Application Note

CD-0003

Application using Model FDCD-465 Highly-Sensitive FDCD Measurement Accessory

[Introduction]
Fluorescence Detected Circular Dichroism(FDCD) is a method to measure the difference in intensities of fluorescence that arise when optically active sample is excited by left-hand and right-hand circularly polarized light. FDCD gives equivalent information to absorption CD while utilizing the superior sensitivity and selectivity of fluorescence detection. When FDCD is actually measured, CD value can be calculated from the results of FDCD measurement by the following equation.

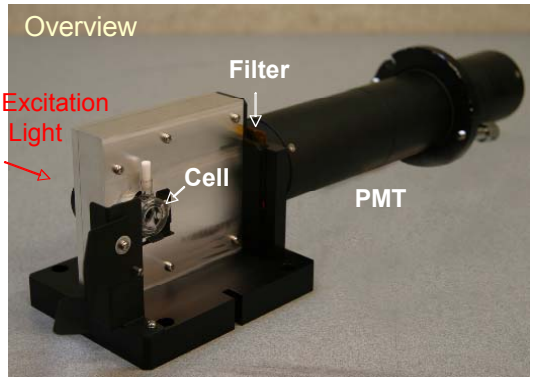
$$\Delta\varepsilon = \varepsilon_l - \varepsilon_r = (3.032 \times 10^{-5}) \times S \times (1 - 10^{-A}) / (cd10^{-A}) \text{ ---------- (1)}$$

where;

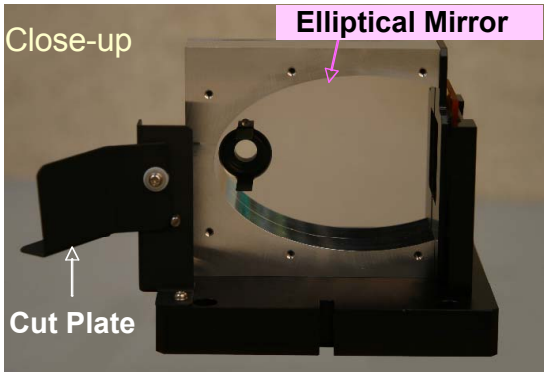
A: absorbance of sample, c: molar concentration of sample,
d: cell length(cm), S: FDCD value measured(mdeg)

[Principle of Measurement]
FDCD-465 is a highly sensitive FDCD measurement accessory combining both a cylindrical cell and an elliptical cylinder mirror. This accessory is designed to collect all radiated fluorescence light emitted in circumferential direction from the cell, resulting not only in the improvement of light collection efficiency, but also the ability to eliminate the effect from artifacts caused by fluorescence anisotropy. [2)3)]

FDCD-465 Outer view



Inside of FDCD-465



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FDCD-465 is designed to minimize the influence from artifacts due to fluorescence anisotropy, however, using solvents with high viscosity such as glycerol may cause such an influence to some degree. In order to eliminate such an influence, the FDCD-465 is provided with Balancing Mask as described below.

The fluorescence light in F-y direction does not reach the detector due to the blockage caused by the cell itself. At this time, the intensity of fluorescence of (F+x + F-x) becomes slightly larger than the (F+y + F-y), creating the possible cause of the artifact. By using the Balancing Mask, the balance of intensity of these fluorescence can be maintained to eliminate the artifacts even when utilizing solvents with high viscosity.

Mask (mm)

[FDCD measurement of ammonium d-10-camphorsulfonic acid water solution]

Figure 1 displays FDCD, CD, and UV spectrum of ammonium d-10-camphorsulfonic acid (d-10-ACS) water solution of 0.0024M(0.06%w/v). Since the FDCD is an excitation spectrum, the excitation and emission spectra need to be obtained by a spectrofluorometer beforehand. The fluorescence peak, when d-10-ACS water solution was excited by 285 nm, was at 427 nm. Here, J-820, FDCD-465, L38 excitation light cut filter, and 1cm cell were used to obtain FDCD spectrum in the range of 350-220 nm.

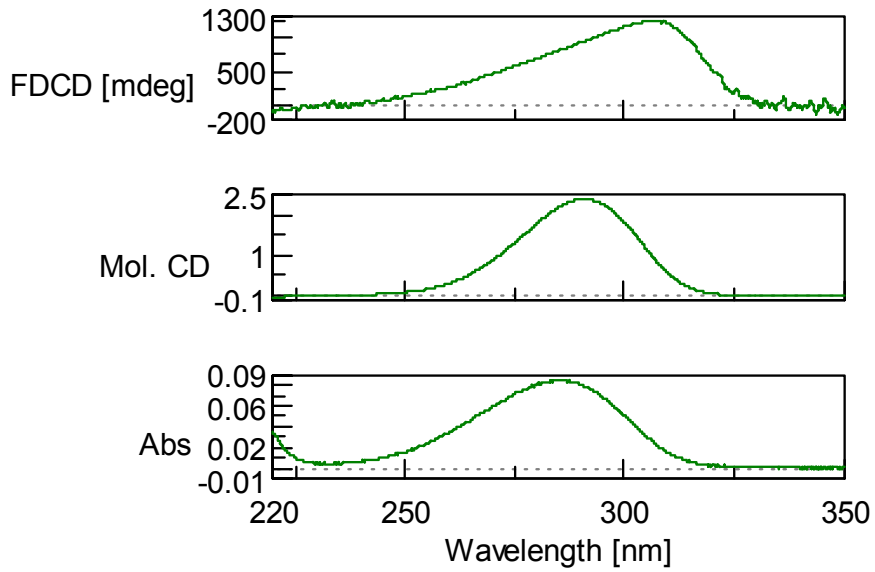

Figure 1: d-10-ACS (0.0024M) water solution's FDCD (top), CD (middle),UV (bottom) spectrum

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Based on the obtained FDCD, CD, and UV spectrum, utilizing the equation(1), CD spectrum converted from FDCD was compared with the measured CD spectrum. As shown in Figure 2, the CD spectrum obtained from FDCD was in good agreement with the measured CD spectrum within an experimental error.

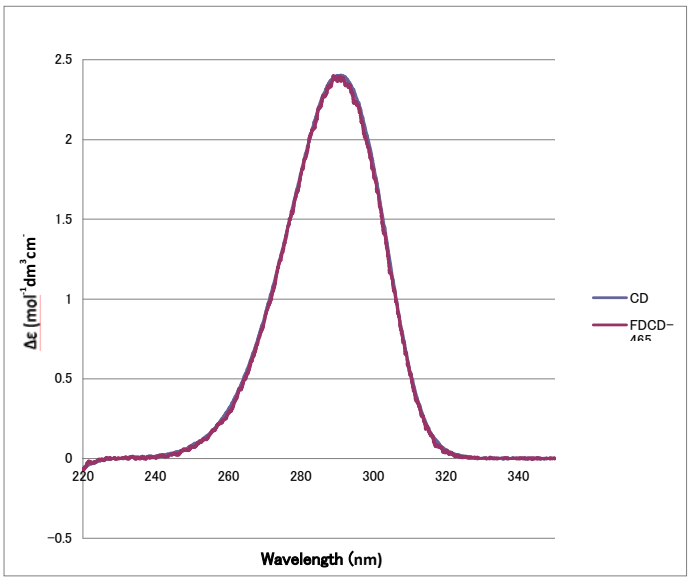

Figure.2: The CD spectrum obtained from FDCD was in good agreement with the measured CD spectrum

[Comparison of sensitivity in FDCD and CD measurement]

Figure 3 and Figure 4 show FDCD spectrum and CD spectrum of (1S, 2S)-trans- cyclohexanediol bis (6-methoxy-2-naphthoate)/ acetonitrile solution with very low concentration conditions, respectively.

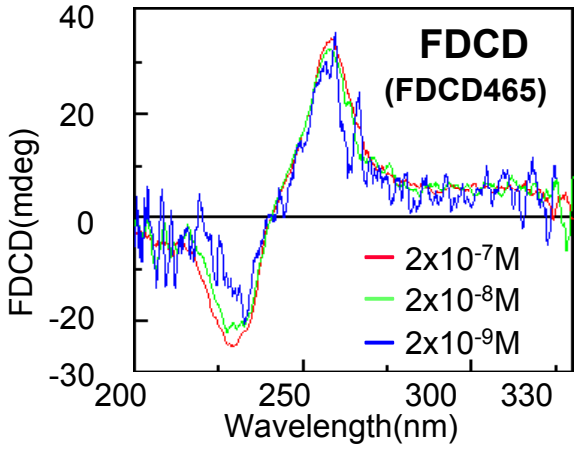

Figure 3: FDCD Spectrum

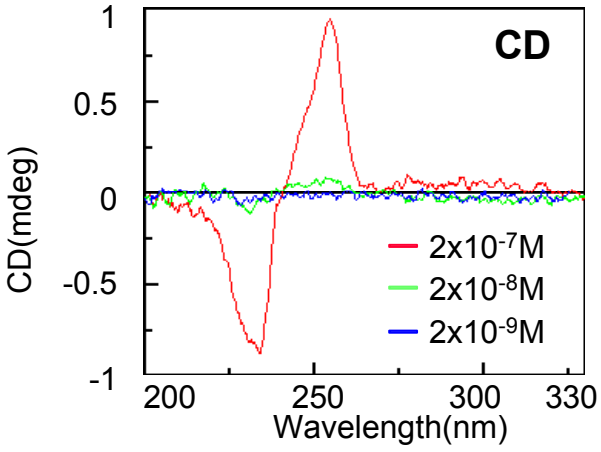

Figure 4: CD Spectrum

Measurement Conditions

FDCD: SBW 4nm, 16 times as accumulation (2×10^{-7}M)

CD: SBW 2nm, 8 times as accumulation (2×10^{-7}M)

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In the actual FDCD data obtained, the difference in intensity of fluorescence excited by right-hand circularly polarized light and left-hand circularly polarized light is normalized against the intensity of total fluorescence. Therefore, as shown in Figure 3, the FDCD value (mdeg) displays a constant value, regardless of sample concentration.

In the CD measurement, as shown in Figure 4, CD signals were observed with 2×10^{-7}M, but CD signal could not be observed with 2×10^{-8}M. On the other hand, by using the FDCD-465 for measurement, signals were clearly observed with lower concentration of 2×10^{-9}M.

As explained, if the sample is suitable for FDCD measurement, much higher sensitivity such as several tenfold to a hundred times can be achieved than standard CD measurement.
Information regarding (1S, 2S)-trans-cyclohexanediol bis (6-methoxy-2naphthoate) has been provided by Tatsuo Nehira, Hiroshima University, and Katsunori Tanaka, Osaka University.

<Reference>

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