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Third-generation glucose biosensor incorporated in a conducting printing ink

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Abstract

A biosensor based on a conducting screen-printing ink for the direct amperometric measurement of glucose is described. Carbon electrode prints, containing the work, reference and auxiliary electrodes, are used as the substrate for the sensor. The active parts of the carbon ink are the redox enzyme glucose oxidase and the conducting polymer polypyrrole, which can communicate directly with each other. As a result, the biosensor is largely independent of the oxygen concentration. The feasibility of the described biosensor ink for screen printing is shown.

Introduction

The employment of conducting polymers in the field of biosensors has been extensive [1-4]. Most of the work has been focused on the enzyme immobilising properties of the polymers and only a few examples of large redox enzymes communicating directly with electrode materials are known today [5, 6]. The poor electrochemistry of enzymes and other biological receptors is ascribed to the fact that the redox-active centres of these molecules are usually surrounded by a thick, insulating protein shell [7, 8]. To realise direct electrochemical interaction an electrode material is required that penetrates the insulating shell and communicates effectively with the active centre inside. Polypyrrole has been suggested to be such a material and, therefore, has been studied by many researchers for this purpose with varying success [6, 9-13].

Recently, we reported on the direct interaction of glucose oxidase adsorbed on the internal surface of polypyrrole microporous structures [14-16]. We have constructed third-generation glucose sensors from these conducting polymer microstructures [17, 18], which detect glucose amperometrically, without the need of oxygen or artificial mediators as is the case in first [19] and second [11] generation biosensors.

One of our third-generation glucose sensor systems is based on sub-micron latex beads in which polypyrrole is deposited electrochemically [16]. Glucose oxidase has been adsorbed irreversibly inside the microporous structure of this composite membrane. The catalytic activity of the enzyme is retained in this structure and it was shown that glucose oxidase communicates directly with the conducting polymer [20]. In order to be more flexible in the future production of biosensors, we had the idea to incorporate the active parts of the biosensor into a conducting printing ink. When this is accomplished, mass production of third-generation glucose sensors, using screen-printing techniques is feasible [21-23]. In this paper we present results on the behaviour of the biosensor system when this is incorporated into a conducting carbon ink. It will be shown that the properties of the ink-based sensor are essentially the same as those of the original biosensor system.

Experimental

Glucose oxidase (EC 1.1.3.4) type X-S (122 000 U/g) from Aspergillus niger and catalase (EC 1.11.1.6) from bovine liver were obtained from Sigma. Benzoquinone was from Aldrich (Germany) and was sublimed prior to use. Pyrrole was from Merck and was distilled before use. Latex suspensions (2.5% solid, particle size 250 nm) were from Polysciences. Agarose type VIII was purchased from Sigma. Electrodag 421 SS graphite-based polymer thick film ink was a gift from Acheson Colloiden bv, Netherlands. All other reagents were of analytical grade.

The used galvanostat was a home-made instrument. Its current output was monitored with a digital multimeter (Fluke 77). The enzyme activity assay was
performed using an Autolab potentiostat controlled by an Olivetti M24 personal computer and General Purpose Electrochemical System (GPES) software (Eco Chemie, Netherlands). The amperometric biosensor measurements were performed using an Antec EC Controller (Antec Leyden, Netherlands). Current output was recorded on a Yew 3056 pen recorder.

Preparation of glucose sensor ink

The latex-poly(pyrrole) biosensors were prepared as described previously [16]. Glass slides (φ 29 mm) were used as the substrate for casting the latex membranes. The prepared biosensor membranes were removed from the glass slides, mortared and sieved through a 63 μm sieve. The membrane materials was weighed and mixed with the appropriate amount of conducting ink. To construct a glucose sensor from the biosensor ink, the latter was applied to the working electrode of a pre-printed carbon electrode print (Fig. 1). The reference electrode consisted of an Ag/AgCl-ink layer which was placed on top of the carbon layer. The biosensing ink layer was dried at room temperature overnight.

Enzyme activity assay

Enzyme activity was assayed with a three-electrode cell containing 5 mM benzoquinone and 0.5 M glucose in 20 ml PBS (pH 7.5). Prior to use, the glucose solution was allowed to mutarotate for at least 24 h. The assay was performed with a platinum rotating-disk electrode (RDE) (6 mm diameter) equipped with an Electrocraft Corporation model E550 motor and E552 speed control unit. The platinum working electrode was set at a potential of 0.350 V (versus Ag/AgCl reference electrode) and was rotated at a speed of 2000 rpm. A platinum wire was used as the auxiliary electrode. The solution was flushed with nitrogen before each experiment. During the assay nitrogen was blanketed over the solution. The actual assay was performed by monitoring the current output of the RDE while immersing a sample containing the enzyme into the solution.

Amperometric biosensor measurements

To perform amperometric measurements, the printed glucose sensors were connected to a potentiostat. The potential of the sensor was set at the desired value. The printed biosensor was placed in a stirred buffer solution. When the background current had diminished sufficiently, the buffer solution was replaced by a glucose solution and the current response of the biosensor was recorded.

Results and discussion

Preparation of biosensor ink

Latex membranes were cast on freshly sputtered platinum layers from an aqueous solution of 0.05 wt.% of agarose and 1.25 wt.% of the suspended latex beads [16, 24]. The membrane was subsequently dried at low temperature (277 K), which resulted in a uniform layer without cracks. After heat treatment (333 K, 1 h) a very strong layer was obtained. Constant current electropolymerisation of poly(pyrrole) was performed with the latex-membrane electrodes by supplying a current of 20 mA/cm² to the membrane while immersing it in 0.3 M pyrrole in phosphate-buffered saline solution (PBS) for the appropriate amount of time [20]. After rinsing in PBS, the polymer-modified electrode was immersed in a solution of glucose oxidase (5 mg/ml) for several hours, after which it was dried overnight. The prepared latex-poly(pyrrole) biosensors were removed from the substrate material, ground and sieved (63 mesh). The sieved material was weighed and mixed with the appropriate amount of graphite ink (vide infra). The biosensor ink was stored at 253 K when not used immediately. To construct glucose sensors, the active material was screen printed on the carbon working electrode of a pre-printed electrode configuration, which also contained the reference (Ag/AgCl ink) and the auxiliary (carbon ink) electrode (Fig. 1). After printing, the biosensors were dried at room temperature overnight.

Five different compositions of biosensor ink were prepared by varying the amount of biosensor material, viz. 1, 2, 4, 10 and 50% (dry weight of biosensor:weight of graphite ink), respectively. Except for the last mentioned one, the printing properties of these compositions could not be distinguished from the plain carbon ink. All the ink responded well to glucose (vide infra).

Sensor activity assay

The conducting ink containing the biosensor material was tested separately (i.e. independent of the amperometric biosensor performance) for enzymatic activity by means of an assay, described previously [25, 26]. In this assay the natural co-substrate for glucose oxidase is replaced by the artificial electron acceptor benzoquinone. This acceptor is reduced to hydroquinone, which can be detected electrochemically at a rotating-
disk electrode. The resulting current is a measure for the enzymatic activity of the sample. In Fig. 2 the enzyme activity is shown of a 27 mg sample of a 50% biosensor:ink composite. The current of the rotating-disk electrode increases immediately upon introduction of the ink sample into the measurement cell (Fig. 2, point 1). Withdrawal of the sample causes the slope of the current to return to the initial value (spontaneous oxidation of glucose by benzoquinone). This shows that the enzyme is properly immobilised inside the biosensor ink. Improperly immobilised enzyme would have stayed in solution, causing the current slope to be higher after removal of the sample (point 2 in Fig. 2). Repeated introduction of the sample did not lead to a change in the amount of active, immobilised enzyme present (points 1–4). A semi-quantitative calibration of the measured enzyme activity was achieved by introduction of a known amount of glucose oxidase into the cell (120 mIU, point 5 in Fig. 2). It was concluded that approximately 60 mIU of active glucose oxidase was present in the measured quantity of the 50% biosensor ink. The fact that the biosensor–ink composition is enzymatically active and remains stable shows the feasibility of the process.

**Amperometric measurements**

Biosensors were constructed by applying the enzymatically active ink on the various printed working electrodes (Fig. 1). The ink was allowed to dry (room temperature) after which the three-electrode print was inserted in buffer and connected to a potentiostat. The current response of the biosensor to glucose was measured at various potentials. The results are shown in Fig. 1.

![Fig. 1](image1)

**Fig. 1**. Measurement of the enzyme activity of a 50% biosensor ink by monitoring enzymatically produced hydroquinone at a rotating-disk electrode. 1 and 3: Introduction of ink sample; 2 and 4: withdrawal of ink sample; 5: introduction of 120 mIU of glucose oxidase in the solution.

![Fig. 2](image2)

**Fig. 2.** Measurement of the enzyme activity of a 50% biosensor ink by monitoring enzymatically produced hydroquinone at a rotating-disk electrode. 1 and 3: Introduction of ink sample; 2 and 4: withdrawal of ink sample; 5: introduction of 120 mIU of glucose oxidase in the solution.

Fig. 2. It can be seen from the Figure that at low potentials the response to glucose is strongly dependent on the applied potential. At higher potential the dependence is less dramatic. In accordance with previous results [14, 16, 27] we found that at a potential of 100 mV versus Ag/AgCl reference the current due to the addition of glucose still resulted in a positive current response, whereas the deliberate addition of a small amount of hydrogen peroxide (0.0025 wt.%) resulted in a strongly negative response [28–30]. This experiment shows that the response to glucose must be the result of direct electronic contact between the enzyme molecules and the conducting polymer indicating that oxygen mediation is not an important mechanism (see also below).

In a second series of experiments, the sensors were poised at a potential of 0.35 V and placed in solutions containing different concentrations of glucose. The results for concentrations up to 15 mM are shown in Fig. 4. In this Figure the response under nitrogen atmosphere is shown, as well as under ambient atmosphere. The former measurements were conducted in the presence of the enzyme catalase. This enzyme was added to eliminate any enzymatically produced hydrogen peroxide. It can be seen that there is only a slight difference in current response between the two measurements, viz. only at high glucose concentrations. Although oxygen mediation is present, we conclude, taking into account the hydrogen peroxide experiment at low potential *(vide supra)*, that direct communication between the redox enzyme and the conducting polymer is the major mechanism in our sensors [31].
The technology described above can be applied to produce disposable biosensors based on a number of commercially interesting redox enzymes and we are currently investigating this in more detail.

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References


Biographies

Cees Koopal studied chemistry at the University of Utrecht and received his Ph.D. degree in organic chemistry from the University of Nijmegen in 1992. He joined the Department of Microbiology at TNO-Nutrition in 1993 and his current research is on projects concerning biological quality and safety.

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