



Available online at www.sciencedirect.com



Procedia Engineering 00 (2011) 000–000

**Procedia
Engineering**

www.elsevier.com/locate/procedia

Proc. Eurosenors XXV, September 4-7, 2011, Athens, Greece

Toward a hydrogen peroxide sensor for exhaled breath analysis

J. Wiedemair, H. D. S. van Dorp, W. Olthuis, A. van den Berg a*

MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217, 7500AE Enschede, The Netherlands

Abstract

In this contribution a chip-integrated amperometric sensor for the detection of H_2O_2 in exhaled breath condensate (EBC) is reported. The electrode chip is characterized, and detection of H_2O_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_2O_2 in the gaseous phase.

© 2011 Published by Elsevier Ltd.

Keywords: hydrogen peroxide; amperometric sensor; gas phase detection; exhaled breath condensate

1. Introduction

H_2O_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_2O_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_2O_2 in EBC have been reported, such as spectrophotometry [2], or electrochemistry [3]. Electrochemical sensors are particularly attractive due to e.g. ease of

* Corresponding author. Tel.: +31 53 489 2755; fax: +31 53 489 3595.
E-mail address: j.wiedemair@utwente.nl.

miniaturization and low cost. Amperometric H_2O_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_2O_2 to the electrode surface, (electrochemical) conversion results in a concentration dependent current signal. Although such approaches for electrochemical H_2O_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_2O_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. 6.2mm².

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 $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH7) and 0.1M KCl. H_2O_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_2O_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_2O_2 uptake into the membrane was measured by amperometry in a closed electrochemical cell enabling exposure to H_2O_2 vapor. All chemicals were obtained from Aldrich, and deionized H_2O was used to prepare solutions.

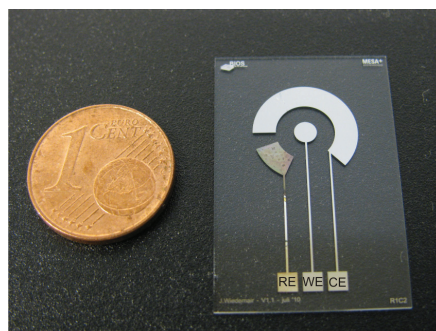


Fig. 1. Photograph of a microfabricated chip. Notations at the contact pads mark electrode type.

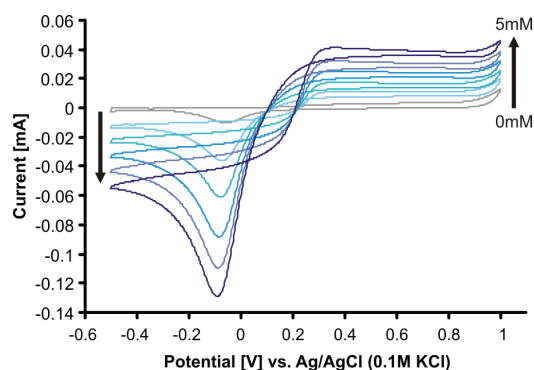


Fig. 2. Cyclic voltammograms in 0.1M $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7) and 0.1M KCl (grey trace), and after addition of 1-5mM H_2O_2 (blue traces; scan rate: 50mV/s). Arrows indicate the current increase upon H_2O_2 addition.

3. Results and discussion

To study the electrochemical behavior of H_2O_2 and select appropriate potentials for consecutive amperometry CV was conducted. Fig. 2 shows cyclic voltammograms (CVs) recorded at varying H_2O_2 levels (0-5mM) in the chosen supporting electrolyte (0.1M $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH7) and 0.1M KCl). As expected addition of H_2O_2 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. H_2O_2 oxidation was preferred over H_2O_2 reduction due to the targeted sensor application in an O_2 rich environment.

Fig. 3A shows a series of chrono-amperometric response curves obtained at different levels of H_2O_2 . Although the calibration curve derived from these results is linear at high H_2O_2 concentrations, detection of H_2O_2 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 $\sim 10\mu\text{M}$ to $\sim 1\mu\text{M}$. This is visualized in the averaged calibration curves depicted in Fig. 3B.

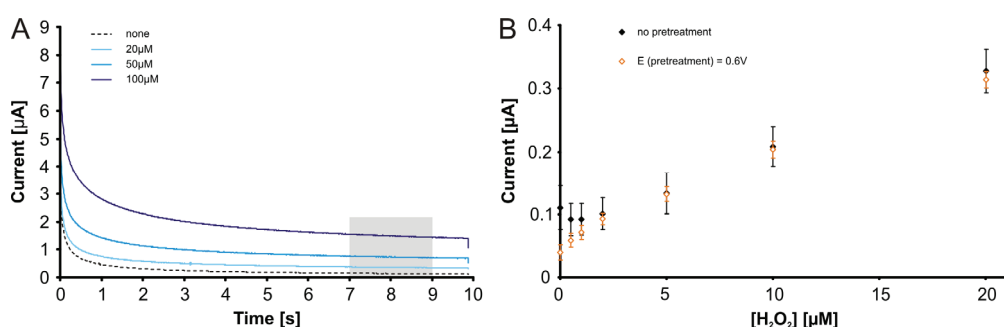


Fig. 3. (A) Chrono-amperometric response curves and (B) resulting calibration curves obtained while biasing chip-integrated WEs at 0.5V vs. Ag/AgCl RE for 10s and adding H_2O_2 to 0.1M $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7) and 0.1M KCl. Averaged current values in the grey shaded area of (A) were used to extract calibration curves shown in (B). Averaged calibration curves ($n=9$) for untreated and electrochemically pretreated (5min at 0.6V vs. Ag/AgCl RE) electrode chips are compared.

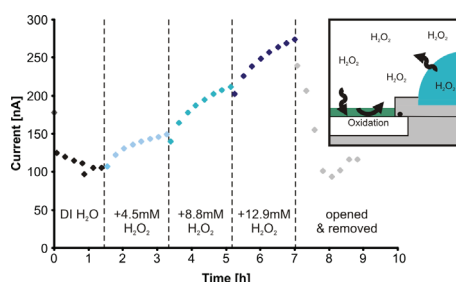


Fig. 4. Current response of agarose-coated electrode chips to increasing concentrations of H_2O_2 vapor. Current values are extracted from amperometry recorded while varying the H_2O_2 concentration (0–12.9mM) in the H_2O droplet, as well as removing the droplet for membrane regeneration. The inset shows a scheme of some of the processes occurring in the closed cell.

For the detection of H_2O_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_2O_2 (see schematic inset in Fig. 4) was used to test sensor functionality. A H_2O droplet containing increasing amounts of H_2O_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_2O_2 in the droplet leads to a corresponding current response due to H_2O_2 uptake of the membrane. Opening the cell and removal of the droplet leads to a H_2O_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_2O_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.

Acknowledgements

This project is part of the Nano4Vitality program, financed by the Ministry of Economic Affairs and the provinces Gelderland and Overijssel.

References

- [1] Montuschi P. Exhaled breath condensate analysis in patients with COPD. *Clin Chim Acta* 2005;**356**:22–34.
- [2] Dekhuijzen PN, Aben KK, Dekker I, Aarts LP, Wielders PL, van Herwaarden CL, et al. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1996;**154**: 813–6.
- [3] Marek E, Muckenhoff K, Streckert HJ, Becher G, Marek W. Measurements of L-lactate and H_2O_2 in exhaled breath condensate at rest and mild to moderate exercise in young and healthy subjects. *Pneumologie* 2008;**62**:541–7.
- [4] Kulys J. Flow-through amperometric sensor for hydrogen peroxide monitoring in gaseous media. *Sens Act B* 1992;**9**:143–7.
- [5] Benedet J, Lu D, Cizek K, La Belle J, Wang J. Amperometric sensing of hydrogen peroxide vapor for security screening. *Anal Bioanal Chem* 2009;**395**:371–6.