Measuring Rapid Kinetics Data with a Commercial Spectrophotometer Using a Stopped-Flow Accessory

HI-TECH SCIENTIFIC

APPLICATION NOTE

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MEASUREMENT OF THE REDUCTION OF POTASSIUM FERRICYANIDE BY L-ASCORBIC ACID WITH A SHIMADZU UV-1700 SPECTROPHOTOMETER USING A STOPPED-FLOW ACCESSORY.

The reduction of Potassium Ferricyanide by Ascorbic Acid is a well known kinetic reaction that was first published by Tonomura *et al.*[1] The speed of this reaction is dependent on the pH value of the solution which makes this reaction a very useful one for testing the performance of kinetic instruments.

Keywords: Stopped-Flow Accessory, Shimadzu UV-1700, Rapid Kinetics

Introduction

When Potassium Ferricyanide ($K_3Fe(CN)_6$) is dissolved and brought into solution with L-Ascorbic Acid (Vitamin C, $C_6H_8O_6$), the Ferricyanide ($Fe(CN)_6^{3-}$) can be reduced by the Ascorbic Acid (AA) to form $Fe(CN)_6^{4-}$. Being an acid AA is present in the solution in the form of AA, AA^- and AA^{2-}, the ratio of these depending on the pH of the solution.

$$AA \leftrightarrow AA^- + H^+$$
$$AA^- \leftrightarrow AA^{2-} + 2H^+$$

This also means that the speed of the reaction is determined by the pH of the solution. Two of the forms in which AA is present in the solution, AA^- and AA^{2-}, can react with Ferricyanide.

Methodology

The reaction mechanisms for the reduction of Ferricyanide by Ascorbic Acid are:

$$AA^- + Fe(CN)_6^{3-} \xrightarrow{k_1} AA\cdot + Fe(CN)_6^{4-}$$

and

$$AA\cdot + Fe(CN)_6^{3-} \xrightarrow{k_2} AA^o + Fe(CN)_6^{4-} + H^+$$

for the AA^- form, and:

$$AA^{2-} + Fe(CN)_6^{3-} \xrightarrow{k_3} AA^-\cdot + Fe(CN)_6^{4-}$$

and

$$AA^-\cdot + Fe(CN)_6^{3-} \xrightarrow{k_4} AA^o + Fe(CN)_6^{4-}$$

for the AA^{2-} form. In these equations AA^o is the oxidised form of AA, $C_6H_6O_6$.

The free radicals formed in this reaction instantaneously react with $Fe(CN)_6^{3-}$ and only k_2 and k_4 contribute to the pseudo first order rate constant *k*, which can be used if [AA] >> $[Fe(CN)_6^{3-}]$. The concentration of $Fe(CN)_6^{3-}$ during the reaction is then given by:

$$[Fe(CN)_6^{3-}] = [Fe(CN)_6^{3-}]_0 \cdot \exp^{-kt}$$

Experimental

Potassium Ferricyanide has an absorbance maximum at 420 nm, this can be seen in the spectrum of $K_3Fe(CN)_6^{3-}$ shown in Figure 1.

All kinetic traces were collected at this wavelength with an AA syringe solution concentration of 20 mM and a $Fe(CN)_6^{3-}$

concentration of 1 mM in the syringes. The experiments were performed at room temperature and the pH value was determined by the Ascorbic Acid concentration (pH ≈ 3).

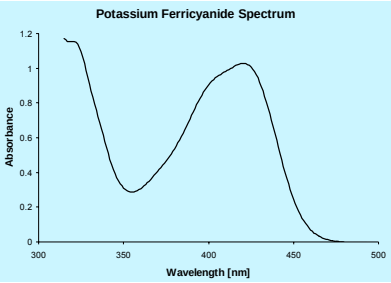

Figure 1: Spectrum of $K_3Fe(CN)_6$

First the acquisition process was started on the Shimadzu UV-1700 spectrophotometer and subsequently the syringes on the SFA-20 were pushed by hand to start the reaction in the cell between Ferricyanide and Ascorbic Acid. The SFA-20 stopped-flow accessory used for these measurements with the Shimadzu UV-1700 was equipped with a standard cell. Kinetic traces were collected for 10 seconds with a time resolution of 100 ms per data point and 101 data points in total were recorded for every trace.

Results

Traces of 14 separate experimental shots were recorded with very good reproducibility. This can be seen in Figure 2 where all the individual traces are shown for the first 3.5 seconds only, because the reaction was over after 3-4 seconds.

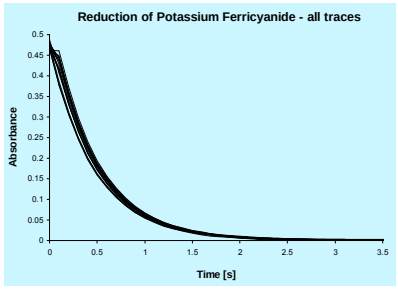

Figure 2: All kinetic traces collected for the reduction of $K_3Fe(CN)_6$

A single trace was then used to fit first order reaction kinetics. Both the original data and the fit to the data are shown in Figure 3.

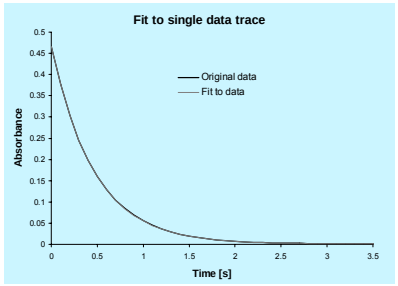

Figure 3: Kinetic trace for $K_3Fe(CN)_6$ reduction with a fit to the data.

The first order rate constant found for the reaction was k = 2.15 s^{-1}, which is in good agreement with the value found in the literature[1].

Conclusion

The first order kinetics assumption provides a very good fit to the original data, proving that it was safe to make this assumption with the used concentrations of reagents. Also, the quality of the data collected with the Shimadzu UV-1700 spectrophotometer was very good and consistent. These experiments therefore prove that the Shimadzu UV-1700 is a very suitable spectrophotometer to measure fast kinetic reactions reliably in conjunction with the SFA-20 stopped-flow accessory.

References

[1]B. Tonomura *et al.*, Analytical Biochemistry 84 (1978) 370-383.

Acknowledgements

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