

Characterization of the Electroenzymatic Reduction of H_2O_2 in Carbon Dioxide by Voltammetry with a Chemically-Modified Microelectrode

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Recently, a broad array of enzymes have been found to retain their activity in a variety of unconventional media,^{1a} including CO_2 .^{1b,c} Voltammetry has not been previously applied to the study of enzyme catalysts in CO_2 -based fluids because their highly resistive nature limits the use of conventional voltammetric methods. One approach to voltammetry in resistive fluids involves the use of a self-contained electrochemical cell coated with a conductive film.² We show here that a self-contained cell coated with a redox polymer containing horseradish peroxidase (HRP) can be used for the electrocatalytic reduction of H_2O_2 in low density, water-modified CO_2 ($T = 30^\circ\text{C}$, $P = 850$ psi, $\rho = 0.165$ g/mL).³ This is the first example of electrochemical biocatalysis in a CO_2 -based fluid near its critical point ($T_c = 31^\circ\text{C}$, $P_c = 1073$ psi).

A self-contained electrochemical cell was made by sealing a pair of platinum disks into the end of a glass tube.² One disk had a radius of $5\ \mu\text{m}$ and was used as the working electrode, while the other had a radius of $125\ \mu\text{m}$ and was used as the counter electrode. The cell was coated with a cross-linked redox polymer film containing HRP. The cross-linkable redox polymer⁴ consisted of poly(vinyl pyridine) (MW 50 000, Polysciences, Warrington, PA) modified with pendant $\text{Os}(\text{bpy})_2\text{Cl}$ ($\text{bpy} = 2,2'$ -bipyridine) and ethylamine groups. The entire face of the cell was coated with $12\ \mu\text{L}$ of phosphate buffer (pH 7.4) containing the redox polymer (0.55 mg/mL), HRP (used as received from Sigma, St. Louis, Mo., 0.36 mg/mL), and the cross-linking agent (poly(ethylene glycol)diglycidyl ether, Polysciences, 0.27 mg/ml). The film was cured in air for 48 h, soaked in ultrapure H_2O for 15 min, and redried in air for 1 h. The coated cell was mounted in a 15-mL stainless steel vessel² which contained $60\ \mu\text{L}$ of ultrapure H_2O , which is enough to saturate the CO_2 .⁵ The vessel was purged three times with SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) and then pressurized to 850 psi at 30°C , which is in the single-phase region of the CO_2 phase diagram,³ as confirmed with a view cell. Cyclic voltammetry was performed with the working electrode lead of the potentiostat (EI400, Ensmann Instruments, Bloomington, IN) connected to the microdisk electrode and both the reference and auxiliary leads connected to the counter electrode of the self-contained cell.

After the fluid was pressurized, cyclic voltammograms of the redox polymer were recorded at a sweep rate of $0.05\ \text{V/s}$ in the absence of H_2O_2 . Initially, the voltammograms exhibited severe resistive distortion, which decreased rapidly over the first 10–15 min of the experiment. Figure 1A shows the voltammogram obtained after 30 min. The voltammogram is predominantly peak-shaped, but a steady-state oxidation current flows at an

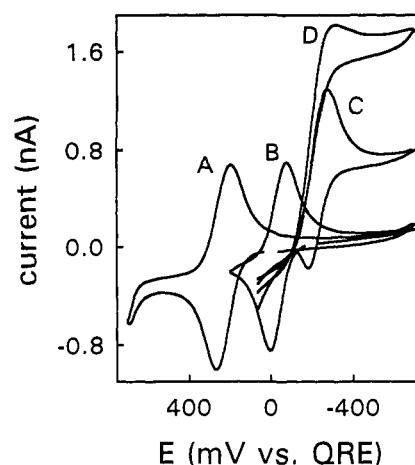


Figure 1. Cyclic voltammograms of the redox polymer with immobilized HRP in CO_2 at 850 psi and 30°C in the absence of H_2O_2 (A), and 12 min (B), 22 min (C), and 32 min (D) after repressurization of the vessel with H_2O_2 added (sweep rate = $0.05\ \text{V/s}$).

applied potential of 500–600 mV. This mixed type of voltammetric response is characteristic of a thin electroactive film at an inlaid microdisk electrode.⁶ The resistive distortion initially observed was due to an insufficient amount of water in the film during the early stages of the experiment, as evidenced by our inability to obtain a voltammogram without adding water to the fluid. The importance of the water content of the film was also demonstrated by experiments in air. The voltammogram in humid air resembled Figure 1A, while no voltammogram could be obtained in dry air. These results show that ions in these films must be solvated for electron hopping⁷ to occur.

Once the voltammogram shown in Figure 1A was obtained, the vessel was vented, $3.86\ \mu\text{L}$ of 6% H_2O_2 was added, and the vessel was again purged three times and pressurized with CO_2 . The amount of H_2O_2 added would give a concentration of 0.5 mM if it were completely dissolved in the fluid, which is unlikely (*vide infra*). No information on the solubility of H_2O_2 in water-modified CO_2 exists, so the actual concentration of H_2O_2 is unknown. Following repressurization of the vessel, the voltammograms again exhibited resistive distortion which decreased with time. Figure 1B shows that the voltammogram obtained after 12 min is identical to that obtained before addition of H_2O_2 (Figure 1A), except for a shift of the half-wave potential to a more negative value. The potential shift also occurred during analogous experiments in aqueous solution. No shift occurred, however, when a conventional SCE was used instead of the counter electrode of the self-contained cell. This shows that the shift is due to a change in the potential of the counter electrode resulting from oxidation of the redox polymer by H_2O_2 . Voltammograms C and D in Figure 1 were obtained by starting the potential sweep at a value positive of the redox polymer wave and sweeping initially in the negative direction. Over a period of 40 min, the voltammogram changed from the peaked shape typical of diffusion in a thin film to the sigmoidal shape characteristic of an electrocatalytic process.⁸

Verification that the change in the shape of the voltammogram from peaked (Figure 1A) to sigmoidal (Figure 1D) was due to the onset of an electrocatalytic process was obtained in three ways. First, when the vessel was vented, repressurized, and reequilibrated for ca. 10 min without addition of peroxide, no significant change in the shape, amplitude, or half-wave potential of the voltammogram occurred for 2 h (the longest time tested).

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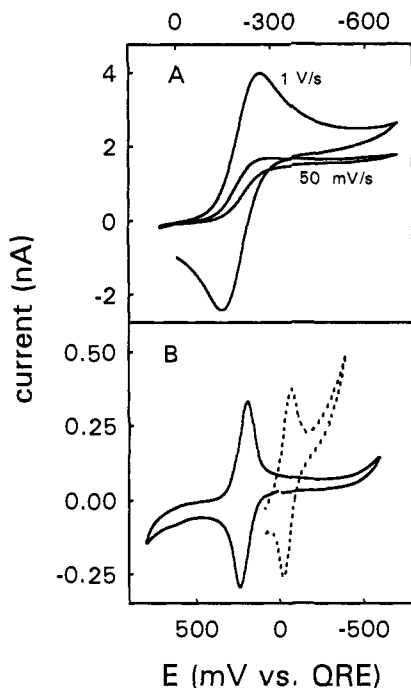


Figure 2. (A) Dependence of the catalytic response on sweep rate. The voltammogram at 1 V/s was obtained after the steady-state response was observed at 0.05 V/s. (B) Cyclic voltammograms of the redox polymer without HRP in CO₂ at 850 psi and 30 °C before (solid line) and 40 min after (dashed line) addition of peroxide to the vessel (sweep rate = 0.05 V/s).

Second, in an experiment in which the sigmoidal voltammogram was first established at 0.05 V/s in the presence of H₂O₂, a cyclic voltammogram was recorded at 1 V/s. Figure 2A shows that at the elevated sweep rate the voltammogram takes on a diffusion-controlled appearance, which is consistent with the behavior of electrocatalytic processes.⁸ Third, experiments identical to that described in Figure 1 were performed with films lacking HRP. Figure 2B shows voltammograms obtained before and 40 min after introduction of H₂O₂ to the vessel. No electrocatalysis occurred in the absence of HRP, even though the shift in the half-wave potential of the redox polymer and some direct reduction of H₂O₂ at the electrode did occur. Figure 2B shows that H₂O₂ oxidation of the redox polymer in the absence of HRP occurs too slowly to be observed by cyclic voltammetry at a sweep rate of 0.05 V/s. These three control experiments each support the conclusion that the sigmoidal voltammogram in Figure 1D is due to the electrocatalytic reduction of H₂O₂.

The intense interest in fluids near their critical point is derived in large part from their enhanced mass transport properties, i.e., high diffusivity and low viscosity.⁹ Considering this, it is

surprising, at first glance, that the catalytic response to H₂O₂ took several minutes to appear and almost 40 min to stabilize. The slow time course is easily rationalized, however, by noting that the solubility of H₂O₂ in low-density CO₂ is expected to be very low, H₂O₂ being a polar species. The low solubility of H₂O₂ in CO₂ limits the quantity of H₂O₂ available to undergo reactions in the film. In the early stages of the experiment, H₂O₂ entering the film is consumed in the process of oxidizing the redox polymer. This explains why the shift in half-wave potential occurs before the onset of catalysis. Once the film is completely oxidized, H₂O₂ builds up in the film and catalysis is observed. Furthermore, the voltammogram in Figure 1D shows complete kinetic control, so diffusion of peroxide must be fast on the time scale of cyclic voltammetry at 0.05 V/s. Therefore, the 40-min period required for stabilization of the response to H₂O₂ cannot be a consequence of a slow diffusion process.

One possible advantage of using low-density fluids, such as CO₂ near its critical point, for electrochemistry with polymer-immobilized enzymes is the acceleration of the apparent rate of catalysis by overcoming the barrier to diffusion of substrate posed by the polymer matrix. It is intriguing to note that the sigmoidal voltammogram in Figure 1, which is indicative of complete kinetic control, was obtained at 0.05 V/s. This is a rapid sweep rate by typical enzyme electrochemistry standards.¹⁰ This rapid scan rate implies a correspondingly rapid catalytic rate constant since the dimensionless kinetic parameter for electrocatalysis is $\lambda = (k^*C_2^*/\nu)(RT/nF)$, using the nomenclature of ref 8. Sigmoidally-shaped voltammograms are expected only when λ is greater than 1. We have studied the HRP/polymer combination in aqueous solution with both macro- and microelectrodes and using various recipes for electrode modification. Even after optimization of HRP-based sensors,¹¹ we have never observed sigmoidally-shaped voltammograms in aqueous solutions at such a rapid sweep rate. Thus, all preliminary indications are that the use of low-density CO₂ accelerates the apparent enzyme kinetics.

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