New Materials for Electrochemical Sensing: Glucose Biosensors Based on Rigid Carbon-Polymer Biocomposites

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Summary

The development of composites based on conductive phases dispersed in polymeric matrices has permitted important advances in analytical electrochemistry particularly in sensor devices. The present work reviews the use of biocomposite materials in the construction of glucose amperometric biosensors. These composites are bulk--modified biologically (adding enzymes and cofactors) and chemically (blending mediators and catalysts). The review covers all references found in literature up to 1996.

Keywords: electrochemical biosensors, biosensors for glucose, carbon-polymer biocomposites

Introduction

At present, environmental, clinical and industrial samples show great diversity and analytical complexity. At the same time, competitiveness in industrial context is heigthened increasingly. Most strategies in analytical chemistry today call for complex instrumentation and considerable support. This support includes special laboratory facilities and highly skilled personnel. Chemical sensors and biosensors are a key element of novel strategies applied to analytical instrumentation. Sensors and sensor-based devices provide original solutions without the need of complex instruments or a huge support infrastructure. Chemical sensors and biosensors are devices that are small, robust, portable and easy to use. Additionally, they do not need reagents to operate and they can yield reliable information continuosly.

Amperometric biosensors formed usually by biologically surface-modified voltammetric electrodes are gaining increasing importance. This corresponds to their high reliability, robustness and sensitivity (1). Additionally, new materials and immobilization techniques are assayed for the mass production of these devices. In this context, the present review covers recent work in the field of glucose amperometric biosensors based on a new type of materials known as biocomposites. These materials are formed by rigid conductive composites based on carbon-polymer matrices where the biological material (enzymes) as well as other modifiers (cofactors, me-diators, catalysts, additives, etc.) are jointly bulk-immobilized.

Conducting composites for amperometric sensing

A composite results from the combination of two or more dissimilar materials. Each individual component keeps its original nature while giving the composite distinctive chemical, mechanical and physical qualities. These qualities are different from those shown by the individual components of the composite (2). The electrical properties of the composite depend on the nature of each of the components, their relative quantities and their distribution. The electrical resistance is determined by the connectivity of the conducting particles inside the polymeric matrix. This means that the relative quantity of each composite component has to be studied to determine the optimal composition. This entails the construction of percolation curves (3).

Carbon-based materials are used frequently as the conductive phase in composites used for electrochemical sensors. These materials are available in different morphologies such as graphite, carbon black, carbon fibers, etc. Carbon materials show a high chemical inertness which provides a wide range of anodic working potentials. They have a low electrical resistivity (10^{-4} Ω cm ap-

proximately) and they have a highly pure crystal structure. This yields low residual currents and, consequently, a favorable signal-to-noise ratio.

A key feature in any electrochemical sensor is a good detection limit that is associated to a high signal-to-noise ratio. In accordance to the equation derived by Oldham (4), if the area is very small, as in microelectrodes, the perimeter of the surface has a more significant influence in the mass transport than the area itself. This is translated to a better sensing function since a non-linear diffusion is established generating a steady state current that raises the signal-to-noise ratio. This is known as the edge effect. This favourable effect and the reduced size of the electrode are interesting and welcome events. However, the generated currents are very low, calling for sophisticated and expensive voltammetric equipment. Hence, whenever small size is not required, the use of arrays or ensembles of microelectrodes is desirable. These ensembles can be seen, for example, as a macroelectrode formed by a great number of carbon fiber microelectrodes. These microelectrodes are separated by an insulator and connected in parallel. The signal produced by these ensembles of carbon fibers is the sum of the individual currents generated by each microelectrode. The end product is a sensor with a signal comparable to the signal of a macroelectrode, but showing the signal-to-noise ratio of a microelectrode. Weber (5) showed that these electrodes have a maximum efficiency when the separation between microelectrodes is around 0.1 μm. The implicit difficulty of constructing these electrodes is considerable. On the other hand, electrodes based on composites with a random dispersion of very small carbon particles in a polymer matrix are very easy to prepare. If a percolation curve is drawn for these composites, the optimal content of the conductor can be found.

Preparation of the biocomposites and biosensor construction

The biocomposites are prepared very easily. The powdered graphite is dispersed homogeneously by hand with the proper amount of polymer. According to Tallman and Petersen (6), these materials can be classified as dispersed composites since the conductor particles have an equal opportunity to occupy any point throughout the matrix.

The polymer material is activated when its components are blended. The activation happens when a volatile fraction evaporates or when a hardener, catalyst or initiator acts on the resin. The resin may be epoxy, silicone, methacrylate, polyester or polyurethane (see Table 1). The contents of the graphite, the modifier (enzyme, catalyst, mediator), and the additives are optimized for a particular polymer matrix. Graphite particles are smaller than 50 μm (7). The goal is to achieve the maximum electrical conductivity and the highest response quality with an appropriate biocomposite rigidity. Graphite content may vary from 20% (epoxy) to 60% (silicone) (7). As mentioned earlier, there are some commercially available epoxy resins that already contain graphite (8). The homogeneous mixture is introduced 2 to 3 mm into a tube made of PVC, glass, etc. A metal disk coupled to a wire is used to contact the composite inside the tube. The ensemble is left at room temperature or slightly higher

Table 1. Glucose biosensors based on rigid conducting biocomposites

Composite construction (mass fraction/%)				$E_{appl.}/V$ *vs.* Ag/AgCl	pH	Linear concentration response range/mM	Ref.
Enzyme [a]	Carbon	Polymer	Mediator/Catalyst				
GOD (2)	graphite (19)	epoxy (79) (Epo-Tek H77)		+1.15	7.0	0.1– 5	7
GOD (2)	graphite (49)	epoxy (49) (Epo-Tek 302)		+1.15	7.0	0.1– 5	7
GOD (2)	graphite (49)	methacrylate (49)		+1.1	7.0	0.2– 5	7
GOD (2)	graphite (62)	silicone (36)		+1.15	7.0	0.4–20	7
GOD (2)	graphite (36)	polyester (62)		+1.1	7.0	0.1– 5	7
GOD (2)	graphite (60)	polyurethane (38)		+1.15	7.0	0.05– 5	*This review*
GOD (20) covalently bound to graphite	graphite (10)	Teflon (70)		+0.9 +0.8	7.4 7.4	2.5–30 0.2– 1 [b]	9
GOD (1.5)	graphite 15.8	epoxy (63.0) (Epo-Tek H77)	gold (11.8) and palladium (7.9)	+0.9	7.0	0.01– 2	15
GOD (20)	graphite-epoxy (Dylon) (54)		1,1'-dimethyl-ferrocene (26)	+0.5 +0.3	7.4 6.5	1– 6 [b]	8 17
GOD (1.5)	graphite (15.8)	epoxy (63.0) (Epo-Tek H77)	TTF (19.7)	+0.15	7.0	0.1– 2	18
GOD (2)		silicone (28)	TTF, TCNQ (70)	+0.2	7.0	0.1– 5	*This review*

[a] glucose oxidase (GOD) (100–200 U/mg)
[b] flow-injection analysis
TTF = tetrathiafulvalene; TCNQ = tetracyanoquinodimethane

Table 2. Results of glucose determination for eight samples taken from the fermentation broth, using three different analytical methods. Relative standard deviation was bellow 5%.

Sample	Time/h	γ(glucose)/g L^{-1}		
		Biocomposite	YSI	HPLC
1	2.00	7.99	8.10	8.42
2	2.50	7.47	7.79	7.72
3	3.00	6.75	7.08	6.31
4	3.75	6.23	6.31	5.88
5	4.50	5.20	5.20	4.90
6	5.00	4.21	4.25	3.31
7	5.50	3.55	3.22	2.42
8	6.00	1.71	1.69	1.34
Confidence interval 95%*		±0.06	±0.05	±0.05

*For each sample determination (n=3) the confidence interval was smaller than the indicated value

(40 °C) for one or more days as needed by curing of the polymer. When it is hardened, the biocomposite is polished with abrasive papers of decreasing grain size.

If the matrix is Teflon (9), the granular polymer is mixed with graphite in a mass fraction of 70 to 30%, respectively. The biological material is immobilized previously to particles of graphite powder. Once mixed, the material is pressed at 7000 kg/cm^2 producing 2 mm thick disks. These pellets are coupled to a tube to form an electrode. According to Tallman and Petersen (6), these materials can be classified as consolidated composites since the conductor particles extend throughout the matrix in a random, reticulated fashion with regions of pure insulator and pure conductor.

Glucose amperometric biosensors based on rigid carbon-polymer biocomposites

Several glucose biosensors based on biocomposites are reported in literature (see Table 1). Glucose oxidase (GOD) has been used in our laboratory as enzyme model to study the biocatalytic characteristics of rigid conducting biocomposites that feature immobilized enzymes. This oxidase is compatible with matrices of graphite and several polymeric materials such as epoxy resins, polymethacrylate, silicone, polyester, polyurethane and Teflon. These biocomposites have been applied to glucose measurements based on the direct oxidation of the hydrogen peroxide produced by the action of the enzyme. This happens at high positive potentials (0.9 to 1.5 V vs. Ag/AgCl) (see Table 1). When a graphite polymer composite is used, a shift towards more positive potentials is noticed compared to measurements realized with graphite or platinum electrodes (10,11). It is known that carbon electrodes that have metal particles (Pt, Ru, Rh, Pd, Au, etc.) on their surface, show great catalytic action (12,13). The same happens when the metal is dispersed in carbon pastes (14). The addition of catalysts (gold or palladium) to a GOD graphite-epoxy biocomposite for the oxidation of hydrogen peroxide increases the stability of the signal and reduces the response time. Furthermore, the oxidation potential of hydrogen peroxide is lowered by 250 mV (15). This

decrease is also found in experiments with carbon rods where Au-Pd was sputtered to the surface of the electrodes (16). Therefore, metal bulk-modified composites represent more viable alternatives than those surface-modified electrodes produced by sophisticated technologies. However, the inclusion of metal catalysts in the biocomposite does not hinder the action of the usual interferents found in biological samples (ascorbic acid, uric acid, etc.) (15). On the other hand, it has been observed in our laboratory that this material retains the enzymatic activity in dry storage for more than one year.

Artificial electron acceptors may be added to the biocomposite. These substances act as electron mediators between GOD and the electrode and include 1,1'-dimethylferrocene (8,17) and tetrathiafulvalene (18). The addition of these mediators permits the use of working potentials in the range of 0.5–0.15 V. The action of interferents is reduced greatly at these working potentials. In the biocomposite modified with tetrathiafulvalene, ascorbic acid interference is reduced 90% and interference of uric acid is negligible (18).

Fermentation monitoring using a glucose biosensor based on a biocomposite

The combination of systems based on biosensors and flow injection analysis (FIA) are being applied to the monitoring of bioprocesses. One of the key determinants of the stability of a biosensor is the immobilization process used to fix the biological component to the transducer surface. Enzyme loss in a flow system can be imputed to leaching, prolonged use or inhibition of the biological component.

Construction of the graphite-epoxy-Au-Pd-GOD amperometric biosensor. – The biomaterial is made of Epo-Tek H77 epoxy resin, powdered graphite, gold powder, palladium powder and glucose oxidase. The mass fraction of the biomaterial composition was the following: polymer 63.1%, graphite 15.8%, metals 19.7% and enzyme 1.5%.

Microorganism and fermentation medium. – The microorganism employed was the yeast *Candida rugosa* (ATCC 14830) which was preserved in a mixture of malt, peptone and agar at 4 °C. The fermentation was carried out in a chemical medium composed of: $(NH_4)_2SO_4$ (4 g/L), KH_2PO_4 (15 g/L), $MgSO_4 \cdot 7H_2O$ (1 g/L) and $FeCl_3 \cdot 3H_2O$ (19 mg/L) and inositol (4 µg/L), biotine (8 µg/L) and thiamine HCl (0.2 mg/L) as vitamins. The initial glucose mass concentration in the medium was 10 g/L.

Characteristics of the sensing system. – The use of metals permits a lower response time, a higher signal stability, and a lower H_2O_2 oxidation potential (900 mV) when compared to biosensors that do not have the catalyst (1150 mV). These high working potentials are the norm when using electrodes based on conductive composites (10).

Glucose analysis. – Eight samples of approximately 5 mL were extracted from the fermentation vat at different times. Each sample was filtered through a 0.45 µm cellulose acetate membrane (PK) to eliminate biomass. Table 2 shows the results based on the calibration curve. Sampling times are also shown.

Comparison of the results with commercially available analyzers. – The same samples analyzed by the reported

biosensor were analyzed with a commercial glucose analyzer (Yellow Springs Instruments model 2000) and with HPLC. Table 2 shows the results from all three measurements. A statistical analysis using Student's *t*-test for a confidence range of 95% confirms that the proposed method does not diverge significantly from the two comparative methods, and that no interferences were noted during the measurements.

Conclusions

The construction procedure is simple and is carried out using dry chemistry methodologies compatible with thick film technology.

Before curing, these biocomposites are highly mouldable. This permits the easy construction of amperometric sensors of various shapes (cylindrical, planar, tubular, flow-through, etc.) and sizes.

After curing, these materials are very stable from a mechanical point of view. The surface is stable, rigid, polishable and can be drilled, or otherwise altered mechanically.

The components of the active surface can be controlled by defining their content in the bulk. The presence of enzymes, cofactors, mediators, additives, etc. on the sensing surface can be tailored by adjusting their content in the bulk of the biocomposite.

Biosensors prepared with the techniques defined here have great biological stability. The biocomposite acts as an impervious reservoir for the biologically active components. A loss of sensitivity on the surface is recovered by a simple polishing procedure. Each new surface yields reproducible results if all the individual components of the biocomposite are dispersed homogeneously in the bulk.

The morphology, the size and the distribution of the conducting particles define the behaviour of the biosensor as a microelectrode array. These microelectrode arrays or ensembles show an efficient mass transport and a better electrochemical response (higher signal-to-noise ratio, lower detection limits and shorter response times).

The detection range of the biosensors reported fulfill most industrial and clinical requirements for the respective analytes. Short response times make them suitable

for future applications where many samples have to be processed in a given time. These applications include flow-injection analysis (FIA).

The resulting biosensor is inexpensive and can therefore be considered for single-use applications.

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Novi materijal za elektrokemijsko senzoriranje
Biosenzori za glukozu izgrađeni od bioaktivnih tvari u čvrstim matricama od ugljika i polimera

Sažetak

    Razvoj mješovitih čvrstih materijala s vodljivom fazom raspršenom u polimernim matricama omogućio je veliki napredak u analitičkoj elektrokemiji, osobito na području kemijskih i biokemijskih senzora. U radu je dan kratak pregled primjene čvrstih materijala za pripravu amperometrijskih biosenzora. Ti se materijali dobiju umješavanjem ugljika u polimere uz dodatak bioaktivnih tvari (enzimi i kofaktori) i kemijski aktivnih tvari (posrednici elektrona i katalizatori). U prikazu su obuhvaćeni svi navodi nađeni u literaturi do 1996. godine.