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NOTE

# Glucose biosensor with polymer microencapsulated enzyme

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**This paper describes a new technique of immobilization of enzyme on an amperometric biosensor to enhance response as well as stability. Glucose oxidase (GOD) was chosen as the model enzyme and cellulose acetate butyrate (CAB) as the polymer for encapsulation. The encapsulation was achieved by multiple emulsion technique. GOD was also immobilized by cross-linking with glutaradehyde directly on the surface of the electrode and results from a solution of 2 m*M* glucose were compared. The microencapsulated GOD electrodes proved to be superior to cross-linked GOD electrodes both with respect to response and stability.**

Magner[1] has described the development of glucose biosensor starting from early electrodes to present day mediator modified electrodes. An amperometric glucose biosensor was developed by Miao *et al.*[2] using chitosan matrix cross-linked with glutaraldehyde. However very little activity was observed on immobilization of enzymes by microencapsulation. Microencapsulation within thin-wall spheres, the next approach to enzyme immobilization, was introduced several years ago by Chang[3]. The thin-wall of the sphere is semipermiable such that the enzymes are physically prevented from diffusing out of the microcapsule while the reactants and products can readily permeate the encapsulating membrane. In the present work, we have improved the device with the help of microencapsulated GOD in a polymer film (cellulose acetate butyrate, CAB). We have compared the response and activity retention capability with time (two weeks) and shown that microencapsulation of enzymes gives better response and better stability. The method used is unique.

## Results and Discussion

Initial tests were focussed on determining the minimum enzyme activity required to generate appreciable response from the system. A series of tests were performed using GOD immobilized with glutaraldehyde having activities 5, 10, 25, 50, 75, 100, 200 and 400 mU to determine the minimum activity of enzyme required. The strength of glucose test solution was increased from 1 to 40 m*M* by adding calculated volume of 2 *M* glucose solution at an interval of 240 s. The enzyme electrodes with activities from 5–75 mU showed insignificant response. The electrodes with 100 mU enzyme activity showed no substrate saturation within the same concentration range. The 200 mU GOD electrodes showed moderately high values of response. Thus we have decided to keep the enzyme loading in the sensor at 200 mU per electrode. Two different studies were done. A number of electrodes were coated with microencapsulated GOD and also the same number were coated with GOD without microencapsulation. A comparative study was made for both types from the (i) response for a 2 m*M* glucose solution, (ii) stability of the electrodes for up to about 2 weeks by noting the performance with the electrode every day and also with different unused electrodes made on day one. The results are depicted in Figs. 1 and 2, which show that the response of the microencapsulated electrode is higher than ordinary GOD electrode at any point of time. Fig. 1 shows stability of the electrodes with respect to retaining enzyme activity

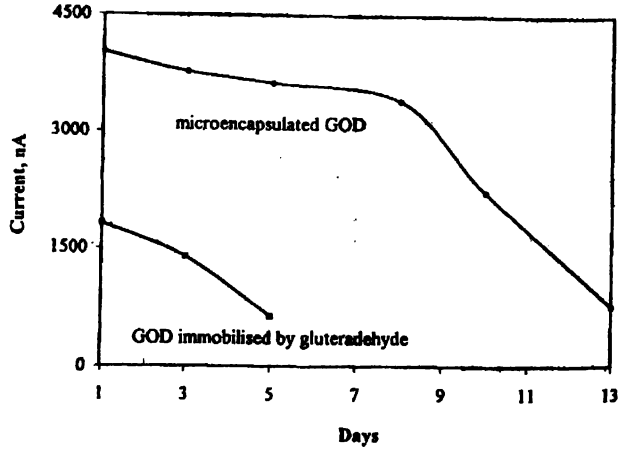


**Fig. 1.** Response and stability study of unused GOD electrodes made on day-1 with immobilization by microencapsulation using CAB and cross-linking with glutaraldehyde for 2 m*M* glucose in 0.1 *M* phosphate buffer, pH 7.4, 0.1 *M* KCl; potential applied 0.35 V vs Ag/AgCl.

for unused electrodes made on day-1. Fig. 2 gives the decline in response for the same used electrode manufactured on day-1. It is clear from both the figures that our process of microencapsulation worked very well without loss of the enzyme activity and the performance is far better than immobilization by cross-linking. It is also seen from Fig. 1 that the electrodes with microencapsulated GOD may be used for 8 days without significant drop in activity as compared to 3 days with ordinary ones.

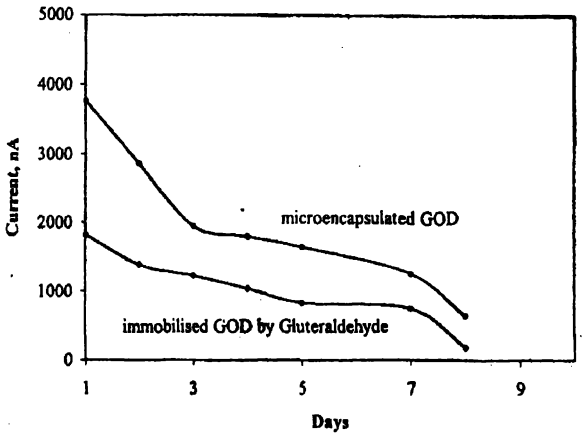


**Fig. 2.** Response and stability study of used GOD electrodes made on day-1 with immobilization by microencapsulation using CAB and cross-linking with glutaraldehyde for 2 m*M* glucose in 0.1 *M* phosphate buffer, pH 7.4, 0.1 *M* KCl; potential applied 0.35 V vs Ag/AgCl.

## Experimental

A buffer of 0.1 *M* $Na_2HPO_4$-$NaH_2PO_4$ (pH 7.4) containing 0.1 *M* KCl was used. Glucose oxidase (GOD, EC 1.1.3.4, from *Aspergillus niger*, specific activity 18500 U $g^{-1}$), glucose and glutaraldehyde (Sigma), cellulose acetate butyrate (CAB, National Chemicals, Vadodara), dichloromethane (DCM, Glaxo India) and Span-85 (Sigma) were used. All buffer solutions were prepared using double-distilled water.

All the test procedures were performed on an Auto Lab electrochemical analyzer ($\mu$AutoLab-Type II) with GPES software (Ecochemie, Utrecht, The Netherlands). The response of an individual electrode was obtained by deducting the base line current, from the response at a particular set of conditions (e.g. enzyme loading concentration of substrate, time of measurement). The average value of this difference was plotted against concentrations or strengths.

*Fabrication of biosensors* : Three-electrode devices were made in-house by a multistage screen-printing process using a DEK 248 machine (DEK, Weymouth, UK). Devices were printed on to 250 $\mu$m thick polyester sheet (Cadilac Plastic, Sweeden, UK). The sensor consisted of a working electrode made of 5% rhodinized carbon, a reference electrode of 15% Ag/AgCl and a counter electrode made of carbon[4].

*Preparation of cross-linked GOD electrodes* : A 10 $\mu$L of GOD solution in PBS with 2% glutaraldehyde strength 200 mU per electrode was used on each WE and dried. The electrodes were washed with PBS and again dried and kept at 4° until further use. An 'O'-ring was secured on the three-electrode assembly and 90 $\mu$L PBS was added and required potential applied. After attaining equilibrium, 20 m*M* glucose solution (10 $\mu$L) was added at equal interval of time. Three sets of readings were taken. Working potential was +350 mV across working electrode. Readings were taken at an interval of 2 min and mean of the three readings was taken. The response was plotted against the corresponding glucose strength.

*Preparation of microencapsulated GOD electrode* : Glucose oxidase (200 mU/electrode) dissolved in water (1.5 ml) was mixed with CAB (0.4277 g) dissolved in dichloromethane (8 ml) with shaking. Then span-85 (30 $\mu$L) was added, and the mixuture was emulsified at low power with a sonicator (Sonics & Materials, USA) till stable emulsion of water-in-oil was formed. The emulsion was examined through microscope (MBL2100, Kruss, Germany) with 400 times magnification. This was then sprayed in a third phase of phosphate buffer and kept suspended till further use. Scanning electron micrograpgh (SEM) analysis of the microcapsules was performed. The particle size ranged from 5 to 30 $\mu$m. A calculated amount of this suspension was added on the electrode surface containing 2% of glutaraldehyde. The electrode was then dried at room temperature. PBS (90 $\mu$L) was added on the 'O'-ring fixed around the three-electrode assembly. After attaining equilibrium, 20 m*M* glucose solution (10 $\mu$L) was added to make the solution of 2 m*M* glucose. Stable current reading was obtained through GPES software.

## Acknowledgement

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## References

1. E. Magner, *Analyst*, 1998, **123**, 1967.
2. Y. Miao, L. S. Chia, N. K. Goh and S. N. Tan, *Electroanalysis*, 2001, **13**, 347.
3. T. M. S. Chang, *Science*, 1964, **146**, 524.
4. S. Kroger and A. P. F. Turner, *Anal. Chim. Acta*, 1997, **347**, 9.