Melisa Carpio is currently a graduating senior at the University of California, Berkeley majoring in Chemical Engineering with an emphasis in Biotechnology. Prior to her appointment in SULI, Melisa held research positions at UCSF's Department of Pathology studying the parasite Trichomonas vaginalis and at LBNL's Physics Department modeling solar funnels. Through SULI, Melisa began working in LBNL's Environmental Energy Technology Division under the guidance of Dr. John Kerr. She still works for the Kerr group researching rhodium catalyst complexes and their application to sensing devices. This research received first place at the 2005 AiChE Western Regional Conference and honorable mention at the 2005 AAAS National Conference. In the future, Melisa aspires to attend graduate school and continue researching in the fields of chemical and bioengineering.

John Kerr is Program Manager in the Advanced Energy Technologies Department, Environmental Energy Technologies Division of Lawrence Berkeley National Laboratory where he has been on staff since 1994. He received his Ph.D. in organic chemistry from the University of Edinburgh in 1978 and has held a number of research and management positions in large companies (Union Carbide, PPG Inductries, Inc.) and small start-up stage companies where he worked on organic electrochemistry for synthesis and energy conversion as well as separation technologies, including the Artificial Gill which used artificial blood systems to extract oxygen from the ocean for underwater power. Since coming to LBNL he has worked on development of polymer membrane separators for lithium batteries, fuel cells and polymer LED systems in addition to combining polymer systems with chemical and biochemical catalysts for synthesis, sensing and separation purposes.

EXAMINING RHODIUM CATALYST COMPLEXES FOR USE WITH CONDUCTING POLYMERS DESIGNED FOR FUEL CELLS IN PREPARING BIOSENSORS

Melisa M. Carpio, John B. Kerr

ABSTRACT

Biosensing devices are important because they can detect, record, and transmit information regarding the presence of, or physiological changes in, different chemical or biological materials in the environment. The goal of this research is to prepare a biosensing device that is effective, quick, and low cost. This is done by examining which chemicals will work best when placed in a biosensor. The first study involved experimenting on a rhodium catalyst complexed with ligands such as bipyridine and imidazole. The rhodium catalyst is important because it is reduced from Rh^{III} to Rh^{I}, forms a hydride by reaction with water and releases the hydride to react with nicotinamide adenine dinucleotide (NAD^+) to selectively produce 1,4-NADH, the reduced form of NAD^+. The second study looked at different types of ketones and enzymes for the enzyme-substrate reaction converting a ketone into an alcohol. Preliminary results showed that the rhodium complexed with bipyridine was able to carry out all the reactions, while the rhodium complexed with imidazole was not able to produce and release hydrides. In addition, the most effective ketone to use is benzylacetone with the enzyme alcohol dehydrogenase from baker's yeast. Future work includes experimenting with bis-imidazole, which mimics the structure of bipyridine to see if it has the capability to reduce and if the reduction rate is comparable to the bipyridine complex. Once all testing is completed, the fastest catalysts will be combined with polymer membranes designed for fuel cells to prepare biosensing devices that can be used in a variety of applications including ones in the medical and environmental fields.

INTRODUCTION

Biosensors are commonly used for the food industry, health care, and even by the government for security purposes [1]. Biosensing devices are used because they can detect, record, and transmit information regarding the presence of, or physiological changes in, different chemical or biological materials in the environment. A basic biosensing device is composed of three parts (Figure 1). The first two steps involve a hydride-forming reduction reaction and a cofactor transformation. The final step is an enzymatic reaction to produce the desired products. In this study, the rhodium in the catalyst complex is reduced chemically or electrochemically from Rh^{III} to Rh^{I}, producing hydrides by reaction with water. The hydrides then react with nictotinamide adenine dinucleotide (NAD^+) to selectively
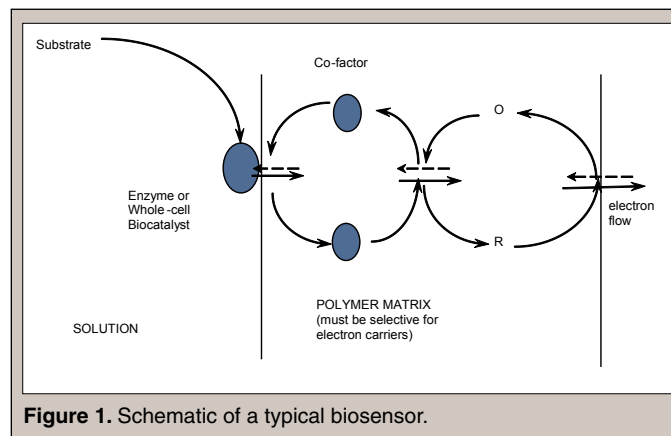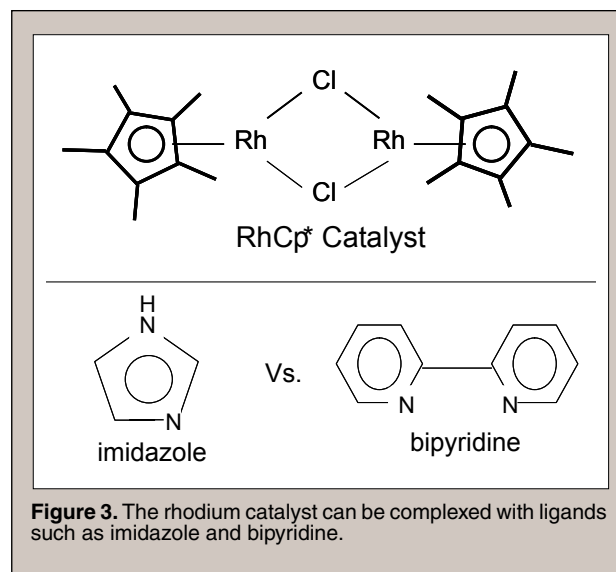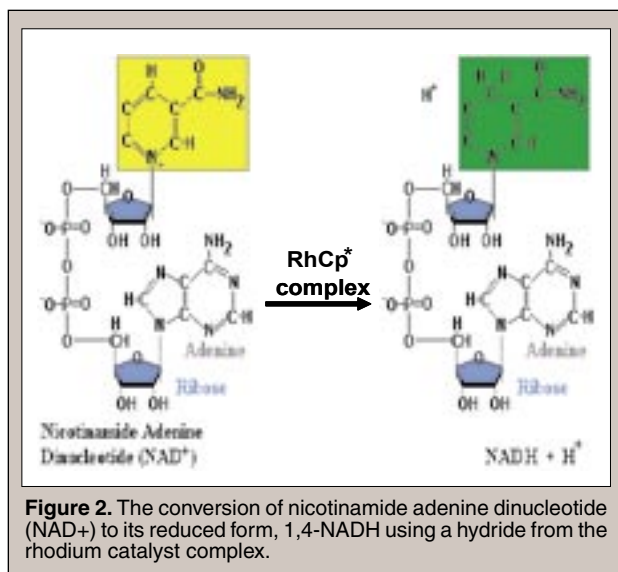

Figure 1. Schematic of a typical biosensor.

Figure 2. The conversion of nicotinamide adenine dinucleotide (NAD+) to its reduced form, 1,4-NADH using a hydride from the rhodium catalyst complex.

Figure 3. The rhodium catalyst can be complexed with ligands such as imidazole and bipyridine.

produce 1,4-NADH, the reduced form of NAD+ (Figure 2). Lastly, the 1,4-NADH reacts through an enzyme catalyst with a ketone to make an alcohol and regenerate NAD+. The goal of this research is to prepare a quick, efficient, and low cost electrochemical biosensor. Such biosensors can be used in environmental applications to detect trace amounts of harmful chemicals in the air or in medical applications to measure amounts of glucose present in a person's bloodstream. This is done by examining different rhodium catalyst complexes as well as different ketones and enzymes to see which one works the best.

Previous experiments have complexed the rhodium (Rh) catalyst with polypyridine or phosphine ligands. Such ligands are necessary for the Rh^{III} complex to get reduced to the Rh^{I} complex which produces a hydride ion by reaction with water [2]. Without the rhodium complex present, NAD+ would react to form (NAD)$_2$ and NADH isomers that are not active with enzymes. 1,4-NADH is the preferred form because it is a biochemically active compound that is a hydride donor in enzymatic reactions, such as the conversion of a ketone into an alcohol or pyruvate to lactate [3,4].
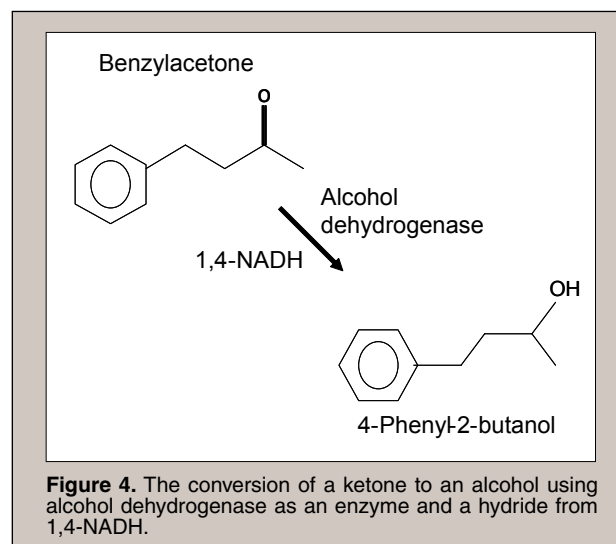
During the reduction of ketone (substrate) into alcohol (reduced product), the 1,4-NADH cofactor donates a hydride to permit reduction of the substrate into the reduced product [5]. Under the same conditions, different ketones reduce at different rates depending on their structure. The oxygen atom must be at a position where it avoids both steric interaction with its own side chains as well as other molecules. During this process, NADH loses a hydride and at the end of the reaction, NADH is oxidized to form NAD+. Then, the process must be reversed, and the NADH must be regenerated. NAD+/1,4-NADH regeneration is a low cost alternative to continually adding NAD+ or 1,4-NADH until the reaction goes to completion [6].

By adding an enzyme like alcohol dehydrogenase to the system, the catalyzed reaction exhibits chemo-, region-, and stereo-selectivity [7]. The chiral alcohols produced are important in pharmaceuticals and in making specialty chemicals. Such chirality can be detected using gas chromatography (GC) and capillary electrophoresis (CE). Proper separation of the enantiomers of chiral compounds requires that the GC and CE be operated at right temperatures, the correct buffer is used, and the solutions are at the appropriate concentrations [8].

Once the reaction is complete and the product has been formed it must be separated from the other parts of the system, which can be difficult if the reaction is done in solution. One possible way to isolate the product is to immobilize one or more components. This can be done by putting some parts, like the electrode, cofactors, and enzymes in a "membrane reactor," whose walls are permeable only to the substrates and products [9]. It is important for a biosensor that biomolecules are immobilized on conducting surfaces so that their properties remain intact [1]. This is especially important in this study because the conducting surfaces are the core of the reduction reactions. In addition, the three parts of the biosensor depend upon each other, so if one part fails, the whole sensor will not function.

One proposed "membrane reactor" involves using ionically conducting polymers designed for fuel cells. Conducting polymers have a number of properties that allow them to act as effective mobilizers of biomolecules [1]. One such property is that they

Figure 4. The conversion of a ketone to an alcohol using alcohol dehydrogenase as an enzyme and a hydride from 1,4-NADH.

Figure 5. Cyclic voltammograms testing the reduction of the RhCp* catalyst complexed with imidazole versus that with bipyridine. The sweep rate is 50mV/s.

Chemicals

All chemicals were used as received. The chemicals include $[Cp^*RhCl_2]_2$ (Colonial Metals); β-nicotinamide adenine dinucleotide (NAD) and β-nicotinamide adenine dinucleotide, reduced form (1,4-NADH) (Sigma); benzylacetone, 4-phenyl-2-butanol, 2,2'-dipyridyl , alcohol dehydrogenase (both from baker's yeast and equine liver), dichloromethane, HPLC grade water, acetophenone, and 2-pentanone (Aldrich); and sodium phosphate (JT Baker).

Instrumentation

Electrochemical measurements were made by means of cyclic voltammetry using a PAR173 Potentiostat equipped with a Model 276 Computer Interface connected to a standard three electrode cell with a glassy carbon electrode as the cathode, a platinum gauze counter electrode as the anode, and a silver (Ag) reference electrode. Argon gas was bubbled through each sample to deoxygenate the solution before measurements were taken, and a stream of argon was passed over the solution during measurements.

Potentiostatic experiments were done under the same conditions as cyclic voltammetry except a carbon felt electrode replaced the glassy carbon electrode as the cathode. In addition, the argon gas was bubbled throughout the solution for the duration of the experiment. Both the cyclic voltammetry and potentiostatic experiments were controlled by computer software, CorrWare and CorrView (Scribner Associates).

A Hewlett Packard ³DCapillary Electrophoresis (CE) System and an Aglient 6890 Series Gas Chromatography (GC) System coupled with the Aglient 5973*Network* Mass Selective Detector were used for separating and identifying products from solution. The samples for CE were injected straight from the reaction cell. The samples for GC were extracted with dichloromethane before injection.

The pH was monitored using a VWR pHastchek pH meter. Phosphoric acid and sodium hydroxide were used to either lower or raise the pH as necessary.
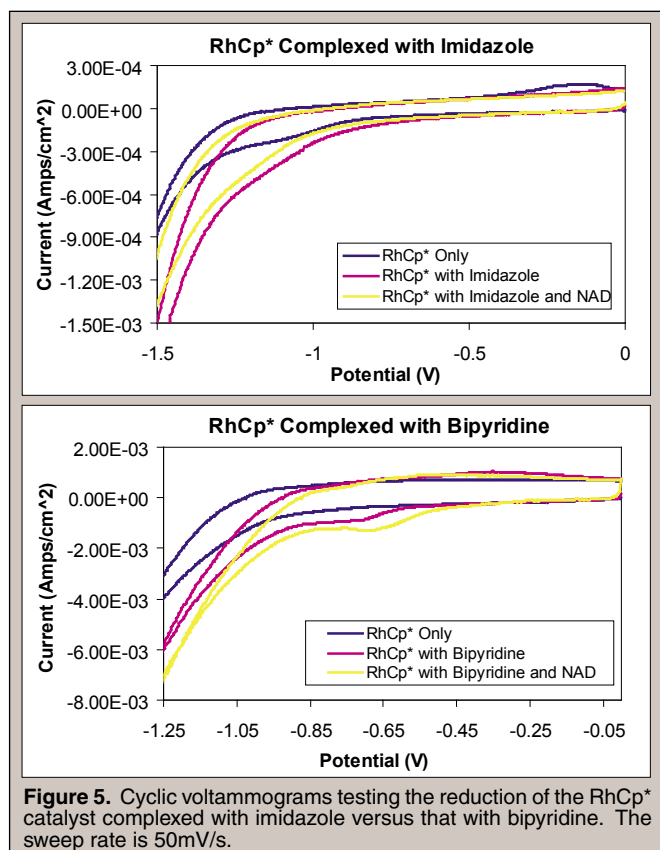
transfer charge rapidly through the polymer membrane. It is ideal to use polymers designed for fuel cells because those are also used with isolation of products [7, 10].

One part of this study will examine rhodium catalysts complexed with bipyridine and imidazole to compare reduction rate (Figure 3). The prediction is that the imidazole complex will work faster because imidazole is often what nature uses when it needs a base. In addition, the imidazole complex has unbound nitrogen atoms that may participate in the reduction reaction through hydrogen bonding to solvent water molecules as well as attract and bind the complex to the conducting polymer. The other part of the study deals with examining four different types of ketones and two different types of enzymes to determine which combination will work the fastest and have the highest conversion (Figure 4).

By preparing biosensing devices using regenerated NAD⁺ and NADH in a system incorporating the rhodium complex to catalyze enzymatic reactions, benefits of both electrochemical and enzymatic strategies can be obtained. Such benefits include high selectivity when producing 1,4-NADH, readily controlled redox potential, easy product isolation, and easy monitoring of reaction progress [11]. In addition, by combining the reaction in a conducting polymer membrane, the product is easily separated and used.

Figure 6. Cyclic voltammogram monitoring the reduction of each step leading up to the enzymatic reaction.
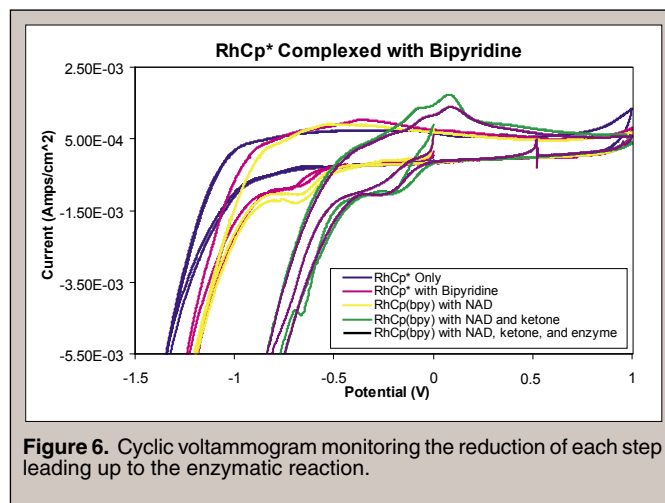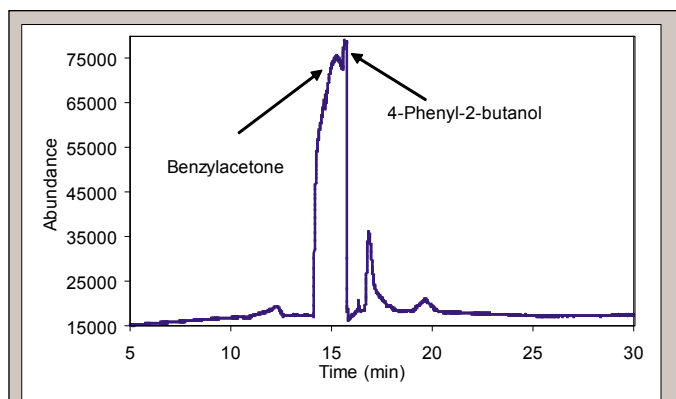
Figure 7. Resulting plot from gas chromatography (GC) analysis of the cyclic voltammetry solution. The identity of the peaks comes from the mass spectrophotometer attached to the GC.
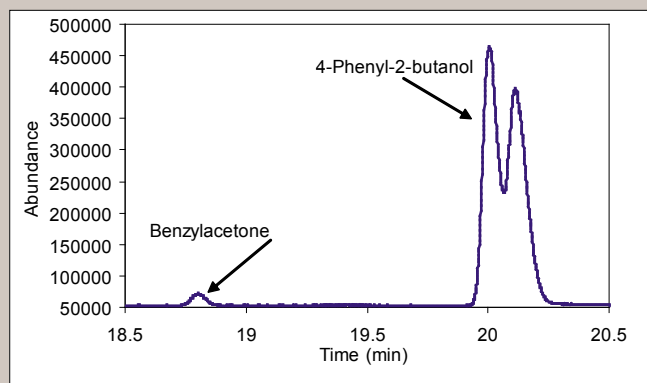
Figure 8. Resulting plot from the GC using a column with cyclodextrin.

UV-Spectra were measured with a Hewlett Packard model 8452A diode-array UV-vis spectrophotometer. Solutions were diluted to 0.25 mM and deoxygenated with argon gas before being placed in the spectrophotometer.

General procedure for the enzymatic reaction converting ketone to alcohol using NAD+/NADH regeneration and the [Cp*RhCl2]2 catalyst

An electrochemical cell was set up with 100 mL of 50 mM phosphate buffer. One millimolar $[Cp^*RhCl_2]_2$ catalyst, two equivalents 2,2'-dipyridyl, and one equivalent NAD$^+$ were added to the solution. Between each addition, a cyclic voltammogram was taken and 1 mL of sample was removed for CE and UV-Vis analysis. A one hour potentiostatic experiment was run at a constant voltage corresponding to the cyclic voltammetry peak to get complete conversion of NAD$^+$ to 1,4-NADH. Ten equivalents of benzylacetone (ketone) and one equivalent alcohol dehydrogenase (enzyme) were then added. Cyclic voltammetry was performed and 1 mL was removed for analysis between each addition. A three hour potentiostatic experiment was run to completely convert the ketone to alcohol (Figure 4). A final cyclic voltammetry was performed and another sample was taken before the remaining solution was extracted with dichloromethane for GC analysis. This procedure was repeated with imidazole instead of 2,2'-dipyridyl. The entire reaction was done at room temperature and at a pH of seven.

General procedure for measuring reaction kinetics and enzyme activity

In a reaction cuvette, enough 1,4-NADH was added to 5 mL of 50 mM phosphate buffer to make a 0.25 mM solution, and a UV-Vis spectrum was taken. Next, an equivalent of enzyme was added, and another spectrum was taken. After 10 minutes, an equivalent of ketone was mixed into the solution. Oxygen was removed by holding the solution under vacuumfor 5 minutes, then capping the cuvette. A spectrum was then taken every 10 minutes until the 1,4-NADH peak at an absorbance of 340nm disappeared. Tests were done on four different types of ketones, benzylacetone made in lab, benzylacetone purchased commercially, acetophenone, and 2-pentanone, and two different types of enzymes, alcohol dehydrogenase from baker's yeast and alcohol dehydrogenase from equine liver.

RESULTS

Cyclic voltammograms comparing the rhodium complexed with bipyridine versus the rhodium complexed with imidazole is shown in Figure 5. The voltammogram in Figure 6 tracks the complete enzymatic reaction from the addition of the rhodium catalyst to the conversion of the alcohol. Figures 7 and 8 are gas chromatographs of a successful ketone to alcohol conversion. In Figure 7, a regular GC column coupled with a mass spectrophotometer was used, while in Figure 8, a column with cyclodextrin was used to separate the chiral alcohol products.

A typical UV-Vis spectrum is shown in Figure 9, with the 1,4-NADH peak at 340 nm highlighted. Figure 10 is a plot of the natural log of the absorbance at 340 nm as shown by the spectra versus the elapsed reaction time. The slope of the resulting linear regression line is the rate of 1,4-NADH appearance/disappearance, which is directly related to the rate of alcohol product production. Tables 1 and 2 list the rates of ketone and enzyme activity as determined by plotting spectral data from each sample as described in Figure 10.
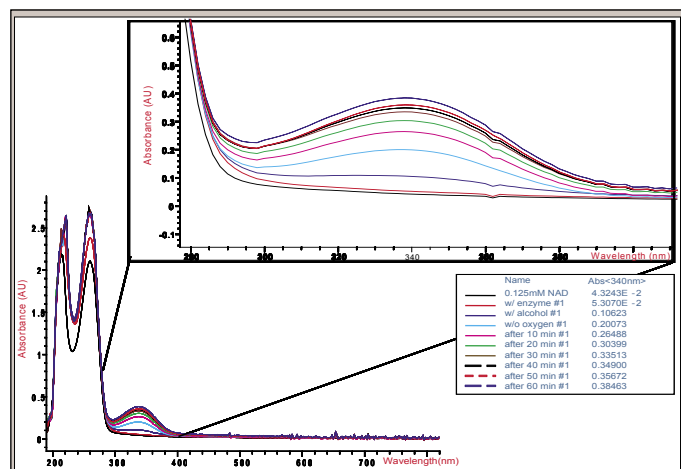
Figure 9. A typical UV-Vis spectrophotometer plot of absorbance versus wavelength. 1,4-NADH peak appearance or disappearance can be monitored as the characteristic peak is at 340 nanometers (nm).

Rate Determination from UV-Vis Data

$$y = 0.0086x + 0.0552$$

Figure 10. By plotting the natural log of the absorbance at 340nm versus the elapsed reaction time, the slope of the resulting linear regression line is the rate of 1,4-NADH appearance/ disappearance.
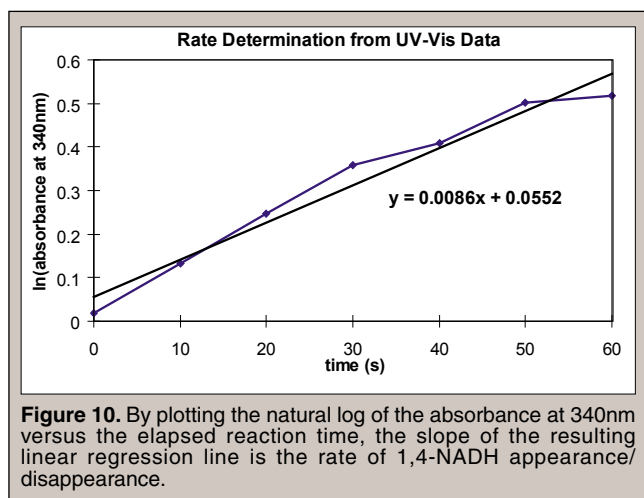
DISCUSSION AND CONCLUSIONS

As shown clearly in Figure 5, the catalyst complexed with bipyridine has a clear reduction peak at -0.9V, and the peak grows with the addition of NAD^+. The catalyst complexed with imidazole does not have a reduction peak, and there is also no reduction peak when NAD^+ is added to the solution. One explanation for the imidazole failing to reduce is that it does not have strong enough bonds with the rhodium catalyst, so the amines from the NAD^+ attract the catalyst rather than the amines from the imidazole. Since the bipyridine was successful in reducing, it is possible that a bis-imidazole compound will have stronger binding than NAD^+, therefore having the ability to reduce the rhodium compound. Another solution is to put the imidazole compounds directly onto the conducting polymer as a chelate effect may mimic the bis-imidazole/bipyridine structure.

Based on the data from Tables 1 and 2, the best ketone to use is benzylacetone prepared in lab and the most efficient enzyme is alcohol dehydrogenase from equine liver. The cyclic voltammetry experiments confirmed that benzylacetone is the best ketone to use. It appears to have the right bulk of carbon atoms and a strategically

Ketone Name	Structure	Rate (s-1)
Benzylacetone (made in lab)		0.044
Benzylacetone (from Aldrich)		0.009
Acetophenone		0.0027
2-Pentanone		0.0001

Table 1. This table lists four different types of ketones and their rates of 1,4-NADH disappearance. Benzylacetone (made in lab) is the fastest of the four.

placed oxygen atom adequate for binding in the enzyme reaction center. However, the only enzyme that successfully converted the ketone into alcohol was the alcohol dehydrogenase from baker's yeast, which contradicts the results from the UV-Vis experiments. It could either be that the alcohol dehydrogenase from baker's yeast only works when a current is passed though the sample, while the one from equine liver does not need current. However, the activities of the enzymes are so sensitive to small variations in the conditions that it is hard to make a definite conclusion.

Preliminary results show that the rhodium complexed with bipyridine is effective in reducing a ketone to an alcohol using enzymatic catalysis and NAD^+/1,4-NADH regeneration. In addition, the ketone benzylacetone and the enzyme alcohol dehydrogenase from baker's yeast is the only successful substrate-enzyme combination that produced a product.

Future work includes repeating the cyclic voltammetry with bis-imidazole ligands on the RhCp* catalyst complex to determine if has the ability to reduce and release hydrides, or trying to put the imidazole directly onto the conducting polymer to test if the chelate effect works. If reduction does occur, the rate of reduction of the

Enzyme Name	Rate (s-1)
Alcohol Dehydrogenase (from Baker's Yeast)	0.0024
Alcohol Dehydrogenase (from Equine Liver)	0.0325

Table 2. This table lists two different types of enzymes and their rates of 1,4-NADH disappearance. Alcohol dehydrogenase (from equine liver) is the faster of the two.

bis-imidazole complex or the imidazole on polymer complex will be compared against the bipyridine complex. Finally, the quickest and most effective of the catalyst complexes will be combined with polymer membranes designed for fuel cells to prepare a biosensor device like the one shown in Figure 1.

ACKNOWLEDGEMENTS

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