

CHEAP AND MASS-PRODUCIBLE ELECTROCHEMICAL SENSOR OF HYDROGEN PEROXIDE

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Hydrogen peroxide is present naturally in any living cell. Although hydrogen peroxide concentration in cell is strictly regulated during normal metabolism, several human diseases lead to increased hydrogen peroxide concentration in body fluids. It is beneficial to monitor hydrogen peroxide levels in human body for early disease diagnostics. This can be done by creation of electronic device, which will use electrochemical sensor in order to perform automated hydrogen peroxide concentration measurement in a sample. The aim of the research is to use printed circuit board (PCB) technology in order to create a replaceable electrochemical hydrogen peroxide sensor that could be cheaply mass-produced to enable further development of automated health diagnostics device.

Keywords: *Electrochemical sensor, healthcare, hydrogen peroxide, mass production, printed circuit board.*

1. INTRODUCTION

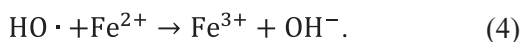
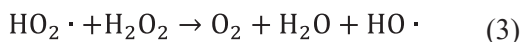
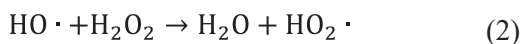
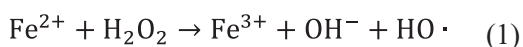
Hydrogen peroxide (H_2O_2) is a breathing process by-product naturally present in every living cell [1], including every part of the human body [2]. Living cells have built hydrogen peroxide regulation mechanisms; thus, in small amounts hydrogen peroxide is harmless. Due to some diseases, hydrogen peroxide regulation may fail and its concentration may increase, leading to vari-

ous forms of damage. Various studies have linked high hydrogen peroxide concentrations in body fluids to Parkinson's and Alzheimer's diseases [3], DNA corruption and premature cell death [4]–[6], cancer and cancer metastasis [7], and brain injuries [8]. Thus, it would be beneficial to develop a cheap and rapid method of hydrogen peroxide detection in body fluids for primary

health assessment. Up to now, hydrogen peroxide has mainly been detected by fluorescence analysis [1], [9]–[11], which requires a specially trained operator and laboratory equipment. Thus, it is an actual task to develop a hydrogen peroxide sensor that could be mass-produced cheaply and further integrated into autonomous analysis devices.

A possible way to make hydrogen peroxide analysis more accessible would be electrochemical analysis automated by computer. Recent studies have shown that electrochemical detection of hydrogen peroxide is possible in electrochemical cell with regular electrodes [12], as well as with various nanostructured electrodes [13]–[19]. It has also been demonstrated that electrochemical sensors could be integrated into small, simple and portable devices [20] that use small sample sizes for analysis. In order to create cheap and accessible hydrogen peroxide detectors, it would be beneficial to develop a cheap method of mass production of electrochemical sensors for use in such portable devices. Printed circuit boards (PCBs) could be used to achieve such goals, as they are widely used in electronics manufacture, thus making them easily available for large-scale production. This approach is very flexible, as PCBs could be designed with arbitrarily shaped, sized and positioned electrodes, as well as with gold coatings, thus making them chemically neutral. Every PCB is produced to exact

dimensions, thus offering good repeatability of electrochemical measurements. It is also possible to make nanostructured PCB electrodes by using electrodeposition methods, as reported in [15]. The possibility of using PCB chips as electrochemical hydrogen peroxide sensors will be evaluated in the article. It is commonly known that hydrogen peroxide actively oxidises ferrous ions, Fe^{2+} , in solution. The reaction between H_2O_2 and Fe^{2+} is a multi-step mechanism that was previously described in [21] and can be seen in equations (1)–(4):



It can be seen that hydrogen peroxide oxidises Fe^{2+} ions to Fe^{3+} , as well as producing large numbers of free radicals. Thus, it is possible to sense the presence of hydrogen peroxide electrochemically in solution by the addition of Fe^{2+} ions and monitoring the Fe^{2+} to Fe^{3+} oxidation peak. It should be noted that it is possible to further enhance the sensitivity of electrochemical sensors by coating its electrodes with free-radical-susceptible materials, as previous studies have shown that coating electrodes with nanostructures greatly improve such sensitivity [17], [22]–[26].

2. EXPERIMENTAL

2.1. Reagents and Materials

This research used $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 97 % ethanol and 30 % H_2O_2 aqueous solution purchased from Sigma-Aldrich. All solu-

tions used were freshly prepared using distilled water.

2.2. Optical Detection of H_2O_2 in FeCl_2 Solution

The solution transmissivity spectrum was analysed to prove the possibility of hydrogen peroxide detection by its reaction with FeCl_2 in solution. The FeCl_2 solutions of target concentration were prepared and divided into two samples, one of which was used as a control. Hydrogen peroxide aqueous solution was added to a flask containing

the other sample of FeCl_2 solution, thoroughly mixed and both samples placed in the dark for 15 minutes. Their transmissivity spectrum was then evaluated by Shimadzu UV-2550 spectrophotometer. The transmissivity spectra were evaluated by transmissivity change compared to the control FeCl_2 solution in the range of 900–300 nm.

2.3. Electrochemical Cell with PCB Electrodes

This research used a custom three-electrode electrochemical cell consisting of a PCB chip, a Ag/AgCl reference electrode (RE), and a sample solution container. Both working electrode (WE) and counter electrode (CE) were placed on a replaceable PCB chip, with the Ag/AgCl RE placed separately from the PCB. The on-chip WE and CE were produced as copper pads

coated with gold by an industry-standard ENIG process. The PCB chip was designed to fit into a quick-change connector in the electrochemical cell, making it easily and quickly replaceable. PCB chips containing the WE and RE were prepared as described elsewhere [27] and replaced before each measurement.

2.4. Electrochemical Detection of H_2O_2 in FeCl_2 Solution

Cyclic voltammetry (CV) was used to detect the presence of hydrogen peroxide in the FeCl_2 solution. Two parts of freshly prepared FeCl_2 aqueous solution were mixed with one part of hydrogen peroxide aqueous solution and mixed thoroughly. The resulting solution consisted of 1 mM FeCl_2 and a

target concentration of hydrogen peroxide and was placed into the sample container of the electrochemical cell and CV measurements performed after 15 minutes by sweeping the potential from -0.6 to $+0.3$ V vs Ag/AgCl at a sweeping rate of 50 mV/s, with starting potential of 0 V vs Ag/AgCl .

3. RESULTS AND DISCUSSION

3.1. Optical Detection of H_2O_2 in FeCl_2 Solution

The possibility of detection of hydrogen peroxide by reaction with FeCl_2 was evaluated by addition of various concentrations of aqueous hydrogen peroxide solution into 1 mM FeCl_2 solution.

As shown in Fig. 1, the addition of hydrogen peroxide greatly reduced trans-

missivity of the FeCl_2 solution in the range of 300–350 nm, which is a clear indication of chemical reaction between hydrogen peroxide and Fe^{2+} ions. Either this reaction itself or the reaction products can further be detected by electrochemical analysis. Experimental data suggests that hydrogen

peroxide concentrations as low as 5 μM can be detected, as they produce notable transmissivity change ($\sim 10\%$).

The effect of FeCl_2 concentration on

measurement result was evaluated by addition of 10 μM H_2O_2 into FeCl_2 solutions of various concentrations.

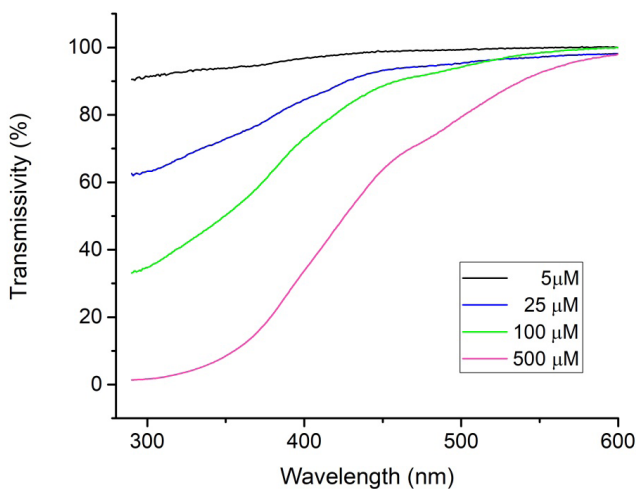


Fig. 1. 1 mM FeCl_2 solution transmissivity spectrum with added H_2O_2 .

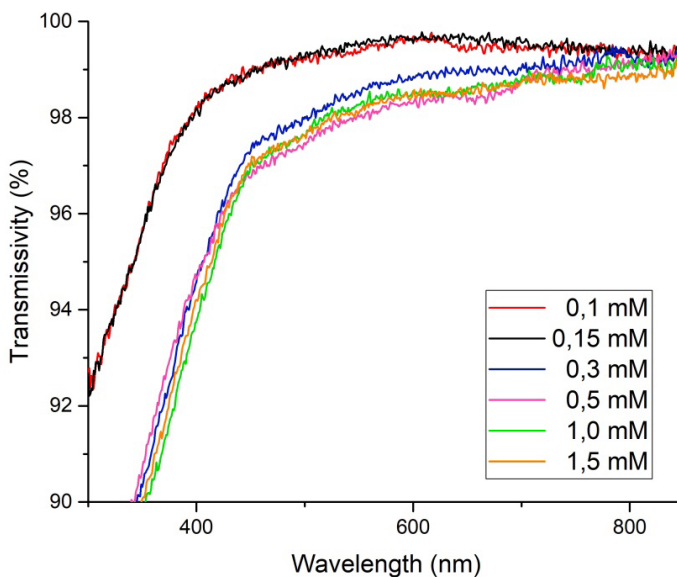


Fig. 2. Various concentrations of FeCl_2 solution transmissivity after 10 μM H_2O_2 addition.

As can be seen in Fig. 2, the reaction of hydrogen peroxide with FeCl_2 at concentrations of 0.1–0.15 mM produced small

changes in transmissivity. An increase in FeCl_2 concentration to 0.3 mM contributes to the appearance of more significant

changes in transmission compared to 0.1–0.15 mM concentration (Fig. 2). However, a further increase in concentration above 0.5 mM does not cause significant changes and the transmission spectra coincide. It can be concluded that in order to achieve maximal detection sensitivity at small hydrogen peroxide concentrations, FeCl_2 should be

present in a concentration at least 30-fold higher than the target hydrogen peroxide concentration. To avoid depletion of the solution, the indicated minimum value for FeCl_2 should be several times higher than H_2O_2 (for example, concentration of 1 mM, which is 100-fold higher than the target concentration of H_2O_2 is used).

3.2. Electrochemical Detection of H_2O_2 in FeCl_2 Solution

Cyclic voltammetry measurements of hydrogen peroxide in 1 mM FeCl_2 solution

were performed as described in the previous section.

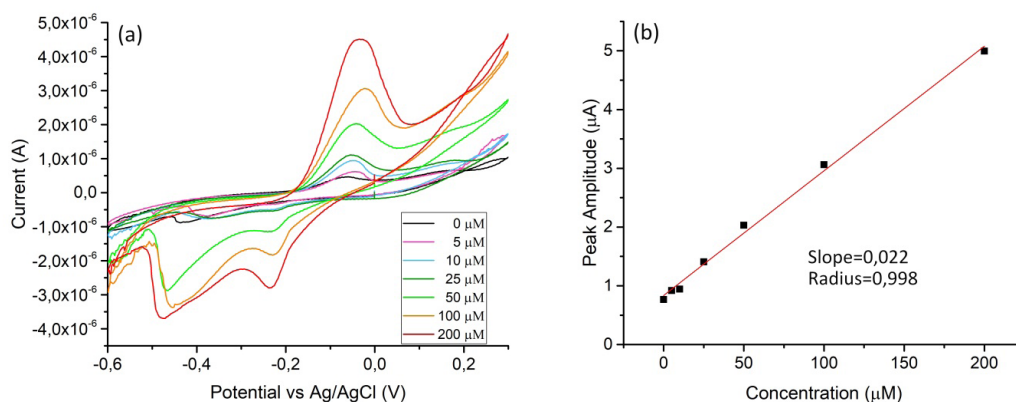


Fig. 3. Cyclic voltammetry result of H_2O_2 in 1 mM FeCl_2 solution (a) and -0.05 V vs Ag/AgCl peak amplitude versus hydrogen peroxide concentration (b).

As can be seen in Fig. 3a, addition of hydrogen peroxide to FeCl_2 solution causes an irreversible electrochemical reaction with the presence of one oxidation peak at -0.05 V and two reduction peaks at -0.2 V and -0.4 V. It should be noted that the presence of hydrogen peroxide in solution creates a peak at -0.2 V that is not present in the voltammogram of pure FeCl_2 . Thus, the peak at -0.2 V could be used to detect the presence of hydrogen peroxide qualitatively, and combined analysis of all peak amplitudes could be used to determine hydrogen peroxide concentration. For the quantitative determination of peroxide, the oxidation peak -0.05 V is used, which corresponds to the oxidation of Fe^{2+} to Fe^{3+} .

The calibration curve of the concentration dependence on the current peak amplitude is shown in Fig. 3b. Taking into account the slope of the calibration curve, the sensitivity of this sensor is $22 \mu\text{A}/\text{mM}$. The calculated limit of detection (LOD) is $7.14 \mu\text{M}$ assuming signal-to-noise ratio = 3.

In the future, the sensitivity of this sensor can be increased by using nanostructured coatings containing iron. The use of such coatings will significantly increase the active surface area of the sensor.

Comparison of the analytical performance of the proposed electrochemical sensor with other Fe-containing H_2O_2 sensors reported previously is presented in Table 1.

Table 1. Comparison of the Analytical Performance of the Proposed Electrochemical Sensor with Other Fe-Containing H₂O₂ Sensors

Electrode		Linear range	Sensitivity	LOD	Source
Fe ₂ O ₃ /GC		10–150 μ M	2.5 μ A/ μ M	7 μ M	[28]
3D micro-snowflake structured α -Fe ₂ O ₃		0.1–5.5 mM	7.16 μ A/mM	10 μ M	[29]
MA- α -Fe ₂ O ₃ nanoflakes		0.05–3.145 mM	422.5 μ A/mM	22 μ M	[30]
Vertical α -FeOOH		0–6.5 mM	194.0 μ A/mM	18 μ M	[31]
γ -Fe ₂ O ₃ /Fe ₃ O ₄		0.0002–8 mM	N/A	0.05 μ M	[32]
Fe ₃ O ₄ /3D G NCs	N/A	274.15mA/mM	0.078 μ M	[33]	
PCB chip in FeCl ₂ solution	0-200 μ M	22 μ A/mM	7.14 μ M	This work	

4. CONCLUSION

Custom-made PCB chips could be used as electrochemical cell electrodes in order to create cheap mass-produced portable electrochemical sensors for rapid and easy detection of excess concentrations of H₂O₂ without involving complex laboratory equipment and specially trained operator. FeCl₂ buffer solution is a viable option for hydrogen peroxide detection by both elec-

trochemical and optical analysis methods. The suggested buffer could be used to detect hydrogen peroxide concentrations as low as 5 μ M by both transmissivity spectrum analysis and CV. In order to achieve maximal sensitivity at low hydrogen peroxide concentrations, FeCl₂ should be present in a concentration at least 30-fold higher than the target hydrogen peroxide concentration.

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