Application Note
Chemical

Monitoring Fast Chemical Reactions Using Stopped Flow Kinetics

Data collection started in 50 ms, with data collected every 12 ms

Authors

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Introduction

There are many factors which control the rate of a chemical reaction. These include the type of metal center, the size and charge of the ligands, the concentration of the reactants, and environmental conditions such as pH and temperature. All these factors can produce systems with half lives in the region of milliseconds to hours to days.

One method of monitoring a reaction is through UV-Vis spectrophotometry. If the reactant or product exhibits a change in absorbance as a function of reaction time, this method can usually be employed. A conventional cell is adequate for reactions with half lives greater than a couple of minutes, however, reactions that are over within a second or milliseconds need specialized equipment. A Rapid Kinetics Accessory (or Stopped-Flow Apparatus) can be used to measure such fast reactions.

This study used a Cary 50 UV-Vis but the Cary 60 UV-Vis, which has superseded the Cary 50 UV-Vis, would also be suitable

Theory

Conventional spectrophotometric techniques cannot be used when investigating reactions that occur at a sub-second rate. If reactants are added manually, and then stirred for a few seconds to allow adequate mixing, the reaction is over and no changes in spectra are recorded. This is overcome by using a Stopped-Flow apparatus (Figure 1) that provides instantaneous mixing and recording of data on a tens-of-millisecond time scale. The technique rapidly mixes two solutions in a flow cell and starts recording data when the mixing ceases. Both the Cary 50 and Cary 60 UV-Vis spectrophotometers can record a data point every 12.5 ms.

Figure 1. Rapid Mix Accessory - SFA20.

The stopped flow apparatus is connected to the spectrophotometer through a remote-send-cable that is attached to an accessory controller port on the instrument. The reactants are placed in two syringes, labeled Solution A and Solution B, as shown in Figure 2. Upon pressing the plunger, the solutions travel separately to the cuvette and only mix upon entering the cell. Previously reacted solution is ejected into a waste syringe, which moves back until hitting a micro switch. The Solution then stops flowing into the cell and data collection begins instantaneously, which eliminates any delay caused by a manual start.

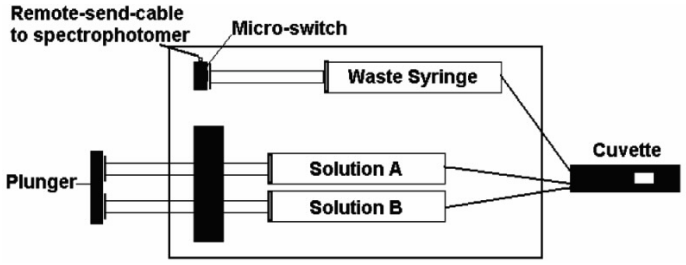

Figure 2. Schematic of Rapid Mix Accessory

The data are processed in much the same way as when using conventional techniques. The in-built algorithms, available in the Cary WinUV Kinetics application, allows zero, first and second order fits to be applied to the experimental data. The fitted curve is displayed on the graph and the calculated parameters appear in the report. It is critical that there are a sufficient number of data points in the initial stage of the reaction as this is where most of the changes in the spectra occur.

Enough data spanning 3-4 half lives should be collected. The Cary 50 or 60 UV-Vis spectrophotometer coupled to a Stopped-Flow apparatus can measure reactions that are over within a couple of hundred milliseconds!

Equipment

- Cary 50[2] UV-Vis spectrophotometer
- Rapid Mix Accessory (SFA-20)
- Green food dye
- Bleach in the form of White King[1]

Experimental

To demonstrate the extremely fast data collection capabilities of the Cary 50[2] UV-Vis, the rate of bleaching of green food dye was investigated. The rate of the reaction was controlled by varying the concentration of bleach, until the limits for measuring this reaction were achieved. First-order kinetic fits were then applied to the data.

A solution of green dye in de-ionized distilled water was prepared so as to give an absorbance of ca. 0.4 (Solution A).

Solution B was prepared by diluting the bleach solution (0.655 mL) with 30 mL of de-ionized distilled water.

[1] *Household laundry bleach liquid with 40 g/L available chlorine present as sodium hypochlorite.*

[2] *The Cary 50 has been superseded by the Cary 60, which has equivalent performance.*

The instrument parameters for the Cary 50 UV-Vis were set up as follows:

Parameter	Setting
Wavelength (nm)	414
Ave Time (s)	0.0125
Y Min	0
Y Max	0.5
Cycle (min)	0
Stop (min)	0.2

Results

The color green is composed of the two primary colors yellow and blue, reflected in the UV-Vis spectrum of green food dye in water, Figure 3. The yellow component has an absorbance at 414 nm and the blue component at 629 nm. The addition of bleach causes a rapid change in the absorbance at 414 nm, following a first order decay path, the rate of which depends on the concentration of bleach. The reaction was monitored at 414 nm.

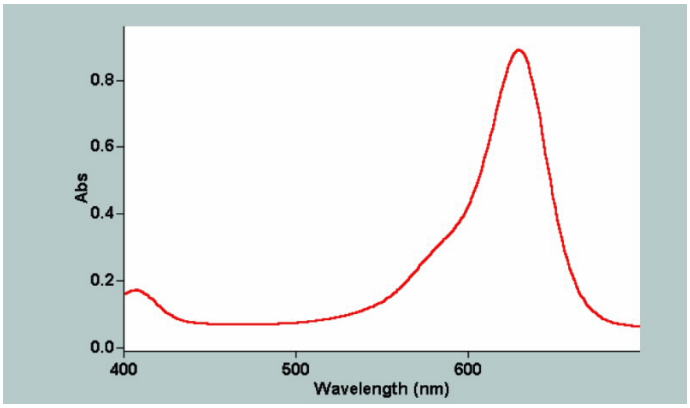

Figure 3. UV-Vis spectrum of green food dye in water.

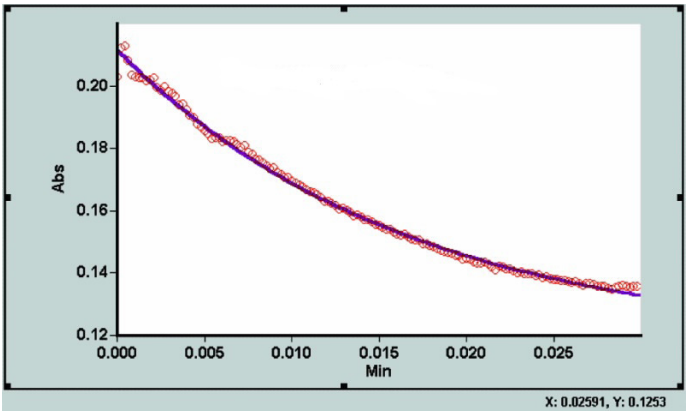

Figure 4. Change in absorbance at 414 nm of green dye and bleach.

Figure 4 shows the change in absorbance over time for the bleaching of green food dye collected on the Cary 50 UV-Vis. The reaction is over within 2 seconds, and the rate constant for a first order decay was calculated to be 60.320 min^{-1} with an SD of 0.0013.

Discussion

In order to accurately monitor reactions with sub-second half-lives, a spectrophotometer must have the following features:

1. Minimum delay time between mixing of reagents and recording the first data point.
2. The ability to acquire enough data points per second to allow accurate fitting of the data.
3. Remote read plug to accommodate a Rapid Mix Accessory.

The Cary 50 and Cary 60 UV-Vis have minimized the waiting period between mixing and collecting the first data point by incorporating a 'Synch Start' function in its software. This essentially 'primes' the software, preparing it for its first reading. The overall delay is less than 50 ms on the Cary 50 and Cary 60 UV-Vis, which means that valuable data are not lost during the initial part of the reaction.

The Cary WinUV Kinetics software provides all the necessary tools to analyze and display the data. Curve fitting is completed within seconds and the fitted curve is overlaid on the experimental data. The option of displaying the experimental data as points and the fitted curve as a solid line results in a professional report, which can be customized by the user.

The instrument parameters used in the data collection and the results of the curve fitting are also presented in the report.

Conclusion

The Cary 50 and 60 UV-Vis are fast wavelength scanning UV-Vis spectrophotometers, collecting data from single or multiple wavelengths at 80 data points per second. The minimum delay between mixing of the reagents and collecting the first data point allows for the collection of more data during the initial part of the reaction, where the greatest change in absorbance occurs. This enables accurate monitoring and analysis of reactions that occur on a sub-second level.

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