Application Note
UV-0019

Reduction Reaction of 2,6-Dichloroindophenol using the Absorption Stopped-Flow System

Introduction

The absorption stopped-flow measurement system is designed to measure rapid enzymatic, catalytic, and oxidation-reduction reactions. The system consists of the FS-110 fast scan spectrophotometer and the SFS-822 stopped-flow accessory and allows for two to four liquid samples to be rapidly mixed. Stopped-flow absorption spectra can be measured in 5 msec intervals to acquire reactions occurring at millisecond timescales.

FS-110
Fast Scan Spectrophotometer

This application note illustrates how the absorption stopped-flow system can determine the reaction rate for the reduction of 2,6-dicholorindeophenol (DCIP), whose color in aqueous solution changes from to blue to colorless as a result of its reaction with L-ascorbic acid.

Keywords

FS-110 fast scan spectrophotometer, UV-Visible/NIR, SFS-852 Stopped-flow system, Stopped-flow Measurement program, Reaction Rate Calculation program

Experimental

Measurement Conditions			
Spectrophotometer		**Stopped-flow System**	
Optical Pathlength	2 mm	Time of solution sending	10 msec
Data Interval	1 nm	Mixing ratio	1:1
Measurement Interval	0.010 sec	Volume of solution sending	50 µL
Measurement Time	0-3 seconds	The measurement is started when the syringe is stopped.	

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L-ascorbic acid was dissolved in NaOH/Na2HPO4 to acquire a final concentration of 20 mmol/L and the pH was adjusted to 7.6. Aqueous solutions of DCIP were used at a concentration of 1 mmol/L.

Results

Figure 1 shows the 3D spectra of the DCIP reaction. When the reaction begins, the spectra illustrates an absorption maximum around 600 nm which coincides with the sample exhibiting a blue color. Within 1 second after starting the measurement, the absorbance drops to approximately zero and the sample changes to colorless.

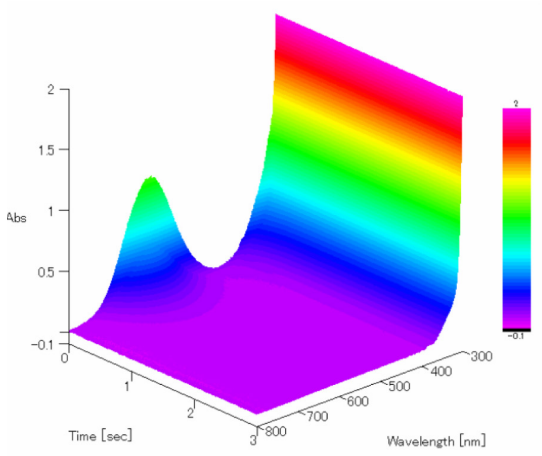
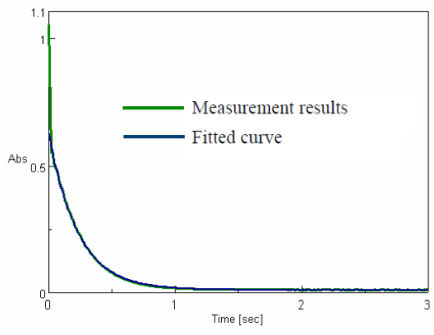


Figure 1. 3D spectra of the reduction of DCIP.

Figure 2 shows the time course absorbance data at the absorption maximum (604 nm) and the curve fitted to the reaction data between 0.03 and 2.0 seconds. The reaction is assumed to be a primary reaction, and the fit results are in excellent agreement with the measurement results. Using the Reaction Rate Calculation program, a reaction rate of 4.3 sec-1 was calculated.



Reaction rate calculation equation:
Y(t) = 0.615066 x exp(-t/0.230571)

Baseline equation:	Y(t) = 0.0105329
Time constant:	0.230967 sec
Rate constant:	4.32963 sec^{-1}
Half-life period:	0.160094 sec

Figure 2. Time course measurement results and the fitted curve for sample absorption measured at 604 nm.

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