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Sol-Gel Derived Ceramic-Carbon Enzyme Electrodes: Glucose Oxidase as a Test Case

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Abstract. Several types of amperometric biosensors comprised of immobilized glucose oxidase in chemically-modified ceramic-carbon matrices are compared. The electrodes are comprised of several building blocks each performing a specific function. Glucose oxidase is used to catalyze the bio-oxidation of glucose; carbon powder imparts conductivity and favorable electrochemical characteristics: the Ormosil network provides rigidity and porosity; and the organic modification of the Ormosil imparts controlled surface polarity. Additionally, hydrophilic chemical modifiers are incorporated in order to control the size of the wetted, electroactive layer; high dispersion of inert metal catalysts is used to enhance hydrogen peroxide oxidation and redox mediators may be co-immobilized when oxygen independent response is desirable. The electrodes can be prepared either in the form of thick supported film, useful for disposable electrodes or as bulk-modified, disk shape electrodes, which can be used as renewable surface electrodes.

Keywords: sol-gel, biosensor, electrode, glucose oxidase

1. Introduction

The pioneering work of Braun et al. [1], demonstrating sol-gel doped xerogels triggered an extensive research in bioceramics, yielding also a large number of sol-gel derived biosensing applications [2–4]. Most of the activity in this field was directed to the development of photometric applications, due to the favorable optical characteristics of silica and organically modified silica (Ormosil) matrices [5–7]. However, the versatility of the sol-gel processing makes it also a most promising route for the production of silica and silica composite electrochemical devices. Indeed, several research groups have reported sol-gel derived electrochemical applications, including films of wet silica gels on inert metal electrodes [8], doped silica powders attached by a plastic membrane to a Clark oxygen electrode [9], and sandwich type electrodes, in which the enzyme is immobilized between two layers of sol-gel derived silica [10]. Kurokawa and coworkers used enzyme doped composite titania-cellulose biofilm electrodes [11] and we have concentrated on the development of conductive matrices such as vanadium pentoxide gels [12] and composite carbon-silica matrices [13, 14].

This paper summarizes the activities of our group on the development of composite carbon-ceramic biosensors. Using the well documented [8–10, 12–17] glucose oxidase enzyme as a common test case, we demonstrate how sol-gel processing can be used to tailor different types of composite, organically modified silica-carbon electrodes. We shall refrain from extensive discussion of the metrological characteristics of each class of biosensors and instead present an overview of different pathways that demonstrate the power of sol-gel technology.

2. Ceramic-Carbon Electrodes (CCEs)

Ceramic-carbon electrodes are made of a dispersion of 1–40% (w) carbon powder in porous, organically modified silica support [18, 19]. Typical preparation procedure of these electrodes is as follows. 1.5 ml

methanol (Frutarom, Laboratory Chemicals, Haifa), 1.0 ml methyltrimethoxysilane (ABCR, Karlsruhe, Germany) and 0.05 ml hydrochloric acid (11 M) catalyst are mixed for 2 minutes, followed by the addition of 1.25 g carbon (e.g., High purity carbon powder (app. 10–20 μm), from Ultra Carbon Corporation, Bay City, Michigan) and shaking for another minute. The mixture is then spread-coated on a supporting plate or filled into a capillary tube and allowed to dry for several days in ambient conditions (app. 25°C). Additional water for the hydrolysis step is provided by air humidity. This procedure yields porous, brittle and conductive material with app. 40% void fraction, and when the carbon loading exceeds the percolation value, the conductivity of the matrix is sufficient for electroanalytical purposes (>1.0 mho $\cdot$ cm^{-1}). The percolation loading of the carbon filler ranges between 1–2% (w) for nanocrystalline carbon powder (Ketjenblack EC-600JD, Akzo Chemie America, Michigan) and up to 22% (w) is needed when high purity, 20–40 μm dimension graphite powder (Ultra Bay carbon) is used. The electrodes can be molded in virtually any configuration including supported or unsupported thick films, disks, rods and even miniature electrodes.

The thickness of the wetted section of the electrode is important from electrochemical point of view. When the size of the active layer is excessively large the double layer capacitive currents and surface Faradaic processes, which are both proportional to the electroactive surface (i.e., the wetted graphite surface) outmask the electrochemical signal contributed by the diffusing target analyte. When the electroactive section of the electrode is too thin then external diffusion may become the rate limiting step, a situation that is prone to hydrodynamic fluctuations. Additionally, when the wetted section is small the amount of exposed, wetted catalyst may become too low, giving weaker conversions and electrochemical signals. In conventional polymer coated electrodes the thickness of the active layer is determined by the dimension of the polymer coating. Control of the thickness of the active section of composite carbon-ceramic electrodes can be done by tuning the surface polarity of its components. When the organofunctional surface group of the Ormosil (R) is hydrophilic (or when tetraalkoxysilane monomer is used for electrode preparation) the resulting CCE is hydrophilic and the matrix is flooded with the electrolyte. When apolar organofunctional alkoxysilanes are used as starting monomers, water is rejected by the hydrophobic surface and only the outer surface

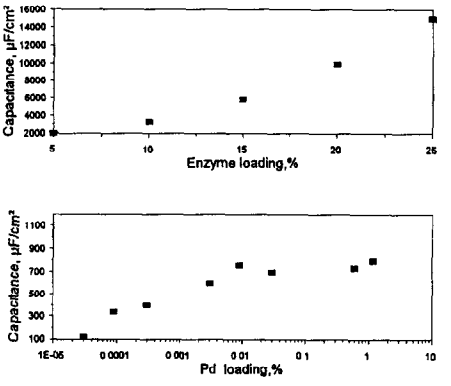

Figure 1. Dependence of the observed capacitance on glucose oxidase (top) and palladium load percent (w%).

of the electrode is wetted by the electrolyte. Thus, CCEs can be used in situations where minimal surface area electrodes are preferred (e.g., in flow configuration and external diffusion control applications) and for situations where the active surface area should be maximized (e.g., in catalysis and biocatalysis). We have recently demonstrated that intermediate situations, where a controlled thickness (10–200 μm) active layer is desirable, are also attainable by the addition of a hydrophilic species to the starting sol-gel solution. The hydrophilic modifiers can be either fine dispersion of inert metal catalysts, bulky hydrophilic substances that remain in the xerogel and modify its cage polarity or readily leachable chemicals such as polyethylene glycol or inorganic salts that can be washed out and leave wide open channels for the penetrating electrolyte. Figure 1 demonstrates the effect of the addition of palladium metal (A) and enzyme loading (B) on the double layer capacitance of hydrophobic CCE. The double layer capacitance is a proportional measure of the electroactive surface of the electrode [13, 20, 21].

This flexibility in shaping the configuration of the CCEs allows ample versatility in designing the electrochemical cells. Thus, it is possible to use CCEs in conventional flag or disk shapes, flow-through, or thin layer flow-cells configurations and even to perform the electrochemical measurement in a sample drop deposited on the surface of the working electrode (Fig. 2). The spherical shape of the drop is stabilized by the strong hydrophobicity of the working CCE. Drop analysis is useful for disposable sensors and when sample minimization is required. Such situation is often encountered in medical diagnostics.

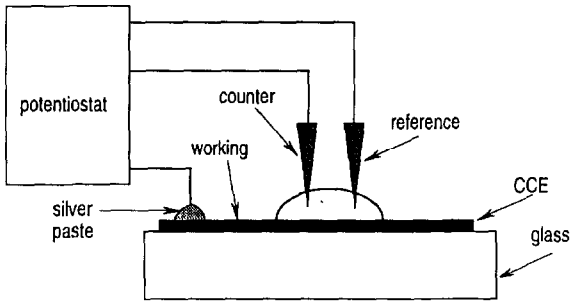

Figure 2. A scheme of drop analysis on a flat hydrophobic CCE.

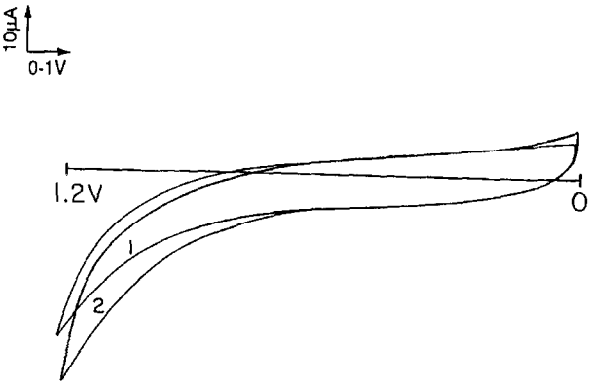

Figure 3. Cyclic voltammograms (10 mV/s, scan rate) of glucose oxidase modified CCE. Curves correspond to 0 and 20 mM glucose (pH 5.6).

3. Examples of Glucose Biosensors

Glucose sensors are often used to demonstrate new biosensing concepts due to the robust nature (high specificity, high stability) and low cost of the glucose oxidase enzyme, and the medical and commercial potential of this application. These and the fact that the activity of the enzyme is well documented, motivated the choice of glucose oxidase as a demonstrative test-case in our studies.

Several classes of polymer coated amperometric glucose electrodes were reported in the literature (see for example, Albery's classification [22]), all of which can be realized in sol-gel derived glucose oxidase modified ceramic-carbon matrices. The preparation protocol of the enzyme modified CCEs is similar to the preparation of the blank CCEs with three minor adjustments, the use of hydrochloric acid catalyst is minimized; methanol solvent is not introduced to the starting solution and gelation and drying are performed in a refrigerator. Briefly, 0.8 ml MTMOS (methyltrimethoxysilane), 0.5 ml water and 0.1 ml of 0.1*M* hydrochloric acid are mixed, sonicated for 10 minutes and stored in a refrigerator. 0.1 g carbon powder is impregnated with 0.005–0.025 g glucose oxidase (EC 1.1.3.4 type VII-S, 250,000 units/g from *Aspergillus niger*, Sigma) and mixed with the ormosil precursors. The mixture is then used to cast electrodes which are then allowed to dry for approximately 3–7 days in a refrigerator. Chemical modifiers can be introduced at each step.

Unmediated Glucose Biosensors

Here, the glucose is oxidized to gluconolactone, the reduced FAD cofactor is regenerated by dissolved oxygen, yielding hydrogen peroxide which can be detected by an electrochemical oxidation step:

$$\text{Glucose} + \text{GOx(FAD)} \rightarrow \text{Gluconolactone} + \text{GOx(FADH}_2\text{)} \quad (1)$$

$$\text{GOx(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{GOx(FAD)} + \text{H}_2\text{O}_2 \quad (2)$$

$$\text{Electrode reaction: } \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{e}^- + 2\text{H}^+ \quad (3)$$

Figure 3 demonstrates typical cyclic voltammograms (CV) of a glucose oxidase loaded carbon ceramic electrode in a buffer solution with or without glucose. The hydrogen peroxide oxidation wave commences at 600 mV/SCE. This wave disappears when the solution is deaerated. These two observations are also the two major drawbacks of the unmediated glucose biosensor. The electrode signal is highly dependent on oxygen tension and the high overvoltage of hydrogen peroxide oxidation on graphite surfaces makes the electrode highly susceptible to interfering reducing agents (e.g., ascorbic acid). In the following, we shall address the conventional methods for abating these drawbacks and demonstrate that these methods can be easily realized in composite ceramic-carbon matrices.

Inert Metal/Glucose Oxidase/CCEs

These electrodes are prepared by impregnation of the carbon powder with inert metal salts (e.g., palladium chloride), followed by reduction by high pressure hydrogen gas or another strong reducing agent. The carbon powder can then be used for the preparation of glucose oxidase/CCE by the conventional preparation protocol depicted above. Typical CV response of 1% palladium metal modified Glucose oxidase/CCE in blank

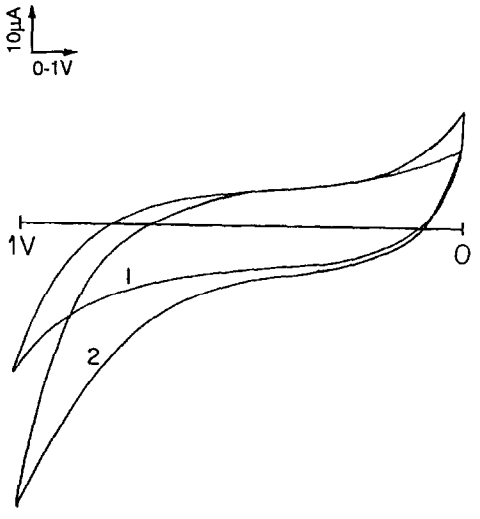

Figure 4. Cyclic voltammogram of Pd/glucose oxidase/CCE. Voltammograms correspond to 0 and 20 mM glucose in pH 5.6 phosphate buffer; scan rate = 10 mV/sec.

and glucose solution are presented in Fig. 4. The anodic wave of hydrogen peroxide oxidation is shifted by app. 300 mV compared with unmodified glucose oxidase/CCE, thus minimizing chemical interferences of reducing compounds. The catalyst modified CCE is sensitive to the level of dissolved oxygen and it still regains its blank response after deaeration.

Supply of Oxygen from the Gas Phase

There are two general methods to eliminate the dependence of the electrode response on the level of dissolved oxygen: either by supplying an alternative electron acceptor (as discussed below), or by providing an external source of oxygen. The high porosity of CCEs and the fact that only a thin layer at the outermost section of the electrode is flooded by the electrolyte suggest that a supply of oxygen through the back of the electrode is a possible solution to this problem. This can be realized by simply leaving the back of the electrode exposed to the ambient atmosphere. Indeed the response of electrodes with exposed back was practically independent of the level of dissolved oxygen. Figure 5 demonstrates a dependence of the response of a Pd/Glucose oxidase/CCE on oxygen partial pressure at the CCE back side.

Mediated Glucose Oxidase/CCE

Redox mediators are frequently used in order to reduce the operating potential of amperometric biosensors

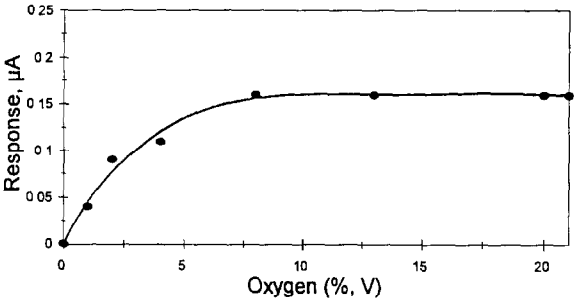

Figure 5. Steady state response of Pd/glucose oxidase/CCE exposed to several levels of oxygen partial pressures at the back of the electrode (deaerated solution, 20 mM glucose, phosphate buffer, pH 5.6).

and for attaining oxygen independent signal. Redox species such as ferrocene or TTF (Tetrathiafulvalene) serve as electron acceptors instead of oxygen and the reduced species is regenerated by electrooxidation on the conductive surface of the electrode.

$$\text{Glucose} + \text{GOx(FAD)} \rightarrow \text{Gluconolactone} + \text{GOx(FADH}_2\text{)} \quad (4)$$

$$\text{GOx(FADH}_2\text{)} + \text{Oxidized Mediator} \rightarrow \text{GOx(FAD)} + \text{Reduced Mediator} \quad (5)$$

$$\text{Electrode reaction: Reduced Mediator} \rightarrow ne^- + \text{Oxidized mediator} \quad (6)$$

Co-immobilization of redox species along with glucose oxidase can be achieved by introducing the redox mediators to the sol-gel starting solution and using conventional preparation protocol. Figure 6 demonstrates typical CV response of a TTF mediated electrode [13] containing approximately 0.1% (w) TTF. The oxidation (and regeneration) of the TTF is observable even at +0.2 V *vs.* SCE, i.e., 400–500 mV lower than the H_2O_2 oxidation on unmediated electrodes. The TTF mediated biosensor was insensitive to the concentration of dissolved oxygen and exhibited the same response in aerated and in nitrogen-bubbled glucose solutions. TTF was chosen as a model mediator due to its strong adsorption on graphite surfaces, which lowers it's leachability from the CCE. Indeed the stability of the TTF mediated electrode was much better than ferrocene modified biosensors. However, since the oxidized form is charged and more soluble we still observed considerable leaching of the mediator during prolonged operation.

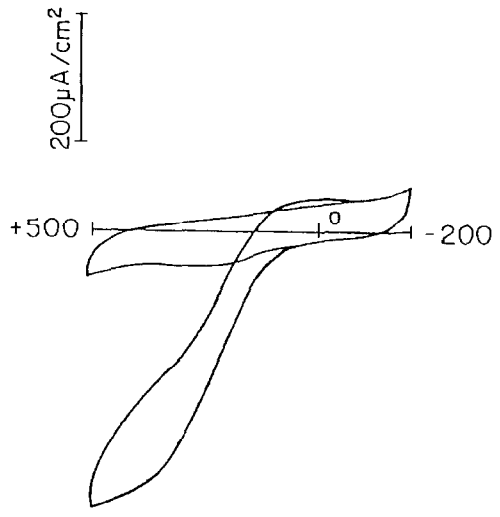

Figure 6. Cyclic voltammogram of TTF mediated glucose oxidase/CCE. Voltammograms correspond to 0 and 20 mM glucose in pH 5.6 phosphate buffer; scan rate = 10 mV/sec; Potential scan between −200 mV and 500 mV vs. SCE.

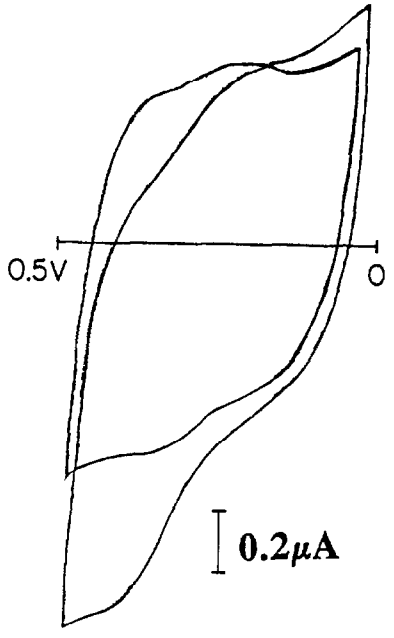

Figure 7. CV of wired ferrocene-glucose oxidase/CCE. Voltammograms correspond to 0 and 20 mM glucose in pH 5.6 phosphate buffer; scan rate = 10 mV/sec.

Redox Modified Glucose Oxidase/CCE

A possible way to reduce leaching of the redox mediators is to anchor the mediators to bulky chemical species that can be more effectively caged in the silica network. A straight forward way to do so is by anchoring the redox mediator directly to the enzyme itself. This concept of "wired enzymes" was introduced by Heller [23] as a method of obtaining direct charge transfer from the enzyme redox center to the electrode surface. Figure 7 demonstrates typical cyclic voltammograms of ferrocene modified glucose oxidase/CCE. The electrochemical response is similar to that of a ferrocene modified glucose biosensor, but the wired electrodes exhibit improved in-use stability. Detailed account of the preparation of "wired" glucose oxidase/CCE will be presented elsewhere [24].

4. Concluding Remarks

The sol-gel and sol-gel doping technologies are powerful tools, suitable for the preparation of a wide range of bioceramic carbon-composite materials.

CCEs are bulk-modified and thus, once contaminated can be renewed by a simple mechanical removal of the outer section of the electrode. Their high resistance to plastic deformation makes it possible to polish away the outer layer of the electrode without clogging the porous structure. Thus, a fresh electroactive layer, rather then a clogged surface, can be exposed after each polishing step. These properties, in addition to the facile ways of shaping any desirable electrode configuration make CCEs suitable for the preparation of thick-film-disposable biosensors, as well as, for the preparation of renewable active-layer enzyme electrodes. The electrodes can be modified by incorporating appropriate carbon powder, choice of the type of Ormosil and chemical dopants, including, active proteins, inert metals, organo-metallic catalysts or redox mediators.

We have avoided detailed discussion of the metrological characteristics of each class of electrodes and reserved such details for specific communications in analytical journals. However, some general metrological characteristics, that are common to all these electrodes should be mentioned in order to illuminate the solid nature of this concept.

The operating voltage, and thus the level of interferences from redox species in the solution can be adjusted by incorporation of a wide selection of redox mediators or by introducing chemical catalyst suitable for hydrogen peroxide oxidation [25, 26].

The shelf life stability of these electrodes is highly dependent on the preparation conditions, but when the electrodes are prepared with no methanol at moderate

pH, they are stable for at least two months at ambient conditions. (This property was dramatically improved since our first publications, in which we reported much inferior shelf life [13, 14]).

The dynamic range of the glucose CCEs depends on the structure and thickness of the active layer and it typically exceeds the required range for biomedical applications. The standard deviation of renewal reproducibilty (by mechanical polishing) is usually less than 5%, which is sufficient for most analytical applications. However, batch to batch and inter-electrode reproducibility is still poor and preparation technology should be improved before this class of electrodes can be used for commercial disposable biosensors.

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