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Hydrogen peroxide detection with improved selectivity and sensitivity using constant current potentiometry

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Abstract

In this paper, a potentiometric H_2O_2 sensor based on a FET structure is presented. The sensor has a redox active gate contact such as Os-polyvinylpyridine (Os-PVP) containing the enzyme horseradish peroxidase (HRP) which has a high sensitivity to H_2O_2. The basic principle of the sensor is to measure hydrogen peroxide concentration by means of measuring the change in the work function of the electroactive gate of the FET due to its redox reaction with H_2O_2. A constant current potentiometric mode is used to improve the sensitivity of the sensor. To avoid the influence of ascorbic acid on the sensor, an additional layer of Nafion is mounted on top of the electroactive gate. The influence of the Nafion concentration on the characteristics of the sensor (sensitivity and selectivity) has also been investigated.

Keywords: Hydrogen peroxide sensor; Constant current potentiometry; Ascorbic acid

1. Introduction

The development of a hydrogen peroxide sensor has been considered important in biomedical and environmental applications. It is necessary to determine hydrogen peroxide, not only in chemical and industrial processes such as disinfection and waste-water treatment, but also as an intermediate product of an enzyme reaction in biochemical processes (for example, glucose and lactate determinations). For this purpose, many types of hydrogen peroxide sensors have been developed. In practice, measuring hydrogen peroxide by using an electrochemical (amperometric and potentiometric) sensor is suitable for quick test applications. In an amperometric sensor, hydrogen peroxide redox reactions occur at the working electrode, for example a bare Pt wire with an immobilized enzyme on top, by applying a suitable potential. One of the disadvantages of the amperometric sensors is the required high overpotential of hydrogen peroxide oxidation, which limits the selectivity of the sensor. A way to circumvent the selectivity problem encountered with amperometric hydrogen peroxide detection is the application of a potentiometric sensor. The potentiometric sensor consists of a metallic wire and a specific electrode material on top of it. This electrode material is electroactive with high catalytic properties for hydrogen peroxide. The signal of the sensor should be measured with respect to a reference electrode. To avoid the complication of using the reference electrode, a potentiometric H_2O_2 sensor based on an ^{E}MOSFET structure having a redox active gate contact such as Os-polyvinylpyridine (Os-PVP) containing the enzyme horseradish peroxidase (HRP) has been developed. However, the sensitivity of the sensor based on the conventional potentiometric principle is limited by the Nernst equation. To improve the sensitivity of the sensor, a method called ''constant current potentiometry'' has been developed [1,2]. When a small dc current is applied between a redox material and the solution, the work function of the redox material is also influenced by that current. The sensitivity of the sensor working in this mode depends on the value of the applied current and is found to be much higher than the Nernstian value. During practical use of this enzyme sensor, there is still a problem of interfering species such as ascorbic acid, which is often found in biological fluidic samples. To eliminate the influence of the ascorbic acid, a few approaches have been shown in literature. One of them is to directly immobilize an ascorbic oxidase into the sensing layer of the sensor. Another approach is to use a polymeric hydrogen peroxide permeable membrane to prevent the interference of the ascorbic acid. This membrane can be used as a matrix for immobilization of the peroxidase such as amphiphilic decyl ester derivatives of the amino acids D- and L-tyrosine [3] and poly-*o*-phenylenediamine [4,5], or can be mounted on top of the sensing layer of the sensor such as a polyelectrolyte multilayer (PEM). It has been suggested that H_2O_2 can diffuse into the PEM film poly(allylamine)/poly(vinylsulfate) or poly(allylamine)/poly(styrenesulfonate)

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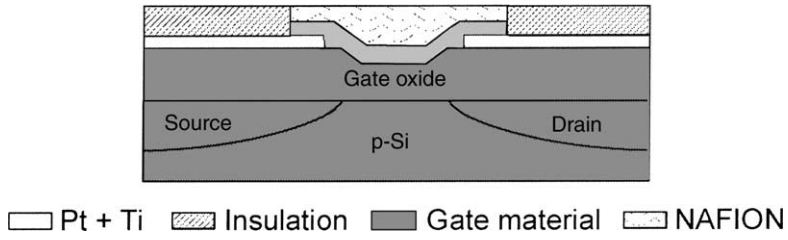

Fig. 1. Schematic cross section of the EMOSFET.

smoothly while the ascorbic acid can not penetrate the film by a size exclusion mechanism. The selectivity of the sensor depends on the difference in permeability of the membranes, due to the different molecular density or packing in the PEM films [6]. Among the materials in the group of those which are permeable for hydrogen peroxide, a negatively charged perfluorinated ionomer Nafion is a good candidate to prevent the interference of ascorbic acid as well as other anionic substances [7]. Moreover, by using Nafion the biofouling effect of the biosensors can be reduced. In this paper, the EMOSFET-based hydrogen peroxide sensor with the additional layer of Nafion on top of the electroactive polymer Os-PVP containing HRP gate will be shown. The influence of the Nafion concentration on the characteristics of the sensor (sensitivity and selectivity) will also be presented.

2. The EMOSFET-based sensor

The schematic cross section of the EMOSFET is shown in Fig. 1. Around the FET gate area, a platinum film called a remote gate electrode is deposited. Next, a thin layer of the redox active gate material, Os-PVP containing horseradish peroxidase (HRP), is deposited on top of the gate oxide of the FET, which covers the Pt remote electrode. This peroxidase is known as an enzyme which can catalyze the reduction of hydrogen peroxide [8]. A Nafion layer is mounted on top of the gate to improve the selectivity of the sensor. The basic principle of the sensor is to measure hydrogen peroxide concentration by means of measuring the change in the work function of the electroactive gate due to its redox reaction with H_2O_2 by measuring the change in the threshold voltage of the MOSFET.

The reduction of hydrogen peroxide under an enzymatic reaction with peroxidase is described as follows:

$$H_2O_2 + 2Os^{2+} + 2H^+ \leftrightarrow 2Os^{3+} + 2H_2O \quad (1)$$

From the theory of the EMOSFET, the threshold voltage, V_T, of the EMOSFET is a function of the work function or the oxidation ratio of the electroactive gate material, i.e. $[Os^{3+}]/[Os^{2+}]$, that reflects its oxidation by the hydrogen peroxide:

$$V_T = \text{const} + \frac{RT}{F\log(e)}\log\frac{(\gamma_{Os^{3+}})[Os^{3+}]}{(\gamma_{Os^{2+}})[Os^{2+}]} = \text{const} + \frac{RT}{2F\log(e)}\log[H_2O_2]$$

where γ is the activity coefficient and $[Os^{n+}]$ is the concentration of the Os^{n+} centers in the polymer, respectively. The effect of pH can be neglected when the measurement is done in a buffer solution.

It has been analyzed in [1] that because of the high catalytic property of the enzyme, the electroactive gate can fully be oxidized by a very small amount of H_2O_2 according to Eq. (1), the threshold voltage reaches very fast a saturated value. In order to increase the sensitive range of the sensor, it is necessary to keep the gate material not fully oxidized by applying an external dc reducing current. When the reducing current is applied between the gate and the solution, the threshold voltage of the EMOSFET depends not only on the oxidation ratio of the gate material, but also on the current density. Now an additional sensitivity of the sensor appears, because the exchange current density of the reaction between the hydrogen peroxide and the Os-PVP also depends on the hydrogen peroxide concentration.

$$V_T = \text{const} + \frac{RT}{2F\log(e)}\log[H_2O_2] - \frac{RT}{F\alpha\log(e)}\log\frac{I}{I_0} \quad (3)$$

where I is the applied current density I_0 is the exchange current density (also a function of $[H_2O_2]$), α is the dimensionless transfer coefficient of the reaction (1).

3. Experimental

3.1. EMOSFET fabrication

The MOSFET-structure having a gate 500 μm wide and 15 μm long was fabricated using standard NMOS processing steps without metal gate [1]. The platinum remote gate electrode was deposited by a sputtering technique. In order to insulate the relevant parts of the sensor from the solution, the sensor was packaged using Hysol® epoxy. To improve the adhesion of the epoxy, a tantalum oxide layer was deposited on top of the sensor using reactive sputtering, followed by a spin-coated polyimide layer. Commercial Os-polyvinylpyridine containing HRP (from BioAnalytical System) was deposited on top of the gate area of the FET by a coating technique. The sensor was dried for 4 h.

3.2. Nafion deposition

The diluted Nafion solutions used for coating, having a concentration in the range from 0.5 to 5%, were prepared by

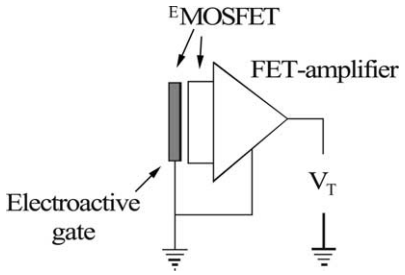

Fig. 2. Set-up used for measuring the threshold voltage of the EMOSFET.

diluting the Nafion 117 (purchased from Fluka) in water. Next, 0.2 μl of the diluted Nafion solution was dropped and spread over the gate area of the EMOSFET. Finally, the sensor was dried for 20 min in air.

3.3. Measurement set-up

Characterization of the electroactive redox gate Os-PVP before and after Nafion coating has been investigated using a PAR263A potentiostat (EG&G). The electrochemical cell consists of the Os-PVP gate electrode as a working electrode and a Radiometer saturated calomel electrode. A platinum plate having an area of 1 cm^2 was used as a counter electrode. For the threshold voltage measurement, the EMOSFET was connected to an ISFET source and drain follower as described in [2]. In this set-up, the electroactive gate was grounded to prevent any external interference (see Fig. 2). In case an external current is applied to the gate of the FET, a floating current source is used with respect to a remote Pt counter electrode. During measurement, the solution was continuously stirred at a constant rate. The solution used for the measurements was a phosphate buffer containing Na_2HPO_4, KH_2PO_4 (pH = 7.1). The stock hydrogen peroxide solution and ascorbic acid were prepared in the same phosphate buffer. All chemicals used (Merk, Fluka) were of analytical reagent grade.

4. Results and discussion

Prior to the investigation of the sensitivity of the sensor to hydrogen peroxide, the redox properties of the sensing layer have been characterized in a phosphate buffer by applying a sweep potential between 0–500 mV with respect to the SCE. Fig. 3 shows a part of the cyclic voltammogram of the redox active layer recorded at a sweep rate of 100 mV/s. The redox peaks, typical for $[Os^{2+}]/[Os^{3+}]$, are clearly visible. After deposition of the Nafion layer, the cathodic and anodic peaks diminished (dashed lines in Fig. 3), resulting from a slight reduction of the redox properties of the Os-PVP.

The response of the sensor having the Nafion layer on the gate to hydrogen peroxide has been studied by adding a small amount of the stock hydrogen peroxide solution. During measurement, a dc reducing current of 25 nA has been applied. As can be seen in Fig. 4, when the hydrogen

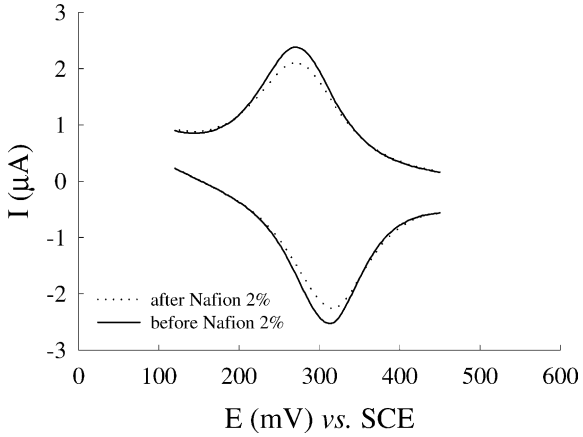

Fig. 3. Cyclic voltammogram of the electroactive gate taken before and after the Nafion coating at a sweep rate of 100 mV/s in phosphate buffer.

peroxide concentration in the solution is changed, the response time of the threshold voltage of the device with the Nafion layer prepared from the 5% Nafion solution is less than 1 min. The same experiment has been done with sensors

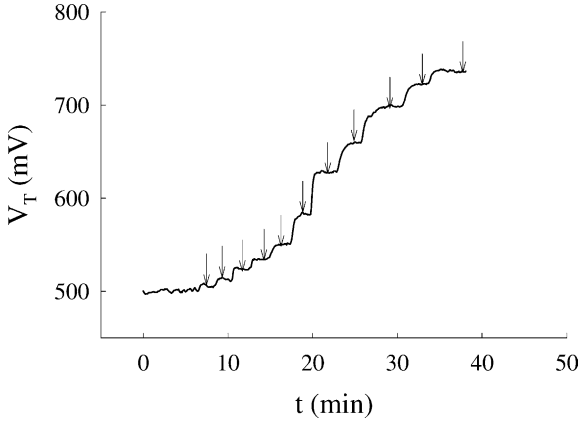

Fig. 4. Threshold voltage as a function of time when the H_2O_2 concentration in solution is changed (Nafion 5%). Arrows above the curve indicate when hydrogen peroxide is added.

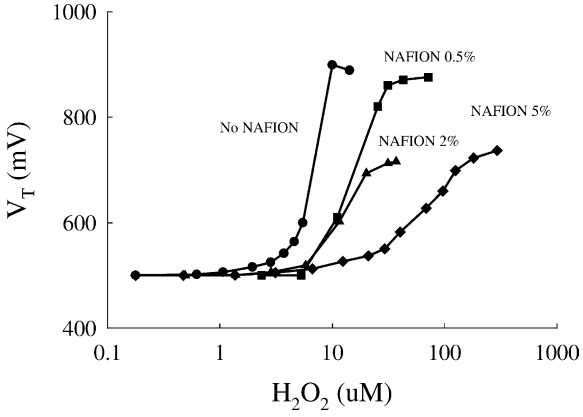

Fig. 5. Sensitivity of the sensor to hydrogen peroxide is dependent on the concentration of the Nafion in the gate (I = 25 nA).

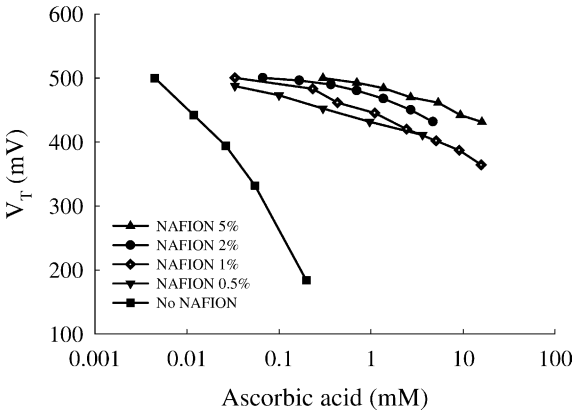

Fig. 6. The interference of ascorbic acid on the threshold voltage of the sensor at different Nafion concentrations ($I = 25$ nA).

Table 1
Some characteristics of the sensor with and without the Nafion layer on the gate

	Sensitivity to H_2O_2 (mV/dec)	V_T response to 13 μM·H_2O_2 (mV)	V_T response to 0.2 mM ascorbic acid (mV)
No Nafion	720	+390	−320
Nafion 0.5%	563	+162	−27
Nafion 2%	320	+124	−3
Nafion 5%	221	+26	0

having a different Nafion concentration. It shows that the response time of the sensor is not influenced by the Nafion concentration in the gate.

The influence of the Nafion concentration on the sensitivity of the sensor to hydrogen peroxide is presented in Fig. 5. It can be seen that the existence of the Nafion layer on the gate changes the sensitivity and the detection limit of the sensor. The sensitivity of the sensor decreases with the increasing concentration of the Nafion in the gate. The detection limit of the sensor is shifted to a higher value of the hydrogen peroxide concentration.

Fig. 6 shows the influence of the ascorbic acid on the threshold voltage of the ^{E}MOSFET. If there is no Nafion layer on the top of the gate, the signal of the sensor would significantly be changed when the ascorbic acid concentration varies in a range of 5–200 μM. With the additional Nafion layer made from the 0.5% Nafion solution, the sensor has no response to ascorbic acid concentration lower than 30 μM. In the mean time, the sensitivity of the sensor to ascorbic acid is reduced in comparison with the case without Nafion layer as shown in Fig. 6. When the concentration of the Nafion increases, the sensitivity of the sensor to the ascorbic acid remains almost the same but the detection limit is much higher. At the Nafion concentration of 5%, the sensor has no influence of the ascorbic acid when its concentration is lower than 1 mM. Some characteristics of the sensor, such as the sensitivity to hydrogen peroxide and the influence of ascorbic acid, are shown in the Table 1.

5. Conclusions

A study on the hydrogen peroxide sensor based on the ^{E}MOSFET which has the electroactive Os-polyvinylpyridine gate containing the horseradish peroxidase, has been shown. The sensor works in the constant current potentiometric mode, i.e. a constant dc current is applied between the solution and the gate of the FET while the threshold voltage of the FET is measured as the signal of the sensor. The influence of ascorbic acid on the sensor is reduced depending on the concentration of the Nafion layer that is deposited on top of the gate. The sensitivity of the sensor to hydrogen peroxide, is much higher than the Nernstian value, even in the case of added Nafion layers.

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