Kinetics Measurements with High Sensitivity Spectroscopy

'Chemical Stoplight' Reaction Monitoring

Yvette Mattley, PhD, Principal Applications Scientist, Ocean Optics

The study of chemical kinetics provides important information on the rate and mechanism of the chemical reactions that occur all around us — from inside the cells of the human body to the ozone layer in the atmosphere. Characterising the impact of parameters such as reactant concentration, temperature, pH and the presence of a catalyst are vital to optimising reaction conditions and understanding the mechanism of the reaction.

In the human body, chemical kinetics measurements are made to characterise the impact of enzyme catalysts on metabolism and to understand the factors critical to the accurate dosing and release of a medication. In an industrial or process setting, detailed knowledge of the chemical kinetics for a reaction enables the use of the optimum conditions and reactant concentrations to maximise product yield while minimising reactant waste.

Absorbance spectroscopy provides a mechanism for characterising and better understanding these key reactions. Spectrometer system sensitivity, and enhanced spectrometer features such as onboard buffering for data integrity are important factors to improve results.

The 'Chemical Stoplight' Reaction - Easy to Visualise Kinetic Reactions

The chemical stoplight reaction, a reversible oxidation reduction reaction featuring the redox indicator dye indigo carmine, is a popular teaching lab demonstration and the star of many online videos. During the reaction, the solution changes from green to red to yellow as the indicator dye is oxidised and then reduced when oxygen levels decrease. Vigorous mixing to reintroduce oxygen restarts the reaction. While this colourful reaction is often used to illustrate the principles of reactions that oscillate, the change in absorbance during the experiment also shows how spectroscopy can be used to characterise kinetics.

To demonstrate this, the following experiment uses a high sensitivity spectrometer to collect the absorbance data needed to characterise the chemical kinetics for a reversible oxidation reduction reaction featuring the indicator dye indigo carmine. Indigo carmine exists in oxidised, reduced and intermediate forms depending on its environment. Each form has a slightly different chemical structure resulting in the absorption of different wavelengths of light.

When indigo carmine is mixed in a flask with a reducing agent (dextrose) in a basic solution (NaOH), it acts as an indicator of the state of the redox (reduction oxidation)

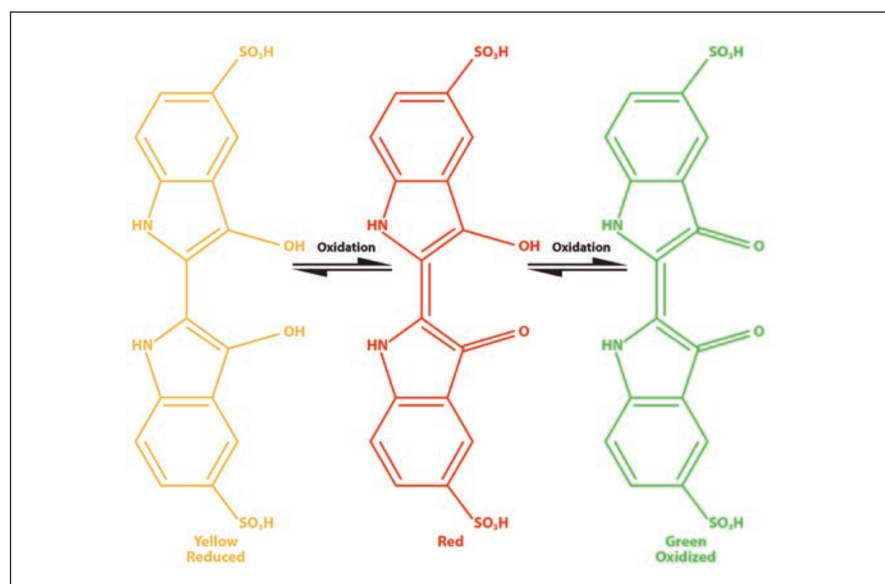

Figure 1. Oxidation of indigo carmine (Source: University of Edinburgh School of Chemistry)

process. When oxygen is introduced to the solution by shaking the flask, the indicator is in its green oxidised form (most exposed to oxygen in the air). The reaction mixture colour changes to red and then to yellow as the indigo carmine goes from an oxidised to a reduced state.

Indigo carmine changes colour as a result of changing levels of oxygen in the solution. The solution is yellow in colour. When the solution is mixed, oxygen dissolves into the mixture oxidising the indicator and changing the colour to red. When the flask is shaken more vigorously, the levels of oxygen increase even more, oxidising the indicator further and causing it to turn green. When the solution is left alone, the oxygen concentration drops due to a reaction with dextrose and the solution returns to its original yellow colour. The formula for this chemical reaction is shown in *Figure 1*.

For kinetics measurements a spectrometer with high dynamic range is essential, as it is better able to detect a wide range of light levels while pulling peaks out of the noise. A high capacity for onboard buffering (ideally up to 15,000 spectra) ensures data integrity. This means no data points are missed during critical stages of the reaction when the computer fails to request a spectrum because it is burdened with other tasks. This buffering is very important for kinetics measurements, where the loss of even a minimal number of spectra can affect the results.

Experimental Conditions

The chemical stoplight reaction mixture is prepared by adding 1.5 mL 2.5% dextrose solution, 1.5 mL 1 M NaOH solution and two drops of 1% indigo carmine solution to a disposable cuvette. Note that the NaOH solution used for this reaction is very caustic. The NaOH solution and mixture should be handled with care and safety precautions should be taken while working with these solutions. A cover is placed on the cuvette, which is then shaken to introduce oxygen into the solution until it turns a greenish colour. The cuvette is then placed into a 1 cm pathlength cuvette holder and absorbance is measured with the back-thinned CCD array QE *Pro* spectrometer (Ocean Optics) using commercial spectroscopy software (OceanView, Ocean Optics). The reaction is monitored using the software's strip chart feature, set to measure absorbance at 553 nm and 759 nm.

When the absorbance of the peaks at 553 nm and 759 nm drops to the baseline, the cuvette is shaken thoroughly to reintroduce oxygen and restart the reaction. The cuvette is again shaken until the mixture is greenish in colour and the indicator dye is oxidised. The reaction can be restarted several times by shaking the cuvette thoroughly to introduce oxygen and reoxidise the indigo carmine. If the reaction occurs too fast or the peak intensities drop too low, the reaction mixture can be refreshed by adding a few additional drops of indigo carmine dye solution to the cuvette.

Results

The visible absorbance spectra measured at different times during the chemical stoplight reaction are shown in *Figure 2*. After starting the reaction by thoroughly mixing the solution to introduce oxygen, the absorbance at 553 nm increases, while the absorbance at 759 nm decreases. The absorbance for the peak at 553 nm increases until the oxygen in the mixture is depleted. As oxygen levels decrease, the absorbance at 553 nm decreases to the starting absorbance level.

The absorbance trends measured during the chemical stoplight reaction for the peaks at 553 nm and 759 nm are shown in *Figure 3*. The absorbance trend over time corresponds to a change in the colour of the solution as the indicator dye is oxidised and reduced. The solution starts out green and slowly turns red as the oxygenation state of the indigo

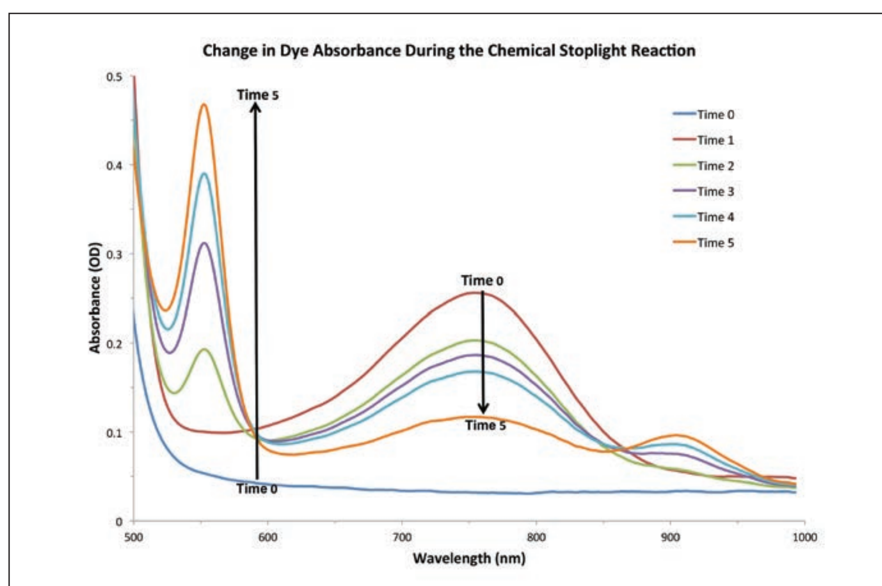

Figure 2: Absorbance spectra measured during the chemical stoplight reaction

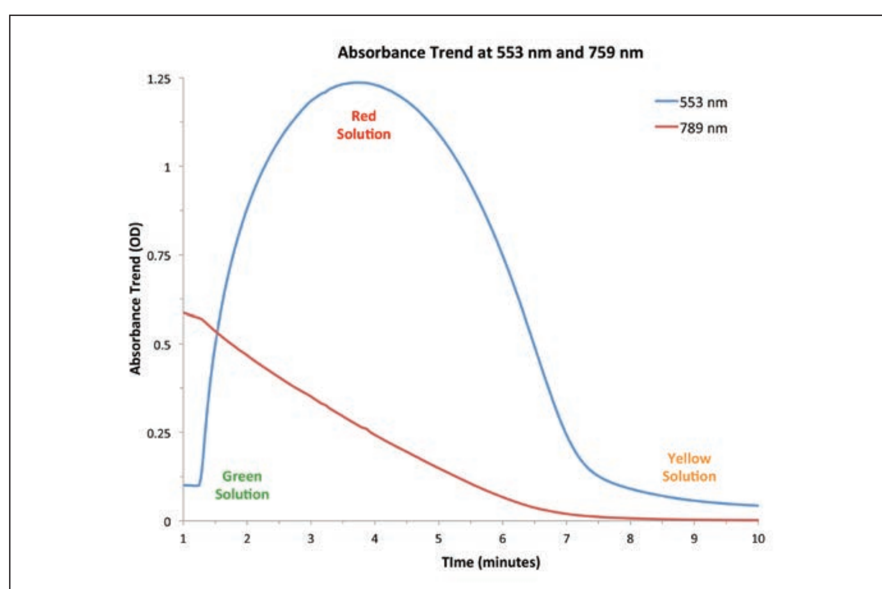

Figure 3: Absorbance trend measured during the chemical stoplight reaction for the peaks at 553 nm and 759 nm

carmine indicator dye changes. The solution then turns to yellow as the oxygen in the mixture reacts with dextrose, decreasing the oxygen available to oxidise the indicator dye. The absorbance trend repeats when oxygen is reintroduced into the reaction mixture via vigorous shaking.

Ensuring Data Integrity

Characterising the chemical kinetics for the stoplight reaction requires changing the reactant concentrations and then repeating the measurements at each concentration level.

To ensure the most accurate kinetics measurements, it is important to collect as many data points as possible during the reaction. Data integrity can be a significant challenge with the multitasking nature of today's computers. While a spectrometer continually acquires data at the acquisition time specified, a computer's capacity to request spectra often falls behind, with the latency resulting in missed scans. Inaccurate reaction rates, rate law and rate constants could result if the computer lags behind during a critical point in the reaction.

With the ability to buffer up to 15,000 spectra onboard, the QE *Pro* overcomes data integrity issues related to computers that fall behind spectrometer acquisition rates. This means there will be no missed scans even if the computer's processing capacity falls behind and fails to ask for spectral data for up to 150 seconds (more than two minutes). The trend shown in *Figure 4* for the number of spectra stored onboard the spectrometer over time was generated for approximately 50 minutes of continuous data acquisition.

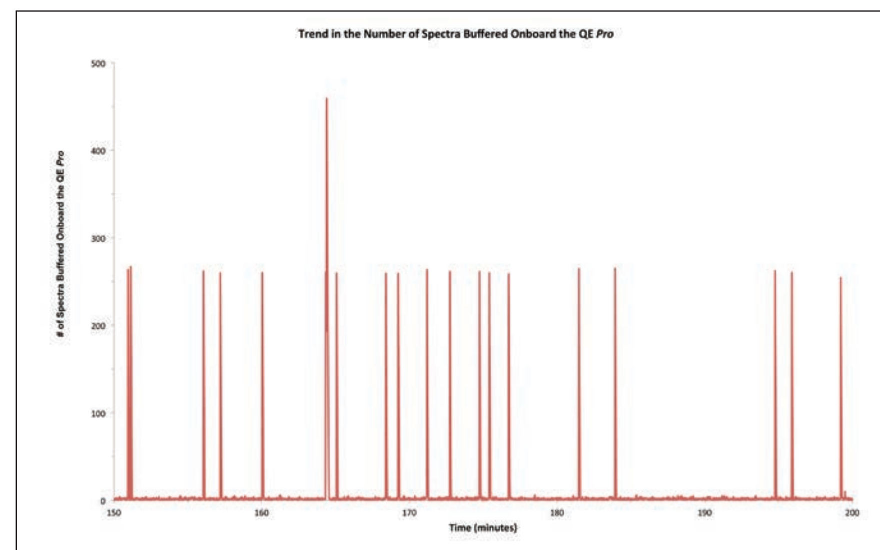

Figure 4: Trend over time for the number of spectra stored in the spectrometer's onboard buffer during continuous acquisition

For spectrometers with minimal onboard buffering, each time the number of spectra stored onboard the spectrometer goes above three spectra (occurring almost 20 times for the data shown), critical kinetics data would be lost as the computer falls behind. Fortunately, no data is lost during this 50-minute acquisition period due to a spacious onboard buffer – ensuring complete data integrity and superior kinetics characterisation.

This is especially significant for chemists and life scientists characterising enzymes (organic) and catalysts (inorganic), each of which affects reaction rates. For example, changes in enzyme function may be associated with diseases and other medical conditions; understanding those changes can be helpful in investigating how drugs may help treat those diseases. Also, catalysts play a role in areas such as chemical processing and environmental monitoring.

Conclusions

The colourful chemical stoplight reaction is a staple in many undergraduate teaching laboratories. The changing colour appeals to the visual senses as the solution transforms. But, as demonstrated by the absorbance data acquired using high-sensitivity spectroscopy equipment during this reaction, there is much more than just a colour change going on in the solution. The changing oxidation state of the indicator dye results in a change in absorbance, which can be measured without losing a single data point during the entire reaction using the right spectrometer. For kinetics measurements like this one, where every data point is critical, a spectrometer with high onboard buffering capability ensures that no data is lost during the reaction.

Indeed, as is the case with examination of other kinetic reactions, each data point is critical. A more complete understanding of various processes – from food decomposition and microorganism growth, to disease diagnoses and drug interactions – can be achieved by harnessing the power of high-sensitivity spectroscopy.