Electroreduction of O_2 to Water on the “Wired” Laccase Cathode[†]

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Oxygen was electrocatalytically reduced to water at a current density of 5 mA/cm^2 and at +0.7 V (NHE) in pH 5 citrate buffer at 37.5 °C. The electrocatalyst was a composite of laccase cross-linked with a redox polymer on a hydrophilic cloth of 10 μm diameter carbon fibers. The redox polymer, PVI−Os(tpy)(dme-bpy)$^{2+/3+}$, [poly-N-vinyl imidazole with 1/5th of the imidazoles complexed with [Os(tpy)(dme-bpy)]$^{2+/3+}$ (tpy=terpyridine; dme-bpy= 4,4′-dimethyl-2,2′-bipyridine)], electrically connected (“wired”) the laccase reaction centers to the fibers.

Introduction

The electroreduction of O_2 to water near neutral pH and at ambient temperature has been one of the longest-standing and best-researched problems of electrochemistry. It has been a missing element of a future biofuel cell with a noncorrosive electrolyte and operating near ambient temperature. The presently used platinum group metal-based cathodes of sub-100 °C H_2/O_2 fuel cells operate near pH 0 and the silver-activated carbon cathodes of ambient-temperature zinc-air batteries operate at pH 14. In contrast, the laccase-based cathode of this paper operates at 37.5 °C and at pH 5 in a citrate buffer. O_2 is electrocatalytically reduced on the cathode to water at a current density of 5 mA/cm^2 and at +0.7 V (NHE).[2]

Laccase cathodes on which O_2 is electroreduced to water were introduced by Tarasevich et al. They formed these by directly adsorbing laccase on Teflon-bound high-surface area carbon-black particles.[1] Their cathode was stable and operated at pH 5 at a current density of 175 μA/cm^2 when poised at 1.10 V. Tarasevich et al. also reported a current density approaching 10 mA/cm^2 at pH 3.5, for an electrode poised at 0.8 V. The electrode, made by loading 1−2 mg/cm^2 of a laccase from the Institute of Biochemistry of the Armenian SSR on 1 cm diameter carbon substrates with various micro-, meso- and macropore ratios[2] was, however, difficult to reproduce in absence of information on the species from which the laccase was derived and on the size and the size distribution of the carbon particles. Papers written in the past decade do not mention the high current density O_2 cathodes, even though some of these were written by members of the team that reported the high current density cathodes. Yaropolov et al. studied the bioelectrocatalysis of O_2 reduction on laccase-modified graphite and carbon electrodes, using laccases from *Rhus vernicifera* (a tree) and from *Coriolus hirsutus* (a fungus). They showed that the tree laccase films catalyzed O_2 electroreduction near neutral pH, but only at potentials negative of 0.4 V. The fungal laccase films catalyzed O_2 electroreduction at pH 5 at potentials as oxidizing as 0.8 V, but the current densities were less than 50 μA/cm^2.[3] Santucci et al. studied the heterogeneous electron transfer to *Rhus* laccase and ascorbate oxidase on gold, showing that trapping of the enzymes in a cross-linked tributylmethyl phosphonium chloride-modified polystyrene membrane enhanced the O_2 electroreduction below 0.4 V at neutral pH. The currents observed were <1 μA/cm^2.[4] Trudeau et al. were the first to “wire” laccase, immobilizing it in a 0.44 V redox potential polymer and forming a pH 4.7 azide sensor. The sensor operated at room temperature at a current density of 100 μA/cm^2 when poised below 0.4 V.[5] Lee et al. reported a series of laccase-based oxygen cathodes, where the enzyme was in direct electrical contact with pyrolytic carbon. They observed a current density of 190 μA/cm^2 at +0.2 V at 22 °C and at pH 3.8.[6] Palmore et al. described a fuel cell, its cathode compartment containing a homogeneous laccase solution and a vitreous carbon electrode poised at 0.4 V, operating at a current density of 120 μA/cm^2 at pH 4.[7] Thuesen and co-workers reported a current density of 13 μA/cm^2 at 0.36 V for a monolayer of surface-immobilized laccase at pH 3.3.[8] Jin et al. electroreduced O_2 at a current density of 0.3 μA/cm^2 on an electrode poised at −0.1V at pH 5.8, using a homogeneous laccase solution and cytochrome *c* as mediator.[9] Tsujimura et al. recently described a bilirubin oxidase-carbon felt electrode operating at pH 7 at a current density of 0.89 mA/cm^2 when poised at 0.22 V.[10]

Our initial communication described the operation and the design principles of the cathode of this paper.[11] The electrocatalyst was a composite of carbon cloth, consisting of 10 μm diameter hydrophilic carbon fibers, coated with the cross-linked electrostatic adduct of laccase from *Coriolus hirsutus* (a polyanion) and an electron-conducting redox polymer (a polycation). The redox polymer electrically connected (“wired”) the laccase reaction centers to the carbon fibers. The structure of the 0.78 V redox potential polymer is shown in Figure 1. The polymer was made of poly-N-vinyl imidazole by coordinating 1/5th of the rings to [Os(tpy)(dme-bpy)]$^{2+/3+}$ (tpy = 2,2′:6′,2″-terpyridine; dme-bpy = 4,4′-dimethyl-2,2′-bipyridine). The catalytic scheme whereby O_2 is electroreduced to water is shown in Scheme 1. The open-circuit potential of an electrode made by directly adsorbing *C. hirsutus* laccase on vitreous carbon is 0.82 V at 1 atm O_2 and at pH 5. The laccase oxidizes the Os^{2+}

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[‡] All reported potentials are vs the potential of the normal hydrogen electrode (NHE).

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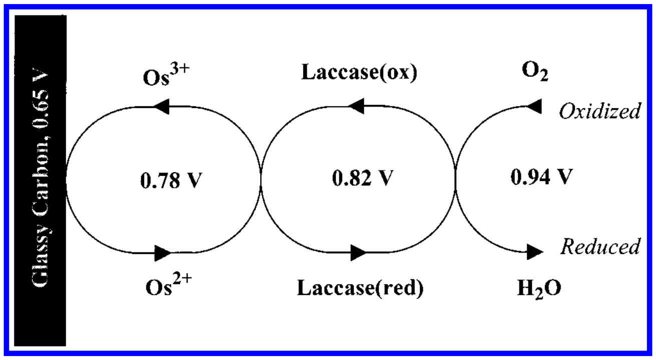
SCHEME 1: The Electrocatalytic Cascade of the Cathode



centers of the electron and ion-conducting redox polymer to Os^{3+}, and the redox polymer is electroreduced at the carbon surface at a potential near 0.65 V.

As will be seen below, the O_2 electroreduction potential and current density of the laccase cathode depend on the redox polymer used to "wire" the enzyme, on the weight fractions of the components of the electrocatalytic composite, on the temperature and O_2 pressure, and on the concentrations of inhibitors of laccase.

Experimental Section

Chemicals. Laccase (EC 1.10.3.2) from *Coriolus hirsutus*, (60 catechol units per mg protein) was received from SynectiQ (Dover, NJ) as a pH 6.5, 3 mg/mL solution in 1:1 glycerol-sodium phosphate buffer. The solution was concentrated to 50 mg/mL by ultrafiltration and washing with distilled water. Carbon cloth (Toray TGPH-030, Japan) was received as a sample from E-TEK (Somerset, NJ). Ultrapure O_2 and air (Praxair), sodium chloride and sodium hydroxide (Aldrich, Milwaukee, WI), and poly(ethylene glycol) diglycidyl ether (PEGDGE, Polysciences Inc., Warrington, PA) were used as received.

Polymer Synthesis. 2,2′:6′,2″-terpyridine (tpy) was complexed to $(NH_4)OsCl_6$ to produce $Os(tpy)Cl_3$ as described[12] except that reactants, at 1:1 molar ratio, were dissolved in ethylene glycol and refluxed under argon for 1 h. The $Os(tpy)Cl_3$ produced was precipitated by pouring the reaction mixture into ethyl ether (yield 90%). The complex was then reacted with 4,4′-dimethyl-2,2′-bipyridine (dme-bpy)[13] at a 1:1 molar ratio to form $[Os(tpy)(dme\text{-}bpy)Cl]Cl_2$, which was also precipitated by pouring the reaction solution into an excess of ethyl ether (yield 85%).[14] The redox polymer was prepared by complexing the reduced complex, $[Os(tpy)(dme\text{-}bpy)Cl]Cl$, with poly(N-vinylimidazole) in a 1:4 molar ratio and was purified as described.[14]

Electrode Preparation. The composite electrodes were assembled on 3 mm diameter vitreous carbon rotating disk electrodes (RDE) mounted in Teflon sleeves. These disks were sanded with ultrafine sand paper, polished with alumina slurry (1 μm) and sonicated for 5 min in deionized water. The polishing step was repeated until no voltammetric features beyond water oxidation were observed in a 50 mV/s scan in phosphate buffer, pH 7 in the range between 0.2 and 1 V. The 350 μm thick carbon cloth had a nominal 78% void fraction and consisted of 10 μm diameter fibers. The cloth was cut into 4 mm diameter disks, which were cemented, using conductive carbon paint (SPI, West Chester, PA), to the surface of the vitreous carbon RDE. The rod-bound cloth was made hydrophilic by exposure to a 1 Torr O_2 plasma for 5 min.[15] For the base-case electrode, 10 μL of an aqueous 7.6 mg/mL redox polymer solution were mixed with 2 μL of 200 mM citrate buffer (pH 5), 2 μL of a 35 mg/mL laccase solution, and 2 μL of 5 mg/mL PEGDGE. An 8 μL aliquot of this mixture was pipetted onto the mounted hydrophilic carbon cloth, which was promptly wetted and penetrated by the solution. The composite mounted disks were cured for >12 h in air at room temperature.

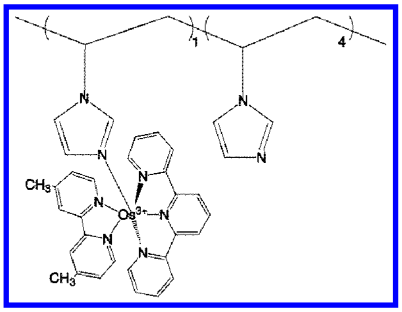


Figure 1. Structure of the laccase wiring redox polymer.

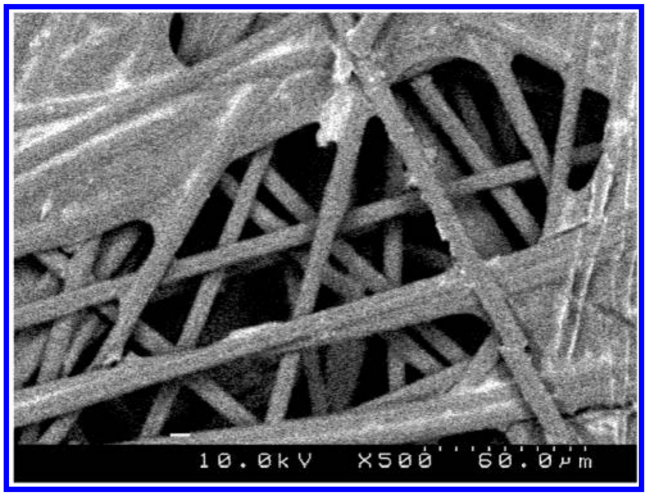


Figure 2. SEM (electron backscattering mode) of the composite 350 μm thick carbon cloth cathode loaded with 0.78 mg/cm^2 "wired" laccase (45 wt % laccase, 49 wt % redox polymer, 6 wt % PEGDGE) The lighter areas are coated with more heavy atoms (osmium).

Instrumentation and Cell. The measurements were performed using a CH−Instruments Electrochemical Detector Model #CHI832 and the data were collected by a dedicated computer. The electrodes were rotated using a Pine Instruments Rotator. The water-jacketed electrochemical cell had a Pt wire or vitreous carbon counter electrode and an Ag/AgCl reference electrode. It contained 100 mL of citrate buffer (200 mM, pH 5) and was maintained at 37.5 °C. To maintain a fixed volume of electrolytic solution in the cell the admitted gases were presaturated with water by passage through a bubbler, which contained a solution similar to that in the cell.

Results

Composite Electrodes. The distribution of osmium in the hydrogel-carbon cloth composite after its dehydration in the evacuated chamber of an electron microscope is shown in electron micrograph of Figure 2. In obtaining the picture, the electron microscope was operated the electron backscattering mode. The figure shows large, solvent accessible pores between the 10 μm fibers. Because the backscattering of electrons increases with atomic mass, regions of high osmium concentration appear as bright patches. The presence of the osmium-containing polymer is particularly pronounced in patches

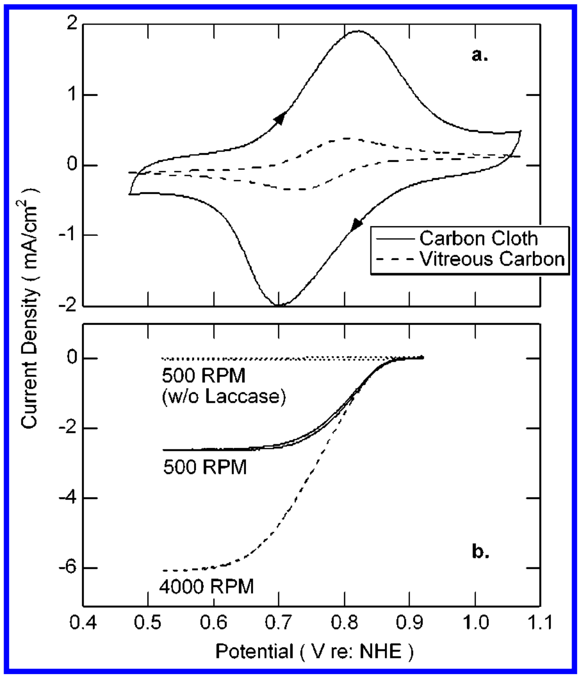


Figure 3. **(a)** Cyclic voltammograms (50 mV/s sweep rate, 500 rpm) of the vitreous carbon and the composite carbon cloth electrodes under argon. **(b)** Potential-dependence of the O_2 electroreduction current density at 1 mV/s sweep rate at 1 atm O_2 pressure. Loading as in Figure 2, 0.2 M citrate buffer, pH 5, 37 °C. Current densities were based on the projected area of the 4-mm carbon cloth disk (0.13 cm^2).

clinging to the binder that glues the carbon fibers of the cloth. Figure 3a shows cyclic voltammograms of a plain vitreous carbon electrode and of the carbon-cloth composite electrode under argon at 50 mV/s sweep rate. The only voltammetric waves observed were those associated with the electrooxidation/reduction of PVI[Os(tpy)(dme-bpy)]$^{2+/3+}$, centered at 0.77 V. The waves are about an order of magnitude larger for the carbon-cloth composite electrode than for the polished vitreous carbon electrode, suggesting an order of magnitude difference in the electrochemically accessible, redox-polymer coated surface area.

The polarization curves of the carbon cloth composite and the polished vitreous carbon cathodes, both under 1 atm O_2 rotated at 500 and 4000 rpm in 0.2 M pH 5 citrate buffer at 37.5 °C, are seen in Figure 3b. In the absence of laccase O_2 is not electroreduced on either electrode. At 0.5 mg/cm^2 laccase loading O_2 is electroreduced when the electrodes are poised at potentials negative of 0.85 V, the potential where the electroreduction of PVI[Os(tpy)(dme-bpy)]$^{3+}$ becomes significant (Figure 3a). The current density, based on the 0.13 cm^2 carbon cloth disk, increases nearly linearly with decreasing electrode potential until the mass-transport defined plateau is reached. The observed linearity suggests that the current density is limited by ohmic resistance. The O_2 mass transport defined plateau of the current density is a function of the O_2 partial pressure, the temperature and the angular velocity of the rotating electrode below about 10^3 rpm (Figure 4). Above 10^3 rpm, the plateau is defined by the kinetics of the O_2 electroreduction and increases with temperature. The current density reaches a remarkably high value of 10 mA/cm^2 at 1 atm O_2, 37 °C, and 4000 rpm. In a quasi-stagnant, air-saturated solution the current density at 37 °C is lower by 2 orders of magnitude, about 100 μA/cm^2.

The optimal enzyme mass fraction was ~40% when the PEGDGE binder fraction was 6 wt % for both the carbon cloth composite and for the polished vitreous carbon electrodes (Figure 5). Although a more rigorous analysis would reveal day-to-day variations in enzyme activity, this effect was within the repeatability error. Observed current densities at 0.62 V were found to vary directly with both the loading of the wired laccase, for loadings between 0.2 and 1.3 mg/cm^2, and the thickness of the carbon cloth for thicknesses of 90 μm to 350 μm (data not shown).

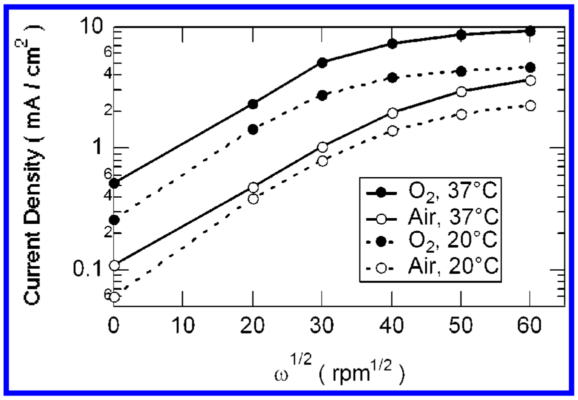


Figure 4. Dependence of the current density of O_2 electroreduction at 0.62 V (NHE) on the square root of the angular velocity of the rotating electrode. Conditions as in Figure 3b.

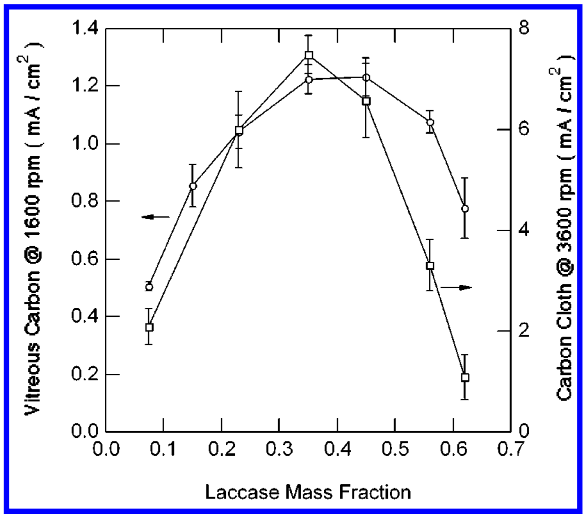


Figure 5. Dependence of the O_2 electroreduction current density at 0.62 V (NHE) on the laccase loading. Total loading was fixed at 0.78 mg/cm^2 and PEGDGE constituted 6 wt % of the total mass. Conditions as in Figure 3b.

When the pH was raised above its optimal value of pH 5 the current density dropped and at pH 7 the electrode was inactive (Figure 6). Chloride also inhibited the electroreduction: The current density declined by 60% when the Cl$^-$ concentration was raised from nil to 0.1 M. Under ~0.1 N/m^2 shear stress, exerted by rotation at 1000 rpm, the carbon-cloth composite cathodes were much more stable than the electrodes made by coating the polished vitreous carbon electrodes with films of wired laccase (Figure 7a). The O_2 electroreduction current of the composite electrodes dropped to 1/3rd of its initial value in 4.7 days and their voltammetric Os$^{2+/3+}$ electrooxidation/reduction peaks declined by 42% ± 5% (Figure 7b). The loss of current was at least three times faster in the noncomposite vitreous carbon electrodes. Although the half-lives of the shear-stressed composite electrodes were of ~3 days, the half-lives of the noncomposite electrodes were only of ~1 day.[16]

Discussion

The results show that at pH 5 and at 37 °C and under conditions of high convection oxygen is electroreduced to water at 0.62 V at a current density exceeding 5 mA/cm^2 on the

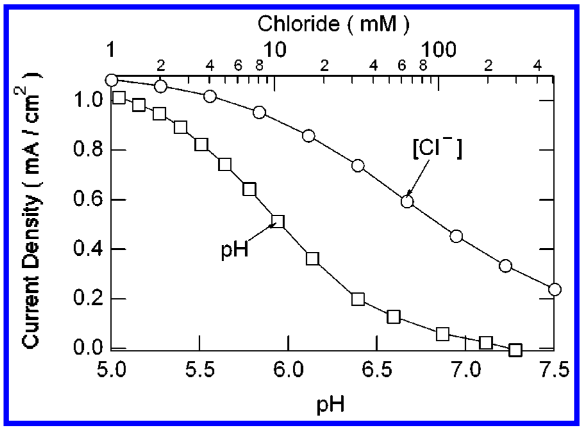


Figure 6. Dependence of the current density of the noncomposite (polished and catalyst coated) vitreous carbon electrodes on pH and on the chloride ion concentration. 0.62 V (NHE), 900 rpm. Conditions as in Figure 3b.

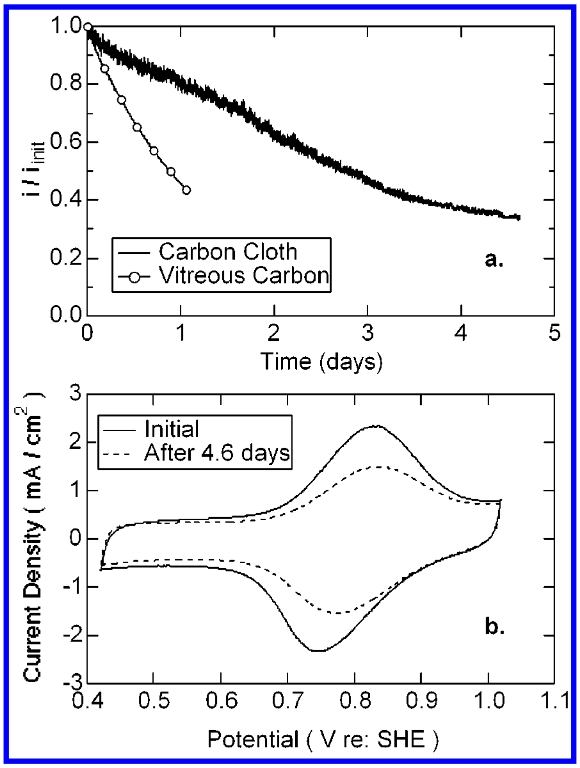


Figure 7. Operational stability of the "wired" laccase electrode. (a.) Time-dependence of the current density of carbon-cloth composite and of the vitreous carbon noncomposite electrodes poised at 0.62 V (NHE). (b.) The change in the cyclic voltammograms of the carbon cloth composite electrode after 4.6 days of operation. Scan rate 50 mV/s. The composition, loading and conditions were as in Figure 3b.

composite "wired" laccase cathode. At 0.8 V, a potential just 0.15 V below the standard pH 5 potential for the four-electron reduction of O_2 to water, the current density exceeds 1 mA/cm^2 (Figure 4). Higher current densities (of 1 A/cm^2) have previously been reached below 100 °C only at pH 0, and only in cathodes based on platinum-group alloy catalysts. To illustrate the power density that might be reached in a future biofuel cell made with the cathode and with a glucose electrooxidizing anode operating at $>$ 2 mA/cm^2 poised at 0.2 V, we consider a cell consisting of a 1 cm long, 0.4 cm diameter hollow tubular anode and a similar cathode. If operation of the cathode would be extended to pH 7, with no inhibition by chloride anions and other blood constituents, then with arterial blood (8 mM hemoglobin-bound O_2, 5 mM glucose) flowing through the cell at a linear velocity of 10 cm/s, the 0.24 cm^3 cell would produce 1.2 mW. It would generate in one week the amount of energy stored in the highest energy density lithium battery of equal volume. A cathode operating in a major blood vessel of an animal would require a wired laccase electrode that would not loose its activity when the pH is raised from 5 to 7 and when Cl^- is present at its physiological concentration. A recent study suggests that the activity of laccase from *Pleurotus ostreatus* declines only moderately upon increasing the pH from 5 to 7.[17]

The observed optimal enzyme mass fraction was ~40% for both the carbon cloth and the vitreous carbon electrodes (Figure 5). The constancy of the ratio suggests that the thicknesses of the electroactive zones were similar for both structures, and that the increased current density with the cloth derives solely from an increase in active surface area when enzyme kinetics and electron transport are not re-balanced.[18]

The wired laccase cathode was reasonably stable even when the electrode was continuously stressed by shearing at 0.1 N/m^2 through rotation at 1000 rpm (Figure 7a). The major cause of loss was the stripping of the electrocatalytic film, suggested by the fact that not only the current density but also the voltammetric peaks declined (Figure 7b). If enzyme deactivation were the dominant cause of loss, only the O_2 electroreduction current, not the voltammetric peaks of $Os^{2+/3+}$ electrooxidation/reduction, would have declined. After 4.7 days of continuous operation, 66% of the O_2 electroreduction current and 42% of the electroactive $Os^{2+/3+}$ centers were lost. This suggests that enzyme degradation caused only ~24% of the current loss in 5 days.

Conclusions

O_2 was electroreduced to water at pH 5 on a rotating (1000 rpm) carbon-cloth composite "wired" laccase (45 wt % laccase, 49 wt % redox polymer) cathode at 5 mA/cm^2 current density at 0.62 V in O_2-saturated pH 5 citrate buffer at 37 °C. In stagnant, air-saturated, citrate buffer the current density was ~100 μA/cm^2. The half-life of the cathode was ~3 days when it was sheared at a stress comparable to that of fast-flowing blood in a major blood vessel. The next necessary step toward a fuel cell implanted in a major blood would be the extension of the cathode's operating pH from pH 5 to the physiological pH 7.2−7.4 range and its operation in the presence of chloride at its physiological concentration.

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