

## APPLICATION OF A PLANAR ENZYME MICROELECTRODE TO BIOPROCESS MONITORING

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Using microelectronic technology it has been possible to develop disposable glucose electrodes (1). The transducer is realised using thin-film deposition and lift-off techniques, and consists of two Pt electrodes (working and counter) and an Ag/AgCl reference electrode. This transducer is covered with an enzyme membrane. The hydrogen peroxide formed by the enzyme catalysed reaction is measured at + 0.6 V.

For use in FIA, such electrodes must meet certain specifications as to response time, sensitivity, reproducibility, accuracy, and electrode lifetime. We have investigated the performance of electrodes in an FIA system with particular reference to the measurement of glucose at low concentration in culture media. Typical performance characteristics are -

- a) a lifetime of 14 days
- b) a sensitivity ( peak height ) of  $30 \mu\text{A M}^{-1}$  at a flow rate of  $1.4 \text{ ml min}^{-1}$  and sample volume of  $190 \mu\text{l}$
- c) a noise level below  $5 \text{ pA}$
- d) a linear response from  $1.0 \mu\text{M}$  to  $1 \text{ mM}$  glucose

The detection limit is determined not by the signal / noise ratio, but by the presence of interfering compounds within the sample. We have determined quantitatively the level of this interference during mammalian cell culture, and have shown it to be much smaller than expected. It is possible to include additional membrane layers in construction of the sensor. These would be designed to reduce such interference and thus increase specificity. Our results suggest that this may not be necessary.

### 1 Introduction

Amperometric biosensors based on FAD containing oxidases are commercially available for use in FIA systems. The sensor usually consists of a two or three electrode cell. This cell is covered with a multi-layer enzyme membrane, which needs to be replaced at regular intervals. An alternative strategy would be to construct a disposable electrode. This would have the advantage of potential mass production; and possible advantages of microelectrode performance compared to normal size electrodes.

## 2 Materials and methods

### 2.1 Electrode fabrication

Planar electrode cells were fabricated as described (1). A platinum working electrode is separated from platinum counter electrode by a Ag/AgCl reference electrode. A glutaraldehyde cross linked glucose oxidase / albumen layer was formed across all three electrodes as described (2); or by spreading 1 - 2  $\mu\text{l}$  of a buffered (0.1 M NaPhosphate pH 7.0) solution of glucose oxidase (50mg/ml Boehringer Type 1) and albumen (80 mg/ml Sigma Fraction V) over the surface of the electrode and subsequent exposure to glutaraldehyde vapour (50% aqueous solution) in a covered petri dish at r.t. for two hours. Electrodes were stored in 50 mM sodium phosphate pH 7.0, 0.15 M KCl, 1.0 mM sodium azide (buffer A). Electrodes were housed in one of two flow cells, the first of which was equipped with an external reference electrode (Metrohm) situated downstream of the microelectrode.

### 2.2 Flow injection conditions

The flow injection unit used was constructed in this institute (3). Buffer A served as carrier stream, and was also used to dilute samples and standards. Samples of 190  $\mu\text{l}$  were injected, typically at a flow rate of 1.4 ml  $\text{min}^{-1}$ , and at ambient temperature. Sample throughput was 45  $\text{h}^{-1}$ . The signal from the potentiostat (Metrohm 641) was fed over a chart recorder to the microprocessor, where it was evaluated as peak height and integral. Samples from bioprocesses were sterile filtered (Millipore 0.2  $\mu\text{m}$  cellulose acetate), stored at  $-20^{\circ}\text{C}$  prior to analysis and subsequently diluted with buffer A before injection to give glucose concentrations in the range 2-40  $\mu\text{M}$ .

### 2.3 Removal of glucose from samples

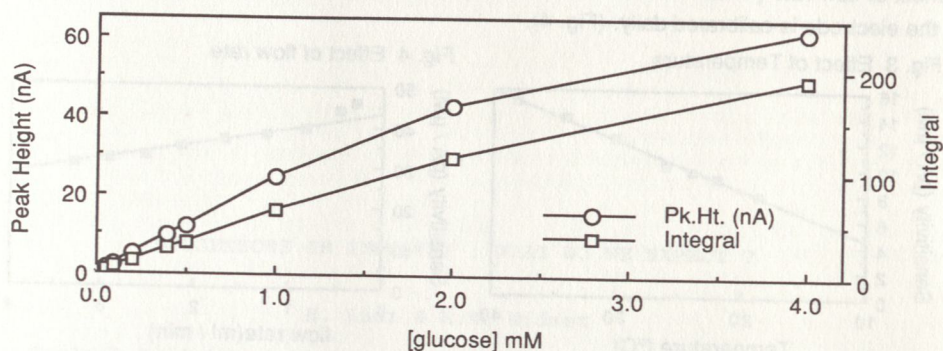
To remove endogenous glucose, samples were diluted (minimum 1 / 10) with buffer A (without Na Azide) to give a glucose concentration below 200  $\mu\text{M}$  (higher concentrations necessitate the repeated saturation with air), and 4.9 ml of this solution was mixed with 0.1 ml glucose oxidase solution (100 mg  $\text{ml}^{-1}$ , Boehringer Grade III), and incubated at room temperature for 20 minutes.

## 3 Results

### 3.1 Electrode sensitivity, accuracy, dynamic range and signal to noise ratio.

A typical calibration curve is shown in Fig. 1. The sensitivity observed varied in the range 5 - 50 pA /  $\mu\text{M}$ , depending upon the thickness of the enzyme layer, thin layers showing higher sensitivity. The noise level was 1-4 pA, allowing routine measurements at 1.0 nA full scale (typically in the range 1.0 - 40  $\mu\text{M}$  glucose). The relative standard deviation ( $n = 8$ ) was 1.5% and 0.9% for 5  $\mu\text{M}$  and 40  $\mu\text{M}$  standards respectively. For peak height a linear range was observed up to 1.0 mM, allowing one point calibration. Variations due to changes in oxygen tension are avoided by dilution with buffer, and by operating at low glucose levels.

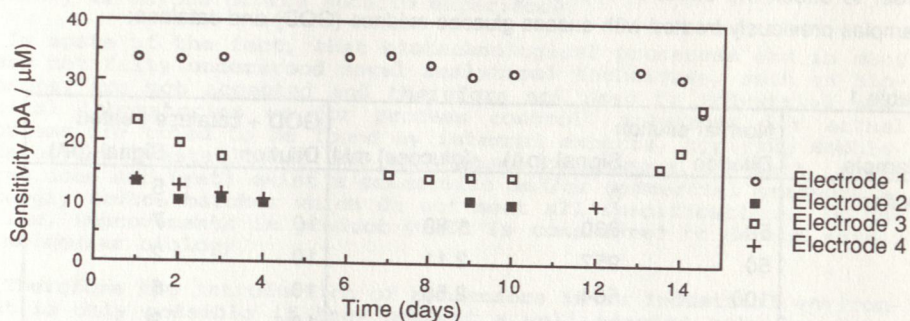
Fig. 1 Glucose calibration curve



### 3.2 Electrode lifetime

The long term stability of four electrodes is depicted in Fig. 2. Some electrodes showed an initial loss in sensitivity over the first two days, presumably due to enzyme wash-out, whereupon a stable response was observed for up to fourteen days when in constant use.

Fig. 2 Long-term stability of electrodes



Changes in the observed day to day sensitivity could be correlated to fluctuations in the ambient temperature. Electrode failure was characterised by a short period of highly variable response, followed by short circuiting. Visual inspection indicated physical and chemical deterioration of the electrodes as the cause. The enzyme layer appeared stable, except in the case of very thin membranes, when a loss of response was observed after several days, which could be recovered by application of a new enzyme layer. Use of the external reference electrode did not increase the lifetime.

### 3.3 Effect of temperature and flow rate upon sensitivity

A linear dependence of the sensitivity upon temperature was recorded in the range 18 - 38°C, with coefficient of  $0.38 \mu\text{A M}^{-1} \text{K}^{-1}$  (Fig. 3). This corresponded to 4.6 % change  $^{\circ}\text{C}^{-1}$  in the response to  $40 \mu\text{M}$  glucose at 20°C. Where the electrode is subject to rapid temperature fluctuations, some form of temperature control or compensation is required. By contrast the

effect of flow rate ( linear variation above  $1.0 \text{ ml min}^{-1}$ ) is of a magnitude that can be neglected if the electrode is calibrated daily. (Fig. 4).

Fig. 3 Effect of Temperature

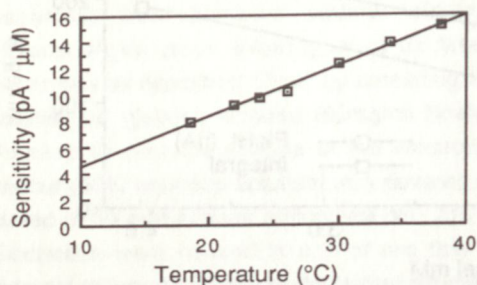
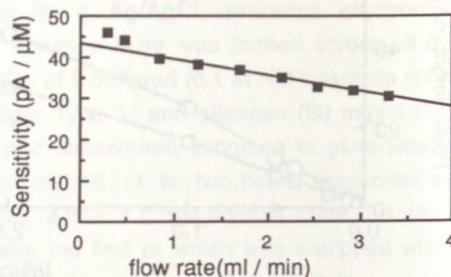


Fig. 4 Effect of flow rate



### 3.4 Measurement of bioprocess samples

In general we observed good correlation between results obtained with our FIA system and with a commercial glucose analyser (Yellow Springs 23 A). Below  $0.5 \text{ mM}$  (sample concentration before dilution), the FIA system gave consistently more reproducible results. In order to check the effect of interfering substances, a series of assays were performed on samples previously treated with excess glucose oxidase (GOD) and catalase:

Table 1

Sample	Normal dilution			GOD + catalase treated	
	Dilution	Signal (pA)	[glucose] mM	Dilution	Signal (pA)
Buffer		0			5
1	50	880	1.89	10	7
2	50	957	2.11	10	2
3	100	584	2.56	10	6
4	150	838	4.66	10	3
5	300	513	2.85	30	8
6	150	824	4.58	10	8

These preliminary results obtained during the cultivation of mammalian cell lines suggest that the level of interference is low enough to enable measurements down to  $10 \mu\text{M}$  during the continuous monitoring of bioprocesses.

### 4 References

- Gernet S. et al Sensors and actuators (1989) 18 59-68
- Koudelka M. et al Ibid (1989) 18 157-165
- Stierli J. et al Accompanying presentation