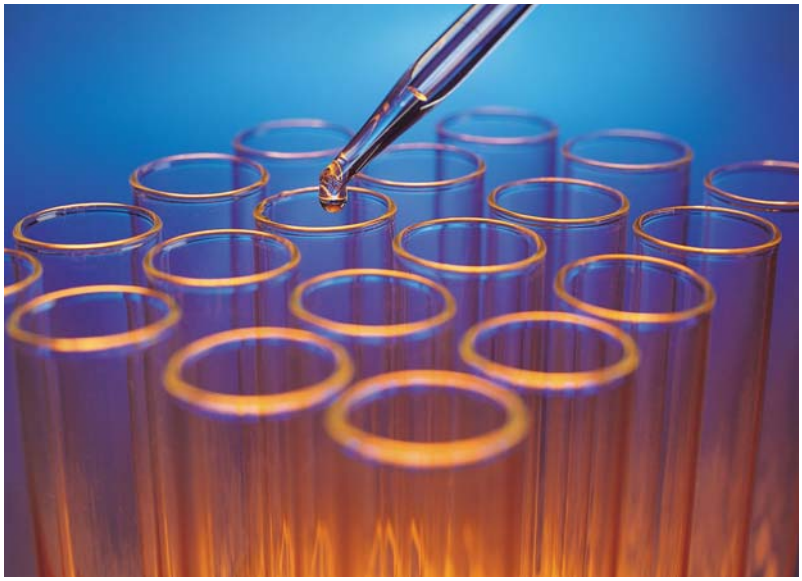
Measuring the formation of DNP using an Agilent Cary 60 UV-Vis spectrophotometer and SFA 20 stopped-flow accessory

Application note

Specialty chemicals



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Abstract

The hydrolysis of 2,4-dinitrophenol acetate by sodium hydroxide has become a standard reaction for testing the performance of fast kinetic instruments. The reaction was first published by Gutfreund[1] in 1969 and can be measured with an Agilent Cary 50/60 UV-Vis spectrophotometer and SFA 20 stopped-flow accessory because of the very fast time-base of this spectrophotometer.

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Introduction

To measure fast reactions like the one presented here, the speed with which the individual data points are collected is very important. In this case the process is further facilitated because of automatic triggering. Via the microswitch on the SFA 20, the Cary 50/60 UV-Vis spectrophotometer can be triggered to collect the data points immediately after the reagents have been mixed and when the chemical reaction starts. This way, all data points will be captured from the beginning of the reaction and averaging multiple traces will be very easy, as the starting point of the reaction is always the first point in the time trace.

Figure 1. Agilent Cary 60 with the SFA 20

Methodology

The reaction used for the experiments shown here is the reaction of DNPA with NaOH, which progresses as follows:

$$\text{DNPA} + \text{NaOH} \xrightarrow{k'} \text{DNP}$$

Sodium acetate is also formed during the course of the reaction, but is not shown in the above equation. For this reaction the change in DNPA concentration is given by:

$$[\text{DNPA}] = [\text{DNPA}]_0 \cdot \exp^{-kt}$$

if [NaOH] >> [DNPA]. The first order rate constant k is given by:

$$k = k' [\text{DNPA}]$$

The DNP that is formed during this reaction absorbs visible light at 400 nm, whereas the DNPA present at the start of the reaction has no absorbance at 400 nm. The reaction can therefore conveniently be followed at this wavelength. This is illustrated in Figure 2, where a spectrum of DNP and DNPA is shown.

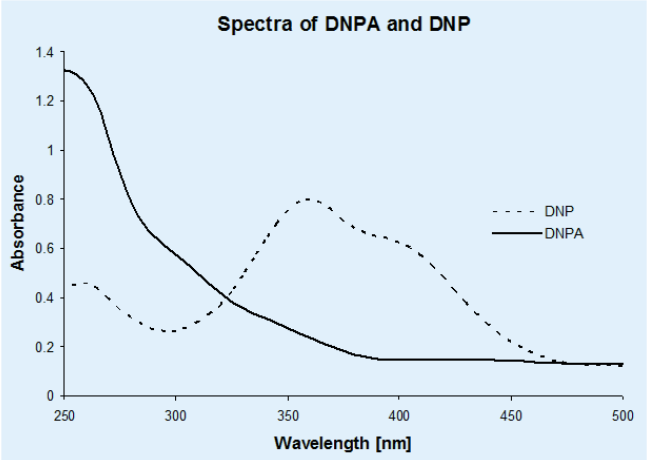

Figure 2. Spectra of DNPA and DNP

Experimental

The syringes of the SFA 20 stopped-flow accessory were loaded with the two reactants; one syringe contained 88 µM DNPA, the other was filled with 0.25 M NaOH. The individual shots were pushed by hand; data acquisition was initialized with the trigger cable assembly. This trigger cable assembly connects the SFA 20 via its microswitch with the Cary 50/60 triggering facility present in the spectrophotometer's sample compartment.

The absorbance measurements were carried out at 400 nm to observe the formation of 2,4-dinitrophenol (DNP). Several traces were collected to be able to average data during processing for better quality. Data was collected for 2 to 5 seconds per trace and a data point was collected every 12.5 ms. This is the highest time resolution the Cary 50/60 spectrophotometer is capable of measuring.

Results

Because of the automatic triggering facility, the averaging of several traces is very easy, and for the kinetics trace shown in Figure 3, eight separate data traces were added and a fit was made to the data to find the rate constant for this reaction. The fit is also shown in Figure 3 with a gray line, whereas the measured data is shown with a black line.

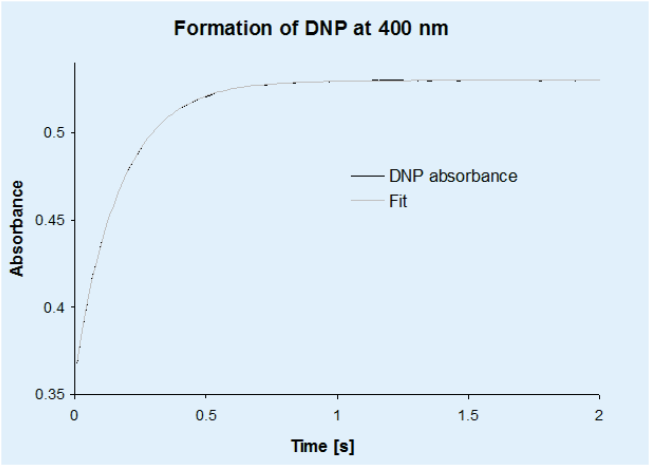

Figure 3. Kinetic trace for DNP formation

From Figure 3, it is apparent that the fit and the measured data are in very good agreement for these experiments. As the concentration of DNPA was negligible (88 µM) compared to the concentration of NaOH (0.25 M), first order reaction kinetics were assumed and from the fit of the kinetics traces a rate constant of k = 5.81 s^{-1} was found. This rate constant is consistent with the one that is quoted in the literature[1].

Conclusion

From these experiments and the fact that the results shown here are the same as those reported in the literature, it can clearly be seen that the Agilent SFA 20 stopped-flow accessory can be used in conjunction with the Agilent Cary 50/60 UV-Vis spectrophotometer to get reliable data for fast kinetic reactions. Because of the fast time base it offers, the Cary 50/60 can make use of the very short SFA 20 dead time of only 6 ms almost completely and be used for fast kinetic reactions.

References

1. Gutfreund, H. (1969) *Methods in Enzymology*, *16*, 229–249.

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