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A novel, disposable, screen-printed amperometric biosensor for glucose in serum fabricated using a water-based carbon ink☆

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Abstract

Screen-printed amperometric glucose biosensors have been fabricated using a water-based carbon ink. The enzyme glucose oxidase (GOD) and the electrocatalyst cobalt phthalocyanine were mixed with the carbon ink prior to the screen-printing process; therefore, biosensors are prepared in a one-step fabrication procedure. Optimisation of the biosensor performance was achieved by studying the effects of pH, buffer strength, and applied potential on the analytical response. Calibration studies were performed under optimum conditions, using amperometry in stirred solution, with an operating potential of +500 mV versus SCE. The sensitivity was found to be 1170 nA mM^{-1}, with a linear range of 0.025–2 mM; the former represents the detection limit. The disposable amperometric biosensor was evaluated by carrying out replicate determinations on a sample of bovine serum. This was achieved by the method of multiple standard additions and included a correction for background currents arising from oxidisable serum components. The mean serum concentration was calculated to be 8.63 mM and compared well with the supplier's value of 8.3 mM; the coefficient of variation was calculated to be 3.3% ($n = 6$).

Keywords: Screen-printed; Amperometric; Biosensor; Hydrogen peroxide; Glucose; Biocomposite ink

1. Introduction

Exact and rapid determinations of glucose are essential in the diagnosis and management of diabetes, therefore, there has been much interest in the design and development of biosensors for this purpose. The first reported biosensor was developed in 1962 by Clark and Lyons (1962), who utilised the decrease in oxygen partial pressure bought about from the oxidation of glucose by the enzyme glucose oxidase (GOD). The use of electrocatalytic mediators can allow detection of enzymatically produced hydrogen peroxide (H_2O_2) at significantly reduced applied potentials, whereby signals from interfering species are minimised. The use of such mediators has led to a wealth of publications on biosensor systems utilising H_2O_2 sensitive base transducers coupled to oxidase enzymes. Carbon, in its different forms, has a wide potential window, is inexpensive to manufacture, and is robust; making it a very popular electrode material. Screen-printed carbon electrodes (SPCEs) can be mass-produced at low cost, and were employed in the fabrication of the commercially successful pen-sized, 30-s blood glucose biosensor (Matthews et al., 1987). In addition, SPCEs have been used for the construction of sensors and biosensors for a wide variety of analytes, as reviewed recently (Hart et al., 2004). However, the fabrication processes involved in most of these devices are quite complex and a simpler process is highly desirable.

Carbon screen-printing inks usually consist of carbon particles, to impart electrical conductance, a polymeric binder, to provide mechanical stability, and a solvent (normally organic), to dissolve the binder. Many enzymes are denatured

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by the organic solvents themselves or by the elevated temperatures required in the curing process, making the bulk incorporation of enzymes into the ink matrix problematic. Water-soluble polymers have been used to incorporate more delicate enzymes such as lactate oxidase (LOD) into water-based inks (Hart et al., 1999), although this results in the need for an additional membrane layer to prevent dissolution of the electrode material by the test solution. Rohm et al. (1996) described an ultraviolet-polymerisable water-based paste containg LOD and graphite. The biosensors were used in a flow–injection system without the need for a membrane. However, separate screen-printed layers were required for the conducting tracks and the biocomposite ink, and a large overpotential was required (+600 mV).

In order to overcome interference problems, electrocatalytic compounds are often used to modify the electrode in order to accelerate the electron transfer kinetics, and thus reduce the required potential. Many different electrocatalysts have been studied, including noble metals, organic dyes, and organometallic complexes. Electro-plating of copper (Kumar and Zen, 2002), sputtering of palladium and gold (Gorton, 1985), and incorporation of platinised carbon particles (Albareda-Sirvent and Hart, 2002) are all examples of metal mediators. Although these systems effectively reduce the required overpotential for H_2O_2 detection, they require several preparation steps, are expensive, and may also catalyse the oxidation of interfering substances. Iron hexacyanoferrate (Prussian Blue) allows peroxide detection at around 0 V (Ricci et al., 2003), but is water soluble, so problems with diffusion into the sample occur. The complex is also unstable above pH 7, so is of little use in analysis of biological fluids. Compounds with low solubility are desirable, such as cupric hexacyanoferrate (Wang and Zhang, 1999), manganese dioxide (Turkusic et al., 2001), and cobalt phthalocyanine (CoPC) (Gilmartin et al., 1995b; Boujtita et al., 2000).

CoPC has been shown to be an effective electrocatalyst for the electrochemical oxidation of H_2O_2; (Gilmartin et al., 1995a). The molecule is insoluble in all aqueous and most organic solvents, so problems with dissolution into the sample are removed. The material can simply be added into carbon ink and screen-printed, to allow simple production of mediated H_2O_2 sensors. CoPC was considered to offer the attraction of a generic approach to biosensor production, as there are many oxidase enzymes that generate H_2O_2. Consequently, the development of biosensors for substrates of these enzymes should be readily achievable.

To our knowledge, there are no reported strategies for the one-step screen-printing of amperometric biosensors suitable for use in stirred conditions, using water-based biocomposite ink. There are considered to be many advantages to the elimination of organic solvents from the printing process, such as improvements in health and safety and environmental impact. This paper describes the characterisation and application of a novel glucose biosensor fabricated using a new water-based carbon ink containing glucose oxidase and cobalt phthalocyanine. The ink employed in the current investigation is a result of a collaborative research project between the University of the West of England and Gwent Electronic Materials Ltd. (GEM product code: C2030901R2).

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of analytical-reagent grade and obtained from Sigma–Aldrich (Gillingham, Dorset, UK). Unless stated otherwise, the supporting electrolyte used throughout was 0.05 M phosphate buffer, which was prepared from stock solutions of 0.2 M sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate. These were mixed to give pH 8.0, and diluted with deionised water (Purite Select HP340, Thame, UK) to 0.05 M. Hydrogen peroxide solutions were prepared daily from a 30% stock solution. The stock solution was periodically checked by titration against standardised potassium permanganate. Standard solutions of β-D-glucose were prepared at least 24 h before required, and allowed to mutarotate at room temperature. Adult bovine serum (Sigma) of known glucose concentration was defrosted on arrival, divided into sterile tubes, and stored at −40 °C until required.

2.2. Apparatus

All electrochemical measurements were carried out using a three-electrode system comprising of a screen-printed biosensor working electrode, a saturated calomel reference electrode (BAS, Indiana, USA), and a platinum wire counter electrode. The cell contents were stirred at a constant rate using a cross-headed magnetic stirring disc and stirrer. The temperature was maintained at 25 °C by means of a water-jacket and circulating water bath (Haake D3, Saddle Brook, USA). pH was monitored using a pH meter (Fisherbrand Hydrus 400) equipped with a glass pH electrode (Russel, UK). The meter was calibrated over an appropriate range using standard buffer tablets. An Autolab PSTAT 10 computer-controlled potentiostat (Windsor Scientific, Slough, UK) was used for all electrochemical studies.

2.3. Procedures

2.3.1. Biosensor fabrication

Biosensors were printed using a DEK 1202 semi-automatic screen-printing machine (Weymouth, UK), equipped with a 156 threads/in. polyester screen, a polyurethane squeegee, and stainless steel flood blade. Biosensors were printed in groups of six onto white PVC card of 0.5 mm thickness, as described previously (Gilmartin et al., 1995b). However, in the present method, no heating step was required in order to cure the ink; the biosensors were simply left to dry overnight at room temperature. When dry, the biosensors were kept over silica gel at 4 °C. Before use, individual