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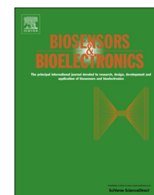

Short communication

Alcohol vapour detection at the three phase interface using enzyme-conducting polymer composites

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ABSTRACT

Immobilisation of enzymes on a breathable electrode can be useful for various applications where the three-phase interface between gas or chemical vapour, electrolyte and electrode is crucial for the reaction. In this paper, we report the further development of the breathable electrode concept by immobilisation of alcohol dehydrogenase into vapour-phase polymerised poly(3,4-ethylene dioxythiophene) that has been coated onto a breathable membrane. Typical alcohol sensing, whereby the coenzyme β-Nicotinamide adenine dinucleotide (NADH) is employed as a redox-mediator, was successfully used as a model reaction for the oxidation of ethanol. This indicates that the ethanol vapour from the backside of the membrane has access to the active enzyme embedded in the electrode. The detecting range of the sensor is suitable for the detection of ethanol in fruit juices and for the baseline breath ethanol concentration of drunken driving. After continuous operation for 4.5 h the system only showed a 20% decrease in the current output. The electrodes maintained 62% in current output after being refrigerated for 76 days. This work is continuing the progress of the immobilisation of specific enzymes for certain electrochemical reactions whereby the three-phase interface has to be maintained and/or the simultaneous separation of gas from liquid is required.

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1. Introduction

Vapour-phase polymerisation (VPP) is seen as a versatile polymerisation method for conducting polymers (CPs) and has been used widely in the past few years. It is based on the polymerisation of condensed monomer from vapour onto an oxidant film. Among the advantages of VPP are that there is no need for the substrate to be conductive, and the produced films are well ordered and highly conductive (Winther-Jensen and West, 2004; Winther-Jensen et al. (2008a).

Enzyme immobilisation is an approach that has previously been carried out using various techniques with the aim of achieving effective electron-transfer either directly to an electrode or through redox mediators (Meredith and Minteer, 2012). Recently, we have developed the 'stuffing method' to simply incorporate enzymes or biological molecules into VPP poly(3,4-ethylene dioxythiophene) (PEDOT) (Chen et al., 2006; Thompson et al., 2010; Thompson et al., 2013) *via* physical entrapment. Direct electron-transfer between a surface-bound enzyme and CP has been observed (Thompson et al., 2010), prompting the development of a solid-state pH sensor based on the same redox system (Thompson et al., 2013). For biological application, the PEDOT-poly (ethylene glycol) and PEDOT-gelatin composites have also been found to be biocompatible and suitable for cell growth (Bongo et al., 2013; Jimison et al., 2012).

In previous works (Winther-Jensen et al. (2008b); Winther-Jensen, MacFarlane (2011); Winther-Jensen et al. (2012)), we have used a porous hydrophobic PTFE-based membrane (Goretex®) to develop an efficient three phase-interface structure for a porous air-electrode. The primary advantage of using such a material as the substrate for an air-electrode is that gas is able to diffuse through the membrane while liquid water cannot. This promotes the formation of an efficient three-phase interface that can be maintained during operation (Winther-Jensen et al., 2008b, 2012). However, the idea of incorporating biological species into the CP electrode was not pursued in these works. The immobilisation of enzymes on porous, structured electrodes in order to increase the surface-area, and subsequently the activity and detection limit is widely developed (Meredith and Minteer, 2012; Tamaki, 2012). There are a few publications demonstrating the successful use of enzyme-based sensors in detecting alcohol or vapour from the headspace (Hämmerle et al., 1996; Hämmerle et al., 2011; Schlangen et al., 2012). In each case, the enzyme was contained in the electrolyte solution adjacent to the working gas diffusion electrode. For bio-fuel cell application, a few works have reported the construction of biocathodes by incorporating enzymes e.g. laccase (Gellett et al., 2010; Gupta et al., 2011a; Rincón et al., 2011),

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Birilubin oxidase (Gupta et al., 2011b), or Copper efflux oxidase for the oxygen reduction reaction (Kontani et al., 2009;Meredith and Minteer, 2012). Bio-sniffers (Gessei et al., 2009;Kudo et al., 2010) have also been developed to detect ethanol and aldehyde. In these reports, the enzyme was immobilised by mixing with either carbon paste or hydrophilic polymer as a 'host' in order to keep the enzyme attached to the conducting platform. These processes frequently involved solvent exposure or mechanical force, conditions which could be too harsh for some biological species. The 'stuffing' method described in this work is considered a 'gentle' approach to the incorporation of an enzyme into the CP for biological purposes.

Here, we report the incorporation of ADH into PEDOT using the 'stuffing' method. The PEDOT/ADH electrode is then further integrated into a breathable electrode configuration. A few alcohols were tested and reduced β-Nicotinamide adenine dinucleotide (NADH) oxidation was used to probe the conversion of alcohols to aldehydes. Scanning electron microscopy (SEM) was used to investigate the presence of embedded ADH in PEDOT. The main purpose of this exercise is to further integrate the breathable membrane concept into biological applications by the incorporation of biological species into CPs.

2. Materials and methods

2.1. Materials

40% Iron (III) para-toluenesulphonate (Fe(III)TOS) in butanol and 3,4-ethylene dioxythiophene were purchased from Yacoo Chemicals Co., Ltd. NADH, β-Nicotinamide adenine dinucleotide hydrate (NAD^+) and ADH from saccharomyces cerevisiae (A3263) were purchased from Sigma Aldrich. Pyridine was from BDH Chemicals. Di-sodium hydrogen phosphate (Na_2HPO_4) and ethanol were purchased from Merck.

2.2. VPP PEDOT and ADH stuffing procedure

VPP PEDOT was prepared from the spin-coating of oxidant solution (24 μl pyridine in 1 ml of 40% Fe(III)TOS) onto FTO glass or Au-coated Goretex® (plasma-treated with maleic anhydride (Ademovic et al., 2005)) and dried in 70 °C oven for 40 s before VPP in EDOT chamber at 70 °C for 30 min. The unwashed PEDOT film was stored in a 45 °C oven to prevent crystallisation of Fe(III) TOS. The enzyme solution was prepared strictly as follows. 15 mg/ml (ADH) was added in 0.01 M phosphate buffer (PB) pH 8.2 and gently mixed to dissolve. 1 M Na_2HPO_4 pH 9 was added to the enzyme solution at 80 μl for every 1 ml of 0.01 M PB pH 8.2. The enzyme solution was cast as a droplet over the unwashed PEDOT film, left for 10 min, and then gently made to wick away from the PEDOT surface using tissue paper. This step was repeated twice before rinsing the PEDOT/ADH electrode with water and drying under N_2. The electrode was either used fresh or stored in a fridge.

2.3. Electrochemical set-up

The electrode was tested in a 3-electrode cell. SCE or Ag/AgCl (3 M NaCl) and Pt wire were used as a reference and counter electrode, respectively. 0.1 M PB pH 8.2 was used as the electrolyte in all experiments as it is the optimal pH for ADH (Liu and Cai, 2007).

2.4. Breathable experiment set-up

The PEDOT/ADH electrode on Au-coated Goretex® was laminated with a gold strip leaving an exposed working area of 0.5 × 0.7 cm^2. The laminated electrode was then assembled as a separator between two plastic chambers as illustrated in the scheme in Fig. 1A. 0.1 M PB pH 8.2 containing 0.8 mM NAD^+ was used as an electrolyte in the front chamber. The back chamber was sealed with an attached syringe ready for ethanol injection. The electrode was then held at 0.25 V until a steady-state current was reached. Various concentrations of aqueous ethanol solution were injected into the back chamber. The total volume of the back chamber was constant at 60 ml while the injected ethanol solution ranged from 3 ml to 6 ml.

2.5. SEM

The samples were sputter-coated with a thin gold layer and SEM was performed using a JEOL 7100F Field Emission Gun Scanning Electron Microscope at 5 kV.

3. Results and discussion

3.1. Enzyme incorporation and alcohol oxidation reaction

A schematic showing the oxidation of alcohol occurring at the three-phase interface between the NAD^+/NADH redox couple, PEDOT/ADH electrode, and ethanol vapour entering from the backside of the Goretex® membrane is given in Fig. 1B. SEM images of the ADH-stuffed PEDOT film clearly shows enzyme globules and a large amount of surface agglomeration occurring

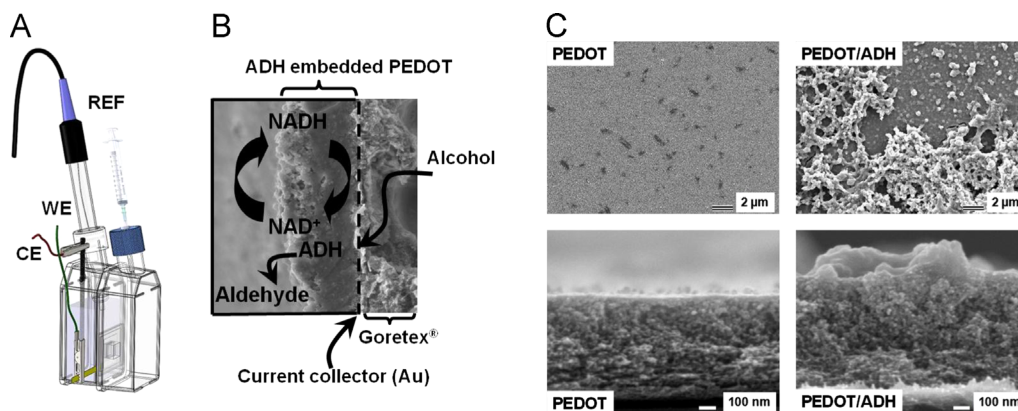

Fig. 1. (A) Schematic of the experiment set-up showing the electrochemical chamber on the left attached to the alcohol vapour supply chamber on the right, (B) Schematic of the three phase-interface reaction between PEDOT/ADH, alcohol vapour and NAD^+/NADH redox couple in the electrolyte, and (C) SEM images of the PEDOT (left column) and PEDOT/ADH (right column) films on FTO glass.

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