Visible Absorbance Spectroscopy of Glucose Oxidase Enzyme Kinetics in a Cuvette

Overview

One of the most unique features of OpenSPR is that it can also function as cuvette based visible spectrometer. This is possible because unlike traditional surface plasmon resonance instruments, OpenSPR uses a broad band white light source. With the specially designed cuvette holder, OpenSPR can quickly become a powerful time resolved visible spectrometer best suited for measurements of solution based reactions. The software can save absorbance spectra over time and can also track peak absorbance or specific wavelength values in real time with a very fast acquisition rate. This is perfect for time resolved measurements like colorimetric assays, absorbance spectroscopy, and more. In this example, OpenSPR is used to determine the Michaelis-Menten kinetic parameters of the glucose oxidase enzyme reaction. These same results can be obtained on the dedicated visible spectroscopy platform, OpenSPEC, by Nicoya Lifesciences.

Materials & Equipment

- OpenSPR Instrument or OpenSPEC Instrument
- Cuvette Holder
- Standard 1 cm path length micro-cuvette
- Standard glucose solution 1 mg/mL
- Glucose Oxidase and Horseradish Peroxidase Reagent (Sigma G3660)
- 3,3',5,5'-tetramethylbenzidine

Safety Notes

Follow the safety precautions outlined in the MSDS for all materials.
Background
Glucose oxidase is a common enzyme used for estimating blood glucose at a high degree of specificity. The oxidation of glucose catalyzed by glucose oxidase will create peroxide and gluconic acid. The rate of this conversion can be monitored in the presence of a peroxidase enzyme and chromophore, the oxidation of which will produce an absorbance signal that can be monitored via spectrometer at a specific wavelength.

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\text{Glucose} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + \text{H}_2\text{O}_2
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\[
\text{H}_2\text{O}_2 + \text{TMB (Colourless)} \xrightarrow{\text{Horseradish Peroxidase}} \text{Oxidized TMB (Blue)}
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3,3',5,5'-tetramethylbenzidine (TMB) was used as the chromophore for this system. The single oxidation product of TMB produces a blue colour with an absorbance peak at 652 nm [1].

Procedure
A 1 mL solution of 2.5 mg/mL TMB containing glucose oxidase and peroxidase was mixed with 1.0 mg/mL standard glucose solution. Absorbance spectra were taken at various time points after the two solutions were mixed to monitor the conversion of glucose into peroxide by glucose oxidase, Figure 1. The peak absorbance at 652 nm was plotted at various glucose concentrations, Figure 2. During data post-processing procedures, the initial velocity of glucose conversion was determined by calculating the slope of the increase in the absorbance at 652 nm over the first 30 seconds after mixing. The initial velocities of experiments conducted at different glucose concentrations were evaluated using the Michaelis-Menten model of enzyme kinetics, Figure 3. A Lineweaver-Burk reciprocal plot, Figure 4, was used to determine the $V_{\text{max}}$ and $K_m$ of glucose oxidase at the experimental conditions, 25°C. $V_{\text{max}}$ is the maximum rate achieved by the enzyme reaction, and $K_m$ is the substrate concentration at which the reaction rate is half of $V_{\text{max}}$. 
Results

Absorbance spectroscopy results during the enzymatic reaction of glucose are shown in Figure 1. Figure 2 shows the peak absorbance value at 652nm over time for each of the glucose concentrations tested.

Figure 1 - Full optical absorbance spectra taken at timed intervals during the oxidation of glucose into peroxide by glucose oxidase. The absorbance peak at 650 nm was produced by the oxidation of TMB.

Figure 2 - Peak optical absorbance values at 652 nm over time at varying concentrations of glucose
The analysis of the above spectral data produced a $V_{\text{max}}$ of 1.35 mM/s and a $K_m$ of 2.97 $\mu$M.

**References**