Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713644168

Immobilization of Glucose Oxidase with Polyurethane on Carbon Support

Ratna s. Phadke[a]; Shridhara Alva[a]; Girjesh Govil[a]
[a] Chemical Physics Group, Tata Institute of Fundamental Research, Bombay, India

First published on: 01 October 1993

To cite this Article Phadke, Ratna s. , Alva, Shridhara and Govil, Girjesh(1993) 'Immobilization of Glucose Oxidase with Polyurethane on Carbon Support', Molecular Crystals and Liquid Crystals, 235: 1, 133 — 137
To link to this Article: DOI: 10.1080/10587259308055185
URL: http://dx.doi.org/10.1080/10587259308055185

Mol. Cryst. Liq. Cryst. 1993, Vol. 235, pp. 133–137
Reprints available directly from the publisher
Photocopying permitted by license only

Printed in the United States of America

IMMOBILIZATION OF GLUCOSE OXIDASE WITH POLYURETHANE ON CARBON SUPPORT

RATNA S. PHADKE, SHRIDHARA ALVA and GIRJESH GOVIL
Chemical Physics Group, Tata Institute of Fundamental Research, Bombay 400 005, India

Abstract Glucose oxidase (GOD) has been immobilized by physical entrapment on polyurethane PU-6 matrix. It has been found that the enzyme acquires greater thermal stability on immobilization. Oxygen acts as a co-substrate for the redox reaction. However, maintaining oxygen concentration constant throughout the experiment is a difficult task. To overcome this we have coimmobilized ferrocene, which can act as an electron acceptor. ^{31}P NMR results indicate that ferrocene is located in the close proximity of the active site of GOD. Moreover, simultaneous use of mediators such as ferricyanide, phenazine methosulphate or flavine mononucleotide have been found to facilitate electron transfer. Platinum, apart from being an expensive metal, is likely to exhibit adverse toxic effects during prolonged 'in-vivo' applications. Electrodes prepared using carbon in the place of platinum, show comparably good response. This opens a new possibility for making cheaper and biocompatible sensors.

Introduction

The performance of a biosensor depends largely upon appropriate interfacing of the biological component with the transducer[1,2]. It is desirable that the immobilizing matrix should be porous, long lasting and easy to prepare. Moreover, the immobilization process should be mild so that the biocatalyst does not lose its activity. Chemical bonding helps in minimizing leaching of biocatalyst from the matrix, but is likely to induce changes in its conformation, resulting in lower catalytic activity. On the other hand, physical entrapment is likely to preserve catalytic activity but is susceptible to substantial leaching out of enzyme. However, leaching can be prevented by a appropriate choice of the immobilizing matrix[3].

Entrapment in natural or synthetic polymer gels is a suitable method for immobilization because of the relative ease of preparation and direct applicability to a variety of systems such as single - or multi - enzyme systems, organelles, cells etc. Entrapment using prepolymers requires mild conditions of operation. Moreover, the method is safe as the prepolymers contain negligible amounts of monomers which are likely to have adverse effects on the biocatalyst. Entrapment of biocatalysts with urethane prepolymers seems to be particularly advantageous because isocyanate functional groups at the terminal ends of the molecule can react with

each other in the presence of water, forming urea linkage and liberating carbon dioxide [4,5]. When prepolymers are mixed with an aqueous solution or aqueous suspension of the biocatalyst, gels are readily formed. The gelation gets more or less complete in 30 min. The hydrophilic / hydrophobic properties of the prepolymer can be controlled by varying the ratio of the poly(ethylene glycol) part and the poly(propylene glycol) part in the polyether diol moiety of the prepolymer. Polyurethane prepolymer, PU-6 has a high content of poly (ethylene glycol) (91%) and hence displays hydrophilic character.

Redox reaction of glucose oxidase (GOD) takes place in conjunction with a redox coenzyme, flavine adenine dinucleotide (FAD). The redox center is not readily accessible due to a mannose coating on the surface of the enzyme [6]. Oxygen is a natural electron acceptor and rate limiting factor in the catalytic action of GOD. Maintaining oxygen tension constant throughout the experiment is difficult. Instead, if a redox couple is in solution, it can function as electron transporter on application of appropriate potential [7]. In this paper, we describe the use of potassium ferricyanide and phenazine methosulphate as electron transporters in solution and ferrocene as relay in immobilizing matrix[1,8].

Materials and Methods

Glucose oxidase (type VII from A.niger, EC 1.1.3.4) and peroxidase (horse raddish) were purchased from Sigma Chemicals MO, USA. Polyurethane prepolymer was a gift from Profs. A. Tanaka and Sonomoto, Kyoto University, Japan. All other chemicals used were of analytical grade and were used without further purification.

Preparation of the working electrode

The carbon plate (1.0mm thick and 3.0sq cm total surface area) was cleaned with ethanol followed by distilled water several times and then dried. About 500 units of the enzyme was dissolved in 0.2ml of sodium phosphate buffer. 0.1ml of the enzyme solution was spread uniformly on one of the surfaces of the electrode. About 5mg of the polyurethane prepolymer PU-6 was uniformly mixed with the enzyme solution. The polymerization starts immediately. The electrode was left undisturbed for 10 min. for the polymerization reaction to complete. The process was repeated on the other side of electrode. The electrode was stored overnight in dark. It was thoroughly washed with phosphate buffer to remove untrapped enzyme or unpolymerized prepolymer that may be present in the matrix. The relay (ferrocene) was added to the prepolymer prior to polymerization. Spectrophotometric monitoring of the washings indicated the absence of significant leaching of ferrocene from the matrix. The thickness of the enzyme film was 0.12 ± 0.02 mm in dry conditions for all electrode preparations.

Characterization of the Immobilized Enzyme

Enzyme activity:

The activity measurements of the enzyme in the free and the immobilized state have been done following standard method[9]. o-Dianisidine has been used as the

coloring complex. The colored complex was monitored at 500nm using SPECTRONIC 1201 system. Immobilized enzyme shows only 48% of solution activity. The decrease in the activity can be attributed to the composite effect of immobilization and leaching of the untrapped enzyme during washings (which is about 10% of the total enzyme used for immobilization).

^{31}P NMR

Clues to localization of relays can be obtained by studying ^{31}P resonance of phosphate moieties of coenzyme FAD.^{31}P nuclear magnetic resonance experiments have been carried out on MSL 300 spectrometer from Bruker having facility for magic angle spinning[10]. Powder aluminium oxide has been used as the filling to achieve better results. The peaks due to FAD of immobilized GOD in PU-6 matrix appear at 11.91 and 10.93ppm positions (ppm values are with respect to orthophosphate) belonging to AMP and FMN moieties coenzyme respectively [11]. On incorporation of ferrocene, an intense peak with a broad shoulder appears at 11.75ppm. This indicates that ferrocene is localized in close proximity of the two FAD phosphate groups. However, the positioning is not symmetrical. Detailed analysis is complex as there are contributions from ring current effects of ferrocene ring as well as magnetic contributions from iron nucleus.

Response of electrode to glucose

The working electrode, counter electrode and the reference electrode have been kept in a glass cylinder. The lid has four inlets: three to insert the three electrodes and fourth for adding substrate (glucose) to the reaction cell. The reaction mixture has been stirred gently and continuously with a magnetic stirrer. A constant potential has been applied between the reference and working electrode using a dry cell. Current between working electrode and the counter electrode has been measured. The mediators have been introduced to the reaction mixture prior to insertion of substrate.

Saturated calomel electrode has been used as reference and platinum electrode as the counter electrode. GOD immobilized on graphite surface serves as the working electrode. The current generated between the working electrode and the counter electrode on addition of glucose has been monitored with time. It is observed that the current increases initially linearly and levels off after about 100s or so. Therefore, we have made all subsequent measurements at the end of 120s to ensure that the equilibrium condition have been attained. Figure 1 depicts the response - glucose concentration curves. The response is linear upto 15mM glucose concentrations for ferrocene coimmobilized electrode (curve a). One notices that the response increases (curve b) when ferricyanide is added to the electrolytic medium. The response is sensitive to the concentration of ferricyanide. The optimum concentration is found to be 0.62mM. The current per unit glucose concentration (Δ i/Δ C) for the same electrode with mediator is three times higher than that without the mediator. Moreover, it is observed that linearity improves with the use of mediator. This implies that the range of utility of the electrode can be enhanced with the appropriate choice of the mediator.

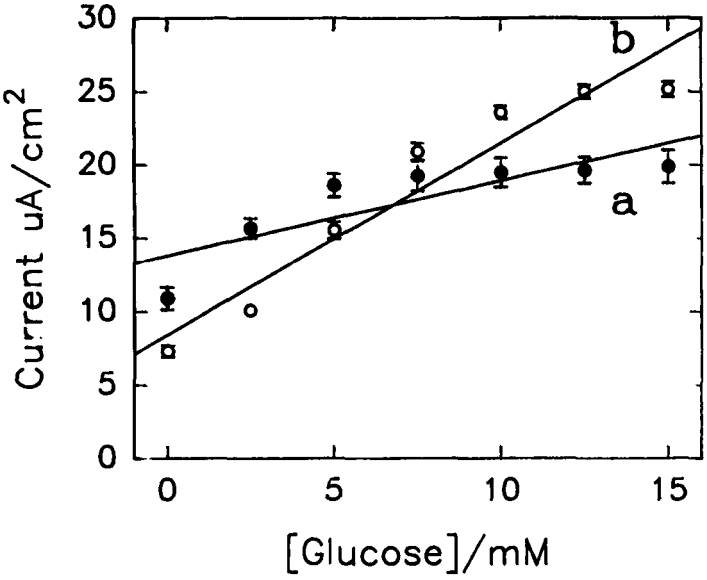

Fig. 1 The current verses glucose concentration for glucose oxidase immobilized in polyurethane matrix on carbon (a) without any mediator[voltage applied -0.8V (CC = 0.84)], (b) with mediator (0.6mM potassium ferricyanide solution)[voltage applied -0.36V (CC = 0.96)]. In both the cases ferrocene was coimmobilized with glucose oxidase. Supporting electrolyte 0.1M sodium phosphate buffer , pH 6.8.

Replacement of aqueous electron shuttle (ferricyanide) by compounds such as phenazine methosulphate (PMS), Chloropentammine cobalt(III) chloride or Flavine mononucleotide (FMN) have not shown significant improvements in response as regards linear range of concentrations or higher slopes (Δ i/Δ C value). The results for different mediators are listed in table I. The measurements done with the electrodes having platinum as supporting electrode are also listed.

Table I A comparative study of the performance of glucose oxidase electrodes with platinum and carbon supporting electrodes with ferrocence coimmobilized in the matrix for different mediators in the eletrolyte. The numbers with the mediator are the concentration and the applied voltage respectively.

	electrode	Current $\mu A/cm^2$			
		$K_3Fe(CN)_6$ 0.6mM 0.36V	$Co(NH_3)_5Cl_3$ 0.2mM 0.45V	PMS 0.2mM 0.15V	FMN 0.2mM 0.25V
[glucose] = 2.5mM	Platinum	3.5	5.5	4.9	3.6
	Carbon	2.8	5.6	7.4	3.9
[Glucose] = 5.0mM	Platinum	9.8	11.0	10.0	8.0
	Carbon	8.2	10.7	14.1	7.2
Δ i/ Δ [glucose]	Platinum	2.99	1.71	1.68	1.01
	Carbon	1.85	1.69	2.05	1.07

One can notice from these results that, carbon based electrodes are equally efficient as platinum based ones and simultaneous use of relays and mediators help to enhance the performance of the electrode under *in vitro* conditions.

Acknowledgements

The authors are grateful to Prof A. Tanaka and K. Sonomoto of Kyoto University, Japan for the supply of polyurethane prepolymer as a gift. The Sophisticated Instruments Facility situated at Indian Institute of Science Bangalore is greatly acknowledged for the use of MSL 300 machine.

References

1. A. E. G. Cass, G. Davis, G. D. Francis, H. A. O. Hill, W. J. Aston, I. J. Higgins, E. V. Plotkin, L. D. L. Scott, and A. P. F. Turner, Anal. Chem., 56, 667 (1984).
2. L. C. Clark, in Biosensors : Fundamentals and applications, ed: A. P. F. Turner, et al., Oxford University Press; Oxford, pp 3 (1989).
3. G. G. Guilbault and G. O. Neto, in Immobilized cells and enzymes : a practical approach, IRL press; Oxford, pp 58 (1985).
4. S. Fukui and A. Tanaka, Adva. bioeng / biotech., 29, 1 (1984).
5. A. Tanaka, I. N. Jin, S. Kawamoto and S. Fukui, Eur. J. Appl Microbiol. Biotechnol., 7, 351 - 354 (1987).
6. Y. Degani and A. Heller, J. Am. Chem. Soc., 110, 2615 (1987).
7. D. L. Williams, A. R. Doig Jr. and A. Korosi, Anal. Chem., 42, 118 (1970).
8. N. C. Foulds and C. R. Lowe Anal. Chem., 60, 2473 (1988).
9. R. Bentley, in The Enzymes, ed: Boyer, P.D., Academic Press; New York, 7, 567 (1963).
10. R. K. Harris in Nuclear Magnetic Resonance Spectroscopy, Bath Press, Avon, pp 144-64 (1986).
11. T. L. James, D. F. Emondson and M. Husain, Biochemistry, 20, 617 (1981).