Detection of Hydrogen Peroxide with Selfmade Prussian Blue Electrodes

PSAPP-002 Electrochemical Experiment

PalmSens
Compact Electrochemical Interfaces

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1 Instructions

The following instructions will guide you to perform the experiment. The theory on which these experiments are based, can easily be checked online at palmsens.com, then search for “Introduction of Detection of Hydrogen Peroxide”. The material needed for the experiments can be ordered via https://www.palmsens.com/product/educational-kit/#contents.

2 Devices and Equipment

- EmStat / EmStatblue / PalmSens
- sensor cable
- sensor connector
- maybe a USB cable
- computing unit (PC, Laptop, notebook, tablet PC (Android), smartphone (Android))
- calculation and plotting software (Excel, Origin, MatLab, Mathematica)
- potentiostat software (PStrace, PStouch)
- ItalSens IS-C
- retort stand
- retort clamp
- beaker (electrochemical cell)
- oven

3 Chemicals

- 0.1 M $K_3[Fe(CN)_6]$ + 0.01 M HCl (prepared from solid iron salts and 0.01 M HCl solution)
- 0.1 M $FeCl_3$ + 0.01 M HCl (prepared from solid iron salts and 0.01 M HCl solution)
- 0.01 M HCl (prepared from HCl standard solution and demineralized water)
- 0.1 M KCl + 0.05 M K_2HPO_4 (pH = 5.4, prepared from solid KCl, K_2HPO_4, demineralized water and 6 M HCl to set the pH)
- H_2O_2 solution to prepare standard solutions

Remark: If you use the 50 mL beaker, you will need 40 mL of a solution for a measurement in the beaker. Smaller vessels work as well.

Note: Hydrogen peroxide decomposes at room temperature and when exposed to light. If solutions are not used right away, store them in a dark and cool place (fridge).

4 Instructions

4.1 Preparing the Prussian Blue Modified Electrode

1. Take an ItalSens IS-C electrode and cut away access plastic. Take care not to damage the lines or electrodes. The electrode should not be touched with bare hands to keep it clean.
2. Place the electrode on a flat ground and add 5 µL of 0.1 M $K_3[Fe(CN)_6]$ + 0.01 M HCl on the working electrode (WE, black dot in the middle) and afterwards 5 µL of 0.1 M $FeCl_3$ + 0.01 M HCl to the first drop. The solution should not touch the reference electrode (RE, silver stripe) or counter electrode (CE, black stripe). To mix the droplets just pump the solution a few times with a pipette.
3. Let the solution react for 10 min.
4. Rinse the electrode with 1 or 2 mL of 0.01 M HCl.
5. Put the electrode in an oven at 120 °C for an hour.
6. Store the electrode dark and dry.

7. If you have to dispose the solution keep in mind: Solutions containing iron need to be collected in a container for heavy metal containing liquid waste. The pH of the collected heavy metal solution should be alkaline (pH > 9). The container should be disposed according to local laws. The cell should be cleaned with demineralized water. Otherwise the drying solution will leave salt stains.

4.2 Characterization of the sensor

Two characterizations are made. Evaluating the behavior of the electrode in CVs with different scan rates shows if Prussian Blue is immobilized at the surface. CVs at different H_2O_2 concentrations show in which potential regions H_2O_2 can be detected.

1. Insert the Prussian Blue electrode into the sensor connector. Connect it to the potentiostat and immerse the electrode in 0.1 M KCl + 0.05 M K_2HPO_4 (pH = 5.4).
2. If a method for performing the Prussian Blue characterization was already prepared for you, load the method. If not choose *Cyclic Voltammetry* from the drop down menu. Choose the current ranges *1 µA, 10 µA* and *100 µA*. The fields *Sample* and *Sensor* are for your own notes. Since we do not want a pre-treatment of the electrode set *t condition* and *t deposition* to 0. Set the other parameters to:
    a. *t equilibrium* = 8 s
    b. *E start* = -0.3 V
    c. *E vertex1* = 0.5 V
    d. *E vertex2* = -0.3 V
    e. *E step* = 0.002 V
    f. *Scan rate* = 0.01 V/s
    g. *Number of Cycles* = 1
3. Start the measurement. Set the drop down list next to the run button from *New* to *Overlay*. Repeat the measurement for 0.02 V/s, 0.05 V/s, 0.1 V/s, 0.2 V/s, and 0.5 V/s. For the last measurement the scan rate is too high for auto-ranging. The current range 100 µA alone needs to be active.
4. Save all the curves.
5. Evaluate the data. Is Prussian Blue adsorbed at the surface? Chapter **Error! Reference source not found.** will provide some help to make a judgement. Calculate the amount of Prussian Blue on the electrode assuming the reaction is happening according to equation **Error! Reference source not found.**.
6. Set the scan rate to 0.05 V/s.
7. Prepare in 0.1 M KCl + 0.05 M K_2HPO_4 (pH = 5.4) solutions of H_2O_2 with a concentration of 20, 50, 100, 200, and 500 µM.
8. Record a CV in 0.1 M KCl + 0.05 M K_2HPO_4 and choose again *Overlay* from the drop down list. Repeat the CV for each of the solutions.
9. What effect has H_2O_2 on the CV? What potentials are suitable for H_2O_2 detection?

4.3 Make a calibration curve

1. Immerse the Prussian Blue modified electrode in 0.1 M KCl + 0.05 M K_2HPO_4 (pH 5.4).
2. If a method for performing the hydrogen peroxide detection was already prepared for you, load the method. If not choose *Amperometric Detection / Chronoamperometry* from the drop down menu. Choose the current range *1 nA* to *10 µA*. The fields *Sample* and *Sensor* are for your own notes. Since we do not want a pre-treatment of the electrode set *t condition* and *t deposition* to 0. Set the other parameters to:
    a. *t equilibrium* = 8 s
    b. *E dc* = -0.1 V
    c. *t interval* = 0.1 s
    d. *t run* = 60 s

    In this way a potential of -0.1 V versus the internal reference electrode will be applied. After 8 s the recording of the data will start. The data will be recorded for 60 s every 0.1 s.
3. Note down the current value at the end of the 60 s.
4. Repeat the measurement with 0.1 M KCl + 0.05 M K2HPO4 pH 5.4 containing 20 µM, 50 µM, 100 µM, 200 µM and 500 µM. Note down the measured current for each concentration.
5. Plot the current versus the concentration. Make a linear fit. The line is your calibration curve.

4.4 Quantification of Hydrogen Peroxide

Different samples can be tested now. Your samples should be in the same concentration range as your calibration measurements (20 µM to 500 µM).

1. Prepare your sample solution by diluting it with 0.1 M KCl + 0.05 M K2HPO4.
2. If a method for performing the hydrogen peroxide detection was already prepared for you, load the method. If not choose *Amperometric Detection / Chronoamperometry* from the drop down menu. Choose the current range *1 nA* to *10 µA*. The fields *Sample* and *Sensor* are for your own notes. Since we do not want a pre-treatment of the electrode set *t condition* and *t deposition* to 0. Set the other parameters to:
    a. *t equilibrium* = 8 s
    b. *E dc* = -0.1 V
    c. *t interval* = 0.1 s
    d. *t run* = 60 s
3. Note down the current at the end of the 60 s.
4. By using the calibration curve and the measured value calculate the concentration in the diluted sample and in the sample.
   Note: Possible samples could be a solution of hydrogen peroxide prepared by your instructor, bleach for removing stains from cloths (hydrogen peroxide solution from a drugstore) or hair dyes (around 6 %). Although hydrogen peroxide is used as a disinfectant, most freely available disinfectants are based on alcohol and not hydrogen peroxide. If you intend to use one as a sample, check the ingredients before. In glow sticks hydrogen peroxide is present as well, but be aware that they need to be disassembled carefully and that it contains hydrogen peroxide in high concentrations.
5. To dispose the used solutions neutralize them, so HCl cannot pose a threat. Afterwards boil the solution for 10 min. The hydrogen peroxide will decompose and thus the solution can be disposed in the sink.

In this experiment you detected the amount of hydrogen using a calibration curve.

Please note that teachers can request the answers to the question in the instructions, using https://www.palmsens.com/contact/