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Glucose biosensors based on thick film technology

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Abstract: Various techniques for the fabrication of thick film biosensors are described. With electrodes printed on conventional thick film substrates, glucose is measured via the formation of hydrogen peroxide using glucose oxidase. Additional diffusion barriers extend the linear range of the sensor up to 15 mM. Using a new, flexible, unfired substrate material sensors with a three-dimensional structure can be made. The resulting cavities can be filled with carbon paste. Hence, the mediator technique and the use of dehydrogenases was applied to the fabrication of thick film biosensors. The linear range, sensitivity and stability of these sensors is strongly dependent on preparation methods such as the immobilization technique (mediated electrode) and the amount of cofactor and enzyme. Without any diffusion barrier the linear range is limited to about 1 mM for the sensor based on the dehydrogenase.

Keywords: thick film, disposable sensors, enzyme electrode, amperometric device, glucose, glucose oxidase, glucose dehydrogenase, mediator.

INTRODUCTION

In the field of electronics thick film technology is known to be a versatile tool for the fabrication of miniaturized electronic circuits. It is based on the screen printing and firing of various pastes differing in composition and electrical properties and classified as conductor, resistor, dielectric and soldering pastes (Reichl, 1988). Recently the application of thick film technology has been extended to other fields, particularly to sensors and transducers, as it became apparent that it offers a number of special options: firstly, by using suitable pastes, electrodes can be made comparable to 'normal' noble metal electrodes. Hence it should be possible to transfer electroanalytical methods to thick film sensors (Baumbach, 1981). Secondly, some pastes have special physical properties, such as piezoresistance, which can be used in non-electrochemical sensors (Prudenziati & Morten, 1986). Thirdly, the necessary electrical parts of the sensor can be fabricated by the same process, so that miniaturized intelligent sensors can be made (Hoffheins *et al.*, 1987). Fourthly, the whole device is cheaper than those prepared using other technologies (e.g. thin film) applicable to the construction of 'integrated sensors' and it is possible to produce disposable sensors at a rather low price even on a medium scale. Some electrochemical thick film sensors have already been described in literature. They are either potentiometric ion-sensitive sensors (Belford *et al.*, 1987; Bechthold *et al.*, 1989) or amperometric devices for the detection of oxygen (Karagounis *et al.*, 1986; Schönauer, 1989) and other electro-

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chemically active substances (Lambrechts *et al.*, 1988). Some of these sensors were used for the detection of glucose, immobilizing glucose oxidase on the working electrode (Lambrechts *et al.*, 1987; Liu, 1987). This principle can be extended to the detection of substrates of other oxygen-dependent enzymes.

Disposable biosensors for medical applications based on electrochemical methods are already known (Matthews, 1987; Weetall & Hotaling, 1987/1988), but they are fabricated by special techniques and materials. In this paper we demonstrate the application of thick film technology, as it is already being applied in some companies, to the construction of biosensors with commercially available thick film materials and its flexibility by implementing new thick film substrate materials. The fabrication of multilayer sensors enables the application of this technology to be extended to mediated systems and to sensors based on cofactor dependent dehydrogenases. Glucose was chosen as a model analyte in all cases.

EXPERIMENTAL

Thick film electrodes

All thick film electrodes were obtained from Siegert GmbH, Cadolzburg, FRG. The layout is shown schematically in Fig. 1. Strips with 4–5 electrodes were used, each being a working electrode in the electrochemical circuit. Hence, they were printed using noble metal pastes, namely platinum, gold or palladium. The fabrication was according to two different techniques:

(a) Conventional thick film technology
Platinum paste was printed on substrates made of fired Al_2O_3 ceramics. After firing at 850°C, the conducting paths were isolated by printing and firing a dielectric paste. The structures obtained by this process are more or less two-dimensional (thickness of the two layers 10–20 μm each) and nearly all procedures known for the fabrication of enzyme electrodes can be applied.

(b) 'Green tape' technology
The substrate material 'green tape' (DuPont) is a non-fired, flexible ceramic which is drillable, punchable and millable. Special thick film pastes

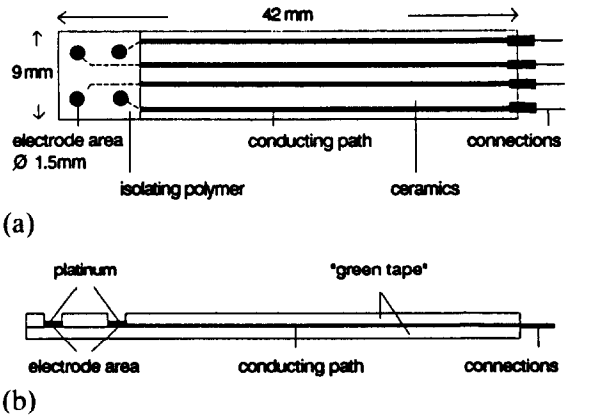

Fig. 1. Schematic layout of the thick film sensors obtained from Siegert GmbH, Cadolzburg, FRG: (a) top view of a conventional thick film electrode; (b) longitudinal section of a 'green tape' electrode.

are printed on this material and covered by a second substrate layer to isolate the conducting paths and to generate cavities on top of the electrode areas. The whole structure is pressed and fired so that a very compact three-dimensional device results. It has already been shown that this process is suitable for the fabrication of potentiometric sensors (Bechthold *et al.*, 1989), as the cavities (depth 0·2 mm) can be filled with an ion-selective membrane. This idea was adopted and the cavities were filled with modified carbon paste, resulting in a wide flexibility of thick film sensors.

Enzyme electrodes

Enzyme electrodes were made by three different methods, depending on the detection principle:

(a) H_2O_2 measurement
Platinum electrodes were printed on conventional thick film substrates. To improve the adhesion of the enzyme layer these electrodes were modified by oxidation at 2·5 V for 5 min and derivatization for 30 min in a solution (10%) of 3-aminopropyltriethoxysilane (Aldrich) in toluene or phosphate buffer. After washing 3 μl of a solution of glucose oxidase (GOD; EC 1.1.3.4; from *Penicillium amagasakiense*; Nagase Biochem. Ltd., Tokyo, Japan), bovine serum albumin (BSA; fraction V, Boehringer) and glutaraldehyde (GA, 2·5%, Merck) (1·2 mg GOD, 2·5 mg BSA, 15·7 μl GA in 100 μl phosphate buffer, pH 7·0) were placed on the electrodes. To increase the linear range of the sensor, it can be

covered with a diffusional barrier by dipcoating in a dispersion of polymethacrylic acid esters in water (Eudragit NE 30D; Röhm Pharma GmbH, Weiterstadt).

The measurements were done at 600 mV versus an Ag/AgCl reference electrode, the electrochemical set-up being completed by a platinum counter-electrode and a potentiostat from Metrohm, model VA 641.

(b) Use of mediators

The mediator technique was applied by using the so-called 'green-tape' electrodes. The cavities on the sensor strips were filled with carbon paste (Deutsche Metrohm GmbH, Filderstadt). Hence, known techniques could be applied: the mediator tetrathiafulvalene (TTF, Fluka) was adsorbed by using a solution in toluene (20 mg/ml) and the glucose oxidase was immobilized either by carbodiimide (Aston, 1987), through a Schiff base (Bradley *et al.*, 1989) or by cross-linking with glutaraldehyde (as described above). As usual, the measurements were done at 220 mV versus an Ag/AgCl electrode.

(c) NADH oxidation

Glucose dehydrogenase (EC 1.1.1.47; from Bacillum megaterium; Merck) and b-nicotinamide-adenine-dinucleotide (NAD; Biomol, Hamburg) were mixed with carbon paste (Deutsche Metrohm GmbH, Filderstadt), resulting in an overall composition of 5% enzyme and 10% cofactor. This mixture was pressed in the cavities of the 'green tape' electrodes. The NADH formed by the enzyme reaction was re-oxidized at 600 mV versus an Ag/AgCl reference electrode.

All measurements were done in phosphate buffer (0·1 M; pH 6·7 for glucose oxidase and pH 7·5 for glucose dehydrogenase) by adding various amounts of a glucose stock solution.

RESULTS AND DISCUSSION

In thick film technology many pastes are known to be suitable for the printing of electrodes. These pastes are conventionally used for conducting paths and contain a metal powder as conductor which may be silver, gold, platinum and/or palladium. Figure 2 shows several calibration curves obtained with thick film platinum electrodes based on glucose oxidase immobilized by cross-linking with glutaraldehyde. The

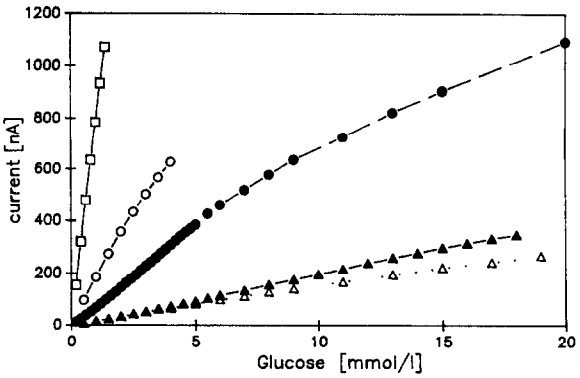

Fig. 2. Calibration curves obtained with thick film platinum electrodes. The enzyme layer is covered with a diffusion barrier to increase the linear range. Eudragit solutions of different compositions were used: □, without membrane; ○, 3%; ●, 6%; △, 9%; ▲, 12% dispersion.

response time of these sensors was 12 s (95% of the steady-state value) and the standard deviation between sensors made in the same process was 7%. The linear range of these glucose electrodes could be extended from 1 mM (without diffusional barrier) to about 15 mM by using a membrane made of a 12% solution of Eudragit, but at the same time sensitivity was drastically decreased.

This principle of sensor fabrication can be extended to the use of other oxidases, as it is based on the oxidation of H_2O_2. To reduce the influence of other electrochemically active compounds such as ascorbic acid which can be oxidized at the same electrode potential, either additional membranes are necessary, for example cellulose acetate, or the applied potential has to be reduced. This can be done by the use of mediators which substitute the oxygen in the enzyme reaction and are re-oxidized at a lower potential than H_2O_2. Known mediators are tetrathiafulvalene (TTF) (Turner *et al.*, 1987), ferrocene and its derivatives (Cass *et al.*, 1984) and tetracyanoquinodimethane (Kulys & Cenas, 1983). As these compounds are insoluble in water they can be used to modify electrodes by physical adsorption, with graphite being the most favourable electrode material. Using commercially available thick film pastes, so far none of the printed electrodes resulted in a stable, mediated enzyme electrode. Hence, another technique was chosen for the application of mediators to thick film sensors. They were made of the 'green tape' material and carbon paste was pressed in the resulting cavities. Figure 3 shows a typical calibration curve

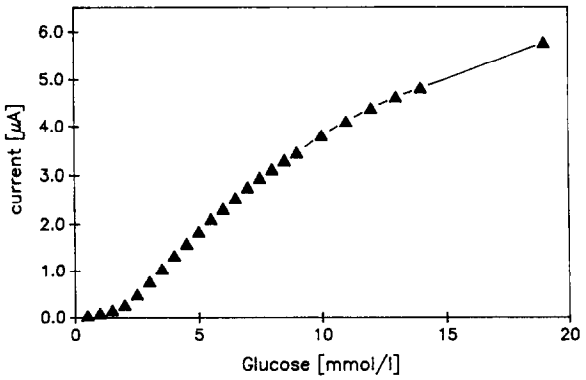

Fig. 3. Calibration curve obtained with a 'green tape' electrode, using TTF as a mediator. Glucose oxidase is immobilized via a Schiff base.

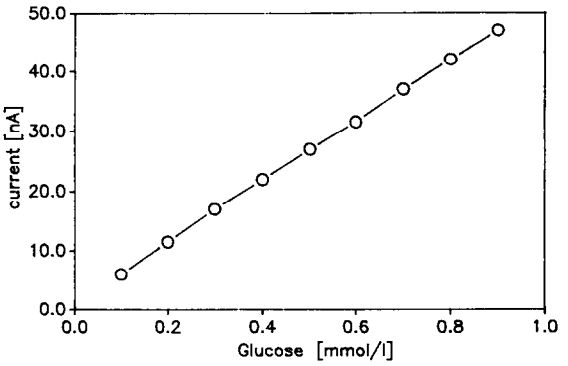

Fig. 5. Calibration curve obtained with a 'green tape' biosensor using a carbon paste electrode modified with glucose dehydrogenase and the cofactor NAD.

obtained with TTF as mediator and glucose oxidase immobilized via a Schiff base. The sensitivity, linear range and long-term stability were strongly dependent on the immobilization method (Fig. 4). The best results with respect to the long-term stability were obtained by cross-linking the enzyme with glutaraldehyde. However, these sensors showed the poorest sensitivity and the shortest linear range (up to 2 mM).

In some cases it may be necessary or at least advantageous to use a dehydrogenase as the biological part of the biosensor instead of the oxidase. 'Reagentless' sensors can be made by combining 'green tape' electrodes with modified carbon paste, i.e. the cofactor does not have to be added to the analyte. Figure 5 shows part of a typical calibration curve. The sensitivity was mainly dependent on the amount of cofactor within the paste (increasing with increasing amount of cofactor), which may be less than 1%. Without any diffusion barrier the linear range is limited to about 1 mM. The long-term stability of these sensors was influenced by the amount of enzyme within the paste. It could be stored in the refrigerator for several weeks, if it contained about 5% enzyme, and was ready for use after re-calibration. To reduce the potential which has to be applied to the electrodes, chemical modifiers can be added to the paste, for example the organic metal NMP^+ $TCNQ^-$ (Kulys, 1981).

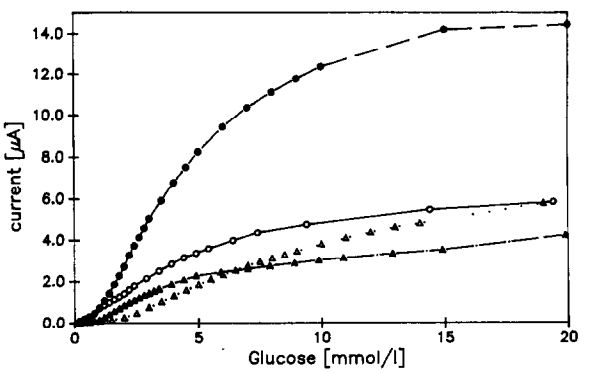

Fig. 4. Comparison of sensitivity and linear range of 'green tape' sensors using TTF as a mediator. Glucose oxidase is immobilized by different methods: O, adsorption on carbon paste; ●, immobilization using carbodiimide; △, immobilization via a Schiff base; ▲, cross-linking with glutaraldehyde.

CONCLUSION

Thick film technology is a well-known technique in the field of electronics and was successfully applied to the fabrication of biosensors. It could be demonstrated that most of the known techniques for immobilizing enzymes, extending the linear range, minimizing the influence of interfering substances and modifying electrodes can be adopted to thick film biosensors. Additionally this technology allows the fabrication of multi-enzyme sensors, as the electrode area may be less than 1 mm^2 and hence several working electrodes can be printed on one strip together with the reference and the counter-electrode.

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