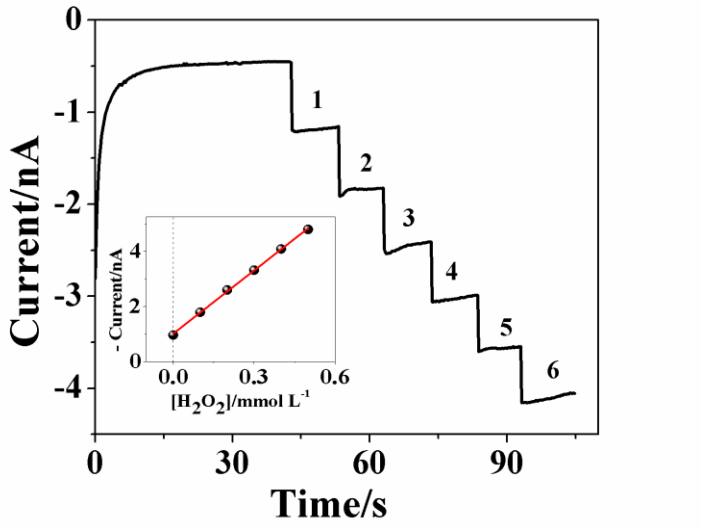

Fig. 6. Amperometric responses recorded with a copper microelectrode during addition of sample 1 (1) and a H_2O_2 standard solution (2 to 6) to 0.1 mol L^{-1} phosphate buffer (pH 7.0). E = -0.2 V. The inset shows the respective analytical curve.

By comparing the slopes of the calibration plot (Fig. 4) with the one obtained in the standard addition experiment (Fig. 6), a significant difference was noticed. A possible explanation for these results is the presence of surfactants in the commercial sample. The adsorption behavior of surfactants at the solid/solution interface can form a barrier on the copper surface, reducing the signal from the sample. [36] Hence, the standard addition method must be used in this case to avoid the effect of the sample matrix.

Values of H_2O_2 concentration in 4 commercial samples were obtained by extrapolating the data of standard addition plots corresponding to the variation in current with added H_2O_2 concentration. The results obtained with the amperometric method were compared with those obtained by a conventional titrimetric procedure, as shows Fig. 7. The

paired t-test indicated that there was no significant difference between the results obtained with both methods at a 95% confidence level and confirms the usefulness of the Cu microelectrode as a simple and straightforward amperometric detector for hydrogen peroxide determination.

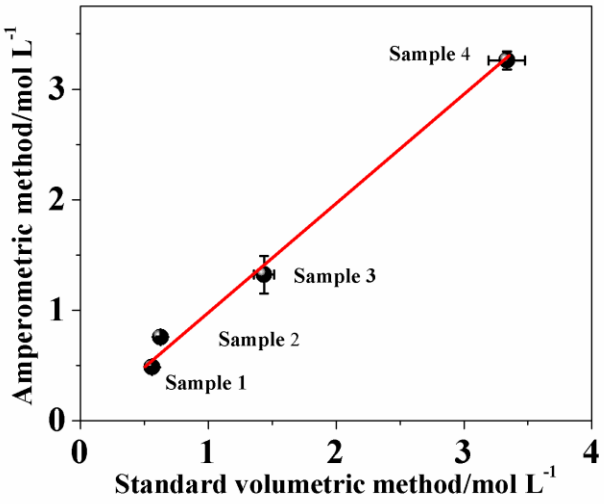

Fig. 7: Correlation between the H_2O_2 contents in commercial samples determined by using the developed sensor and the volumetric method. The regression line is indicated by the red trace.

The analytical performance of the developed copper microelectrode sensor towards H_2O_2 detection was compared with that of different copper sensors described in the literature, and the results were summarised in Table 1. It can be observed that the proposed sensor has an extended linear concentration range and a good detection limit, but this latter value is not as low as a few of those reported in the table. Nevertheless, important features of microelectrodes such as the attainment of steady state conditions at very short times, the ability to perform electrochemical measurements in high resistive solutions, the detection in flowing liquids and the monitoring of compounds in very low sample volumes are advantageous and justify the use of copper microelectrodes as powerful sensors for H_2O_2.

Table 1. Comparison of the Cu microelectrode with other electrochemical sensors for H_2O_2.

Sensor	Detection potential (V)	Linear range (mmol L^{-1})	Detection limit (µmol L^{-1})	Ref.
Cu (d = 3.1 mm)	-0.25	0.0006 – 2.04	1.2	26
CuO flower/GC	-0.2	0.005 – 0.18	1.6	27
CuO nanowires /GC	-0.2	0.010 – 28.87	-	28
Cu (d = 12.5 µm)	-0.2	0.015 – 1.82	2.7	This work

Conclusions

The amperometric detection of hydrogen peroxide was carried out at -0.2 V in phosphate buffer solution (pH 7.0), a suitable potential as neither the oxygen reduction nor the oxidation of easily oxidizable compounds are likely to occur. The microelectrode can be constructed in a simple and inexpensive way, and a fresh and reproducible electrode surface can be easily obtained by mechanical polishing. Values found for H_2O_2 concentration in commercial samples were in good agreement with those obtained by using an independent methodology. Hence, this work has successfully demonstrated that copper microelectrodesare promising H_2O_2 sensors especially because of the unique advantages of electrodes with micrometric dimensions.

Acknowledgments

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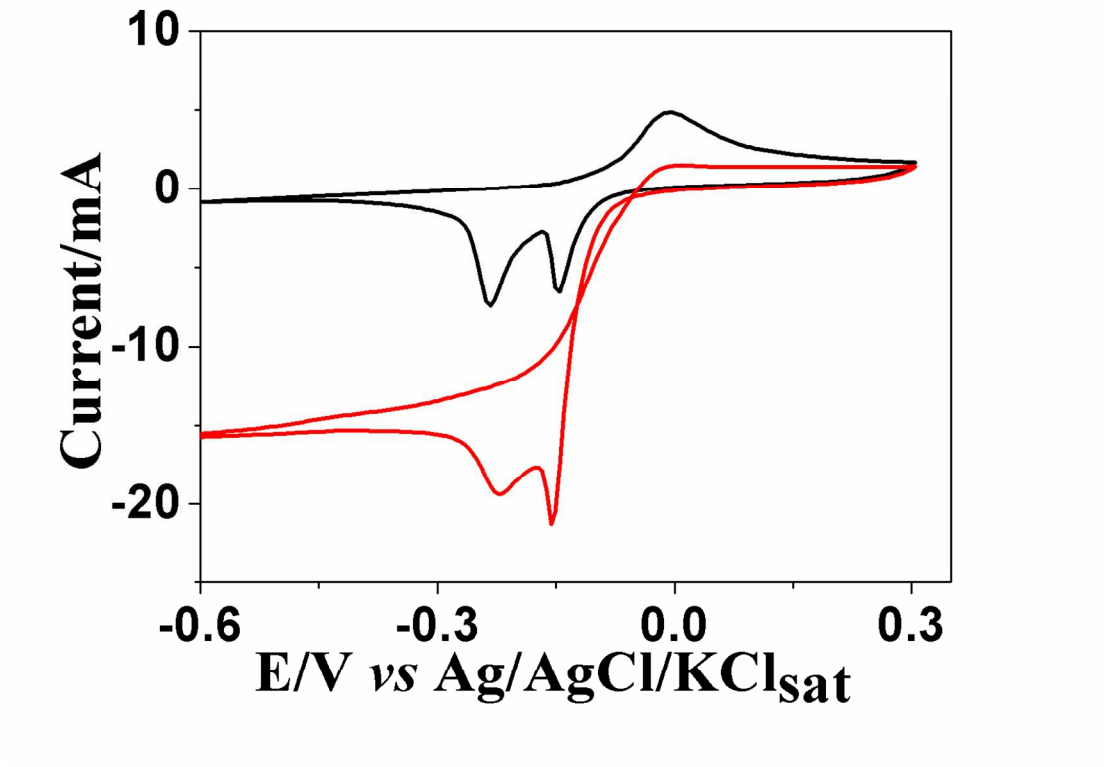
Figure



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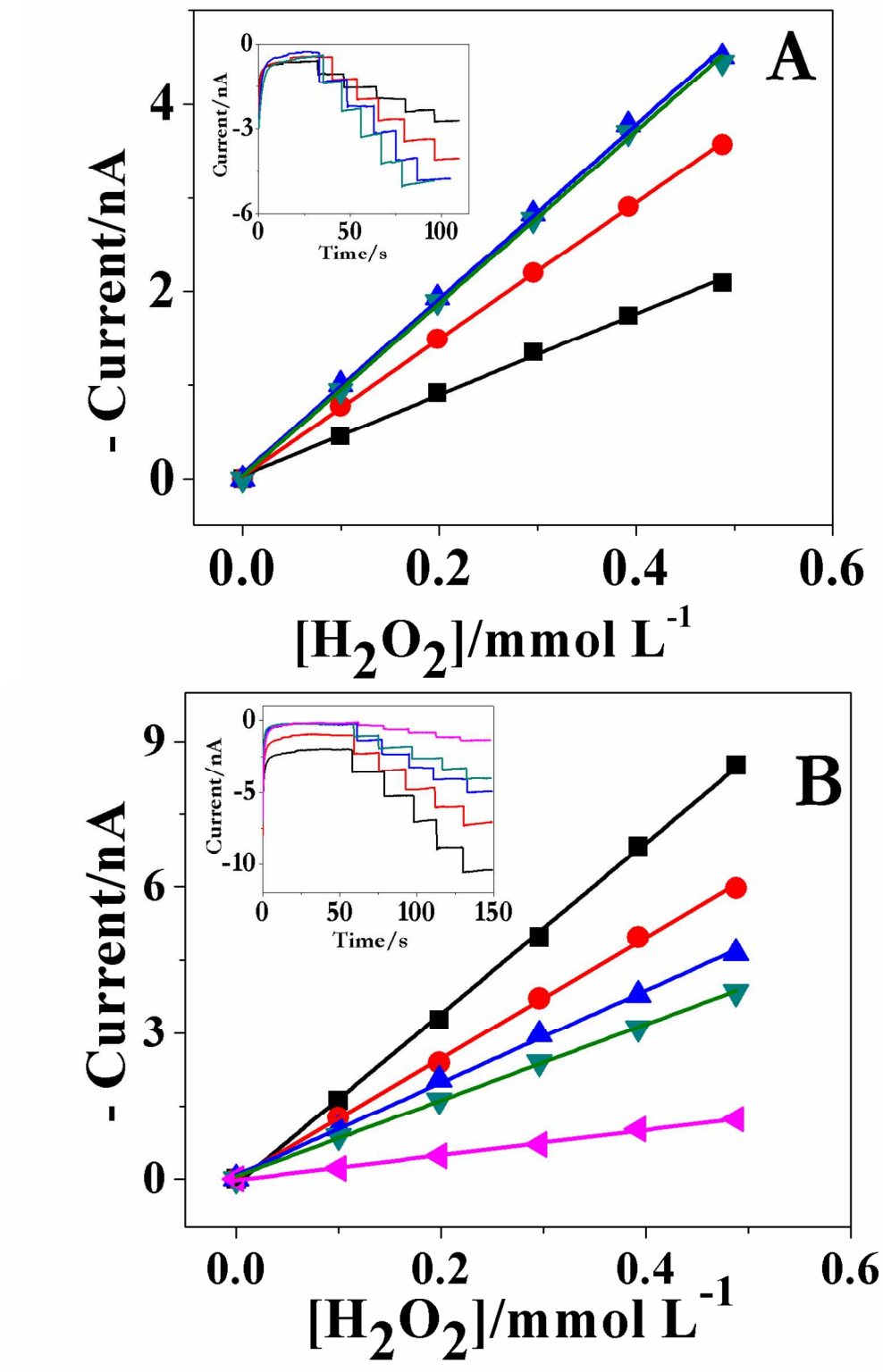

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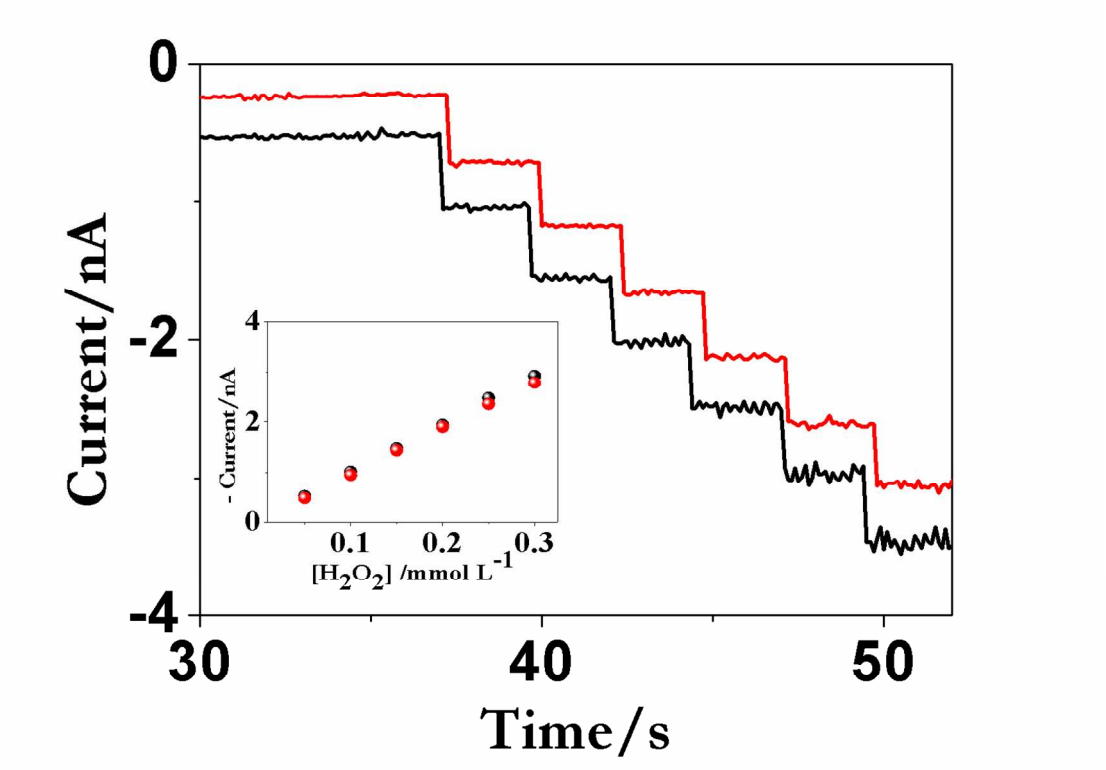

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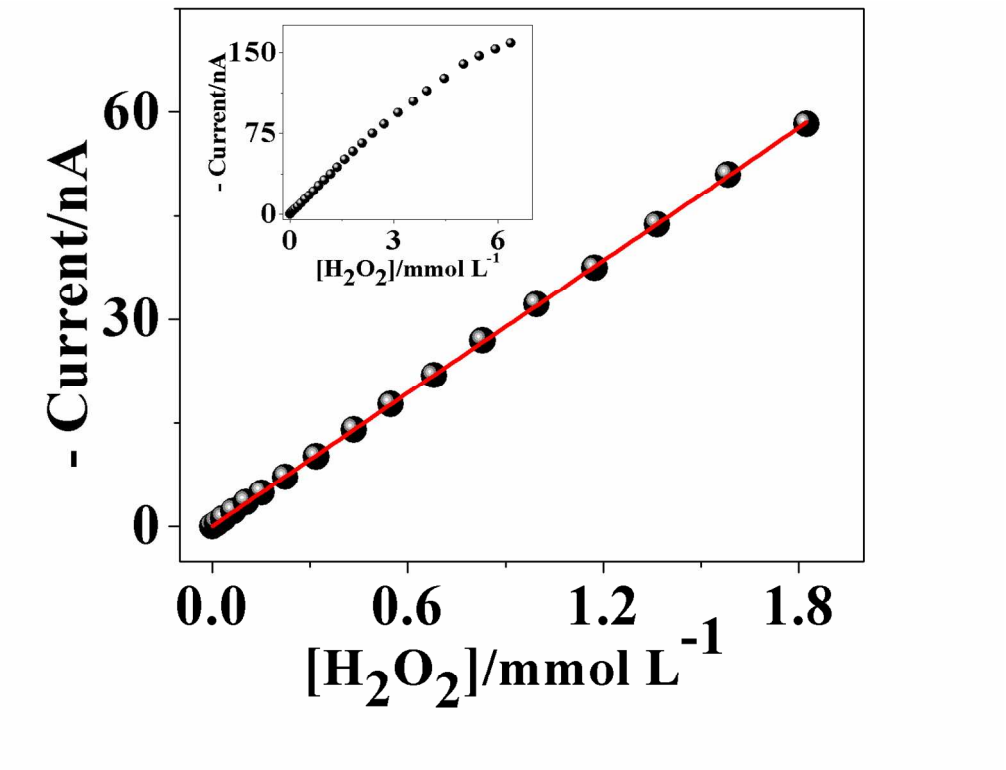

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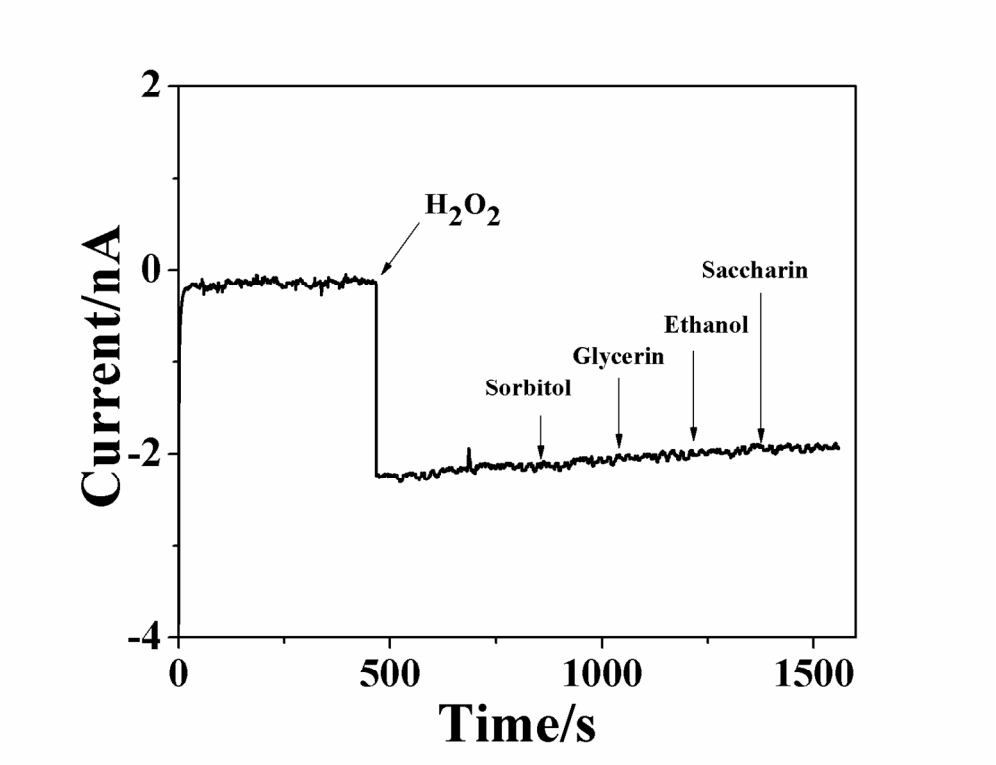
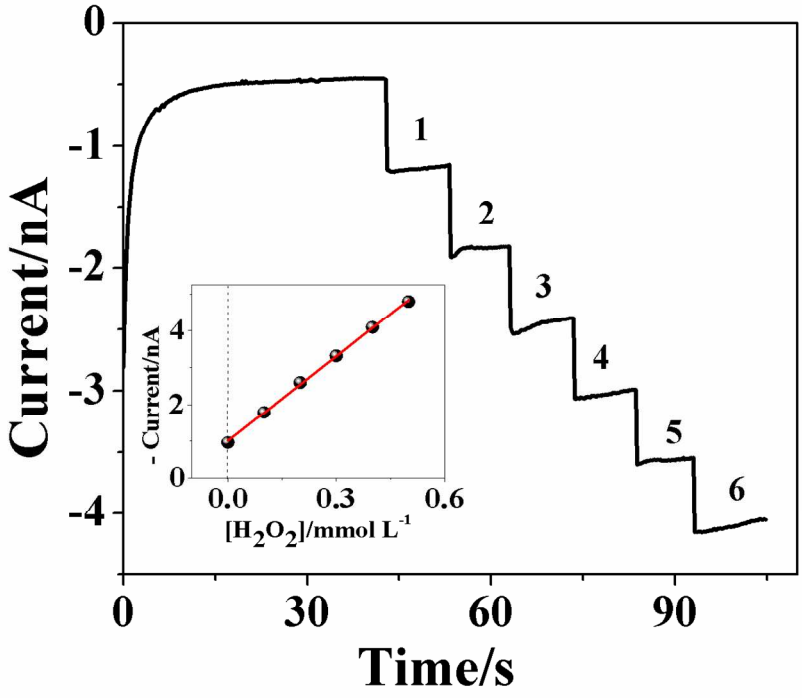


Fig. 5.



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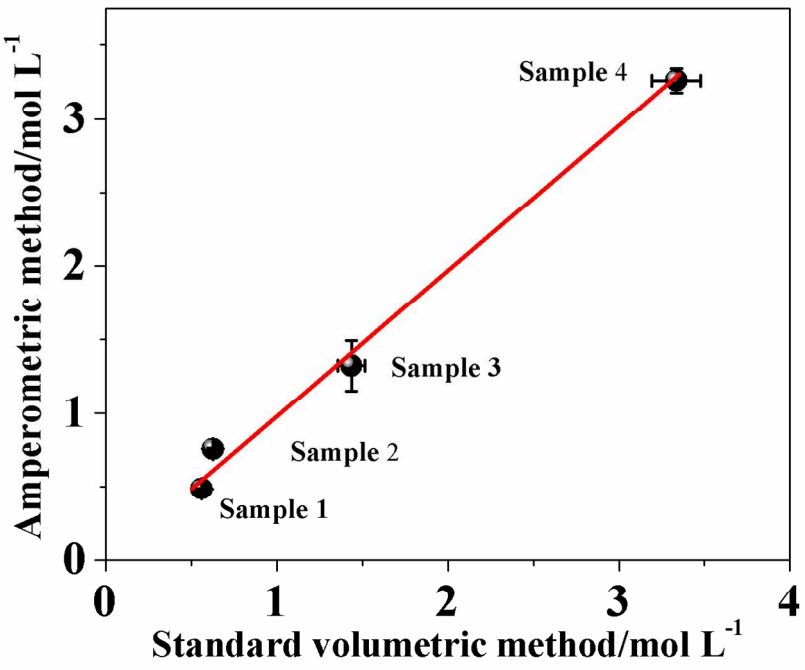

Fig. 7.

Table

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