Benefits of the Agilent Cary 8454 UV-Vis Diode Array for Multi-Wavelength Kinetics

Application note

Speciality Chemicals

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Introduction

The reaction of p-nitrophenyl acetate (pNPA) hydrolysis can be followed spectrophotometrically by monitoring either the decrease in reaction substrates, or the increase in reaction products. Monitoring the rate at which the concentration changes enables the rate of the reaction to be determined.

The Agilent Cary 8454 UV-Visible diode array spectrophotometer can measure a full spectra in as little as 0.1 seconds. Absorbance changes with time can be extracted as often as necessary from the stored spectral data as required. As all the data across the entire wavelength range is acquired simultaneously during the same experiment, the results can be interpreted accurately at different wavelengths providing meaningful insight into the reaction mechanism.

With conventional scanning spectrophotometers, it is usually convenient—or necessary in the case of very fast reactions—to follow the reaction kinetics at a single or select few wavelengths. The hydrolysis of pNPA can be followed at 270 nm for pNPA consumption or 405 nm for p-nitrophenol production as the reaction progresses. However, by not monitoring the entire spectral range, important data can be omitted that is needed to perform a more accurate analysis of the data.

In addition to fast, accurate measurements, accurate temperature control is vital for good kinetic measurements. Depending on the reaction being studied, a variation of only 1° C can cause significant changes in the observed reaction rate. The Agilent Cary 8454 has available a Peltier temperature control accessory (part number 89090A) that provides accurate temperature control and can both heat and cool the sample, as well as measure the temperature of samples using temperature probes.

Experimental

In alkaline solution pNPA undergoes hydrolysis to p-nitrophenol according to the equation:

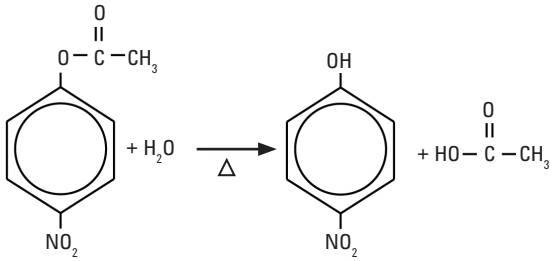

Figure 1. The pNPA hydrolysis reaction

In principle, this is a second-order reaction, but if water is in excess it can show pseudo first-order kinetics. The reaction rate is sensitive to pH and to temperature. The experiments were performed by adding 2.9 mL of 0.1 M phosphate buffer (pH 8.5) to a cuvette, which was placed into a thermostatted cell holder and allowed to equilibrate. The optional external temperature sensor, which is small, and glass coated for inertness, was used to determine the exact temperature of the sample.

The Peltier temperature controller controls the temperature of an aluminium block surrounding the cell containing the sample. The cell is held in close physical contact with the aluminium block to minimize any temperature differential. There is only a very small difference between the preset temperature and the actual sample temperature, which was determined by adding 3 mL of distilled water to a cuvette and using the external sensor to determine the differences at various temperatures (Figure 2). Nevertheless, the temperature that was monitored was that of the actual sample through the external temperature sensor, which insured that the highest accuracy was obtained.

When the reaction temperature had equilibrated, the reaction was started by the addition of 50 µL of p-nitrophenyl in dry acetonitrile stock solution, and a stirrer was used at 700 rpm to mix the reaction. Spectra were automatically acquired at specific intervals that were adjusted according to temperature and the speed of the reaction. In Figure 3, the reaction spectrum at 55°C is shown, with spectra measured at 125-second intervals.

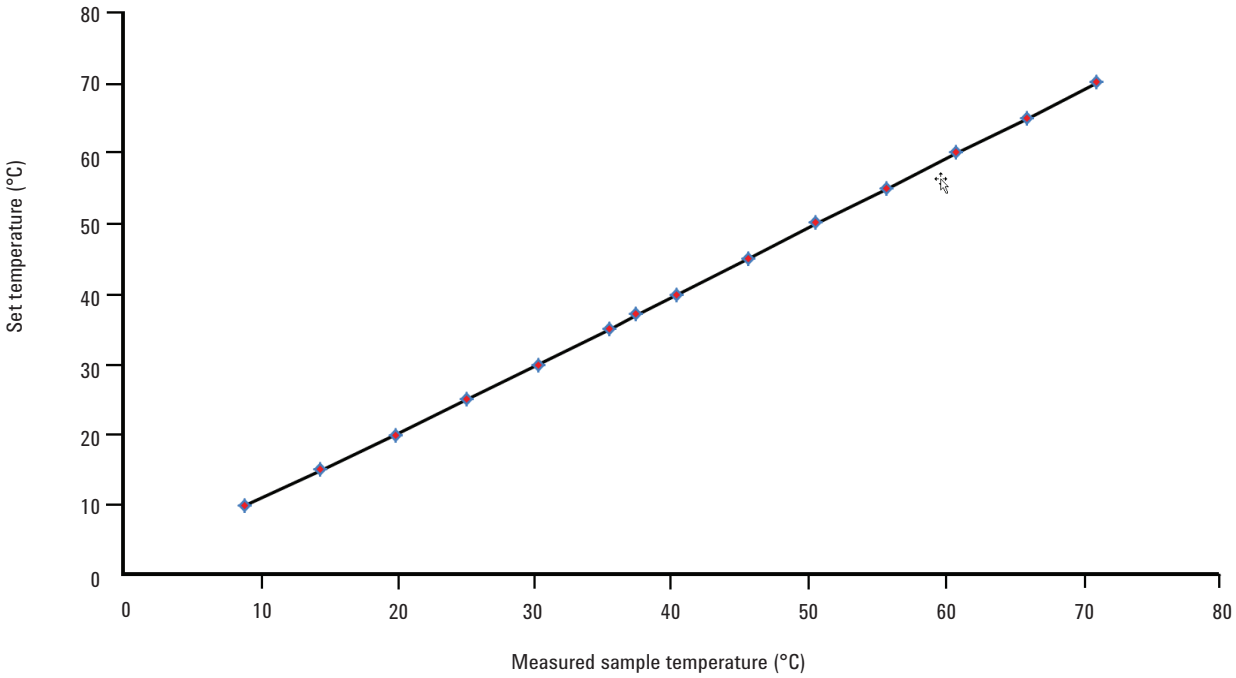

Figure 2. Correlation between set (cell holder) temperature and measured sample temperature.

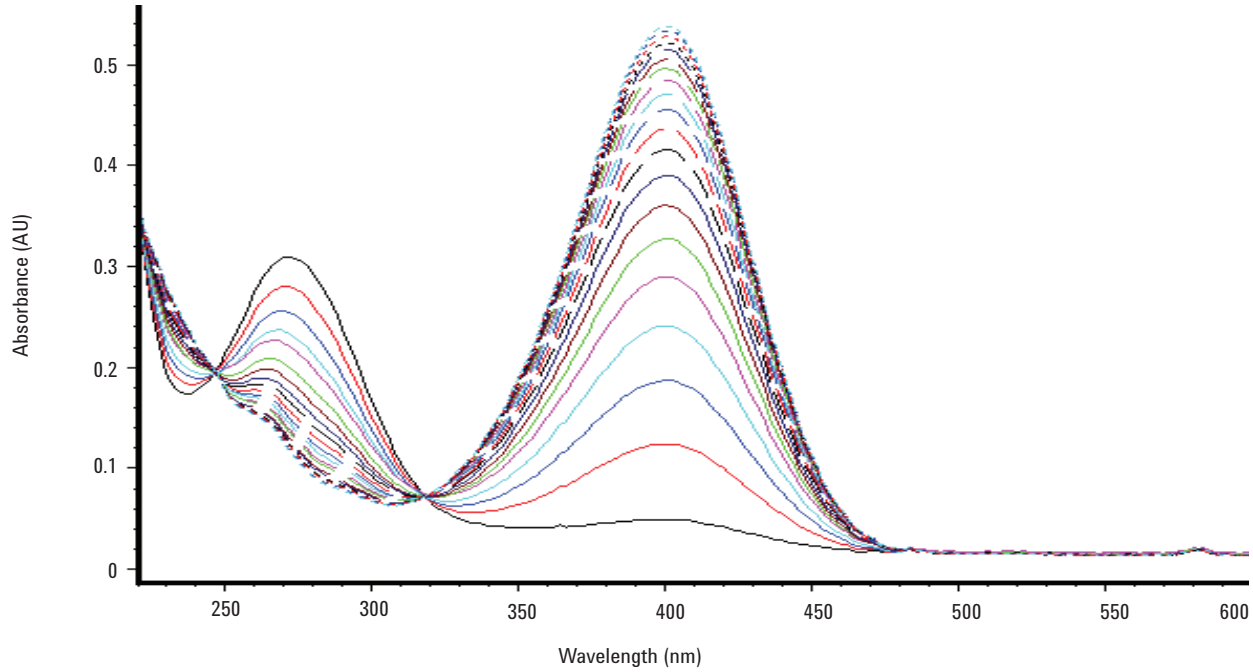

Figure 3. The reaction spectrum of p-nitrophenyl acetate hydrolysis at 55°C.

If a changing absorption spectrum is recorded repetitively over a wavelength range, the spectra might intersect at one or more wavelengths (Figure 3). These points of intersection are known as isosbestic points, and indicate that a uniform reaction between reactants and products (ie no side-reactions or consecutive reactions) are taking place. The precise isosbestic points in Figure 3 are indicative of the optical and temperature stability of the Agilent Cary 8454 UV-Visible spectrophotometer over the reaction time.

Using UV-Visible ChemStation, it is easy to open existing collected data and reanalyze the data at any wavelength. In this case, after data collection, the kinetics of the reaction can be determined by extracting the data for the formation of p-nitrophenol at a single wavelength (405 nm) where no other component absorbs (Figure 4).

Using the Kinetics mode of the UV-Visible ChemStation software, which is available as part of the BioChemical Analysis add-on (G1117AA), the pseudo-first order rate constant was calculated to be 1.24×10^{-3} s^{-1} at 55° C.

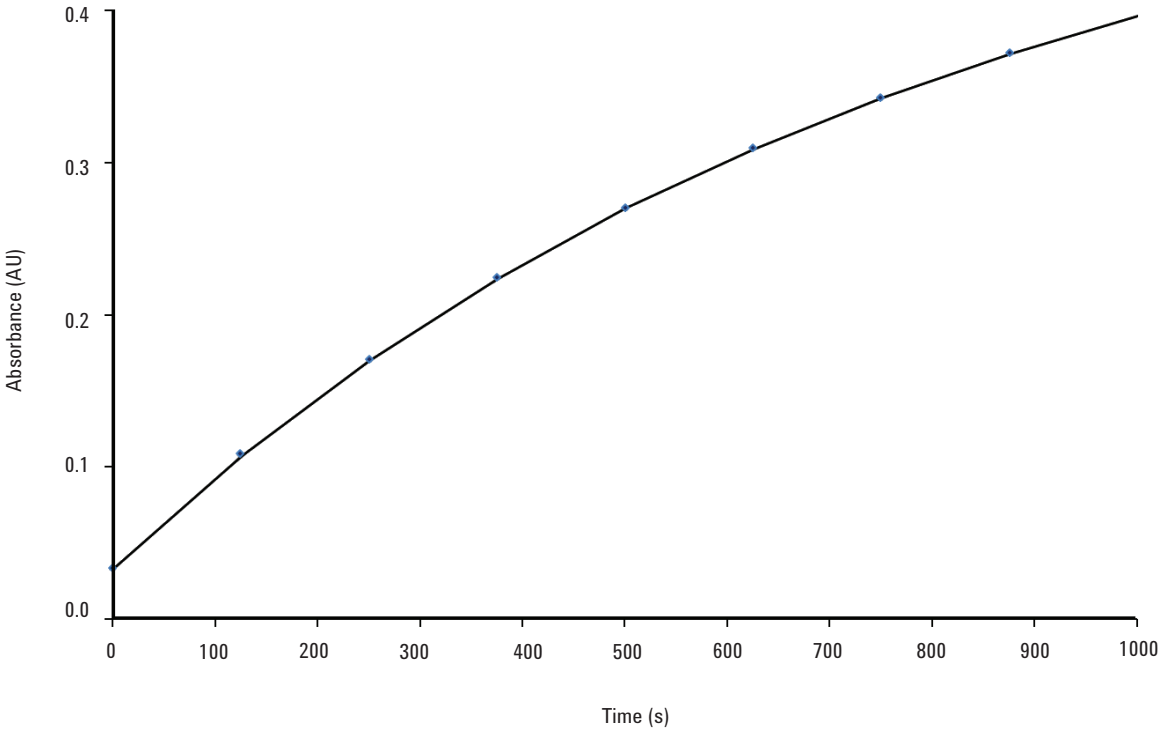

Figure 4. The rate of the reaction was determined from the change in absorbance at 405 nm.

Conclusion

The Agilent Cary 8454 UV-Visible spectrophotometer is ideal for the analysis of the kinetics of a reaction, whether enzyme catalyzed or a chemical reaction. We have demonstrated here the effectiveness of the Peltier temperature accessory for reliably controlling the temperature of the reaction, while the instrument shows exceptional stability over time for kinetic analyses.

The advantages of using a diode array spectrophotometer include the fast acquisition of a full spectrum down to as little as 0.1 seconds per spectrum!

Collecting a full spectrum provides the user with all the data available for the reaction. It is possible to go back after data collection and review the reaction rate for any wavelength easily and quickly. On the other hand, by monitoring across the wavelength range, the spectra could be subjected to multi component analysis to determine the concentrations of both substrates and products at each measurement time point.

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Published March 13, 2014
Publication number: 5991-4283EN

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