Application Note

Bioanalysis, Basic & Fine Chemicals

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A Fast Method of Studying the Impact of Temperature on Chemical Reactions

Reduce experimental time by performing kinetic experiments at four temperatures simultaneously

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Introduction

Many life science and chemical applications require a thorough understanding of the dynamics of reaction processes. Variables such as temperature, pH, pressure and the presence of additional chemical components and macromolecules can have a significant effect on reaction rates. Understanding the influence of such parameters is key for a wide variety of applications including enzyme characterization, chemical synthesis, food manufacture and industries that rely on optimized product storage and stability conditions. UV-Vis spectrophotometers are routinely used to help characterize and quantify the kinetics of reactions as they can continuously measure changes in the concentration over time as determined by the change in absorbance over time.

Examining the effects of different temperatures on reaction rates is time-consuming where experiments must be repeated at different temperatures and requires specialized equipment to be installed in the sample compartment of the spectrophotometer. Such equipment often uses recirculating water to maintain the temperature of samples. This introduces flood-risk, noise, and adds to the maintenance burden of the laboratory.

Recent advances in temperature-based spectrophotometric instrumentation offer significant time-savings and higher accuracy temperature control. The Agilent Cary 3500 Multizone UV-Vis spectrophotometer allows samples to be measured at four different temperatures in one experiment. The Cary 3500 can use integrated in-cuvette temperature probes to accurately control the temperature of the solutions during the experiment, or can be operated via the block temperature, which is highly suited for static temperature experiments. The multicell holder is built into the instrument and uses water-free, air-cooled Peltiers to control the temperature of samples between 0 and 110 °C.

This study aimed to examine the time-saving potential of performing kinetics rate measurements at four different temperatures in one experiment. The hydrolysis of p-nitrophenyl acetate (pNPA) was used for this purpose. It is a well understood reaction, and the rate of the reaction varies as a function of temperature.

Experimental

In an alkaline solution, p-nitrophenyl acetate (pNPA) readily undergoes hydrolysis to p-nitrophenol (PNP). pNPA has an absorbance maximum at 270 nm and PNP an absorbance maximum between 405 and 410 nm, depending on temperature. Wavelength scans over time were performed to monitor both pNPA consumption and PNP production as the reaction progressed. The experiment was performed at pH 7 and the reaction rate of the 80 °C sample was determined.

Samples

A solution of 0.0001 M p-NPA in methanol was prepared. A phosphate buffer solution (PBS) of 100 mM NaCl, 0.1 nM EDTA and 10 mM sodium phosphate was also prepared and adjusted to pH 7.0.

Undiluted PBS was used to establish a baseline and was also used as the reference during each measurement. Standard, 3.5 mL 10 mm optical pathlength quartz cuvettes were used for this experiment with star type magnetic stirrers stirring at 500 rpm.

Instrumentation and method

A Cary 3500 Multizone UV-Vis spectrophotometer was used for all measurements (Figure 1). The method parameters are shown in Table 1.

Figure 1. The sample compartment of the Cary 3500 Multizone UV-Vis spectrophotometer has a built-in multicell holder. Each sample/reference pair can be held at a different temperature.

Table 1. The instrument parameters.

Parameter	Setting
Wavelength range (nm)	220 – 520 nm (scan)
Scan rate (nm/min)	1200
Spectral bandwidth (nm)	5
Signal averaging time (s)	0.1
Data interval (nm)	2
Stirring speed (rpm)	500
No. temperature zones	4
Temperatures (°C)	20, 40, 60, 80
Temperature control	Block

Each sample cuvette was filled with 2980 µL of phosphate buffer solution and placed in the multicell holder (Figure 1). Ten minutes was allowed for temperature equilibration, then 20 µL of the p-NPA in methanol solution was added.

Absorbance scans across the wavelength range of 220 to 520 nm, were collected every 30 seconds for 30 minutes. These measurements were performed simultaneously for each temperature set. The Cary UV Workstation in-built kinetic analysis features were used to generate a kinetic curve and determine the reaction rate.

Results

The hydrolysis of pNPA involves the removal of the acetate group under basic conditions. When the conditions are set such that water is in excess, then the reaction can be considered as pseudo first order. As the pH of the PBS buffer was set to 7, a slower reaction rate can be expected, and the second order behavior dominates.

Temperature effect

Wavelength scans of pNPA hydrolysis at four different temperatures are shown in Figure 2. There is a clear relationship between the temperature of the sample and the reaction rate as measured by PNP production (Figure 2). At 80 °C the isosbestic points are evident, indicating a direct conversion from pNPA to PNP. The wavelength scanning kinetics data for the 80 °C experiment was used to produce an absorbance versus time plot, using the PNP peak at 408 nm (Figure 3). The second order rate calculation (built in to Cary UV Workstation software) was then used to determine the second order rate constant (k) for the reaction as k = 883.194 (1/[min.mol]).

Wavelength scans

By performing wavelength scans over time, the consumption of pNPA and PNP production can be observed (Figure 2). The entire wavelength range provides additional information that might otherwise be missed if only a single wavelength was analyzed. For example, the possible presence of reaction intermediates, as well as subtle changes in the sample, and detection of isosbestic points as shown in Figure 2.

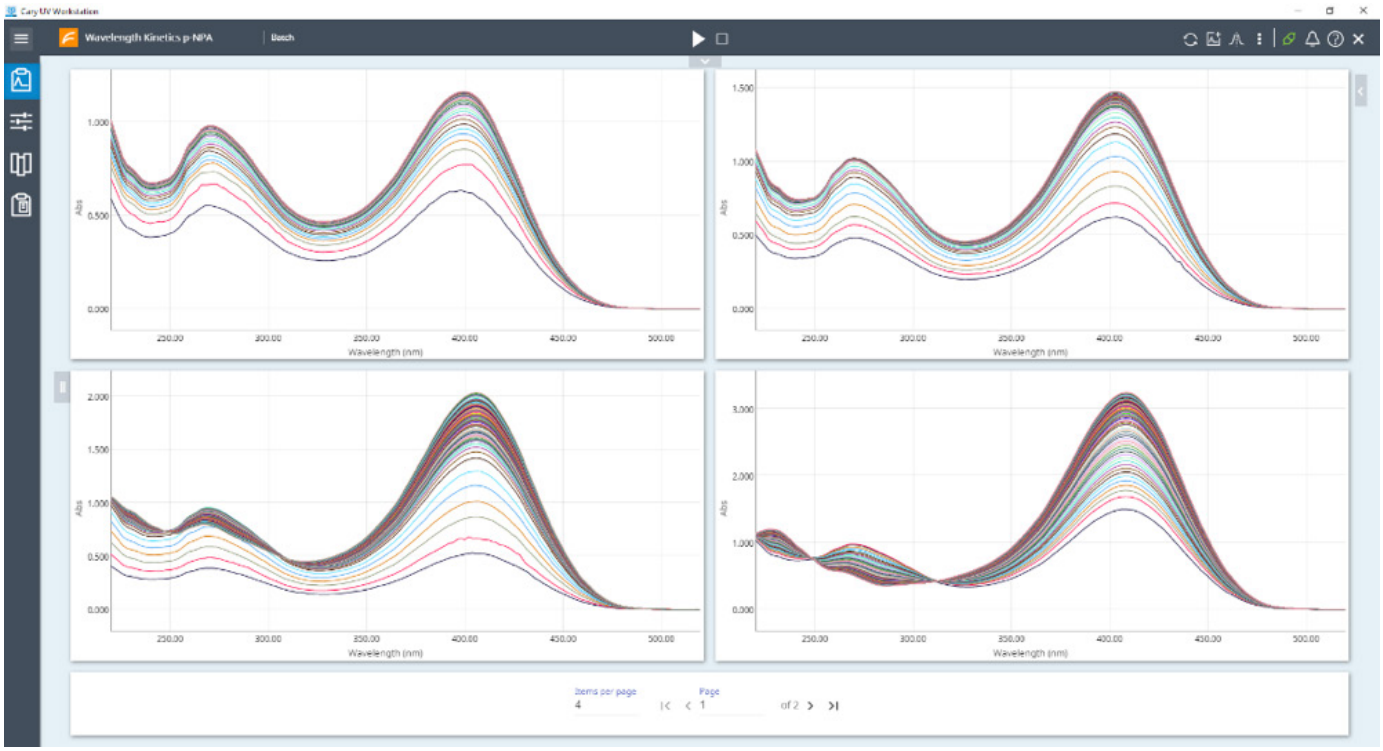

Figure 2. Wavelength scans over time over the wavelength range 220 - 520 nm, collected for 30 minutes after the reaction was initiated by mixing the two reagents. Top left is at 20 °C, top right is 40 °C, bottom left is 60 °C and bottom right is 80°C.

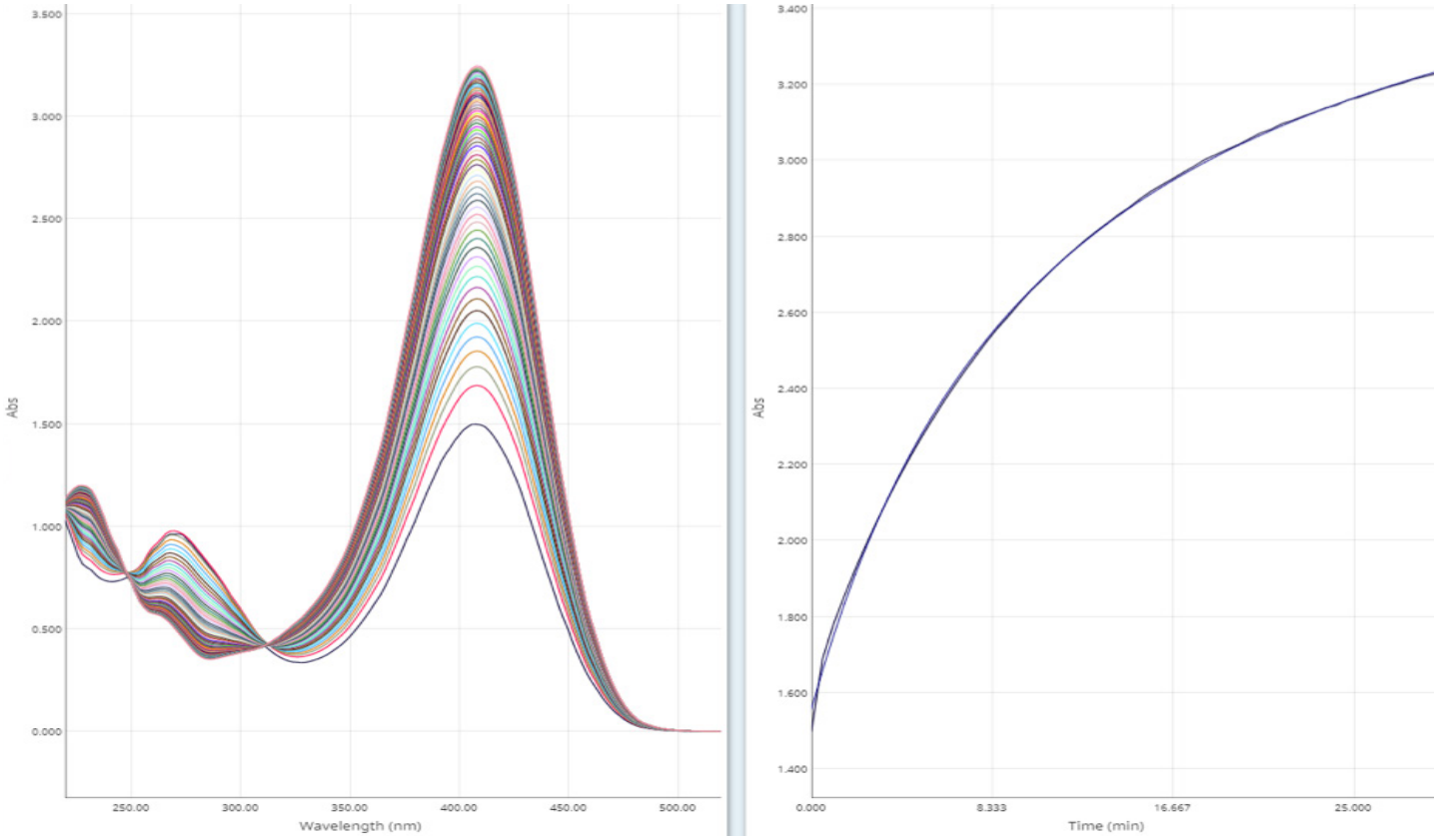

Figure 3. The spectra for the reaction performed at 80 °C with characteristic isosbestic points (left). The change in absorbance over time at 408 nm (right) was plotted within the Cary UV Workstation software and used to determine the reaction rate.

Conclusions

The Cary 3500 Multizone UV-Vis spectrophotometer enabled the hydrolysis of pNPA to be monitored at four different temperatures in a single experiment. The effects of temperature on reaction rates was demonstrated at 20 °C, 40 °C, 60 °C and 80 °C, simultaneously, in only one experiment that took 30 minutes.

Rapidly collecting wavelength spectra during the experiment also allowed the data to be interpreted at different wavelengths. While a reaction rate can be determined for all four temperatures, the reaction mechanism may be proceeding differently. Observing the full wavelength range for the reaction can provide useful insights into the reaction mechanism.

An improved understanding of reaction kinetics is fundamental for better comprehension of chemical interaction and reaction processes. Although highly informative, undertaking detailed experiments to investigate the temperature dependence of reaction processes can be extremely time-consuming. Using the unique multiple-temperature functionality of the Agilent Cary 3500 Multizone UV-Vis, the kinetics data could be collected in 25 % of the time it would take with traditional UV-Vis systems presenting unique time-saving benefits for the laboratory.

www.agilent.com/chem/cary3500uv-vis

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