

Sol-Gel Vanadium Pentaoxide Glucose Biosensor

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The supporting matrix of amperometric enzyme electrodes determines the metrological characteristics of biosensors and therefore attracts large scientific efforts.¹ Currently, supporting matrices are constructed of conductive² or nonconductive polymers,³ conductive organic salts,⁴ or layers of (modified or unmodified) enzymes adsorbed onto electrode surfaces.⁵ In this communication we present a new class of enzyme electrodes made of conductive, porous vanadium pentaoxide prepared by the sol-gel doping procedure. Both the synthesis of conductive bioceramic material and the demonstration of sol-gel enzyme electrode are reported here for the first time. The well-studied, cheap, stable, and practically useful glucose oxidase was selected as a model enzyme.

Sol-gel is a low-temperature technology for the production of ceramic materials through the formation of colloidal suspension of metal oxides.⁶ Avnir et al.⁷ demonstrated that it is possible to immobilize organic compounds in inorganic supports by introducing the organic compounds with the polymerization precursors. Indeed, this new class of organic-inorganic materials found a plethora of diagnostic⁸ and other applications.⁹ Braun et al.¹⁰ demonstrated the possibility of protein immobilization in

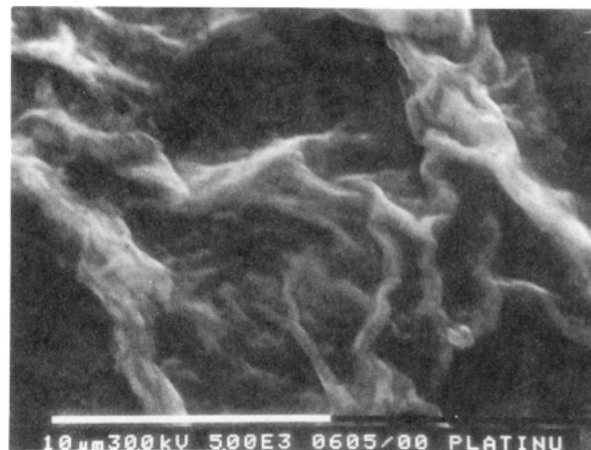


Figure 1. SEM micrographs of platinum electrode coated with a thin film of vanadium pentoxide doped with glucose oxidase. White bar indicates 10 μm scale.

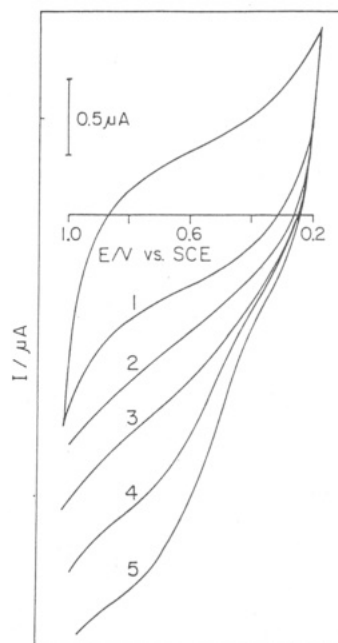


Figure 2. Voltammograms of glucose biosensor immersed in (1) blank solution; (2) 1.2 mM; (3) 3.5 mM; (4) 5.5 mM; and (5) 8.3 mM glucose solutions, buffered at pH = 5.6 (phosphate buffer); scan rate 10 mV/s.

sol-gel silica matrices. This class of bioceramic materials was further applied to produce silica-based photometric and flow injection analysis detectors.^{10–12} Porous inorganic xerogels are particularly attractive matrices for electrochemical biosensors since they combine physical rigidity, negligible swelling in aqueous solutions, chemical inertness, and high photochemical, biodegradational, and thermal stability. The physical and chemical characteristics of metal oxides can be easily manipulated, giving excellent control over the polarity, rigidity, pore size distribution, and, finally, ionic (e.g., $\text{Li}_2\text{O-SiO}_2$ and Nasicon-type ionic conductors) and electronic (e.g., $\text{In}_2\text{O}_3\text{-SnO}_2$, $\text{SnCl}_2\cdot 2\text{H}_2\text{O}$, or V_2O_5) conductivity.¹³ Particularly, the high conductivity of sol-gel vanadium pentaoxide attracted scientific attention.^{14,15}

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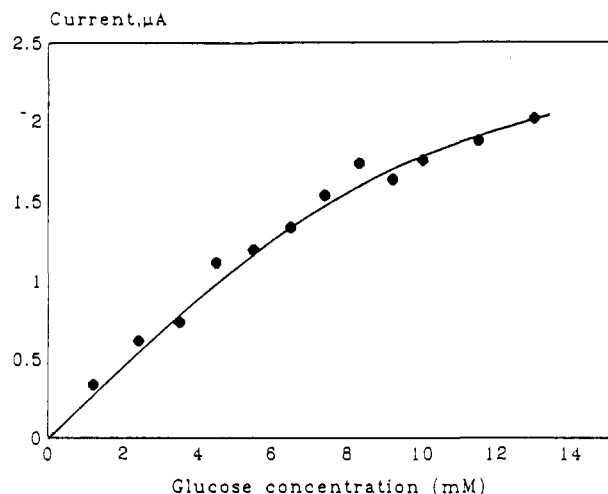


Figure 3. Calibration curve of the glucose biosensor (taken at 700 mV vs SCE).

The following molding procedure was used in order to entrap glucose oxidase in the vanadium oxide matrix. First, 3 g of vanadium pentoxide (BDH) was fused at 850 °C for 3 h (to increase the V^{4+}/V^{5+} ratio), and it was then quenched in 5 mL of distilled water, forming dark brown vanadium pentoxide gel. Approximately 1 g of the gel was dissolved in 3 mL of triply distilled water, and the diluted sol was then mixed with 0.25 mL of 6000 units/mL of glucose oxidase solution (EC 1.1.3.4 type VII-S from *Aspergillus niger*, Sigma Chemical Co.), giving a dark brown colloidal suspension. Enzyme electrodes were constructed by dip coating 0.25-mm-diameter Pt wire (Goodfellow, 99.99%) in the vanadium pentoxide-glucose oxidase solution, followed by overnight drying in ambient conditions.

The immobilization of glucose oxidase in vanadium pentoxide matrices was carried out by employing a modified protocol of the reported procedures to produce undoped vanadium oxide.¹³⁻¹⁵ The enzyme is best added to the colloidal suspension of vanadium oxide before its gelation. Additionally, organic solvents must be excluded from the starting solution due to the insolubility (and denaturation) of enzymes in aprotic solvents. For example, addition of enzyme solution to colloidal suspension of vanadium oxide in acetone-water (as used by Livage et al.¹⁵) was found to break the suspension and to form voluminous precipitate. Finally, a high-temperature sintering step must be avoided, even at the expense of higher electric resistivity.

Figure 1 is a typical SEM (scanning electron microscopy) photograph of a Pt flag electrode covered by a thin film of vanadium pentoxide-glucose oxidase in which the porous structure of the vanadium pentoxide is seen. The same structure (not shown here) is obtained when pure, undoped vanadium pentoxide xerogel is molded using the same synthetic procedure. The observed porous microstructure is that of bare vanadium pentoxide surface. Since vanadium pentoxide is conductive, sputtering of the sample with gold was not necessary for SEM studied.

Figure 2 depicts typical room-temperature cyclic voltammetry studies using a PARC EG&G Model 273 potentiostat and three electrode cells, equipped with a Pt-flag counter electrode and saturated calomel electrode (SCE). The applied scan rate was 10 mV/s. The cyclic voltammograms taken at several glucose¹⁶ concentrations show the increase of the current in the potential range of hydrogen peroxide oxidation (ca. 300–600 mV vs SCE). Experiments under nitrogen bubbling did not show any current

increase even under high glucose concentrations. This indicates that the biosensing mechanism is via hydrogen peroxide oxidation (generated by enzymatic oxygen reduction) and is not governed by direct charge transfer from the FADH coenzyme to the conductive oxide. Figure 3 demonstrates a typical calibration curve showing the response of the vanadium pentoxide glucose biosensor (taken at 700 mV vs SCE) to the dissolved glucose level. The metrological characteristics of the vanadium oxide biosensors such as detection range and sensitivity are similar to those of the commercial glucose electrodes.¹ The calibration curve of the vanadium pentoxide biosensor was stable during prolonged storage, and the electrode did not lose any activity during 10 days of storage at 4 °C. During this period the response of the electrode was checked daily by cyclic voltammetry in blank and in glucose sample solutions.

The sol-gel biosensors, which are exemplified here by the vanadium pentoxide amperometric prototype, promise to compete well with traditional polymer matrices, currently used in commercial electrochemical biosensors. Although, at this stage, only vanadium pentoxide-based biosensor is exemplified, the versatility of the sol-gel process promises that other inorganic biosensors will soon emerge, benefiting from the favorable properties of the enormous class of ceramic materials that can be processed by the sol-gel technology.

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Syntheses and Structure of 8-, 7-, and 6-Membered Silacycloallenes

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Decreasing the ring size of a cyclic allene to rings of fewer than 10 carbons results in deviation of both the normal $C=C=C$ linearity and the orthogonality of the dihedral angle.¹ To date, the smallest isolable cycloallene is 1-*tert*-butyl-1,2-cyclooctadiene,² and the smallest for which structural information is available is the phenylurethane derivative of cyclonona-2,3-dien-1-ol,³ which is bent to 168° and twisted to a dihedral angle of 79.8°. Encouraged by our recent success in the synthesis and structure determination of a tetrasilacyclohexyne,⁴ we have pursued the syntheses of strained silacycloallenes and report herein the first examples of isolable 6- and 7-membered rings containing 1,2-diene units.

A key factor in our synthetic success was the finding that 1,3-bis(trimethylsilyl)-1-propyne (**1**) is quantitatively converted to the allenyl dianion **2** upon treatment with 2 equiv of *n*-BuLi in ether.⁵ Quenching of **2** with α,ω -dichloropolysilanes **3–5** leads in each case to good yields of the corresponding cyclic allenes **6–8** (Scheme I).

Selected spectral data for **6** (only the second example of an isolable 8-membered cyclic allene), **7**, and **8** (the first examples

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