Toward a hydrogen peroxide sensor for exhaled breath analysis

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Abstract

In this contribution a chip-integrated amperometric sensor for the detection of H$_2$O$_2$ in exhaled breath condensate (EBC) is reported. The electrode chip is characterized, and detection of H$_2$O$_2$ in an aqueous phase is shown by means of cyclic voltammetry (CV) and amperometry. Variation of conditions such as the composition of the supporting electrolyte largely influences the obtained electrochemical response. Also it is found that electrochemical pretreatment of the platinum working electrode aiming at surface oxidation improves the detection limit of the sensor. Finally, the device is applied to measurement of H$_2$O$_2$ in the gaseous phase.

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1. Introduction

H$_2$O$_2$ has been reported at elevated levels in the EBC of individuals affected by disorders such as chronic obstructive pulmonary disease (COPD) [1]. To date typical measurement protocols encompass collection of the exhaled breath in condensation units, and subsequent H$_2$O$_2$ detection. Relevant levels of detection can be reached, however such off-line protocols are typically time and labor intensive. Thus achievement of reliable point-of-care detection is desirable, and has the potential for providing improvement in the monitoring and treatment of affected patients.

Different measurement techniques for H$_2$O$_2$ in EBC have been reported, such as spectrophotometry [2], or electrochemistry [3]. Electrochemical sensors are particularly attractive due to e.g. ease of...
miniaturization and low cost. Amperometric H$_2$O$_2$ sensors for liquid- and gas-based detection have been described. Sensors for gas analysis rely for example on a gas permeable membrane covering a supporting electrolyte solution [4], or on a polymer membrane deposited directly on the electrodes [5]. After uptake and diffusion of H$_2$O$_2$ to the electrode surface, (electrochemical) conversion results in a concentration dependent current signal. Although such approaches for electrochemical H$_2$O$_2$ sensors have been reported, no design targeted at integration with a breath sampling system has been realized. In this work we present an amperometric H$_2$O$_2$ sensor feasible for such integration.

2. Experimental

A process combining conventional lithography, metallization, and lift-off was utilized for the fabrication of electrode chips. Borofloat wafers were used as substrates. To accommodate for different electrode materials, two consecutive processes were conducted. Three electrodes were incorporated into the chips, namely a working electrode (WE), a counter electrode (CE), and a reference electrode (RE). The WE and CE consisted of a layered structure of Ta and Pt (total thickness approx. 200nm), and the RE of Ti, Pd, and Ag (total thickness approx. 550nm). Ta/Ti and Pd were used as adhesion promoters and diffusional barrier, respectively. Ag layers were chloridized through 1min immersion in 0.1M FeCl$_3$ thereby forming a Ag/AgCl RE. Fig. 1 shows a photograph of a microfabricated electrode chip. The radius of the disk-shaped WE was 1.25mm; including the contact line the total area of the WE was approx. 6.2mm$^2$.

Electrochemical measurements were performed in custom-made electrochemical cells with a Biologic potentiostat. The supporting electrolyte used during tests in solution consisted of a mixture of 0.1M KH$_2$PO$_4$/K$_2$HPO$_4$ (pH7) and 0.1M KCl. H$_2$O$_2$ was added to this solution step-wise at varying concentrations for CV and amperometry. Calibration curves were obtained by averaging current signals from amperometric response curves and plotting against respective H$_2$O$_2$ concentrations. Average current values were obtained after triplicate measurements at three different electrode chips ($n_{\text{total}}=9$). For select cases electrochemical pretreatment was performed directly before amperometry by polarizing the WE for 5min at 0.6V vs. the chip-integrated RE. For gas-based experiments a thin layer of agarose was used as a membrane material. For this purpose agarose was dispensed in the supporting electrolyte at a concentration of 2%, heated, and spin coated at the electrode chips at 500rpm for 20s leading to solidification. H$_2$O$_2$ uptake into the membrane was measured by amperometry in a closed electrochemical cell enabling exposure to H$_2$O$_2$ vapor. All chemicals were obtained from Aldrich, and deionized H$_2$O was used to prepare solutions.

Fig. 1. Photograph of a microfabricated chip. Notations at the contact pads mark electrode type.
3. Results and discussion

To study the electrochemical behavior of H₂O₂ and select appropriate potentials for consecutive amperometry CV was conducted. Fig. 2 shows cyclic voltammograms (CVs) recorded at varying H₂O₂ levels (0-5mM) in the chosen supporting electrolyte (0.1M KH₂PO₄/K₂HPO₄ (pH 7) and 0.1M KCl). As expected addition of H₂O₂ leads to an increase in current level. Preceding experiments showed that using a phosphate-buffered environment led to a favorable decrease in oxidation potential. Moreover addition of KCl to the supporting electrolyte guarantees stability of the Ag/AgCl RE. Based on CVs shown in Fig. 2, a working potential in the range of 0.4-0.5V vs. the chip-integrated RE was selected for amperometry. H₂O₂ oxidation was preferred over H₂O₂ reduction due to the targeted sensor application in an O₂ rich environment.

Fig. 3 shows a series of chrono-amperometric response curves obtained at different levels of H₂O₂. Although the calibration curve derived from these results is linear at high H₂O₂ concentrations, detection of H₂O₂ at low concentrations is limited for untreated platinum electrodes. It was discovered that electrochemical pretreatment aiming at oxidation of the platinum electrode by application of a constant potential enhances the detection limit from ~10μM to ~1μM. This is visualized in the averaged calibration curves depicted in Fig. 3B.
For the detection of H$_2$O$_2$ in the gas phase the electrode chips were coated with an agarose layer containing the optimized supporting electrolyte. A custom-built closed electrochemical cell allowing for generation of gaseous H$_2$O$_2$ (see schematic inset in Fig. 4) was used to test sensor functionality. A H$_2$O droplet containing increasing amounts of H$_2$O$_2$ served as a source for establishment of an equilibrium following Henry’s law. It can be seen in Fig. 4 that increasing the concentration of H$_2$O$_2$ in the droplet leads to a corresponding current response due to H$_2$O$_2$ uptake of the membrane. Opening the cell and removal of the droplet leads to a H$_2$O$_2$ free environment regenerating the sensor response. It is important to note that the time scale is not a measure of sensor response time since it overlays with the establishment of the equilibrium including mass transport in the gas phase.

4. Conclusions and outlook

Summarizing we have presented a chip-integrated amperometric device for detection of liquid and vapor phase H$_2$O$_2$. Electrochemical pretreatment of the WE improves the detection limit of the sensor. Current experiments are targeted at the integration of the gas sensor with a flow-through sampling system incorporating a cooling element for condensation of the (exhaled breath) sample at the sensor location.

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References