**Characterization of Oxygen Biocathodes**

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Although enzymes are highly efficient catalysts, there have been problems incorporating them into fuel cells. Early enzyme-based fuel cells contained enzymes in solution rather than immobilized on the electrode surface (1 and references within). Enzymes in solutions are only stable for days, whereas immobilized enzymes can be stable for months. One of the main obstacles of enzyme-based biofuel cells has been to immobilize the enzyme in a membrane at the electrode surface that will extend the lifetime of the enzyme and form a mechanically and chemically stable layer, while not forming a capacitive region at the electrode surface. In most $H_2/O_2$ fuel cells, the binder that holds the catalyst at the electrode surface is Nafion. Nafion is a perfluorinated ion exchange polymer that has excellent properties as an ion conductor. However, Nafion has not been successful at immobilizing enzymes at the surface of biofuel cell electrodes because Nafion forms an acidic membrane that decreases the lifetime and activity of the enzyme.

Previous studies in our lab have shown that mixture-cast films of quaternary ammonium salts (such as: tetrabutylammonium bromide) and Nafion have increased the mass transport of small analytes through the films and decreased the selectivity of the membrane against anions (2). These membranes have very similar conductivities as unmodified Nafion, but they have a much higher preference to the quaternary ammonium bromide than to the proton, as shown by titrating the number of available exchange sites to protons in the membranes. Therefore, these films have similar electrical properties, but very different acid/base properties. The treated membranes maintain their neutral pH over a wide range of buffer pHs.

In order to make more stable and reproducible quaternary ammonium salt-treated Nafion membranes, the excess bromide salts must be removed from the casting solution. This salt-extracted membrane is formed by re-casting the mixture-cast membranes after the excess quaternary ammonium bromide and HBr salts have been extracted from the original membranes. Salt-extracted membranes retain the presence of the quaternary ammonium cations at the sulfonic acid exchange sites, but eliminates complications from excess salt that may be trapped in the pore or may cause voids in the equilibrated membrane. Our research group has employed voltammetry, ion exchange capacity measurements, and fluorescence microscopy to characterize the chemical and physical properties of the salt-extracted membranes before enzyme immobilization (3).

The research described in this paper focuses on the development a biocathode by replacing the platinum catalyst at a traditional cathode with an enzymatic catalyst system. The biocathode consists of carbon cloth coated with a modified Nafion membrane containing bilirubin and bilirubin oxidase. The coated carbon cloth is then soaked in 1mM $Ru(bpy)_3^{+2}$ to allow the redox mediator $Ru(bpy)_3^{+2}$ to preconcentrate in the membrane.

The modified membrane retains the electrical properties of Nafion, but the pH is buffered within the membrane and the size of the pore is increased for easier immobilization of large and fragile biomolecules, such as proteins and enzymes. Since Nafion is an ion exchange polymer with a high concentration of sulfonic acid groups, it was further modified to be an electron conductor by introducing $Ru(bpy)_3^{+2}$ complex to act as the redox mediator for the cathode. The introduction of $Ru(bpy)_3^{+2}$ was accomplished by ion exchange of the species into the enzyme immobilization membrane after polymer casting. $Ru(bpy)_3^{+2}$ preconcentrates to concentrations between 0.5-1.5M in Nafion membranes. The high concentration of $Ru(bpy)_3^{+2}$ molecules within the pore structure of the modified Nafion membrane allows for self-exchange based conduction of electrons between the enzyme and the electrode. This results in a biocathode with a membrane that acts both to stabilize and entrap the enzyme while shuttling the electrons between the enzyme and the electrode. Initial testing of the biocathode as a half-cell showed a 102-fold increase in current when a modified Nafion membrane contained both bilirubin oxidase and bilirubin, as compared to a modified Nafion membrane containing only bilirubin. The background current of a buffer solution was $2.03 \times 10^{-5}$ A/cm$^2$ at a bilirubin-modified electrode, where as the current at a bilirubin/bilirubin oxidase modified electrode was $2.04 \times 10^{-3}$ A/cm$^2$. Similar studies were done at bilirubin/bilirubin oxidase modified electrodes that were in degassed solution and in oxygenated solution. The responses of bilirubin/bilirubin oxidase modified electrodes that were tested in degassed (oxygen-free) solutions were not statistically different from bilirubin-modified electrodes. The biocathode has been characterized using cyclic voltammetry and RDE experiments. Further characterization of the selectivity and active lifetime of the biocathodes have been performed to ensure that the enzyme will be active for greater than one month.

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