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Procedia Engineering 120 (2015) 540 – 543

**Procedia  
Engineering**[www.elsevier.com/locate/procedia](http://www.elsevier.com/locate/procedia)

EUROSENSORS 2015

## Detection of adrenaline based on substrate recycling amplification

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### Abstract

An amperometric enzyme biosensor has been applied for the detection of adrenaline. The adrenaline biosensor has been prepared by modification of an oxygen electrode with the enzyme laccase that operates at a broad pH range between pH 3.5 to pH 8. The enzyme molecules were immobilized via cross-linking with glutaraldehyde. The sensitivity of the developed adrenaline biosensor in different pH buffer solutions has been studied.

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Peer-review under responsibility of the organizing committee of EUROSENSORS 2015

*Keywords:* Adrenaline; laccase; biosensor; substrate recycling;

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### 1. Introduction

Catecholamines such as adrenaline, noradrenaline and dopamine play a significant role in the central nervous system as neurotransmitter. They are of rising interest in medical diagnostics and can serve as biomarker. For example, adrenal gland tumors can overproduce hormones and are a frequent cause of hypertension. An adrenal venous sampling (AVS) procedure is used for tumor localization and differential diagnosis. Since adrenaline concentration in adrenal veins is much higher than in the periphery, the concentration gradient of adrenaline can be used as an indicator for the correct insertion and positioning of the catheter in the adrenal vein and successful AVS procedure.

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Different analytical methods have been realized for the determination of catecholamines such as adrenaline, including high-performance liquid chromatography (HPLC) that is the most used technique for analysis, because of its high sensitivity and selectivity [1]. Fluorescence spectroscopy [2, 3], mass spectrometry [4] and chemiluminescence [5] are further detection methods. Although, these methods generally have good sensitivity and reproducibility, nevertheless, they require expensive instruments, well-controlled experimental conditions and extensive sample pretreatment. Therefore, in the last few years, more attention has been paid to develop simple electrochemical tools for the adrenaline detection. The electrocatalytic oxidation of neurotransmitters at different kinds of electrodes, like a glassy carbon electrode modified with a cobalt(II) hexacyanoferrate film or single-wall carbon nanotubes as well as a semiconductor tin(IV) oxide electrodes modified with a luminol film has been described in [6-8]. Electrochemical detection techniques offer several advantages such as remarkable sensitivity, substantial miniaturization and portability [9]. However, these techniques are insufficient for the detection of catecholamine in the concentration range of biological media ranging from micromolar to nanomolar levels.

The detection limit of adrenaline can be improved by the application of a substrate recycling principle using a two-enzyme system: a laccase for the oxidation of adrenaline and a pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (GDH) from *Acinetobacter calcoaceticus* for reduction reaction of the quinone species [10]. However, the optimum working pH of the adrenaline sensor described in [10] was around pH 6 that limits its application for the adrenaline detection in biological solutions. In the presented work, we report on adrenaline detection using the enzyme laccase from the company AB Enzymes, which has been modified to have a broad activity at pH values between pH 3.5 to pH 8. The results of the investigation of the adrenaline sensitivity in different pH buffer solutions are presented.

## 2. Materials and methods

### 2.1. Chemicals

Glutaraldehyde, bovine serum albumin (BSA) and the buffer components were purchased from Sigma-Aldrich (USA). The oxygen sensor was from Atlas Scientific (USA). The enzyme laccase was provided by AB Enzymes (Germany). Cellulose acetate filter with a pore size of 0.2  $\mu\text{m}$  was purchased from Sartorius Stedim Biotech GmbH (Germany). Adrenaline was purchased from Sanofi-Aventis GmbH (Germany).

### 2.2. Preparation of the adrenaline biosensor

The adrenaline biosensor was realized by modification of a commercial oxygen sensor with an enzyme membrane (see Fig. 1). The enzyme laccase operating at a broad pH range (pH 3.5 to pH 8) [11] has been chosen in order to apply the sensor in biological media. For the preparation of the enzyme membrane, 20  $\mu\text{L}$  of the enzyme laccase solution was mixed with 20  $\mu\text{L}$  of BSA (10 vol%) and 40  $\mu\text{L}$  of a mixture of glutaraldehyde (2 vol%) and glycerol (10 vol%) solutions. The resulting volumetric ratio of all components was 1/1/2 (enzyme/BSA/glutaraldehyde-glycerol). A total of 60  $\mu\text{L}$  of the membrane cocktail was then dropped onto a Teflon block. After drying for 24 h at 4  $^{\circ}\text{C}$ , the enzyme membrane was fixed with the help of a cellulose acetate filter (dialysis membrane) onto the high-density polyethylene (HDPE) layer of the oxygen sensor.

### 2.3. Electrochemical sensor characterization

For the electrochemical characterization, the adrenaline biosensor was connected to a potentiometer (2007 Multimeter, Keithley Instruments). The sensor measures the oxygen consumption due to the oxidation of adrenaline by the enzyme laccase. The produced output voltage (delivered by galvanostate oxygen sensor) is proportional to the concentration (partial pressure) of the oxygen in the solution. The sensitivity of the biosensor to adrenaline was investigated in different pH buffer solutions. In addition, the dependence of the sensor signal on adrenaline concentration measured in buffer solution of pH 8 was studied. Therefore, different kinds of buffers covering the pH range between pH 5 and pH 9 were prepared. Citrate buffer (0.1 M) was used for the measurements in the pH range

between 5 and 6, phosphate buffer (0.1 M) in the pH range between 6.5 and 8.4 and tris buffer in the pH range between 8.5 and 9.

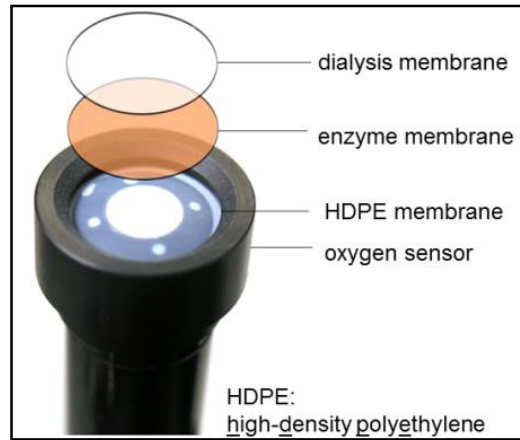


Fig. 1. Adrenaline sensor arrangement with the enzyme membrane.

### 3. Results and discussion

Fig. 2 depicts adrenaline sensitivity of the developed biosensor in buffer solutions with different pH values from pH 5 to pH 9. A maximum sensitivity has been observed in buffer solution of pH 8.

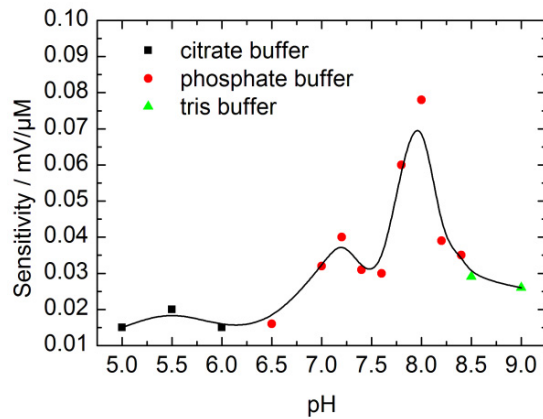


Fig. 2. Determination of pH optimum for adrenaline biosensor with the enzyme laccase.

Fig. 3 demonstrates the dynamic response of the developed adrenaline sensor measured in phosphate buffer solutions (pH 8) with different adrenaline concentrations. The calibration curve was linear in a wide concentration range of 3 –100 μM adrenaline with a slope of -0.079 mV/decade.

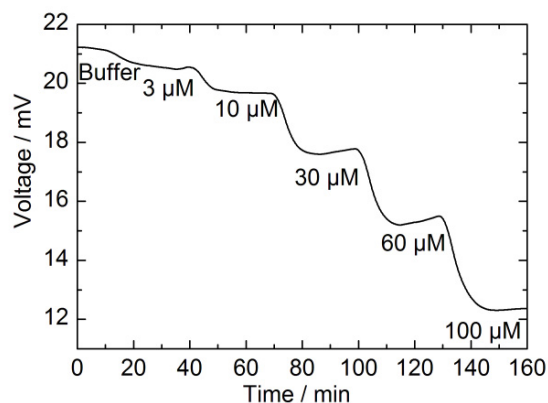


Fig. 3. Dynamic response of the adrenaline sensor measured in phosphate buffer solution (pH 8) with different adrenaline concentrations from 3  $\mu\text{M}$  to 100  $\mu\text{M}$ .

#### 4. Conclusions and outlook

In this work, an amperometric biosensor for the detection of adrenaline has been presented. By using an enzyme laccase from company AB Enzymes, the maximum adrenaline sensitivity has been achieved in a buffer solution with pH value around pH 8. With the developed biosensor, adrenaline could be detected down to 3  $\mu\text{M}$ . In the next step, a two-enzyme adrenaline biosensor based on the substrate recycling principle will be developed to improve the lower detection limit.

#### Acknowledgements

This research is supported by a PhD scholarship from FH Aachen. The authors would like to thank AB Enzymes for providing a fungal laccase.

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