Fast, simple measurement of reaction kinetics using the Agilent Cary 60 UV-Vis spectrophotometer with an SFA 20 stopped-flow accessory

Application note

Pharmaceutical

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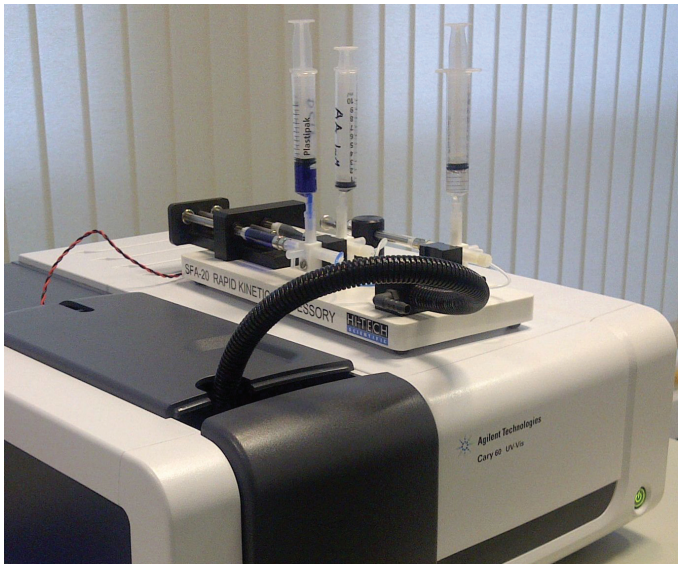

Introduction

Analysis of reaction kinetics is a fundamental chemistry and biochemistry technique that is essential for understanding how these reactions occur. UV-Vis spectrophotometry is often used for this analysis, when the change in reactant or products can be monitored by absorbance at a specific wavelength over time. When chemical or biochemical reactions occur rapidly, specialized equipment is needed. The Agilent Cary 60 UV-Vis spectrophotometer can collect a data point every 12.5 ms, together with the SFA 20 rapid mix accessory, which allows for rapid mixing of reaction components.

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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.

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.

$$AA \leftrightarrow AA^- + H^+$$

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

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 oxidized 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).

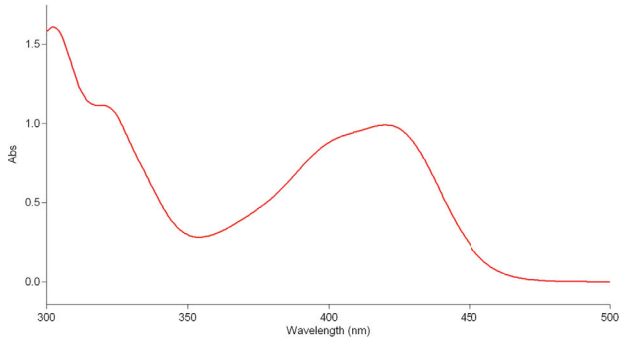

Figure 1. Spectrum of $K_3Fe(CN)_6$

First the acquisition process was started on the Agilent Cary 60 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 Cary 60 was equipped with a standard cell. Kinetic traces were collected for 5 seconds with a time resolution of 12.5 ms per data point and 400 data points in total were recorded for every trace.

Results

Traces of 10 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 5 seconds only, because the reaction was over after 6–7 seconds.

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.

The first order rate constant found for the reaction was $k = 0.83\ s^{-1}$, which is consistent with the value found in the literature[1].

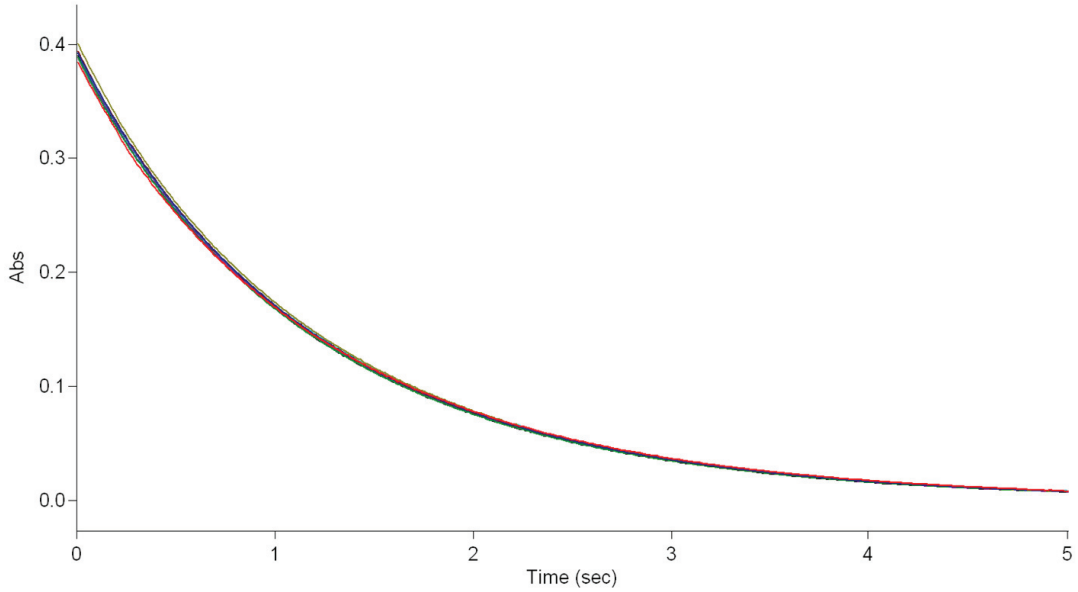

Figure 2. All kinetic traces collected for the reduction of $K_3Fe(CN)_6$

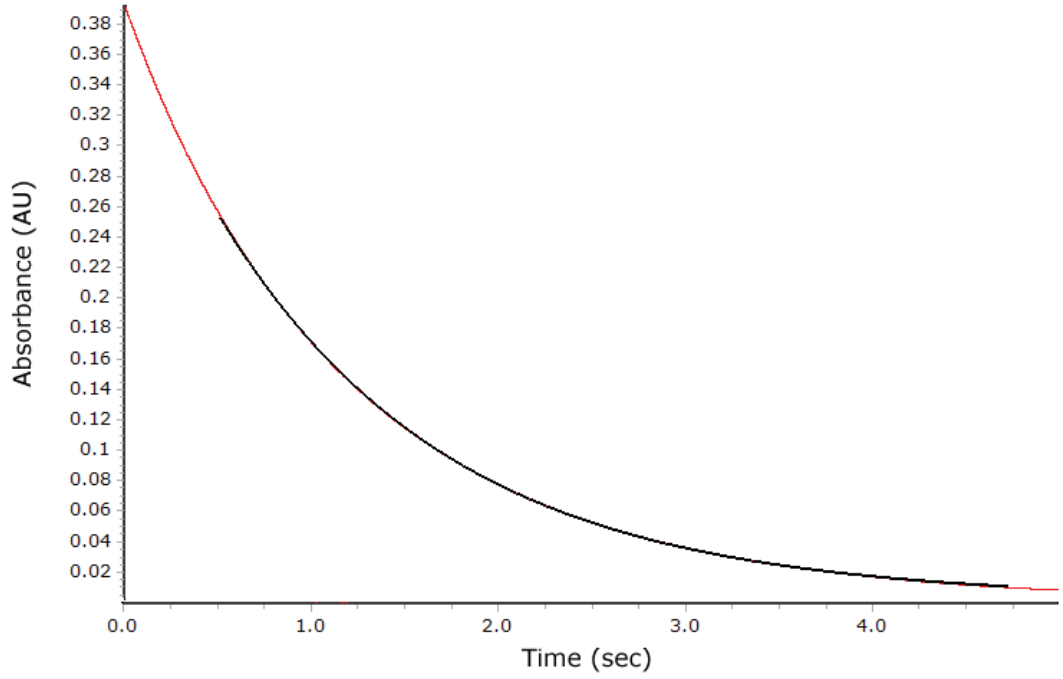

Figure 3. Kinetic trace for $K_3Fe(CN)_6$ reduction with a fit to the data

Conclusion

In both teaching and research laboratories, analyzing reaction kinetics is fundamental to understanding chemical interactions. In this application note, the benefits of using the Cary 60 UV-Vis together with the SFA-20 stopped-flow accessory are shown by the quality and reproducibility of the data collected. The kinetic traces can be fitted to a single exponential model using the Cary WinUV software, consistent with the pseudo first order conditions used. For ease-of-use and data accuracy, the Cary 60 is ideal for measuring fast kinetic reactions with the SFA 20 stopped-flow accessory.

References

1. B. Tonomura *et al*. (1978) Analytical Biochemistry, 370–383.

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