Application Note

UV-0019

Measurement of the reduction reaction of 2,6-dichloroindophenol (DCIP) using the absorption stopped-flow system

Introduction

Using the absorption stopped-flow measurement system, consisting of FS-110 fast scan spectrophotometer and SFS-852 stopped-flow system, two to four kinds of liquid sample can be mixed quickly and the changes in absorption spectra can be measured at intervals of 5 msec. This system allows measurement of rapid enzymatic, catalytic and oxidation-reduction reactions.

This application note illustrates an example of determining the reaction rate by using the absorption stopped-flow system for the reduction of DCIP, whose color in aqueous solution is changed from blue to colorless as a result of reaction with L-ascorbic acid.

Keywords: Stopped-flow, reaction rate, fast scan spectrophotometer

Measurement and analysis system

Absorption stopped-flow measurement system
- FS-110 fast scan spectrophotometer
- SFS-852 stopped-flow system
- Stopped-flow measurement program
- Reaction rate calculation program

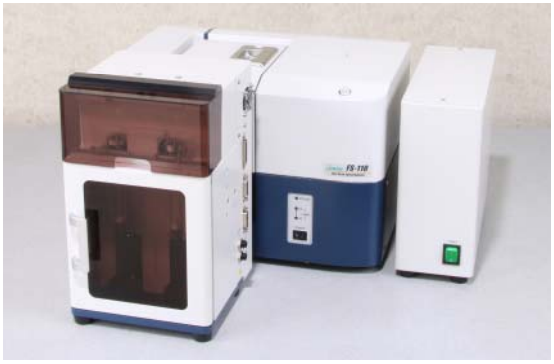

Figure 1 Absorption stopped-flow system

Samples

- 20 mmol/L L-ascorbic acid aqueous solution (Dissolve the L-ascorbic acid with NaOH/Na_2HPO_4 aqueous solution and make it to a constant volume. Then, adjust the pH to 7.6.)
- 1 mmol/L DCIP aqueous solution

Measurement conditions

Spectrophotometer		Stopped-flow system	
Optical pathlength:	2 mm	Time of solution sending:	10 msec
Wavelength range:	300 to 800 nm	Mixing ratio:	1:1
Data interval:	1 nm	Volume of solution sending:	50 µL
Measurement interval:	0.010 sec	The measurement is started when the syringe is stopped.	
Measurement time:	0 to 3 sec		

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Results

Figure 2 shows the 3D spectra of the sample. When the reaction is started, the spectrum indicates an absorption maximum at approximately 600 nm and the sample exhibits a blue color. Then, the absorbance in the visible wavelength range changes to approximately zero within 1 sec after starting the measurement, and the sample turns colorless.

Figure 3 shows the time course data for absorbance at the absorption maximum (604 nm) and the curve fitted to the data between 0.03 and 2.00 sec for the reaction range, assuming the reaction to be a primary reaction. The fitted curve is in excellent agreement with the measurement results. A reaction rate of 4.3 sec^{-1} was calculated.

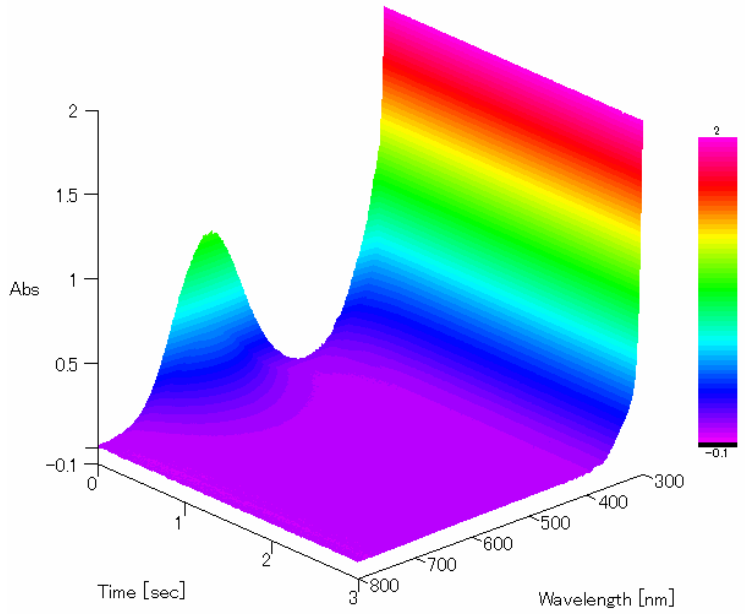

Figure 2 3D spectra of reduction of DCIP

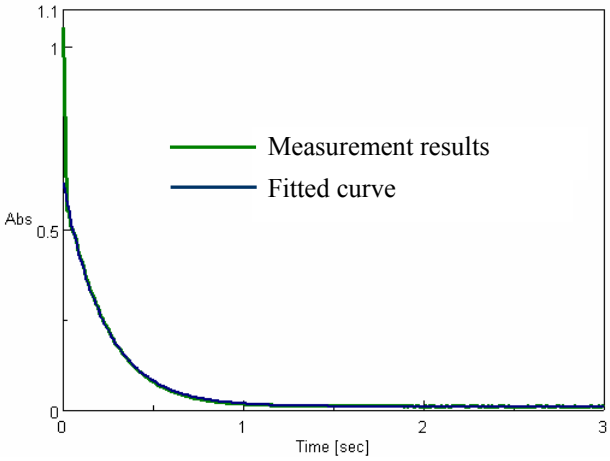

Figure 3 Time-course measurement results and fitted curve for sample absorbance at 604 nm

Curve fitting analysis parameters

Number of reaction steps: 1

Reaction range: 0.03 to 2.00 sec

Curve fitting analysis result

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

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