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review

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A Novel Improved Design for the First-generation Glucose Biosensor

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Summary

The historical development of three generations of enzyme-based amperometric glucose biosensors are compared. A novel design of the first-generation glucose biosensor based on the use of metal-dispersed carbon paste electrode is described. Such a strategy enables remarkable selectivity and improved sensitivity, without excluding or destroying the endogenous electroactive interferences, commonly associated with the first-generation glucose biosensor. It offers a strong, preferential electrocatalytic action towards the enzymatically-liberated hydrogen peroxide due to the proper selection of the transducer metal--dispersed material. On the other hand, a unique, non-polar pasting liquid allows efficient operation of the resulting glucose biosensor under severe depletion of oxygen or under a considerable period of thermal stress by providing an internal oxygen supply and establishing a micro-environment of the enzyme against thermoinactivation, respectively. Metal-dispersed carbon paste enzyme electrodes have thus become a promising new design of the first-generation biosensors, especially for blood glucose measurements and other biothechnical applications due to their remarkable selectivity, high stability, low oxygen-dependence, and good dynamic performance.

Key words: glucose, biosensors, enzyme electrode, metal-dispersed, carbon paste

Medical applications of glucose biosensors have been expanded from clinical laboratories to patients self-control owing to the significance of blood glucose measurements and monitoring for increasing number of diabetics. Together with their applications in food industry and other biotechnological areas, the development of simple and reliable glucose biosensors remains the prime focus of many researchers.

Amperometric enzyme-based bioelectrodes are very suitable for self-testing and *in vivo* monitoring of blood glucose. The glucose amperometric sensor, developed by Updike and Hicks (*1*), represents the first reported use of an enzyme electrode. The so-called first-generation glucose biosensor is commonly based on the entrapment of glucose oxidase (GOD) between dialysis and permselective membranes on a metal or carbon working electrode used as transducer. The liberation of hydrogen peroxide in the enzymatic reaction can be measured amperometrically at the working electrode surface:

$$\text{glucose} + \text{GOD}(\text{FAD}^+) \rightarrow \text{glucolactone} + \text{GOD}(\text{FADH}_2)$$

$$\text{FADH}_2 + \text{O}_2 \rightarrow \text{FAD}^+ + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 \xrightarrow{electrode} 2\ \text{H}^+ + \text{O}_2 + 2\text{e}^-$$

where $\text{FAD}^+/\text{FADH}_2$ is the cofactor of GOD, and O_2 comes from the environment (*e.g.* dissolved oxygen in the solution).

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In the early stage, the enzymatic reaction was followed by monitoring the consumption of the oxygen cosubstrate, but the method was seldom used due to possible variation of oxygen in the environment.

The routine use of a first-generation glucose biosensor has been hampered by two major limitations. The first limitation accrues from the fact that the amperometric monitoring of hydrogen peroxide enzymatically liberated by GOD requires high operating potential where unwanted reactions of coexisting electroactive species in biological fluids, such as ascorbic acid, uric acid and acidaminophenon interfere. The second limitation stems from the restricted solubility of oxygen in biological fluids, that limits the enzymatic reaction, and variations in the oxygen level, especially in the case of implantable glucose biosensors suitable for *in vivo* measurements (*2–5*).

The improvements were achieved by replacing oxygen with a non-physiological electron acceptor, which was able to shuttle electrons from the flavin redox center of the enzyme to the surface of the working electrode. The second-generation of glucose biosensor was developed with the inclusion of such redox mediators. Using redox mediators, the measurements have become insensitive to oxygen fluctuations and can be carried out at lower, more negative potentials where the interfering reactions from physiologically coexisting electroactive species do not interfere. Organic and organometallic redox compounds, such as ferrocene and quinone derivatives, ruthenium complexes, ferricyanide, phenoxazine compounds and organic conducting salts (*6–11*), have been used as electron mediators, with the following working mode:

$$\text{glucose} + \text{GOD}(\text{FAD}^+) \rightarrow \text{gluconolactone} + \text{GOD}(\text{FADH}_2)$$

$$\text{FADH}_2 + 2\ \text{Med}_{(ox)} \rightarrow \text{FAD}^+ + 2\ \text{Med}_{(red)} + 2\ \text{H}^+$$

$$2\ \text{Med}_{(red)} \xrightarrow{electrode} 2\ \text{Med}_{(ox)} + 2\text{e}^-$$

where $\text{Med}_{(ox)}$ and $\text{Med}_{(red)}$ are the oxidizing and reducing forms of the mediator, respectively. This chemistry has led to mass-scale production of commercially available glucose biosensors, especially the development of pen-sized meters for personal glucose measurement. The single-use, disposable strips used with this device are made of polyvinyl chloride and a screen-printed carbon electrode containing a mixture of glucose oxidase and the electron mediator. A fresh drop of undiluted whole blood can thus be assayed for its glucose level in less than 60 seconds. However, the mediated enzyme electrodes may still suffer from ascorbic and uric acid interferences, and the toxicity of many artificial mediators limits their *in vivo* applications.

Another, even more elegant, possibility is to achieve direct, unmediated electrical communication between glucose oxidase an the electrode surface. The modification of glucose oxidase with an appropriate electron relay has been successfully employed for this advanced design. It has been possible to »wire« the enzyme to the electrode with a long chain polymer having a dense array of electron relays, which is flexible enough to fold along the enzyme structure. Ultimately, this third-generation of glucose biosensor would lead to implantable, needle-type devices for continuous *in vivo* monitoring of blood glucose (*12,13*). Such devices would offer an improved control of diabetes, in connection with an internal insulin release system.

It must be considered that glucose biosensors for medical applications have been developed to a promising stage following the more and more sophisticated technical design. In this article, a novel and simple design, based on the first-generation glucose biosensor is described by focusing on transducer. This design is developed in the laboratory of Dr. Joseph Wang at NMSU (Las Cruces, NM, USA) and is based on a metal-dispersed catalytic materials.

What is the basis of an absolutely selective transduction process to the enzymatically-librated hydrogen peroxide in the above-mentioned biosensor? The principles of traditional »first generation« glucose biosensors depends on the immobilization of glucose oxidase onto the carbon or metal transducers and detection of the anodic current associated with the hydrogen peroxide enzymatically generated by glucose and dioxygen substrates. Accordingly, those easily oxidizable endogenous constituents undergo oxidation at a fairly positive potential (>+0.7 V *vs.* Ag|AgCl) required for detection of the hydrogen peroxide. The error caused by the signal overlapping from both the analyte and interfering materials is significant, although the latter exists in physiological fluids at much lower levels compared with the former. A new design, using metallized carbon electrodes instead of the traditional transducers, offers preferential electrocatalytic detection of hydrogen peroxide, in particular yielding the cathodic current response under the lower potentials around 0.0 V, where the endogenous constituents are almost electroinactive, giving very small current response.

A metal-dispersed carbon paste enzyme electrode might be the most important member of these novel, first-generation biosensors. Some examples of ruthenium-, rhodium-, iridium-dispersed carbon paste/GOD biosensors have been reported to offer an amperometric biosensing of glucose, achieving marked selectivity, attractive dynamic properties and reduced sensor configuration complexity (*14–16*).

Three-dimensionally dispersed metal particles in graphite show the unique electrocatalytic action to hydrogen peroxide in comparison with pure metal or carbon surface, greatly lowering the over-potential either in oxidation or reduction direction. Electroreduction of hydrogen peroxide on such metal dispersed carbon paste electrodes is responsible for their absolutely selective amperometric biosensing of glucose, because the unwanted electrooxidation of those endogenous interfering constituents do not occur at so low potential region (approximately 0.0 V) required for the electroreduction of enzymatically generated hydrogen peroxide. In addition, the stable electrocatalytic activity due to the strong adherence of metal centers to the graphite and the considerably lower background signals (similar to that of an ordinary carbon paste) of the resulting electrodes, make them more and more attactive for glucose biosensors. It is worth mentioning that the metal dispersed graphite might be regarded as the transition metals-based »supporting catalysis«. The study of the mech-

anism of their electrocatalytic activity is beneficial not only for the elucidation of the electrode kinetics, but also for the development of glucose biosensors. The bimetallic (ruthenium-platinum) alloy dispersed carbon paste/GOD electrodes have been constructed and compared with the single metal dispersed ones in relation to their selectivity, sensitivity and other dynamic properties (*17*). Figs. 1 and 2 show that the alloy-dispersed carbon/GOD electrode produced a greatly enhanced sensitivity, without compromising the remarkable selectivity inherent to metallized carbon biosensors. The use of carbon-supported alloy particles resulted in greatly improved properties of the biosensor in comparison to biocomposite with dispersion of pure metals. This would be contributive to the understanding of the electrocatalysis of metallized carbon.

The metal-dispersed carbon paste enzyme electrodes, like ordinary carbon paste electrodes, showed the advantages of low background noise, renewed and modified surfaces, miniaturization and easily prepared (sufficiently for the mass-producible, disposable sensors, *e.g.* screen-printed strips), that initiates further interests in them.

Carbon paste used for the preparation of glucose biosensors consisted of a mixture of graphite powder and an organic pasting liquid, commonly mineral oil. The pasting liquid not only serves for filling the crevices between the graphite particles but also prepares an electrode that is fundamentally different from those pure metal electrodes, such as Pt and Au electrodes, commonly used for amperometric transduction. It is well known that the solubility of oxygen is many times greater in some organic solvents than in water. In particular, fluorochemicals have been used as oxygen transporters and blood substitutes in humans and animals in relation to the very high oxygen solubility in such solvents (resembling to that in haemoglobin). Taking advantage of this remarkable oxygen solubility, an oxygen-insensitive first-generation enzyme electrode based on fluorochemical carbon paste has been constructed (*18*), achieving a satisfied oxygen independence which rivals that reported for mediated or wired enzyme electrodes.

As mentioned above, carbon paste enzyme electrodes with the improvements both in graphite and in pasting liquid diminish the major limitations of the first-generation glucose biosensor. In addition, a further investigation showed that a variety of oxidases (including GOD) entrapped within non-polar carbon paste acquired a remarkable stability, especially a resistance to thermoinactivation (*19*). It took an extended period of 4 months to examine the long-term stability of a GOD-containing carbon paste biosensor at the elevated temperature of 60 °C (as shown in Fig. 3). These remarkable

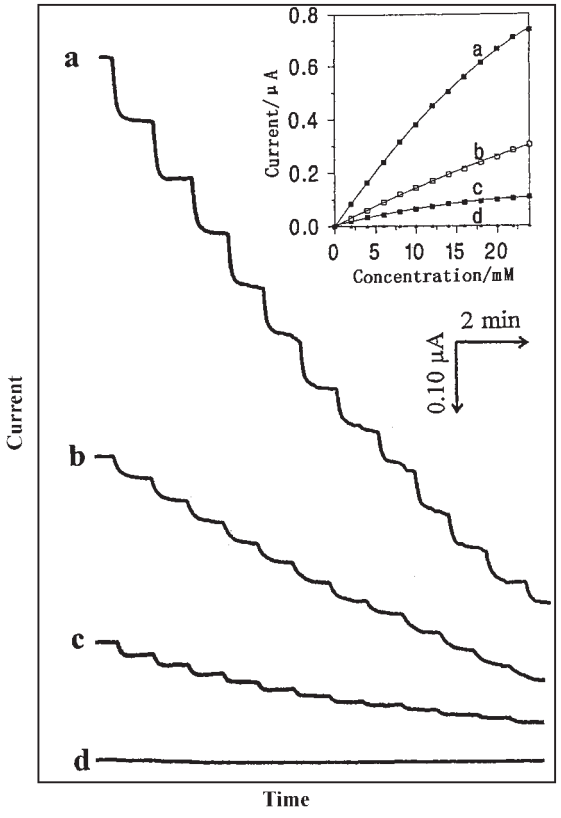

Fig. 1. Amperometric reduction response at the Pt-Ru- (a), Ru- (b), Pt- (c) dispersed carbon and plain carbon (d) enzyme electrodes upon increasing concentration of glucose in 2 mmol/L steps; working potential = –0.05; electrolyte = phosphate buffer (0.05 mol/L, pH=7.4) stirred at 300 rpm. The resulting calibration curves are also shown (inset)

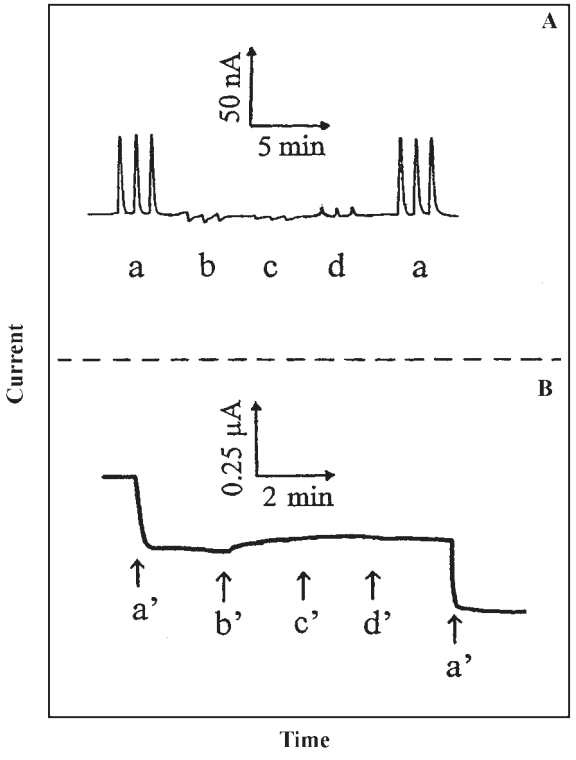

Fig. 2. Flow-injection (A) and batch (B) amperometric signals at the Pt-Ru-dispersed carbon paste glucose oxidase electrode for addition of 8 mmol/L glucose (a); 0.4 mmol/L ascorbic acid (b); 0.4 mmol/L uric acid (c); and 0.4 mmol/L acetaminophenon (d), or 5 mmol/L glucose (a'); 0.1 mmol/L ascorbic acid (b'); 0.1 mmol/L uric acid (c'), and 0.1 mmol/L acetaminophenon (d'). Conditions are the same as in Fig. 1, exept that a flow rate of 1.5 mL/mim was used in (**B**)

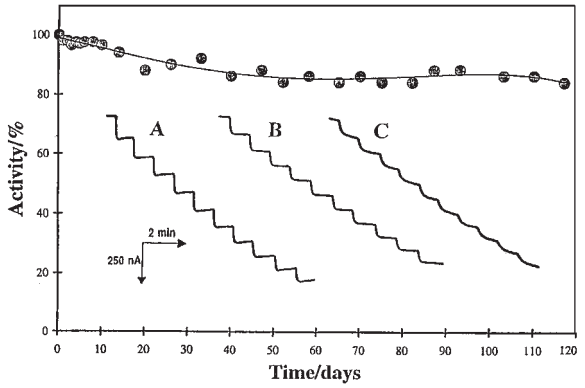

Fig. 3. Long-term time dependence of the activity of GOD entrapped in Rh-dispersed carbon paste and stored at 60 °C for 4 months; insets show the actual response of the Rh/GOD carbon paste electrode at the start (curve A), middle (58th day, curve B), and end (117th day, curve C) of this experiment. The same electrode surface was used throughout. The activity was assayed at the times indicated by amperometric response to 2 mM glucose at the electrode. Working potential = –0.05 V; electrolyte = 0.05 M phosphate buffer (pH = 7.4) stirred at 300 rpm

thermostabilities might be related to the fact that several enzymes are more stable in organic media than in water. Such enhanced resistance towards thermal denaturation stems from the high conformation rigidity of dehydrated enzymes and from the lack of free water molecules, usually required in all enzyme-inactivation processes.

In conclusion, the carbon paste enzyme electrode prepared with a metal- or metal alloy-dispersed graphite and a non-polar pasting liquid which has high oxygen solubility has become a promising design of the first-generation biosensor, especially for blood glucose measurements and other biotechnological applications due to its almost absolute selectivity, high stability, low oxygen-dependence and dynamic performance.

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Nova konstrukcija prve generacije biosenzora za glukozu

Sažetak

Povijesni razvoj na enzimu zasnovanim amperometrijskim biosenzorima za glukozu prikazan je prema njihovoj različitoj izvedbi, a razrađen je u tzv. tri generacije. Uporaba paste koja se sastoji od metala dispergiranog u ugljenu daje poboljšanu prvu generaciju biosenzora za glukozu. Takav način da se postigne bitna selektivnost ne sastoji se u isključivanju ili uništenju endogenih elektroaktivnih interferencija, kao kod svih biosenzora za glukozu prve generacije, već u snažnom, izrazitom elektrokatalitičkom djelovanju prema u enzimskoj reakciji oslobođenom vodikovom peroksidu usredotočivši se na materijal provodnika (transducer). S druge strane, nepolarna tekuća pasta omogućava djelotvoran rad biosenzora za glukozu i pod značajnim nedostatkom kisika osigurava internu dobavu kisika, a pri znatno dugom termalnom stresu uspostavlja mikrookoliš kojim zaštićuje enzim od toplinske inaktivacije. Enzimske elektrode s metalom dispergiranim u ugljenoj pasti postale su perspektivni model za biosenzore prve generacije, osobito za određivanje glukoze u krvi i za druge biokemijske primjene zbog njihove apsolutne selektivnosti, velike stabilnosti, male ovisnosti o kisiku i dobre dinamičke provedbe.