High Sensitivity Direct Mount Photodiode Array for the SX20

- Direct mounting to the cell block for good signal-to-noise ratio even at low light levels that prevent photobleaching
- Investigate enzyme kinetics, biomolecular interactions and inorganic reactions
- Case study involved acid hydrolysis of the complex species tri(ethylamine)nickel (II), $Ni(en)_3^{2+}$

The directly mounted photodiode array accessory ensures highly efficient light collection for stopped-flow absorbance measurements. The improved design ensures that ultimate sensitivity is achieved even at low light levels that may be needed to study species that exhibit photobleaching. As an example of the many applications of this accessory, this application note demonstrates its use to measure the kinetics of an inorganic ligand exchange reaction.

KEYWORDS

- SX20
- Stopped-Flow
- Absorbance Measurements
- Photopdiode Array
- Enzyme Kinetics
- Biomolecular Interactions
- Inorganic Reactions
- Rapid Mixing

Introduction

The Applied Photophysics Ltd. photodiode array (PDA) detector accessory for the SX20 stopped-flow spectrometer is designed to mount directly to the optical cell, ensuring highly efficient light collection. In combination with state-of-the-art electronics, it provides ultimate sensitivity for multi-wavelength stopped-flow absorbance measurements. Even at low light levels, a relatively high signal-to-noise ratio is achieved, meaning the excitation intensity can be reduced if samples are susceptible to photobleaching. The PDA option enables sets of time-resolved spectra to be acquired from a single stopped-flow drive.

The PDA can acquire up to 1000 spectra per second and is available in two wavelength ranges: PDA-UV (185-725 nm) and PDA (330-1100 nm). The accessory is ideal for many applications where spectral changes occur quickly and must be followed over short timescales. As one of the most popular accessories for the SX20, the PDA finds use in several research areas including:

- **Enzyme Kinetics:** For example, redox enzymes often exhibit spectral changes during their catalytic cycle. The PDA accessory can identify and measure intermediate formation on short timescales.
- **Biomolecular interactions:** Spectral changes arising from

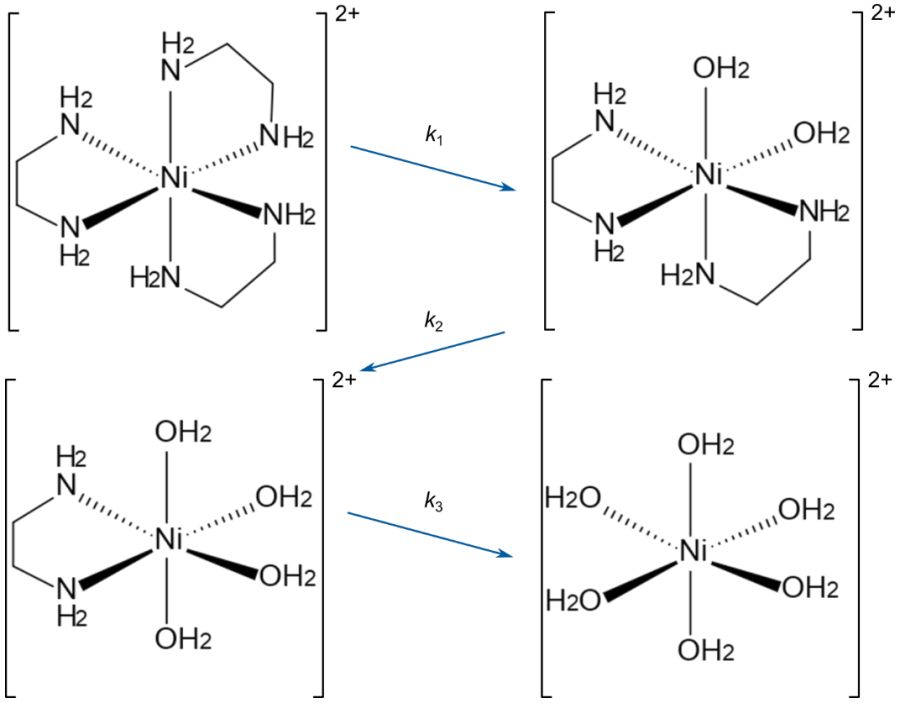

Figure 1: Steps of the $Ni(en)_3^{2+}$ acid hydrolysis reaction

interactions between biomolecules can be monitored using the PDA accessory to obtain key kinetic and mechanistic information about biological systems.

- **Inorganic reactions:** Millisecond-changes in spectral properties resulting from changes in a metal's redox state or ligand are routinely measured using a stopped-flow instrument equipped with the PDA accessory.

Case Study

To demonstrate the utility of the PDA accessory, the acid hydrolysis of the complex species tri(ethylamine)nickel (II), $Ni(en)_3^{2+}$ was followed.

When mixed with acid, the complex species tri(ethylamine)nickel (II), $Ni(en)_3^{2+}$ undergoes hydrolysis to the hydrated hexaaqua $Ni(H_2O)_6^{2+}$ ion via a series of ligand exchange reactions (**Figure 1**).

Each step in this reaction can be measured simultaneously using the SX20 Stopped-Flow Spectrometer operating with a directly mounted PDA detector. On rapidly mixing a solution of $Ni(en)_3^{2+}$ (0.05 M) with hydrochloric acid (1 M), data were acquired over a 42-second sampling period.

Results

The series of spectra obtained is shown in **Figure 2**. All steps were captured by the PDA, allowing to study the overall reaction from a single experiment. Each species in the reaction scheme has a different absorbance spectrum and each reaction step occurs at a different rate. The Pro-K IV fitting software by Applied Photophysics Ltd. was used to analyze the time-dependent spectra. Data was fit with a three-step model to calculate rate constants for each step (**Table 1**) as well as the concentration profiles over the course of the reaction (**Figure 3**). The software also calculated spectra for each species in the reaction scheme (**Figure 3** and **Table 2**).

Table 1: Rate constants calculated for each step. Errors represent standard deviation of 5 repeat measurements.

Rate constant	Value
k_1	57.9 ± 1.5 s^{-1}
k_2	2.73 ± 0.1 s^{-1}
k_3	0.119 ± 0.005 s^{-1}

Conclusions

The directly mounted PDA accessory provides ultimate sensitivity for multi-wavelength stopped-flow absorbance measurements. Direct mounting of the detector means that light collection is highly efficient and so, a good signal-to-noise ratio can be achieved even at low light levels. This is beneficial if samples are susceptible to photobleaching. As an application example, the PDA accessory was used to measure an inorganic ligand exchange reaction. The data was readily fitted to extract kinetic and spectral properties of the species involved, giving insight into the chemical mechanism.

Experimental

Equal volumes of tri(ethylamine)nickel (II) (0.05 M) and hydrochloric acid (1 M) were rapidly mixed together in an SX20

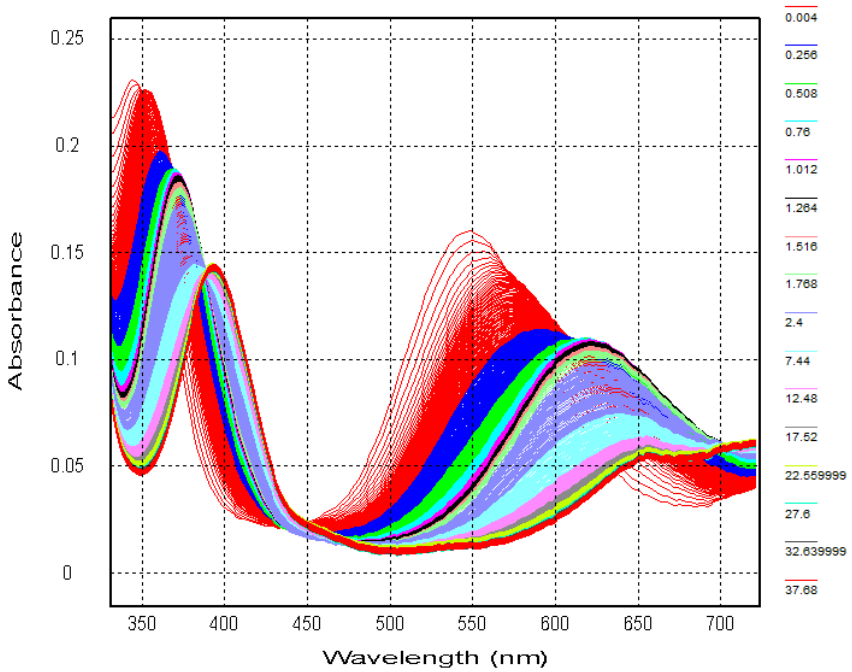

Figure 2: Time resolved spectra measured with the PDA accessory

stopped-flow spectrometer. Data was measured using a directly mounted UV/Vis PDA detector. Data acquisition was performed using a split time base with 500 spectra recorded in the first 2 seconds and 500 spectra measured in the following 40 seconds. 5 repeat drives were performed at 20°C. A three-step reaction model was fit to the data from 350-722 nm using the Pro-K IV kinetic analysis software by Applied Photophysics Limited.

Table 2: Calculated spectral properties for each of the species.

Species	λ_{max} (nm)	ε_{max} (M^{-1} cm^{-1})
$Ni(en)_3^{2+}$	343 546	6.93 4.27
$Ni(en)_2(H_2O)_2^{2+}$	355 567	6.98 3.66
$Ni(en)(H_2O)_4^{2+}$	370 621	5.64 2.98
$Ni(H_2O)_6^{2+}$	393 657	3.6 1.23

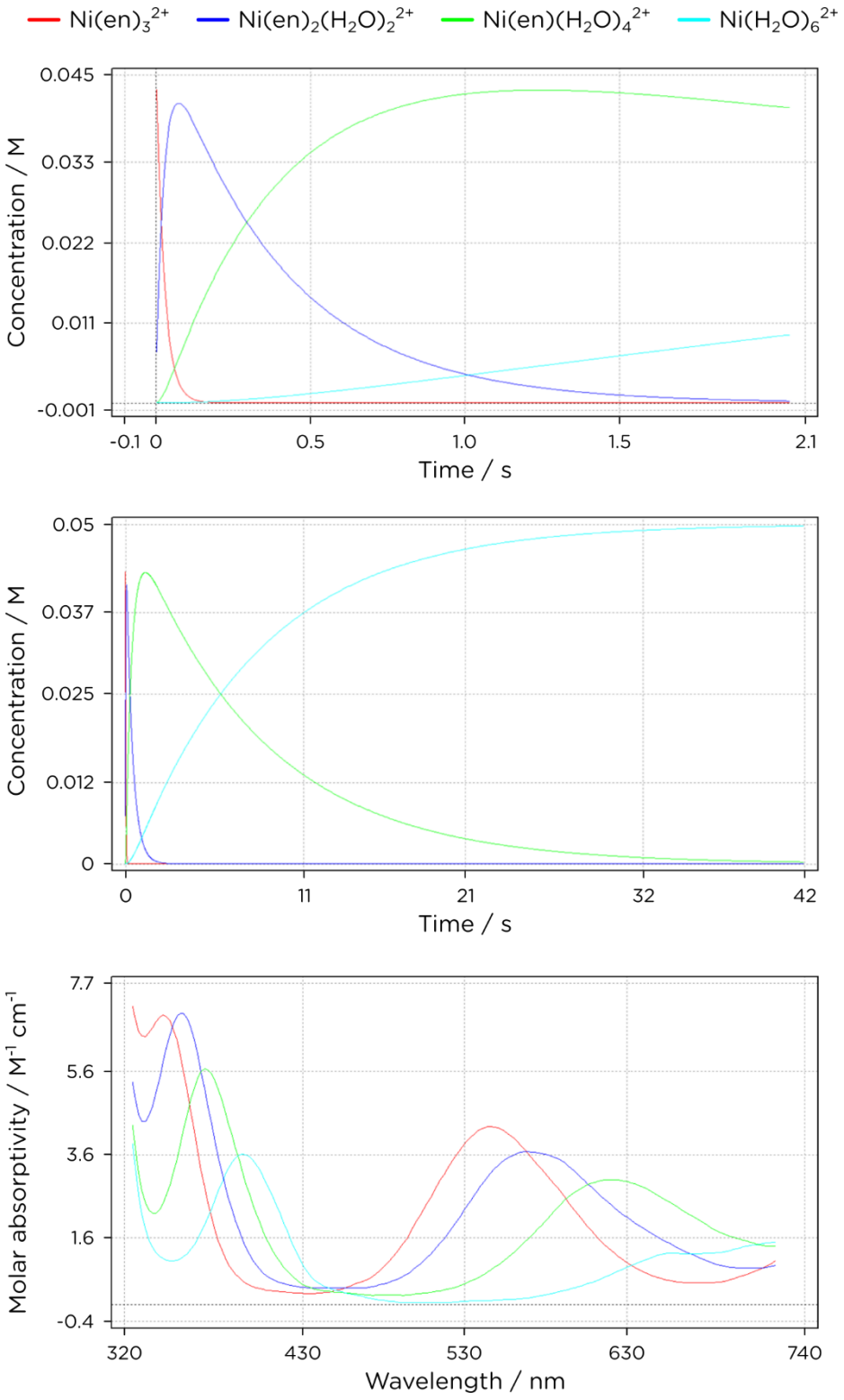

Figure 3: Concentration profiles showing how each species in the reaction varies in the initial 2 seconds (top) and in the entire 42 seconds (middle) and calculated spectra for each species in the reaction (bottom).