CD/ORD

Instruction Manual

Jasco

P/N: 0302-0267D April 2021

Preface

This instruction manual is your guide for using this instrument. It instructs first-time users on how to use the instrument, and serves as a reference for experienced users.

Before using the instrument, please read this instruction manual carefully, and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. When not using the instrument, keep this manual in a safe place. If this instruction manual becomes lost, order a replacement from your local JASCO distributor.

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Contact your local JASCO distributor for instrument servicing. In addition, contact your JASCO distributor before moving the instrument to another location. Consumable parts should be ordered according to part number from your local JASCO distributor. If a part number is unknown, give your JASCO distributor the model name and serial number of your instrument.

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(4) This manual is considered complete and accurate at publication.

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Notation Used

The following notational conventions are used throughout this manual:

General Notation

Notation	Meaning
[Measurement] menu **[Parameters...] command**	Names of menus, commands, and text boxes are enclosed in square brackets '[ ]', followed by a description indicating whether the function is a menu, command, text box, etc. Shortcut keys used to select menus or commands are underlined.
<OK>, <Cancel>	Names of buttons are enclosed in angular brackets '< >'.

Keyboard Operations

Notation	Meaning
Shift **Ctrl**	The key is enclosed in a square and shown in boldface.
Alt **,** **F**	Keys that are to be pressed in succession are separated by commas. In the example shown on the left, the Alt key is to be pressed and released, followed by the F key.
Shift **+** →	Keys that are pressed simultaneously are separated by a "plus" sign. In the example shown on the left, press the → key while holding down the Shift key.

Mouse Operations

Notation	Meaning
Point	Move the mouse pointer to the specified item.
Click	Quickly press and release the mouse button.
Double-click	Click the mouse button twice in rapid succession.
Drag	Point to an item, click and hold down the mouse button. Move the mouse with the button held down, and release the button when the pointer is in the desired position.

Contents

1. Sampling

1.1 Cells and Cell Holders

Three types of ORD/CD cells are available. The material of the cell is fused quartz and all cells are constructed by weld, and therefore can be used not only for ordinary organic solvents but also for acids and bases. Also, these cells are used with each exclusive cell holder. Table 1.1 shows the various types of cells for ORD/CD measurements and their specifications. First, the features of each cell and cautions in handling them will be described and then general precautions in handling the cells will be given.

Table 1.1 Various Types of ORD/CD Cells

Type		Light path length (mm)	Sample volume required (mL)	Light path error	Cell Blank (ORD)
Standard	Cylindrical quartz cell	100 50 20 10 5 2 1 0.5 0.2 0.1	28.3 14.2 5.7 2.8 2.08 1.39 1.16 1.05 0.98 0.96	± 0.01 mm	± 0.005
	Cylindrical water-jacket quartz cell	100 50 20 10 5 2 1 0.5 0.2 0.1	7.85 3.62 1.57 0.79 0.36 0.16 0.1 0.1 0.1 0.1	± 0.01 mm	only for CD
Special	Rectangular quartz cell (with Teflon cap)	20 10 5 2 1	4 2 1 0.4 0.2	± 0.05 mm	only for CD

Cylindrical quartz cell (Fig. 1.1)

(1) This is the standard cell for ORD/CD measurement and features the small cell blank.

(2) The cell holders include the standard type (for 0.1 mm to 20 mm cell) and the cell holders for long cells (50 mm and 100 mm). The cell is set on the cell holder as shown in Fig. 1.1 and is fixed with a Teflon stopper.

(3) The cells of 0.1 mm and 0.2 mm are used only for organic solvents. When they are used for aqueous solutions, the sample injection and cell washing are very difficult because of high viscosity and bubbles.

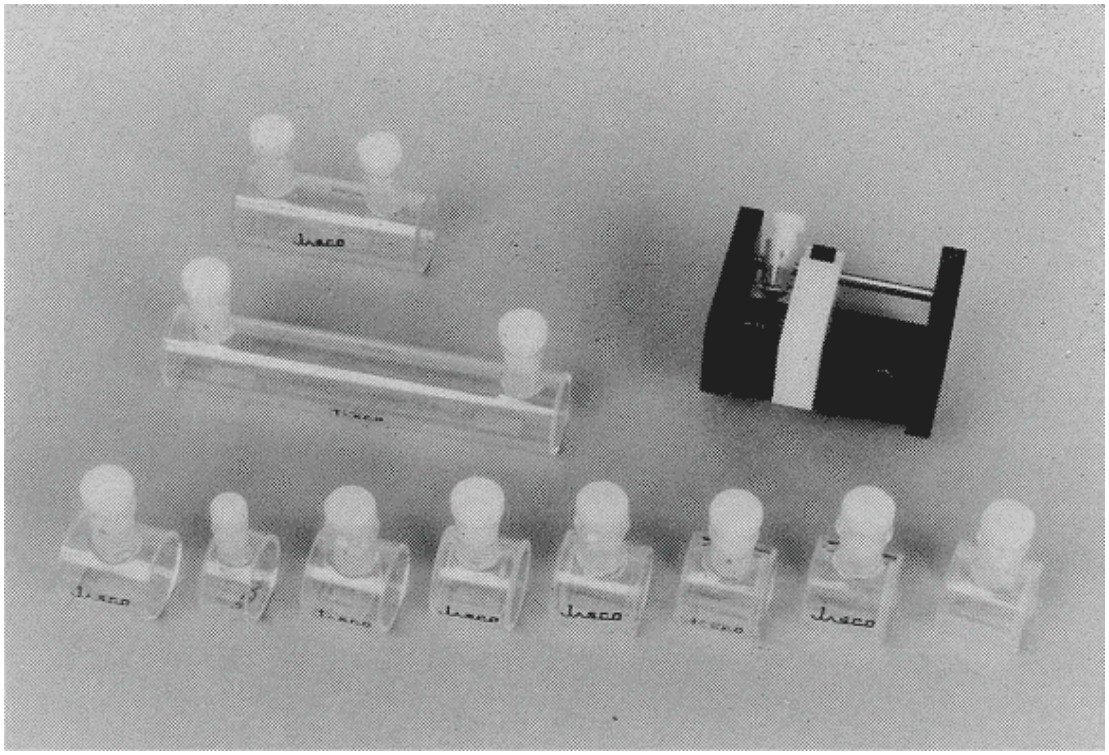


Figure 1.1 Cylindrical cells and the holders

Cylindrical water-jacket quartz cell (Fig. 1.2)

(1) This is a cylindrical cell with a water-jacket having a constant temperature water flow.

(2) The cell holder is the same as that used for the cylindrical quartz cell.

(3) The cells of 0.1 mm and 0.2 mm are used mainly for organic solvent. However, in case of use for aqueous solutions, the sample injection and cell washing are less difficult than the cylindrical quartz cell above.

(4) Be careful not to apply a strong force when connecting the rubber pipe to the connector of the water-jacket. A very flexible rubber pipe for the inlet of constant temperature water should be used.

(5) This cell is suitable for CD measurements (when it is used for ORD, the cell blank sometimes reaches a value of 10 to 100 times).

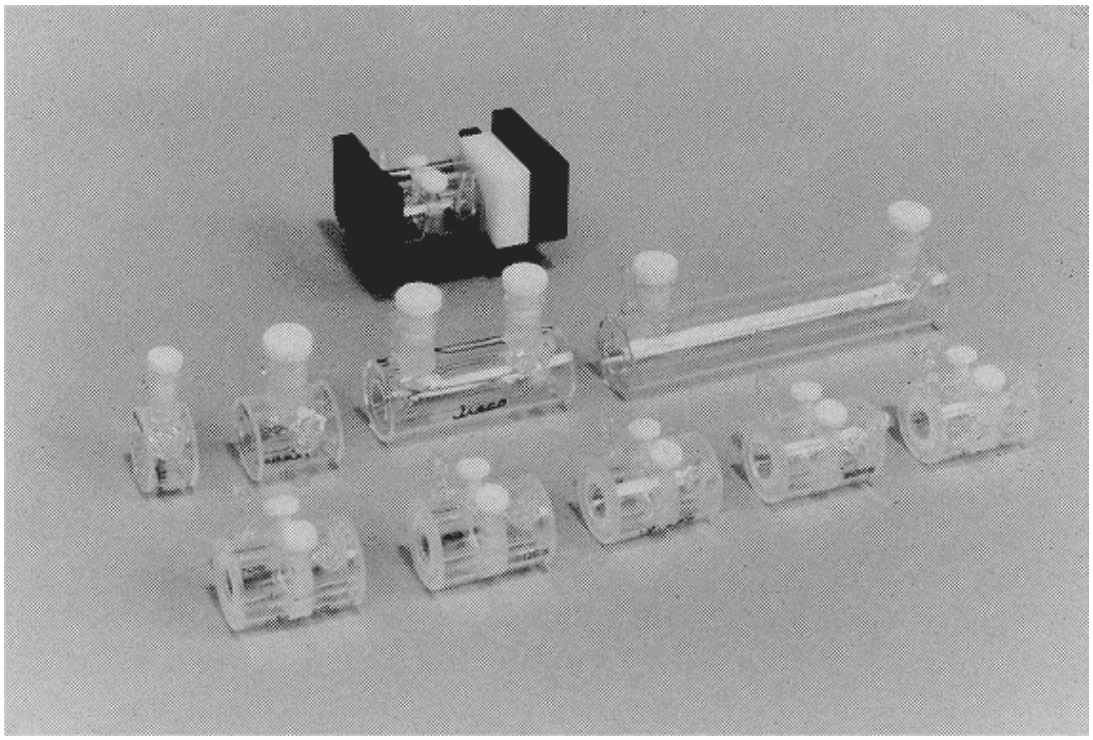


Figure 1.2 Cylindrical water-jacket quartz cells and the holders

Rectangular quartz cell (Fig. 1.3)

(1) This cell allows easy sample handling.

(2) The cell blanks are larger than cylindrical cells.

(3) The cell holder is the same as that used for the cylindrical cell.

(4) A heating holder for the rectangular cell (thermostatic water circulating) is also available.

(5) This cell is suitable for CD measurements (when it is used for ORD, the cell blank sometimes reaches a value of 100 times or more).

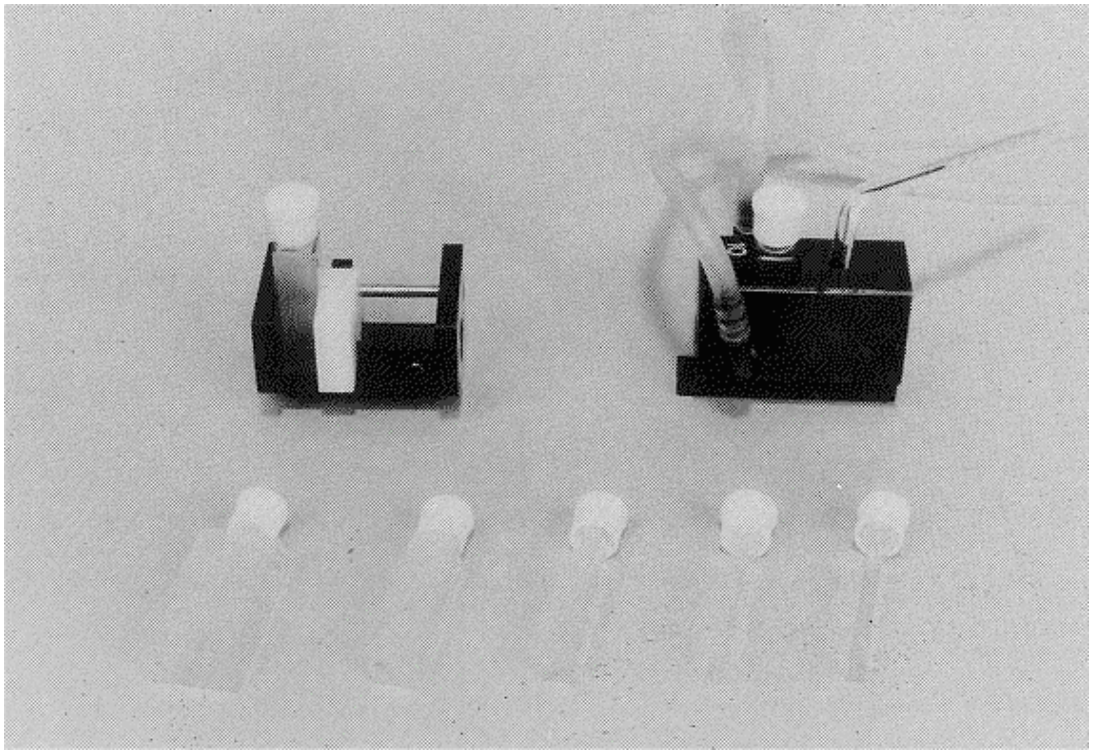


Figure 1.3 Rectangular quartz cells and the holders

General Cautions in Handling the Cell

(1) Be careful not to touch the surface of the cell.

(2) For washing and drying the cell, first, wash the cell with water or solvent. Next wash it with a volatile solvent such as acetone, and then blow air in into the cell using a rubber bulb with a reservoir for drying the cell.

(3) For removing the dirt absorbed to the cell window, use a commercially available washing agent for precision glass tools. Moreover, it is also possible to use concentrated nitric acid or aqua regia, etc.

(4) To simply clean and dry the cell using protein having a strong absorbing power, clean them with distilled water before pouring a 2~3% solution of sodium lauryl sulfate into the cell and leaving them as they are for 2 to 3 min. Then rinse the cell with distilled water approx. 10 times before rinsing it with ethanol and then acetone three times each to dry it.

(5) Although quartz is resistant to chemicals, the optical window is slowly attacked if immersed in alkali solution for a long time. Hydrofluoric acid and phosphoric acid cannot be used.

(6) When the cell is not being used, wrap it with gauze or the like and store it with care so that the optical window is not damaged.

1.2 Solvent

For ORD/CD measurement, solvents used in the ordinary absorption measurement will suffice, however the following should be taken into consideration when selecting a solvent.

(1) Solubility

(2) Transparency in the measuring wavelength region

(3) Optically inactive (especially in ORD)

(4) The sample is not denatured or decomposed.

(5) Polarity of solvent (interaction between the solvent and sample).

For ORD/CD measurement, the solvent for absorption spectrum measurement(UV grade) is most desirable, however solvent for liquid chromatography(LC grade) is also generally acceptable on transparency.

It is to be noted that distilled water kept in a polyethylene bottle for a long time may have deteriorated in transparency in the ultraviolet region because of the eluted polymer additives. When a buffer solution is used, note the wavelength region in which the salts can be used.

On the other hand, in the low temperature measurement, it is necessary to prepare the mixed solvent immediately before use and also to dehydrate and dry the solvent. Ordinarily, it is recommended to flow solvent through an activated alumina (neutral) column.

Table 1.2 Various Solvent and Their Short Wavelength Limits*

Solvent	Usable short wavelength limit(nm)			Remarks
	1cm cell	1mm cell	0.1mmcell	
n-hexane	~210	~185	~180	Nonpolar, small solubility
Cyclohexane	~210	~185	~180	Nonpolar, small solubility
Isooctane	~210	~185	~180	Nonpolar, small solubility
Dioxane	~220	~210	~202	Nonpolar, commonly used for organic compounds
Benzene	~280	~275	~270	Nonpolar, sometimes used in the measurement of synthetic polymers
Carbon tetrachloride	~250	~240	~230	Nonpolar, special in ORD/CD Intermediate polarity, used in
Chloroform	~240	~230	~220	comparison with NMR data
1,2-dichloroethane	~220	~210	~200	Nonpolar, high solubility Polar, commonly used for organic
Methanol	~210	~195	~185	compounds
Ethanol	~220	~195	~185	Polar, frequently used for organic compounds
Trifluoroacetic acid	~260	~250	~240	Measurement of synthetic polymers; corrosive
Dimethylsulfoxide	~264	~252	~245	Used in the measurement of synthetic polymers
Tetrahydrofuran	~220	~210	~204	Used in the measurement of synthetic polymers
t-decalin	~220			solvent for high temperature measurement(bp+194.6°C)
P5-M1	~220	~210		Isopentane/methylcyclohexane (5:1) mixed solvent, nonpolar low temperature solvent (-196°C)
EPA	~220	~210		ethylether/isopentane/ethanol(5:5:2) mixed solvent, commonly used for low temperature measurement(-196°C)
Ethanol/methanol(4:1)	~220	~200		Polar low temperature solvent (-160ºC)
Distilled water	~185	~180	~175	
10 mM Sodium phosphate		~182		
0.1 M sodium phosphate		~190		
0.1 M Sodium chloride		~195		
0.1 M Tris-HCl		~200		
0.1 M Ammonium citrate		~220		

For the solvent sued to measure the vacuum ultra-violet region (less than 180 nm), see item "2.5.1" of this manual.

1.3 Weighing

Sample for ORD/CD measurement, including natural organic compounds, are so precious that the weight of one sample is on the order of several milligrams. In weighing the sample, it is advisable to use a semimicrobalance or microbalance and volumetric flasks of 10 mL to 1 mL.

1.4 Concentration and Cell Path Length

Because the wavelength region of cotton effect (abnormal dispersion of ORD/CD phenomenon: see "Chapter 5") becomes an absorption band, it is necessary to consider the proper range (OD of approx. 1) of OD in addition to the scale sensitivity of CD and ORD. Though steroids with a small absorptivity and large cotton effect have a relatively large degree of freedom for sampling, carefully measure the cotton effect based on the allowed transition with a strong far-ultraviolet region such as the benzenoid chromophore. Table 1.3 shows the criteria for the concentration and path-length of the cells of main chromophores for your reference.
The following Items (1) through (3) describe the cautions for selection of path-length of cells and adjustment of concentration.

(1) Path-length of cell

1) The standard cell has an optical path length of 1 cm.
2) Though the cells with a small optical path length of 5 mm or less are mainly used to expand the short-wavelength limit of the far-ultraviolet region (see Table 1.2), they are also used to properly decrease the sample OD.
3) The cells with a large optical path length of 2 cm or more are used when the sample concentration is smaller than the standard and also used to accurately measure the wavelength region of the ORD background rotation.
4) The error of the optical path length of cells is approx. +0.01 mm. When using cells with a small optical path length, it is advisable to use the method of calibration by comparison of the CD value of the same sample solution for the cell with the larger optical path length already calibrated. Usually, the standard optical path length is 10 mm. The fact that the CD signal intensity to be observed is proportional to the optical path length of cells is used. The sample should have a large enough CD and small enough OD.

(2) Concentration

On CD and ORD measurement, it is undesirable that high concentration is used to get large signal. For, high OD must introduce large noise. Therefore, it is most important to keep OD within the optimum range.

1) Because terpenoid ketones and lactones have a small enough OD even if they have a concentration of 0.05 to 0.1%(W/V), preferable CD/ORD spectrums can be obtained.

2) For a benzenoid chromophore having a strong absorptivity, adjust the concentration so that the OD in the purposed wavelength region will be equal to approx. 1.

3) Note that, if the OD reaches approx. 2, noises increase and the CD/ORD spectrums may be distorted due to luminescence, depending on the sample.

(3) Others

Usually, concentration is likely to increase in the near-infrared region. Therefore, for sampling, check the concentration in advance for an OD of approx. 1 with the absorption spectrum of the sample. In addition, absorption of the solvent occurs in the near-infrared region. Therefore, to use the cell with a large optical path length of 5 cm or more, check the usable wavelength region in advance. To measure the ORD background rotation, use the cell with a large optical path length of 2 cm or more according to necessity, because a concentration of approx. 1%. is recommended.

Table 1.3 Concentration and path length

Chromophore		Wavelength region(nm)	Concentration (%)	Path length (mm)
－C≡C－		$\pi\rightarrow\pi^*$ 220~190	0.1	1~0.5
－C＝C－		$\pi\rightarrow\pi^*$ 200~185	0.1	0.5~0.1
－C＝C－C＝C－		$\pi\rightarrow\pi^*$ 300~250	0.01~0.005	10
＞C＝O,－CHO		$n\rightarrow\pi^*$ 350~240	0.1	10
－C＝C－C＝O		$n\rightarrow\pi^*$ 400~260	0.1	10
		$\pi\rightarrow\pi^*$ 280~200	0.1	1~0.2
-COOH, lactone, ester		$n\rightarrow\pi^*$ 250~200	0.1	10~1
Aromatic	Side chain	$\pi\rightarrow\pi^*$ 300~250	0.01	10
		$\pi\rightarrow\pi^*$ 250~200	0.01	1~0.5
	Skelton of terpene	$\pi\rightarrow\pi^*$ 300~250	0.1	10
		$\pi\rightarrow\pi^*$ 250~200	0.1	1~0.2
S－S		$n\rightarrow\sigma^*$ 300~200	0.1	10
Protein, poly-peptide	Aromatic	$\pi\rightarrow\pi^*$ 350~250	0.1	5
	Amide transition	$\pi\rightarrow\pi^*$ 260~200	0.1	1~0.5
		$\pi\rightarrow\pi^*$ 260~185	0.02	1~0.5
DNA, RNA		$\pi\rightarrow\pi^*$ 300~200	0.1	1~10
Co-chelate complex		$d\rightarrow d^*$ 700~300	0.1	10
		CT 300~180	0.01	1

1.5 Cautions on Special Samples

(1) ORD is generally more affected by the turbidity of the solution than CD. If possible, it is desirable to filter the solution. When a filtration is not essentially suitable in the case of biological molecules, the solution is measured without filtering, however in ORD the reproducibility of the dispersion curve lowers in addition to the increase in the noise, as the turbidity becomes higher. In such a case, use a shorter cell or dilute the solution, if the scale sensitivity can still be changed. Although CD is not so much affected by turbidity as ORD, use a short cell if the photomultiplier voltage exceeds 400 V. The slit width should preferably be more than 2 nm.

(2) In the case of isotropic thin films and pellets, it is important that the sample is homogeneous. The pellet must also be transparent. It is desirable for the shift or deformation of the spectrum to be very small when the pellet is rotated on the optical axis. Another important requirement of the isotropic thin film is for the absorption not to be too strong (less than 3 in absorbance). Generally speaking, CD measurement is easier than ORD.

2. CD/ORD Measurement

The following describes cautions to be used when actually measuring the sample for CD/ORD instrument. For the actual operation method, see the hardware and the software manuals for the CD spectrometer.

2.1 Warming up of CD/ORD Instrument

Warm up the instrument until it is stabilized after starting it

(1) For measurement in normal wavelength region (more than 190 nm), the proper flow rate of nitrogen gas is 3 ~ 5 lit/min.

(2) The required warm-up time is more than 30 min.

(3) Measure often the zero drift of the instrument while warming it up, and the stabilizing time can be obtained. To measure zero drift, set the measurement mode to T-scan, the wavelength to a proper value in the wavelength region to be measured on the day, the measurement time to 2 hr (7200 sec), the data full scale to 20 mdeg, and the response to 2 sec.

(4) Checking of CD-value stability: Check often the CD-value stability with the sample while warming up the instrument. Pour an aqueous solution of 0.06% (W/V) ammonium d-10-camphorsulfonate into a 1 cm cell and set the cell in the instrument to make measurements with a wavelength of 291 nm for approx. 2 hr in the T-scan mode. If the stabilized CD value (value after base correction) remains within +190.4 mdeg (±1%), it is normal.

(5) Checking of ORD value stability: Especially for the ORDE-307W accessory, check the value stability using the sample for warming up.

To set the wavelength to a visible region such as 589.3 nm, previously measure the photomultiplier-high-tension-voltage of the neodymium glass with a wavelength scan to check if the peak wavelength remains at 586 nm +0.5 nm.
Then pour an aqueous solution of 5% (W/V) saccharose into a 1 cm cell and set the cell to the instrument to make measurements in the T-scan mode.

Wavelength	: 589.3 nm
CD full scale	: 500 mdeg
Slit width	: 1 nm
Response	: 2 sec
Measurement time	: 2 hr

If the stabilized ORD value (after water blank correction) remains at +0.3325 deg (±1%), it is normal.
For the ORD-M accessory, the value basically does not change with the elapse of time. A slight variation may occur due to the zero drift of the instrument.

Note: If more than the normal range of zero drift or instability (see the operation manual) is found, contact your local distributor.

2.2 Setting of Sample and Measuring Conditions

(1) Cleaning of cell window plate: Clean the plate surface by dripping ethanol from a pipette onto the surface and wiping it lightly with tissue paper or cleaning paper. Before setting the cell in the cell holder, be sure to check the cleaning condition of the plate surface by holding the cell before a room lamp.

(2) Setting of cell: Set a cylindrical cell in a cell holder as shown in Fig. 2.1. In this case, place the sample port of the cylindrical cell in contact with the guide bar of the cell holder and press it lightly against the front mask with a Teflon cell retainer to secure the cell.

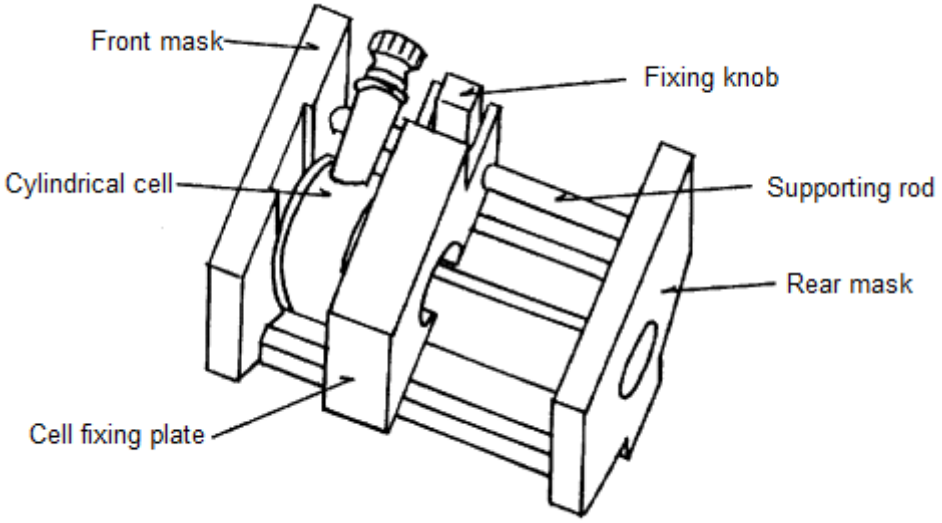


Figure 2.1 Cylindrical cell and cell holder

(3) Checking of sample concentration {optical density (OD)}: After setting the sample, properly move wavelength in the measurement wavelength region to check the high tension voltage (HT) of the photomultiplier detector. Usually, if the OD of the sample in the absorption maximum wavelength is approx. "1", the optimum S/N (signal-to-noise ratio) condition is satisfied and HT in this case ranges between 300 and 350 V. (250 ~ 600 nm; SBW = 1 mm) For nonfluorescent sample, an HT of 400 to 450 V (OD of approx. 2) is accepted. However, an HT of 500 V or more is not recommended because noises increase with the exponential function of the OD when the HT increased.

(4) Setting of measuring conditions (Setting of parameters)

1) Setting of full scale indication: Check if the current full scale indication is proper simultaneously with checking of concentration in Item (3) of this section. If the indicated value of data shows the scale-over or it is extremely small, change the full scale.

Comment: For ORD with ORD-E and CD, the full scale to be set by parameters is defined as one necessary to display the data stored in a memory through an A/D converter as the result of measurement. The full scale for signals to be digital-converted through the A/D converter(hardware full scale) includes 100 mdeg and 1,000 mdeg. When setting the full scale to 1 ~ 100

mdeg, data up to ±200 mdeg can be stored every with 0.01 mdeg step (the hardware full scale is set to 100 mdeg.).

When setting the full scale to 200 ~ 1,000 mdeg, data up to ±2,000 mdeg can be stored with every 0.1 mdeg step (the hardware full scale is set to 1,000 mdeg.).

Meanwhile, for the ORD-M accessory using the optical null method, the data is measured as the absolute value of optical rotation. Data up to ±90,000 mdeg can be stored with every 0.5 mdeg step independently of the indicated full scale.

2) Slit width (SBW): The standard slit width (SBW) is 1 nm. For high-sensitivity measurement, SBW may be expanded to 2 nm in order to improve the S/N ratio of the spectrum.

However, note that the spectrum may be distorted due to scattered light such as fluorescence if the slit width is expanded to more than 2 nm in order to improve the S/N ratio of a sample with a high OD.

3) Setting of wavelength range: For CD, the start wavelength is frequently set to the side of the wavelength 20 ~ 50 nm longer than the wavelength in which the bottom of the spectrum rises and the end wavelength is frequently set to the side of the short wavelength in which the HT voltage is approx. 700 V. Though ORD is the same as CD, it is usually set to a wavelength range wider than CD, because it essentially has signals for any wavelength.

To increase the wavelength range, select the step resolution of wavelength so that the data points will not exceed 2,001.

Table 2.1 shows the maximum wavelength interval to be set corresponding to step resolution.

Table 2.1 Maximum wavelength interval to be set

Step resolution(nm)	Maximum wavelength interval to be set
10	Overall wavelength interval of instrument**
5	Same as the above
2	Same as the above
1	Same as the above
0.5	Same as the above
0.2	400 nm
0.1	200 nm
0.05*	100 nm

* Only for the continuous scan mode

** Photomultiplier for short wavelength side: 167 ~ 800 nm
Photomultiplier for long wavelength side: 400 ~ 1000 nm

4) Step resolution, response, and scan speed are described in the next Item "2.3 Selection of wavelength scan modes" because they are closely related to the wavelength scan modes (continuous-scan and step-scan).

2.3 Selection of Wavelength Scan Modes

In the wavelength scanning mode of the CD/ORD spectrometer, the continuous-scan and step-scan modes can be selected. For the definition of the above two modes, see the operation manual.
This document provides the features of the two modes and the reference to actually use them. Note that the data stored in a memory can be processed independently of these scan modes.

Features of continuous-scan mode:

(1) This mode allows you to select the response by giving priority to the scanning speed. In addition, measurement time does not depend on step resolution. Therefore, this mode is suitable for obtaining normal data with a better S/N in a short time.

(2) The mode is also suitable for high-speed scan.

(3) For an end wavelength with a large HT, this mode may have more noise than the step-scan mode (because data is averaged including those with wavelengths shorter than the end one).

Features of step-scan mode:

(1) Because this mode performs time-averaging of data by stopping wavelength, data corresponding to the wavelength can be obtained basically.

(2) However, this mode is not suitable for the measurement to improve the S/N or high-speed scan because the more the response is lengthened, and the narrower the step resolution is set, the more the measurement time increases.

2.3.1 Measuring Conditions for Continuous-scan

(1) Set the step resolution according to Table 2.1 in the preceding Item so that the measurement wavelength range will not exceed 2,001 points. The standard step resolution is 0.2 nm.

(2) Criteria for selection of scan speeds

For the CD/ORD instrument, scan speeds can be selected between 5,000 and 1 nm/min. Scan speeds are classified into the following four types of a) ~ d) for convenience sake. Select a scan speed according to the measurement purpose and spectral condition of the sample.

a) High-speed scan: 5,000 ~ 500 nm/min

- ⋆Used to measure the CD spectrum of relatively quick reaction, such as stopped flow.
- ⋆Used to measure unstable samples with temperature and light as quickly as possible.
- ⋆Used when the high tension voltage(HT) of a photomultiplier hardly or slightly changes due to wavelength because of the light absorption of the sample.

b) Moderate-speed scan: 200 ~ 100 nm/min

⋆Used to measure sample free from sudden change of HT because of a small light absorption. For example, optically active chelate complex (d-d transition) of cobalt (III) and terpenoid ketone are typical.

c) Normal scan: 50 ~ 10 nm/min

⋆Used when HT changes greatly for measurement of a general sample having a strong absorption band with OD of 1 to 2, measurement of wavelength limit of transparency of solvent, and measurement of instrument within the usable wavelength limit.

d) Low-speed scan: 5 ~ 1 nm/min

⋆Used to measure high-resolution spectrum with long response.

(3) Criteria for selection of responses

Set the response for the continuous-scan mode by considering the following Items a) ~ c).

a) S/N is improved proportionally to the square root of the response. (Common to step scan)

b) Data stored in a memory is the average value of signals with the narrow wavelength range scanned within the response time (this is called "response-wavelength-width" for convenience sake). The response-wavelength-width is obtained by multiplying the scanning speed (nm/sec) by the response (sec).

c) To prevent distortion in the measured spectrum, the response-wavelength-width should be kept at less than 1/10 of the half height width.

d) Use a response of 0.5 to 8 msec for high-speed scan with the scan speed range of 5,000 ~ 1,000 nm/min.

Tables 2.2 ~ 2.5 show the response and its response wavelength width from high-speed to low-speed scan for reference.

Table 2.2 Response-wavelength-width for high-speed-scan
(Scan mode: Continuous-scan)

Response	Response-wavelength-width (nm)*			
	Scan speed(nm/min)			
	5000	2000	1000	500
0.5 sec	0.04	0.02	0.01	0.00
1 msec	0.08	0.03	0.02	0.01
2 msec	0.17	0.07	0.03	0.02
4 msec	0.33	0.13	0.07	0.03
8 msec	0.67	0.27	0.13	0.07
16 msec	1.33	0.53	0.27	0.13
32 msec	2.67	1.07	0.53	0.27
64 msec	5.33	2.13	1.07	0.53
0.125 sec		4.27	2.13	1.07
0.25 sec		8.53	4.27	2.13
0.5 sec			8.53	4.27
1 sec				8.53

* Each value is shown by rounding to three decimal places.

Comments:

(1) The optimum response region in Table 2.2 is between the two underlined values in each column. (Corresponding to the CD-spectrum half width of 5 ~ 20 nm)

(2) For scan speeds of 2,000 nm/min and 5,000 nm/min, set the response to 8 msec or less.

Table 2.3 Response-wavelength-width for moderate-speed-scan
(Scan mode: Continuous scan)

Response	Response-wavelength-width (nm)*	
	Scan speed(nm/min)	
	200	100
16 msec	0.05	0.03
32 msec	0.11	0.05
64 msec	<u>0.21</u>	0.11
0.125 sec	0.43	<u>0.21</u>
0.25 sec	0.85	0.43
0.5 sec	<u>1.71</u>	0.85
1 sec	3.41	<u>1.71</u>
2 sec	6.83	3.41
4 sec		6.83

* Each value is shown by rounding to three decimal places.

Comments:

(1) Each value in the optimum response region in Table 2.3 corresponds to the spectral half-width of 5 to 20 nm.

(2) This mode is usually used to measure large CD or ORD with low HT (small OD).

Example: For the CD spectrum of ammonium d-10-camphorsulfonate (peak value at 291 nm, half-width of 31 nm, water), select the set of scan speed and response so that the response-wavelength-width will not exceed 31/10=3.1 nm. (For 100 nm/min, 1 sec is optimum).

Table 2.4 Response-wavelength-width for normal-scan
(Scan mode: Continuous scan)

Response	Response-wavelength-width (nm)*		
	Scan speed(nm/min)		
	50	20	10
16 msec	0.01	0.01	0.00
32 msec	0.03	0.01	0.01
64 msec	0.05	0.02	0.01
0.125 sec	<u>0.11</u>	0.04	0.02
0.25 sec	0.21	0.09	0.04
0.5 sec	0.43	<u>0.17</u>	0.09
1 sec	0.85	0.34	<u>0.17</u>
2 sec	<u>1.71</u>	0.68	0.34
4 sec	3.41	<u>1.37</u>	0.68
8 sec	6.83	2.73	<u>1.37</u>
16 sec		5.46	2.73

* Each value is shown by rounding to three decimal places.

Comments:

(1) This mode is used for a measurement with an OD of 1 or more.

(2) Make the measurement with a higher scan speed for a broad absorption band and with a lower scan speed for a sharp absorption band.

(3) Each value in the optimum response region in the above table corresponds to a spectral half-width of 5 ~ 20 nm.

Table 2.5 Response-wavelength-width for low-speed-scan
(Scan mode: Continuous scan)

Response	Response-wavelength-width (nm)*		
	Scan speed(nm/min)		
	5	2	1
16 msec	0.00	0.00	0.00
32 msec	0.00	0.00	0.00
64 msec	0.01	0.00	0.00
0.125 sec	0.01	0.00	0.00
0.25 sec	0.02	0.01	0.00
0.5 sec	0.04	0.02	0.01
1 sec	0.09	0.03	0.02
2 sec	0.17	0.07	0.03
4 sec	0.34	0.14	0.07
8 sec	0.68	0.27	0.14
16 sec	1.37	0.55	0.27

* Each value is shown by rounding to three decimal places.

Comments:

(1) This mode is mainly used to measure sharp spectrum with a half-width of 2 nm or less by increasing response. (The optimum region is not shown because it depends on cases.)

(2) Select the set of the scan-speed and response so that the response-wavelength-width is less than 1/10 of spectral half-width. It should be that the spectral half-width is roughly known before measurement.

2.3.2 Measuring Conditions for Step-scan

Step-scan does not include the function of scan speed. Measurement time is determined by the sum of the scanning time of the monochromator and the total integration time of data (product of response and number of data points). Therefore, the measurement time increases as the step resolution becomes narrower and the response is lengthened. However, the S/N improvement effect in the same response is almost same as that of the continuous-scan.
To obtain the measurement time for the step-scan, Table 2.6 shows the scanning time of the monochromator for a measurement wavelength interval of 100 nm and Table 2.7 shows the indicated and true values of response and the total integration time corresponding to the number of data points.

Table 2.6 Step resolution and scanning time of monochromator (Step scan)

Step resolution	Scanning time of monochromator (wavelength interval of 100 nm)
10 nm/step	6 sec (1000 nm/min)
5 nm/step	6.7 sec (900 nm/min)
2 nm/step	8.2 sec (730 nm/min)
1 nm/step	10.5 sec (570 nm/min)
0.5 nm/step	14.0 sec (430 nm/min)
0.2 nm/step	23.1 sec (260 nm/min)
0.1 nm/step	51.5 sec (117 nm/min)

Table 2.7 True value of response and total integration time for each number of data points

Response		Total integration time (sec)*				
Indicated value	True value	101 points	201 points	501 points	1001 points	2001 points
0.5 msec	0.5 msec	0.0505	0.1005	0.2505	0.5005	1.0005
1 msec	1 msec	0.101	0.201	0.501	1.001	2.001
2 msec	2 msec	0.202	0.402	1.002	2.002	4.002
4 msec	4 msec	0.404	0.804	2.004	4.004	8.004
8 msec	8 msec	0.808	1.608	4.008	8.008	16.008
16 msec	16 msec	1.616	3.216	8.016	16.016	32.016
32 msec	32 msec	3.232	6.432	16.032	32.032	64.032
64 msec	64 msec	6.464	12.864	32.064	64.064	128.06
0.125 sec	0.128 sec	12.928	25.728	64.128	128.13	256.13
0.25 sec	0.256 sec	25.856	51.456	128.26	256.26	512.26
0.5 sec	0.512 sec	51.712	102.91	256.51	512.51	1024.5
1 sec	1.024 sec	103.42	205.682	513.02	1025.0	2049.0
2 sec	2.048 sec	206.85	411.65	1026.0	2050.0	4098.0
4 sec	4.096 sec	413.70	823.30	2052.1	4100.1	8196.1
8 sec	8.192 sec	827.39	1646.6	4104.2	8200.2	16392
16 sec	16.348sec	1654.8	3293.2	8208.4	16400	32784

* Total integration time = Response(True value)×Number of data points

The measurement time is able to be practically calculated by using Tables 2-6 and 2-7 as follows:
For example, for a wavelength interval of 200 nm, step resolution of 1 nm, and response of 2 sec, a wavelength scanning time of 21 sec. is obtained (10.5 sec x 200/100) by referring to Table 3-7. Then the total integration time of 411.648 sec. is obtained by multiplying the number of data points (201) by the true value of response (2.048 sec). Thus the measurement time of approx. 433 sec. is obtained (7 min 13 sec) from the sum of both values.

2.4 Number of Accumulations

In general, the spectrum S/N is proportional to the square root of the product of response R and number N of accumulation, which is expressed as follows:

$$S/N \propto (R \times N) 1/2$$

Therefore, to improve the S/N ratio, the same effect is obtained by increasing response and by increasing number of accumulation. However, because relatively longer cycle components are mixed in noises on CD and ORD, it is advisable to average with accumulation.
For normal measurement, select a response within the range of 0.125 to 4 sec and set the number of accumulation so that the measurement time will not be too large.

(1) Though the S/N improvement effect increases as the number of accumulation increases, the measurement time also increases proportionally to the number. The total measurement time of less than 30 min. is a tentative criterion to set the number of accumulation.

(2) Note that, if the measurement time exceeds 1 hr, the spectrum may be affected by the base line drift of instrument. Especially for high-sensitive measurement, the above drift can be corrected by subtraction by use of a sample alternator (optional).

(3) For the sample with a small intensity of CD and ORD, the measurement is often carried out with an OD of 2 or more. However, this is not recommended in view of S/N improvement because very large noise. It should be noticed that the measurement with OD of approx. 1 gives optimum S/N, and therefore, that the number of accumulation is able to be decreased.

2.5 Measurement to 170 nm

In this wavelength region, it is an important factor to substitute oxygen in the optical system included the monochromator with nitrogen gas , and to select the solvent and path-length of the cell.

2.5.1 Solvent and Path-length of Cell

The number of transparent solvents up to 180 nm is very limited, even if using a thin cell with an optical path length of 0.1 mm.

In addition, for some solvent, the short-wavelength limit may greatly be affected by impurities, depending on the grade of the reagent (even the solvent for the absorption spectrum grade may not be useful).

Table 2.8 shows the measurable short-wavelength limits in the vacuum ultraviolet region of various solvents.

Table 2.8 Measurable short-wavelength limit in vacuum ultraviolet region of various solvents

Solvent	Short-wavelength limit (nm)			
	Path-length of cell			
	1 mm	0.2 mm	0.1 mm	0.05 mm
Distilled water	180	176	175	174
Heavy water (For NMR, 99.75%)	175	172	171	170
n-Hexane (for fluorescence)	172		169	168
Trifluoroethanol (For NMR, 99.5%)	177		170	

For solvents such as water and heavy water having low volatility, a demountable cell is often used to expand the short-wavelength limit.

2.5.2 Flow Rate of Nitrogen Gas

For measurement of longer-wavelength region of 190 nm or more, 3 ~ 5 L/min is enough for the flow rate of nitrogen gas. For measurement of shorter-wavelength region of less than 190 nm, however, it is necessary to increase the flow rate greatly. Table 2.9 shows the tentative criteria for the nitrogen-gas flow rate corresponding to the short-wavelength limit.

Table 2.9 Short-wavelength limit and criteria for nitrogen-gas flow rate

Short-wavelength limit	Nitrogen-gas flow rate(L/min)
190	3 ~ 5
185	10 ~ 15
180	15 ~ 20
175	20 ~ 50
170	50 ~ 100

Comments:

(1) Though 10 min is sufficient for the substituting time of the whole nitrogen gas in the monochromator, the efficiency of using the light energy of the light source is to be sufficiently improved if substitution is made for 30 min.

(2) Though the HT voltage may exceed 1,000 V just after opening the sample chamber to set a sample, it soon drops steeply. If the HT voltage does not drop after more than 20 sec, it should be considered that the sample OD may be too high.

(3) In case of large sample chamber, set the attached plastic case for the nitrogen gas purge in the sample chamber.

(4) While measurement is stopped, the flow rate can be returned to 3 L/min. When returning to the original flow rate, the measurement is able to be carried out within 2 to 3 min.

2.6 Cautions for Measurement

(1) Stability of base line and photometric values

For the above stability, see the instruction manual. If the variation of these items exceeds the range in the specifications, the instrument need to be repaired.

1) Baseline drift: When the higher sensitivity is selected, the measurement is easy to be largely affected by the drift. Though the baseline drift decreases during the warming up, it is advisable to check the base-shift per a certain time at a suitable wavelength by monitoring the change during 2 to 3 hr. The automatic sample alternator (option) is effective for a long-time accumulations at a high sensitivity because the base-drift can be corrected by subtraction.

2) Stability of photometric values: For the ORD-M based on the optical null method, the drift does not basically occur.

For both of CD and ORD-E based on the photo-electric method, however, the drift cannot be avoided. Therefore, the shortest measurement time possible should be

selected. It is desirable that the measuring time is set by considering both the stability and the demanding accuracy.

For highly accurate measurement, it is desirable that the stability is monitored by checking the signal magnitude of standard sample before and after sample measurement.

(2) High OD sample

1) Optimum OD: For the sample having a large CD or ORD per unit OD, it is not necessary to increase the sample OD, and then, an OD of approx. 0.2 is enough. For the sample having a small CD or ORD, however, the measurement is frequently made with a high OD of approx. 3 by increasing the concentration. But this is not preferable in view of the spectrum S/N.

The S/N based on the polarization modulation method like CD and ORD has the following proportional relation with OD.

$$S/N \propto OD\ (10^{-OD/2})$$

The above relation shows that the S/N increases approximately in proportion to OD when OD<<1, while the damping effect of the exponent part of the S/N ratio affects largely with the increase of OD when OD>1.

By differentiating the above formula, the optimum OD to maximize the S/N is 2/ln10 = 0.8686. The numbers of accumulation to achieve the same S/N on various OD are 2.5 times for OD = 2 and 11 times for OD = 3 by assuming 1 time for OD = 1.

2) Trouble of artifact signal at high sensitivity

At a high OD of approx. 3, artifact signals of 1 mdeg or less may be observed for an optically inactive sample, though a slight solid difference due to equipment is present. At an OD of approx. 1 , the artifact signal is remarkably small. In any case, note that influence of the artifact signal cannot be ignored at high sensitivity. For high sensitive measurement (small signal with few mdeg), it is advisable to measure the optically inactive sample (racemic body) similar to the absorption spectrum of the sample as the blank.

3) Distortion of spectrum due to luminescence

For the sample emitting luminescence such as fluorescence and phosphorescence, pay attention to measurement because the spectrum may be distorted. Though the influence increases for higher OD, this problem does almost not arise for OD of 1 or less in general.

However, because some samples emit intensive luminescence, it may be necessary to control the OD to 0.5 or less. It is also needed to find the measuring condition in which the spectrum shape does not depend on the concentration or path-length of the cell.

Note that this distortion is ready to arise when the measurement with a path-length-cell of 5 cm or more is carried out.

(3) High tension voltage (HT) of photomultiplier

For CD and ORD, measurement is frequently carried out at a high HT voltage because of a very narrow slit width, the short-wavelength limit of instrument or solvent. In this case, it is a prerequisite to prevent the spectrum from distortion so that the very large noise signal due to an elevation of the HT voltage does not exceed the dynamic range of the electrical system.

Though the limit HT voltage depends on the combination of full indication and response, Table 2-10 shows the relation between them.

Table 2.10 Relation between the reliable upper limit of the HT voltage, full scale indication, and response

	HT voltage limit	
Response	1~100 mdeg/FS	200 mdeg/FS or more
0.5 ~ 8 msec	500 V or less	900 V or less (Independently of response)
16 ~ 64 msec	600 V or less	
0.125 ~ 0.5 msec	700 V or less	
1 ~ 16 msec	900 V or less	

Note: If the HT is increased due to the sample OD, the trouble of high OD described in the above Item (2) may occur even if the upper limit is satisfied.

3. Standard Substances

Standard substances are available for checking the values of the CD/ORD instrument.

3.1 Standard Substance for CD

Some standard substances for CD calibration have been proposed at some wavelength regions and are used. Principal requirements for CD standard substances include commercial availability, high purity, water-solubility, simple sample handling, stable solution, large signal of CD, easy measurement, etc.

(1) JASCO standard; Ammonium d-10-camphorsulfonate

For calibration of CD instrument, JASCO uses non-hygroscopic, water-soluble ammonium d-10-camphorsulfonate (ACS). The molecular ellipticity of 0.06% aqueous solution, [θ] 291.0 is +7910. When the CD spectrum of this standard solution is measured in the wavelength region of 350 nm to 250 nm in a 1 cm cell with a CD full scale of 200 mdeg/FS (see Fig. 3.1), the CD peak value (291.0 nm) is +190.4 mdeg.
In sampling, ACS of 120.0 mg is accurately weighed in a 200 mL volumetric flask (Class A grade or higher) and dissolved by distilled water at the water temperature of 25 °C.

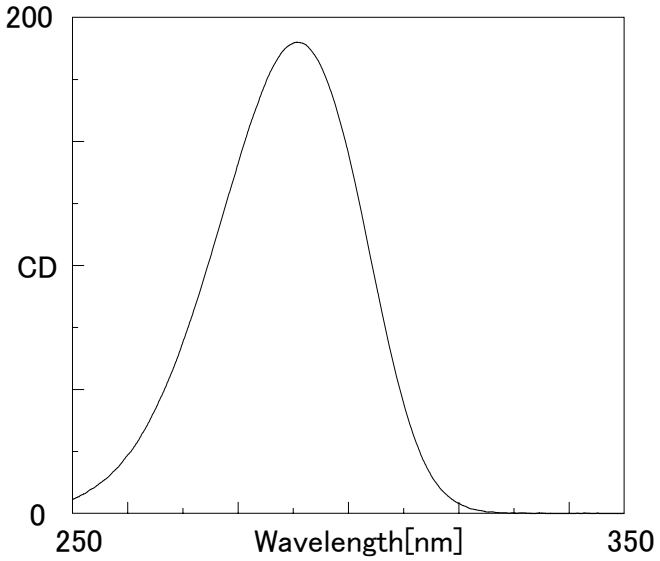


Figure 3.1 CD Spectrum of Ammonium d-10-camphorsulfonate 120 mg/200 mL distilled water, 10 mm cell

This standard solution in a closed glass vessel can also be stably stocked for at least eight months in a refrigerator (4ºC). It is desirable for the CD of this solution to be measured in the temperature-range of 20 ~ 27ºC. ACS is available as CD reagent from Sigma Aldrich. Reference; refer to Section 3.3.

(2) Far-ultra-violet standard; D-(-)-Pantolactone

D-(-)-Pantolactone(PL) have been proposed for CD calibration in the far-UV region by Konno et al(1975) and is widely used now. The mean molecular ellipticity ,[θ]219 = -16140(0.015% water, $[\alpha]_D^{25}$ -50.7;C=2, water) is obtained with the allowable instrumental deviation of within ±2% in the JASCO factory and conforms closely to the absolute CD value [θ]219 -16160 reported by Schippers-Dekkers (1981). Commercial PL should be used after the purity was checked with $[\alpha]_D^{25}$ -50.7 of a pure one.

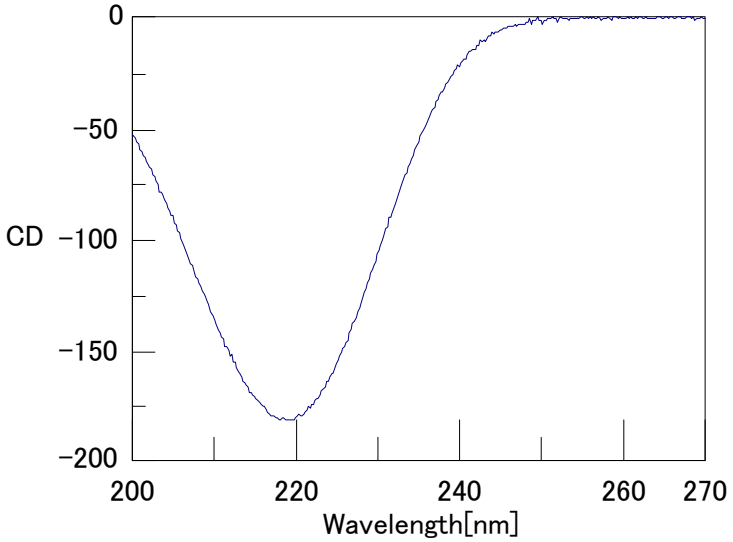


Figure 3.2 CD spectrum of D-(-)-Pantolactone 30 mg/200mL, distilled water 10 mm cell

LP of 30.0 mg is accurately weighed in a volumetric flask of 200 mL and dissolved by distilled water. When the CD spectrum in the wavelength region of 270 nm to 190 nm is measured in a 1 cm cell with a CD full scale indication of 200 mdeg/FS (see Fig. 3.2), the peak reading (219 nm) is -186 mdeg (±2%). The solution is relatively unstable at room temperature (a stable period is within 3 days), however, it will remain stable for at least three months in a refrigerator (4ºC).

(3) Primary standard; d-10-camphorsulfonic acid

d-10-Camphorsulfonic acid(CSA) is widely used for a primary CD calibration. Yang's group (1969, 1977), Krueger-Pschigoda (1971) and Detar (1969) had independently proposed the calibration methods of the CD value of CSA standard solution from the accurate ORD value with analyses of kronig-Kramers transform (K-K) relation. Among them, as the most reliable calibration method Chen-Yang (1977) had encouraged the use of the following relation,

$$[\theta]corr = kx([M]305 - [M]270)$$

Where [θ]corr is a calibrated CD value, [M]s are molar rotations of CSA and k is the calibration constant based on the analysis of KK relation. (k=0.77 (Yang) or k=0.774 (Krueger et al) had been suggested as reliable one (the deviation between both value is 0.5%). On the other hand Schippers-Dkkers (1981) had reported the absolute CD value of CSA, [θ]291 +7820 which was found in the range of the value calibrated from ORD (+7775 ~ +8216).

However, CSA is the deliquescent and tends to cause an error of concentration. Therefore non-hygroscopic above ACS is conveniently used as the secondary standard substance to replace CSA. Now, the error of the CSA standard value had been estimated to about 1%. A more accurate CD-calibration will be the subject in the future with the absolute CD calibration by Norden (1985).

(4) Visible-CD standard

As a visible CD standard, optically active tris (ethylenediamine) cobalt (III) ion is usually used by metal complex chemists. The circular dichroic molar absorptivity of the Λ (+)-isomer is $\Delta\varepsilon$= +1.89 at 490 nm which had been reported by McCaffery-Mason (1963).

Recently, the calibrated values with the absolute measurements had been reported. Schippers et al(1981) had reported [θ] = -5940 with Δ(-)-$[Co(en)_3]I_3$ H_2O ($[\alpha]_D^{24}$ -87.9, $\mathcal{E}_{466}$ 88.6) Norden had also reported $\Delta\varepsilon$ = +1.90 with Λ(+)-isomer.

Unfortunately, $[Co(en)_3]^{3+}$ complex are not still now commercially available.

(5) CD standard sample for long wavelength region

No easily-available and stable CD standard substance is found also in the longer-wavelength region of 600 nm or more. Therefore, Konno et al. (1981) had proposed to use easily-available Ni(II)/tartrate-mixed aqueous solution for the CD standard substance in this wavelength region though it is slightly unstable.

The following describes the result of our studying a specific method of using the above solution as the standard sample.

Accurately weigh nickel-sulfate hexa-hydrate (special class of FUJIFILM Wako Pure Chemical Corporation) to prepare a 0.24M $NiSO_4$ aqueous solution. Calibrate the Ni(II) concentration by measurement of the OD. (The OD (721 nm) of the concentration-calibrated 0.2400M $NiS0_4 \cdot 6H_2O$ is 0.520.)

Accurately weigh Rochelle salt (special class of FUJIFILM Wako Pure Chemical Corporation) as tartrate to prepare a 0.36M solution. Accurately mix both solutions at the rate of 1:1 before using them (coefficient of cubic expansion: 1.0026).

$$\text{Molar ellipticity } [\theta] = 1.0026 \times \theta/[10(C/2)\ell]$$

Where, "θ" represents the measured value of ellipticity (mdeg), C the molar concentration of $NiSO_4 \cdot 6H_2O$ (before mixture), and "ℓ" the path-length of cell (cm).

The following are the [θ] values at the temperature of +25ºC. Note that the value in parentheses show the peak wavelength (nm).

[θ]; -108.9(777), -98.9(718), +8.4(471), +35.3(428), -46.7(400), +49.4(371)

Because these [θ] values show the variations of ±0.1 ~ ±0.6%, resulting from a change of 1ºC in the measurement temperature, it is necessary to make the measurement at +25ºC.

Note that the [θ] value of the nickel sulfate is slightly different for hepta-hydrate. Also pay attention to hexa-hydrate if using a reagent other than the above.

3.2 Standard Substance for ORD

Table 3.1 shows the ORD readings of sucrose solutions for scale calibrations of the CD/ORD instrument.

Table 3.1 ORD Scale checking by use of a sucrose solution

ORD scale (mdeg/FS)	Concentration (g/100 ml)	Path length (cm)	Rotation (mdeg) (589.3 nm)
1000	10.000	1	+665
500	5.000	1	+332.5
200	2.000	1	+133
100	1.000	1	+66.5
50	0.500	1	+33.25
20	0.200	1	+13.3
10	0.100	1	+6.65

For ORD scale calibration, JASCO uses Sucrose (FUJIFILM Wako Pure Chemical Corporation, JIS special grade).

In the measuring operation, it is desirable for the instrument to be warmed up for more than 30 minutes before checking the wavelength. The check of the wavelength is carried out by use of the photomultiplier voltage peak (HT peak) corresponding to the absorption peak at 586 nm of neodymium glass. If the wavelength deviates more than 0.8 nm, wavelength re-adjustment should be carried out in accordance with the instruction manual for the instrument. Then the ORD of the above sucrose standard solution is measured with each ORD scale (600 to 550 nm) and the water blank is also measured. The optical rotation at 589.3 nm is read from the rotation difference between the solution and blank. For reference, the ORD of sucrose is illustrated to Figure 3.3.

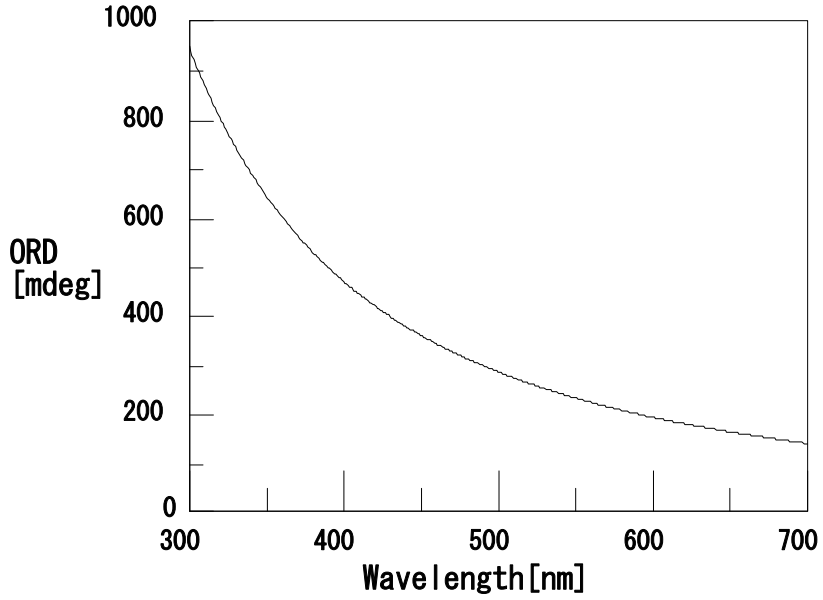


Figure 3.3 ORD of Sucrose, 3.000 g/100 mL distilled water, 10-mm cell

For reference, Table 3.2 also shows the specific rotations of the sucrose aqueous solution given by T.M. Lowry.

Table 3.2 Specific Rotation $[\alpha]_{\lambda}^{20}$ (C= 26) of Sucrose Aqueous Solution (20°C)

Line λ(Å)	$[a]_{obs}$ Degree	$[a]_{calc}$ Degree	$[a]_{obs}$- $[a]_{calc}$ Degree	Line λ(Å)	$[a]_{obs}$ Degree	$[a]_{calc}$ Degree	$[a]_{obs}$- $[a]_{calc}$ Degree
Li 6708	50.51	50.50	+0.01	Cd 4678	109.69	109.58	+0.11
Cd 6438	55.04	55.05	-0.01	Fe 4384*	126.5	126.7	-0.2
Zn 6362	56.51	56.45	+0.06	Fe 4376*	127.2	127.2	0.0
Na 5893	66.45	66.44	+0.01	Hg 4358	128.49	128.38**	+0.1**
Cu 5782	69.10	69.16	-0.06	Fe 4353*	128.5	128.7	-0.2
Hg 5780	69.22	69.21	+0.01	Fe 4337*	129.8	129.8	0.0
Cu 5700	71.24	71.30	-0.06	Fe 4315*	130.7	131.3	-0.6
Hg 5461	78.16	78.18	-0.02	Fe 4282*	133.6	133.6	0.0
Cu 5218	86.21	86.25	-0.04	Fe 4272*	134.2	134.3	-0.1
Cu 5153	88.68	88.63	+0.05	Fe 4261*	134.9	135.1	-0.2
Cu 5106	90.46	90.44	+0.02	Fe 4191*	140.0	140.2	-0.2
Cd 5086	91.16	91.20	-0.04	Fe 4144*	144.2	143.9	+0.3
Zn 4811	103.07	103.03	+0.04	Fe 3889*	166.7	166.7	0.0
Cd 4800	103.62	103.53	+0.09	Fe 3833*	171.8	172.3	-0.5
Zn 4722	107.38	107.33	+0.05	Fe 3826*	173.1	173.2	-0.1
Zn 4680	109.49	109.48	+0.01				

$[a]_{calc}$=21.648/(λ^2-0.0213) λ: μm

*Note 1: Data marked with * are those obtained by photographic readings.*
*Note 2: Values marked with ** are those corrected by JASCO because of printing mistakes in the original reference.*
T.M. Lowry: "Optical Rotary Power", page 131,Longmans, Green & Co. (1935)

According to the Handbook of Chemistry and Physics, 42nd edition, p. 3019 '60 ~ '61), the concentration effect and temperature effect upon specific rotation of the sucrose solution are as follows.

$$[\alpha]_{\lambda}^{20} = +66.412+0.01267d-0.000376d^2 \quad (d=0 \sim 50)$$

$$\alpha_D^t = \alpha_D^{20} \{1-0, 00037 (t-20)\} \quad (t=14 \sim 30^oC)$$

3.3 References on CD standards

(1) Ammonium d-10-camphorsulfonate (JASCO Standard)

T. Takakuwa, T. Konno, H. Meguro; Anal. Sci., 1 , 215 (1985)
"A New Standard Substance for Calibration of Circular Dichroism: Ammonium d-10-camphorsulfonate"

(2) d-10-Camphorsulfonic Acid (Primary Standard)

J. Y. Cassim and J. T. Yang: Biochemistry 8 (5), 1947 ~ 1951 (1969)
"A Computerized Calibration of the Circular Dichrometer"
D. F. DeTar: Anal, Chem. 41 (11), 1406 ~ 1408 (1969)
"Suggested Preliminary Standards for Calibration of Optical Rotatory Dispersion and Circular Dichroism Instruments".
W. C. Krueger and L.M. Pschigoda: Anal. Chem. 43 (6), 675 ~ 677 (1971)
"Circular Dichrometer Calibration by Kramers-Kronig Transform methods".
G. C. Chen and J. T. Yang: Analytical Lett. 10 (14), 1195 ~ 1207 (1977)
"Two-Point Calibration of Circular Dichrometer with d-10-Camphorsulfonic Acid"

(3) (-)-Pantolactone (Far-UV-Standard)

T. Konno, H. Meguro, and K. Tuzimura: Anal. Biochem. 67, 226 ~ 232 (1975)
"D-Pantolactone as a Circular Dichroism (CD) Calibration"

(4) (+)-$[Co(en)_3]^{3+}$ (Visible wavelength region)

A. J. McCaffery, S. F. Mason; Mol. Phys. 6, 359 (1963)
"The electronic spectra, optical rotatory power and absolute configuration of metal complexes. The dextro-tris (ethylenediamine)cobalt(III) ion"
Nickel Tartrate Solution (Tentative visible standard)

(5) Nickel Tartrate Solution (Long-wavelength region)

T. Konno, H. Meguro, T. Murakami, M. Hatano: Chem. Lett., (1981), 953
"A Critical Study on Circular Dichroism Measurement in Longer Side of Visible Region"

(6) 3-point-CD-Calibration D(-)-Pantolactone, d-10-Camphorsulfonic Acid (+)-$[Co(en)_3]I_3 \cdot H_2O$

K. Tuzimura, T.Konno, H. Meguro, M. Hatano, T. Murakami, K. Kashiwabara, K. Saito, Y. Kondo, and T.M. Suzuki: Anal. Biochem. 81, 167 ~ 174 (1977)
"A Critical Study of the Measurement and Calibration of Circular Dichroism"

(7) Absolute CD Measurements and Calibration (D-(-)-Pantolactone, d-10-Camphorsulfonic Acid, (-)-$[Co(en)_3]I_3 \bullet H_2O$

P.H. Schippers, H.P.J.M. Dekkers; Anal. Chem., 53, 778 (1981)
"Direct T.M. Lowry: "Optical Rotary Power", page 131, Longmans, Green & Co. (1935)
Determination of Absolute Circular Dichroism Data and Calibration of Commercial Instruments"

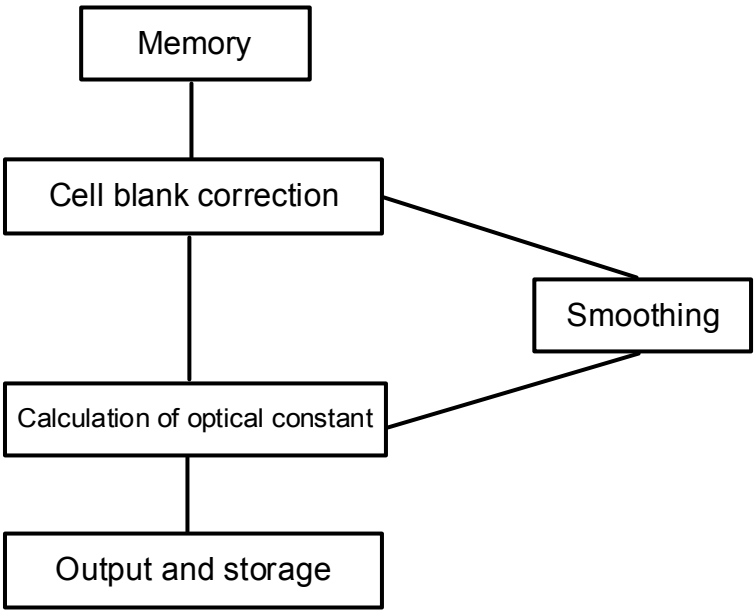
(8) Absolute CD and LD Calibration (2-(+)-$[Co(en)_3]Cl_3NaCl•6H_2O$)

B. Norden, S. Seth; Appl. Spectrosc., 39, 647 (1985)
"Critical Aspects of Measurement of Circular and Linear Dichroism: A Device for Absolute Calibration

4. Data Processing

This chapter deals with cautions for calculation of the optical constant with CD/ORD measurement. For practical operation, see the operation manual. The following shows the general flow on data arrangement.

Data processing



Cautions for data arrangement are described in Sections 4.1 ~ 4.5.

4.1 Memory Data

Data processing is executed with measured data or data read from a disk to the memory of the computer.

4.2 Correction of Cell Blank

CD and ORD themselves are high-sensitive measurement, and the base line of the instrument (only the cell holder is set in the sample chamber) is bent and the cell has the cell blank.
Therefore, when the CD and ORD of a sample are measured, the data includes the signals due to instrument blank and cell blank in addition to the signal due to the sample.
Therefore, for CD and ORD, the method in which the solvent blank (cell blank and instrument blank) is subtracted from the measured data of the sample (including cell blank and instrument blank) is used as the correction method of blanks.
Cautions for cell blank are described in the following Items (1) ~ (4).

(1) For the cell blank, CD is generally smaller than ORD. When the standard cylindrical cell is used, ORD reaches ±5 mdeg, but CD is kept within ±2 mdeg at most.

(2) If a rectangular cell is used, the cell blank of ORD may almost reach to more than ±10 mdeg, though it of CD is kept within ±2 mdeg at most. (Especially, the measurement of ORD in the short wavelength region less than 300 nm cannot be practically measured.)

(3) For the above subtraction method as the cell blank correction, there is no problem when the absolute value of cell blank is small (several tens of mdeg at most).

(4) Other cautions for cell blank include cleanliness of cell surface, standardization of cell direction and cell-holder setting method, and short interval time between sample measurement and blank measurement (for lowering the influence of baseline drift) especially for high-sensitive measurement.

4.3 Smoothing (FFT)

Though the Accumulation method is basically used for improvement of the spectrum S/N, the smoothing method is effective for eliminating remaining noises in view of the measurement time. It is also effective for reading values with a cursor and printing out the data.
The following are cautions for smoothing.

(1) The set of cutting frequencies is usually proper that the high frequency is set to 2 ~ 3 times of low one. Check if smoothing data are correct by overlapping-displaying original and smoothing one.

(2) If noises increase extremely at the end of a wavelength region (mainly for short wavelength), the spectrum near the end may frequently be distorted. In this case, partially cut with the "Truncate Data" function before smoothing, and preferable data may usually be obtained.

(3) For data with insufficient accumulation, no preferable results can be obtained because a noise signal with a long cycle is superimposed on the original spectrum.

In this case, it is advisable to continue accumulation until long cycle components in noises are lost (until they become random noise).

(4) When broad and sharp spectrums are coexistent, if the cutting frequency is adjusted to the sharp spectrum, noises are not sufficiently eliminated, though the spectrum is not distorted. If the cutting frequency is adjusted to the broad spectrum, the sharp spectrum is distorted but noises are sufficiently eliminated.

4.4 Optical Constant

Measured CD and ORD data can be converted into the optical constant as the physical constant by calculation.
The ORD magnitude is expressed as the angle of rotation of the plane of polarization (optical rotation). The CD magnitude is expressed in following both ways; as the optical density difference of left- and right-handed circularly polarized light (circular dichroic absorptivity) and as ellipticity (the extent that linearly polarized light is converted to elliptically polarized light).
The following shows the calculation formula and the input Parameters for the optical constant installed in the CD/ORD instrument.

4.4.1 CD Data

For the CD/ORD instrument, measured original CD data is expressed as the ellipticity (One mdeg equals 0.001 deg). Therefore, the method is used to calculate the optical constant with the original ellipticity data, and the software to mutually convert the ellipticity and the circular dichroic absorptivity is installed.

(1) Specific ellipticity; $[\Psi]$ (deg • cm^2 • $decagrame^{-1}$)

$[\Psi] = \theta / (100\ C'\ \ell)$

θ: Ellipticity (mdeg)

C': Concentration (g/mL)

ℓ: Path-length of cell (cm)

The parameters to be input include the concentration C', and the path length ℓ.

(2) Magneto-specific ellipticity : $[\Psi]M$ (deg • cm^2 • $decagrame^{-1}$ • T^{-1})

$[\Psi]_M = \theta / (10\ C'\ \ell\ H)$

θ: Ellipticity (mdeg)

C': Concentration (g/mL)

ℓ: Path-length of cell (cm)

H: Magnetic field (kilogauss)

The parameters to be input include the concentration C' , the path length ℓ and magnetic field H.

(3) Molar ellipticity: $[\theta]$(deg • cm^2 • $decimole^{-1}$)

$[\theta] = \theta / (10\ C\ \ell)$

θ: Ellipticity (mdeg)

C: Molar Concentration (mol/L)

ℓ: Path-length of cell (cm)

The parameters to be input include the concentration C and the path-length of cell ℓ.

Note:	*For macromolecules such as protein, nucleic acid, and polysaccharide: the mean residue (molecular) ellipticity (θ) is used. The calculation formula can be obtained by substituting [θ] with (θ) in the above formula. In this case, however, the concentration C is the mean residue molar concentration. The mean residue molar concentration Cr is obtained by the following formula.*

Cr = n • Cp = 1000 n C' / Mp

n: Number of constructed residues of macromolecule

Cp: Molar concentration of macromolecule (mol/L)

C': Weighing weight (g/mL)

Mp: Molecular weight of polymer

For the CD/ORD instrument, however, no distinction is made between [θ] and (θ).

Because data is stored as molar ellipticity [θ], provide comments to data when necessary.

(4) Magneto-molar ellipticity: $[\theta]_M$ (deg • cm^2 • $decimole^{-1}$ • T^{-1})

$[\theta]_M = \theta / (C \ell H)$

θ: Ellipticity (mdeg)

C': Molar Concentration (mol/L)

ℓ: Path-length of cell (cm)

H: Magnetic field (kilogauss)

The parameters to be input include the concentration C, path-length of cell ℓ, and magnetic field H.

Note:	*Though the magneto-residue ellipticity $(\theta)_M$ is also the parameter for macromolecules, the expression of data and inputting of concentration are shown in (3).*

(5) Molar circular-dichroic absorption:$\Delta\varepsilon$ (cm^2 • $mmole^{-1}$)

$\Delta\varepsilon = \theta / (32980 \ C \ \ell)$

θ : Ellipticity (mdeg)

C': Molar Concentration (mole/L)

ℓ: Path-length of cell (cm)

The parameters to be input include the concentration C and path-length of cell ℓ.

Note:	*The mean residue circular dichroic absorption is also a parameter for macromolecules. Though the inputting of concentration is as shown in (3) , the expression of data, in accordance with the custom is not distinguished from the molar circular-dichroic absorption "$\Delta\varepsilon$".*

(6) Magneto-molar circular dichroic absorption : $\Delta\varepsilon M$ (cm^2 • $mmole^{-1}$ • T^{-1})

$\Delta\varepsilon M = \theta / (3298\ C\ \ell\ H)$

θ: Ellipticity (mdeg)

C: Molar Concentration (mol/L)

ℓ: Path-length of cell (cm)

H: Magnetic field (kilogauss)

The parameters to be input include the concentration C, path-length of cell ℓ, and magnetic field H.

Note:	*Though the magneto residue circular dichroic absorption is also a parameter for macromolecules, the inputting of concentration is as shown in (3), and the expression of data is as shown in (5).*

(7) Circular-dichroic absorption; Δ OD

Convert the ellipticity θ into the circular-dichroic absorptivity (Δ OD)

Data for circular-dichroic absorption can also inversely be converted into ellipticity

Δ OD = θ / 32980

Note:	*It is not necessary to input parameters.*

(8) Mutual conversion between molar ellipticity [θ] and molar circular-diachronic absorption $\Delta\varepsilon$

$[\theta] = 3298 \cdot \Delta\varepsilon$.

$[\theta]_M = 3298 \cdot \Delta\varepsilon M$

Note:	*It is not necessary to input parameters.*

4.4.2 ORD Data

The following describes how to calculate the optical constant with the ORD data measured by the optional accessory of the CD/ORD instrument.

For the ORD data measured with the ORDM-306 accessory using the optical null method, the optical constant can be directly calculated. For the ORD data measured with the ORDE-307 accessory using the photo-electric method, calculate the optical constant after calibrating the wavelength characteristic of the original data with the "ORD-E correction software".

For the ORD data measured with any accessory, the optical rotation is stored in the memory in milli-degrees (mdeg).

(1) Specific rotation ; $[\alpha]$(deg • cm^2 • $decagrame^{-1}$)

$[\alpha] = \alpha /(100\ C'\ \ell)$

α: Optical rotation (mdeg)

C': Concentration (g/mL)

ℓ: Path-length of cell (cm)

The parameters to be input include the concentration C' and path-length of cell ℓ.

(2) Magneto-specific rotation : $[\alpha]M$(deg • cm^2 • $decagrame^{-1}$ • T^{-1})

$[\alpha]M = \alpha /(10\ C'\ \ell\ H)$

α: Optical rotation (mdeg)

C': Concentration (g/mL)

ℓ: Path-length of cell (cm)

H: Magnetic field (kilogauss)

The parameters to be input include the concentration C', path-length of cell ℓ and magnetic field H.

(3) Molar rotation: [M] (deg • cm^2 • $decimole^{-1}$)

$[M] = \alpha / (10\ C\ \ell)$

α: Optical rotation (mdeg)

C: Concentration (mol/L)

ℓ: Path-length of cell (cm)

The parameters to be input include the molar concentration C and path-length of cell ℓ.

Note: Though the mean residue rotation (m) is also a parameter for macromolecules, the expression of data and inputting of concentration are shown in (3) (Molar ellipticity) in Section 4.4.1.

(4) Magneto-molar rotation: $[M]_M$ (deg • cm^2 • $decimole^{-1}$ • T^{-1})

$[M]_M = \alpha /(C \ell H)$

α: Optical rotation (mdeg)

C: Concentration (mol/L)

ℓ: Path-length of cell (cm)

H: Magnetic field (kilogauss)

The parameters to be input include the molar concentration C, path-length of cell ℓ and magnetic field H.

Note: Though the magneto residue rotation $(m)_M$ is also a parameter for macromolecules, the expression of data and inputting of concentration are as shown in (3) (Molar ellipticity) in Section 4.4.1.

4.4.3 Optical Constant for Pellet, Glass and Pure Liquid

CD may be measured with KBr pellets as a special case. The following shows how to obtain molar ellipticity and molar circular-dichroic absorption in this case. However, note that the original data is expressed in ellipticity θ (mdeg.).

(1) Molar ellipticity

$[\theta] = \theta$ SM/(10 W)

S: Pellet area (cm^2)

M: Molecular weight of sample

W: Sample mass in pellets (mg)

When assuming the path-length of a cell I as 1 cm for convenience by contrasting the formula in (1) with that in (3) in Section 4.4.1 for the parameters to be input, the molar concentration C is expressed by the following formula.

C = W/(SM)

(2) Molar circular-dichroic absorption

$\Delta\varepsilon = \theta$ SM / (32980 W)

The parameter to be input is the same as that in (1).

*Note: Also for ORD, the molar rotation can be obtained in a manner corresponding to (1). However, because ORD is sensitive to turbidity and distortion of pellets, high-sensitive measurement is much more difficult than that for CD. When the density **p** (or weight/volume) and the optical path length ℓ (cm) of pure liquid are known, the molar ellipticity and molar circular-dichroic absorption can be expressed by the formulae in (3) and (4) respectively.*

(3) Molar ellipticity

$[\theta] = \theta$ M/(1000 ***p*** ℓ)

(4) Molar circular-dichroic absorption

$\Delta\varepsilon = \theta$ M/(329800 ***p*** ℓ)

The parameters to be input in Items (3) in Section 4.4.1 and (4) in Section 4.4.2 include the optical path length ℓ (cm) and molar concentration C of the sample. The molar concentration C is calculated with the following formula.

C = 1000 ***p*** / M

Note: The molar rotation can be obtained with the same operation as that in (3).

4.4.4 Lorentz Correction Factor

This factor is used to convert the measured optical data in a medium such as a solution to one in a vacuum. The Lorentz correction is frequently made to quantitatively compare with the data of various solutions in which each refractive index "n" is largely different.
The Lorentz correction is made by multiplying the measured data; the molar ellipticity $[\theta]_\lambda$ or molar rotation $[M]_\lambda$ by the Lorentz factor $3/(n^2 + 2)$ and is expressed by the following formulae.

Reduced molar ellipticity	$[\theta'] = [\theta] \times 3 / (n^2 + 2)$
Reduced residue ellipticity	$(\theta') = (\theta) \times 3 / (n^2 + 2)$
Reduced molar rotation	$[M'] = [M] \times 3 / (n^2 + 2)$
Reduced residue rotation	$(m') = (m) \times 3 / (n^2 + 2)$

The refractive index of solution includes wavelength dispersion. Therefore, the Lorentz factor also has the characteristic of wavelength dispersion and its accurate value depends on each wavelength.
For this program, the Lorentz correction for the entire wavelength region is not performed.
However, in a small wavelength range this correction can be made by considering that Lorentz factor is roughly constant.
The Lorentz factor of dilute buffer-solution of approx. 0.1 M is frequently considered as that of pure water.
For reference, Table 4.1 shows the Lorentz factor of pure water (at 25ºC) including the relative error of 1%.
Note that no distinction is made between $[\theta]$ and $[\theta']$ or between [M] and [M'] in this program.

Table 4.1 Lorentz correction factor of water

Wavelength range (nm)	$3/(n^2+2)$ (Average)
600 ~ 300	0.7873
300 ~ 240	0.7725
240 ~ 210	0.7595
210 ~ 190	0.742
190 ~ 180	0.726

4.4.5 Density Correction of Solution for Variable Temperature Measurement

To measure CD and ORD for variable temperatures of wide range, note that the concentration of solute changes due to the density change of solution.

Especially, for low-temperature measurement at about liquid nitrogen temperature using low-temperature solvent such as EPA or P5-M1 (see Table 1.2), it is necessary to correct the density of the solution because the condensation of solution is approx. 20%. When assuming the density correction factor to be "V_{25}^{T}" at 25ºC, the apparent molar ellipticity and molar rotation are corrected as follows:

Corrected molar ellipticity $[\theta]corr = [\theta]obs \times V_{25}^{T}$

Corrected molar rotation $[M]corr = [M]obs \times V_{25}^{T}$

Where,

$[\theta]obs$: Apparent molar ellipticity

$[M]obs$: Apparent molar rotation

V_{25}^{T}: Density correction factor at TºC on the basis of 25ºC

Table 4.2 shows the density correction factor V_{25}^{T} Of EPA and P5-M1 for reference.

Table 4.2 Density correction factor of EPA and P5-M1

V_{25}^{T} = Vol. at TºC/Vol. at 25ºC = Density at 25ºC/Density at TºC

Temp. / Solvent	25ºC	-5ºC	-29ºC	-41ºC	-74ºC	-192ºC
EPA	1.000	0.956	0.925	0.911	0.874	0.798
P5-M1	1.000	0.957	0.926	0.911	0.874	0.793

Reference: The above values are calculated as the basis of those at 25ºC by R. Passerini, I.G. Ross, W. Ramsey, and R. Foster, J. Sci. Instr., 30, 274 (1953).

4.5 Blank Correction for Magnetic Circular Dichroism and Magnetic Optical Rotatory Dispersion

4.5.1 Blank Correction for MCD

(1) Even if the same cell is used, the blank is slightly different between CD and MCD.

(2) For optically active substances, MCD is observed as a constructed spectrum of the CD due to naturally optical activity and the net MCD induced by a magnetic field. Therefore, to obtain the net MCD, it is necessary to measure for the following Items 1) ~ 6) by also considering the difference of cell blank characteristics between CD and MCD.

1) MCD measurement of sample solution

2) MCD measurement of solvent (cell blank)

3) CD measurement of sample solution

4) CD measurement of solvent (cell blank)

Then, the cell blank corrections are obtained for the measured MCD and CD respectively through the calculation in the following Items 5) and 6).

5) Cell-blank corrected MCD = 1) - 2)

6) Cell-blank corrected CD = 3) - 6)

Finally, the net MCD is obtained using the calculation in the following 7).

7) Net MCD = 5) - 6)

To calculate the optical constant of the MCD of the optically active substance, the net MCD data in Item vii) should be used.

4.5.2 Blank Correction for MORD

(1) The method used to correct the MORD blank and to obtain the net MORD of optically active substances is the same as that for MCD.

(2) The main difference between MORD and MCD is pointed out in the following items 1) and 2).

1) Cells and solvents essentially have MORD, which especially shows a much bigger value than the sample one in dilute solution.

2) Therefore, the MORD of the sample itself is obtained by subtraction between similar large signals dominant to those of solvents and cells, and then, noises and errors included in both data are frequently enlarged and reflected in the results. Thus, it is generally difficult to detect MORD at a high sensitivity.

5. Application of CD/ORD

5.1 Optical Rotatory Dispersion and Circular Dichroism

When a light beam passes through a medium, the following phenomena, (a) and (b), occur.

(a) The light velocity, v, in the medium differs from the velocity, c, in vacuum, that is, the refractive index n (=c/v) is not equal to 1 (refraction).

(b) The light intensity decreases from the initial intensity I_0 in accordance with the following formula:

$$I = I_0 e^{-kd}$$

Where I is the intensity of the light that has passed through a medium d cm in thickness, and k is the absorption coefficient (absorption).

According to the theory of electromagnetism, refraction and absorption are different expressions of the interaction between the electromagnetic field of light and the valence electrons and are closely related to each other. Fig. 5.1 shows the contribution of electronic transition to absorption and refractive index. Outside the absorption band, the refractive index increases with decreasing wavelength, however in the absorption region, it decreases rapidly with decreasing wavelength (abnormal dispersion). The contribution of electronic transition to the refractive index vanishes at the wavelength of absorption maximum. The correlation between absorption and dispersion can be represented by the integral transformation known as Kronig-Kramers formula. Therefore, if the absorption coefficient is known in the entire wavelength region, it is possible to determine the refractive index for any wavelength.

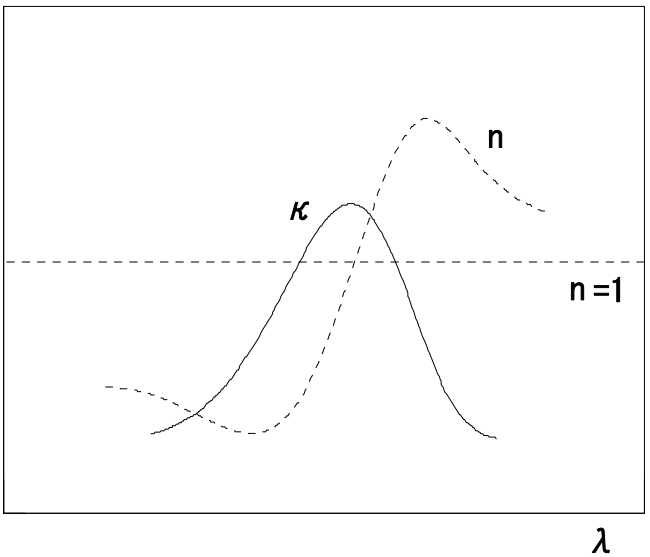


Figure 5.1 Correlation between absorption and refractive index

When linearly polarized light is incident upon a substance, the polarization plane of the transmitted light rotates. Such a substance is called an optically active one. Here, the left- and

right-handed components of the circular polarization of linearly polarized light show phenomena a) and b) in different extents.
When the quantities related to the left- and right-handed polarization are denoted by l and r as subscripts respectively, the following phenomena, (c) and (d) occur.

(c) $n_\ell \neq n_r$
(d) $k_\ell \neq k_r$

If $n_\ell > n_r$, then the left-handed circular component is retarded with respect to the right-handed component in the optically active medium and consequently the polarization plane of the transmitted linearly polarized light composed of both circular components rotates counterclockwise. The angle of rotation per unit length of optically active medium is expressed by the following formula (1) in radian unit.

$$\psi = (\pi/\lambda)\times(n_\ell - n_r) \qquad (1)$$

where λ is the wavelength of the incident light in a vacuum. The specific rotation can be defined by the following formula (2) with an angle α of rotation in degree unit.

$$[\alpha]_\lambda^t = \alpha/(\ell\, c) \qquad (2)$$

where ℓ is the path length in dm, c is the concentration in g/mL, λ is the wavelength, and t is the temperature.
The molecular rotation that is habitually used in comparing the rotations between substances is defined by the following formula (3).

$$[\phi]_\lambda = [\alpha]_\lambda \cdot M/100 \qquad (3)$$

where M is the molecular weight. These formulae indicate that the rotation is proportional to the path length and the concentration of medium. Although these formulae are held within the range of lower concentration, the linearity may be lost at a higher concentration due to the inter-molecular interaction. The rotation, like the refractive index, also varies with the wavelength of the incident light. The wavelength-dependency of the molecular rotation is called optical rotatory dispersion (ORD).
In a given absorption band, the left- and right-handed circular polarized light differ not only in the propagation velocities but also in the absorption coefficients. The latter phenomenon is called circular dichroism (CD; phenomenon (d)). The simplest expression of CD can be given by the difference between the molecular absorption coefficient ε.

$$\Delta\varepsilon = \varepsilon_\ell - \varepsilon_r \qquad (4)$$

where ε is defined by $\ell = I_0\ 10^{-\varepsilon \ell c}$ and c is mole/1. (Absorption coefficient k is nearly equal to $2.303\varepsilon c$.) On the other hand, when linearly polarized light passed through optically active medium in the wavelength region of absorption band, the transmitted light is no longer linearly polarized light and the tip of its electrical field vector traces an ellipse because of phenomenon (d) on both the circularly polarized components. The angle θ whose tangent is equal to the ratio between the minor axis and the major axis of the elliptically polarized light is called ellipticity, and the ellipticity per unit length expressed in radian unit is related to the difference between both the circular absorption coefficients:

$$\theta = (1/4)(\kappa_\ell - \kappa_r) \qquad (5)$$

Similar to the quantities on optical rotation, specific ellipticity is defined by the ellipticity θ expressed in degree unit as follows:

$$[\psi] = \theta / \ell\, c \qquad (6)$$

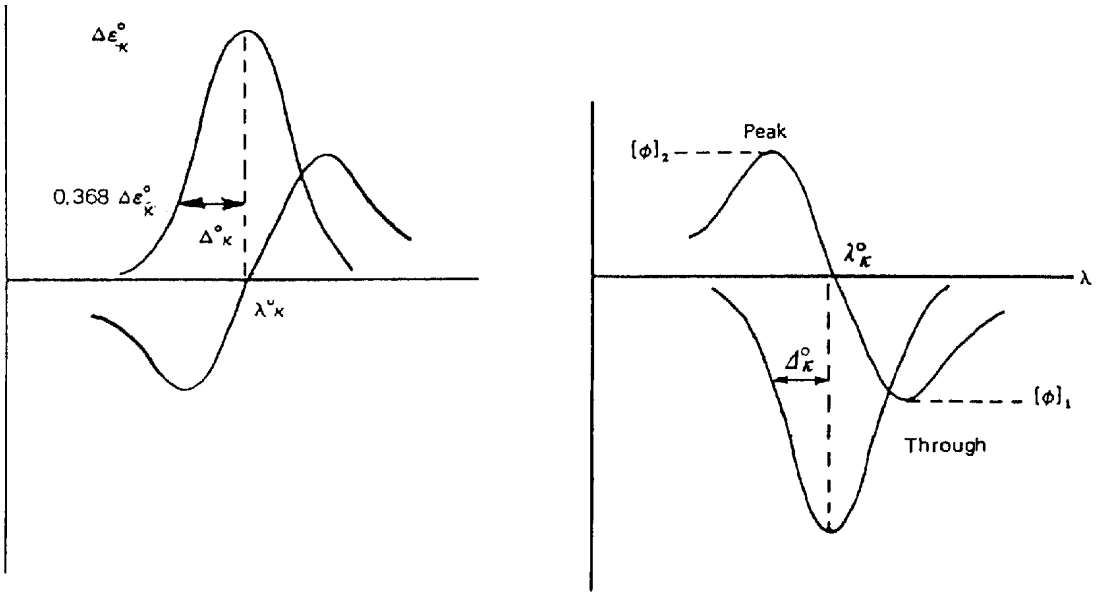
Molecular ellipticity is expressed as follows:

$$[\theta] = [\psi] \cdot M/100 \qquad (7)$$

From Eq. 4 ~ Eq. 7, molecular ellipticity is related to $\Delta\varepsilon$

$$[\theta] = 2.303(4{,}500/\pi)\bullet(\varepsilon_\ell - \varepsilon_r) \approx 3{,}300 \times \Delta\varepsilon \qquad (8)$$

As illustrated in Fig. 5.2, the ORD curve across the absorption band of an optically active substance is a S-shape, while the corresponding CD cure is a convex shape. These phenomena are called the Cotton effect. The Cotton effect in which the ORD curve has a peak on the longer wavelength side is called a positive one (Fig. 5.2 (a)) and that in which the curve has a trough is called a negative one (Fig. 5.2 (b)). The positive Cotton effect gives a positive CD curve with the peak at the inflection point of the S-shaped ORD curve.



(a) Positive (b) Negative

Figure 5.2 Cotton effect

The Cotton effect, which provides much structural information, is characterized by its position, magnitude, sign, and shape of the curve. In the case of a CD curve, the Cotton effect is represented by Δε or [θ] at the maximum wavelength, λ_{max}, and in the case of an ORD curve it is represented by the wavelengths for the peak and trough, molecular rotation, and amplitude, a, defined by the following equation.

$$a = ([\phi]_1 - [\phi]_2)/100 \qquad (9)$$

where $[\phi]_1$ is the extremum of molecular rotation on the longer wavelength side and $[\phi]_2$ is the extremum of molecular rotation on the shorter wavelength side. When a CD curve is approximated by the Gaussian curve, the following equation holds.

$$a = 40\ \Delta\varepsilon_{Max} \qquad (10)$$

To express the Cotton effect more quantitatively, rotational strength, R_k, is defined as follows.

$$R_k = \frac{3hc10^3 \ln 10}{32\pi^3 N} \int_0^\infty \frac{\Delta\varepsilon_k(\lambda)}{\lambda} d\lambda$$
$$= 0.23 \times 10^{-23} \int_0^\infty \frac{\Delta\varepsilon_k(\lambda)}{\lambda} d\lambda \qquad (11)$$

When the CD curve takes the form of a Gaussian curve, R_k, can be expressed as follows.

$$R_k \approx 0.406 \times 10^{-38} \Delta\varepsilon^0{}_k \frac{\Delta_k^0}{\lambda_k} \quad (12)$$

where λ_k is the maximum wavelength, Δ_K^0 is the half band width and $\Delta\varepsilon_K^0$ is the maximum CD value. ORD and CD are related to each other by the Kronig-Kramers equation of integral transformation. If either of both is known for the entire spectral region, another can be found by the following relations.

$$\phi(\lambda) = \frac{2}{\pi}\int_0^\infty \theta(\lambda') \frac{\lambda'^2}{\lambda^2 - \lambda'^2} d\lambda' \quad (13)$$

$$\theta(\lambda) = -\frac{2}{\pi\lambda}\int_0^\infty \phi(\lambda') \frac{\lambda'^2}{\lambda^2 - \lambda'^2} d\lambda' \quad (14)$$

These indicate that ORD and CD are equivalent in usefulness for most structural and stereochemical applications. However, one of them is often more useful than the other one. The ORD curve extends the foot out of the wavelength region of absorption band and, therefore, can be used very effectively when sample has no absorption band in the wavelength region of the measurement. On the other hand, the discontinuity of the CD curve is very useful for the distinction between the overlapped optically active transitions and can be used conveniently in applications where the study of rotational strength carries weight. As discussed above, since ORD and CD have their own advantages and disadvantages respectively, it is desirable to make both measurements at the same time.

5.2 Application of CD/ORD Instruments

The CD/ORD instrument is successfully applied in the field of organic stereochemistry, analytical chemistry including the purity-test of optically active substances and quantitative analyses of pharmaceuticals, natural organic chemistry, complex salt chemistry, biochemistry, and others. Further, the application fields of ORD and CD instruments spread to polymer chemistry, physical chemistry, medical science, agricultural chemistry, and others as a powerful tool for measuring minute changes of the stereochemical structure.

As the measuring technique makes progress, various special measurements are made. These special applications include the observation of the behaviors of substances from low to high temperature utilizing a variable temperature cell, measurement of magnetic circular dichroism (MCD) by attaching a super-conductive magnet, electromagnet, etc. in the sample chamber of the measuring instrument, high sensitivity measurement utilizing a sample alternator attachment, and stopped flow CD measurement using a sample mixing attachment.

These ORD and CD measurements can be made more effectively by utilizing the data processing unit for ORD and CD measurements. The following describes how the instrument is used in fundamental application.

5.2.1 Features and Advantages of ORD and CD

ORD and CD should be used selectively for the intended application depending on their features and advantages. In principle, both techniques always yield identical information and identical results.

CD: The first advantage of CD is that CD maximum nearly coincides with UV maximum with respect to the wavelength, and the signs of the Cotton effect can be determined exactly. In ORD, even such a fundamental check is sometimes not possible because of the disturbance by the background curve. A second advantage of CD is the absence of the background curve. A third advantage is that a CD curve can be approximated by the Gaussian curve so that rotational strength (R_K), which is the intensity factor of ORD and CD, can be easily obtained through calculation, allowing the theoretical treatment of phenomena.

ORD: The advantage of ORD is its characteristic form due to the background rotation specific to each compound. While CD curves are similar type, ORD, which give curves specific to compounds, can be utilized effectively for qualitative analysis and in the first stage of structural studies.

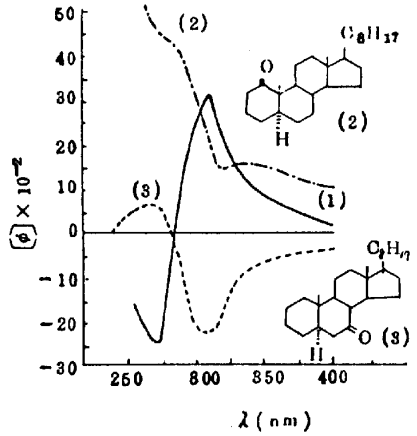


Figure 5.3 ORD of steroid ketones

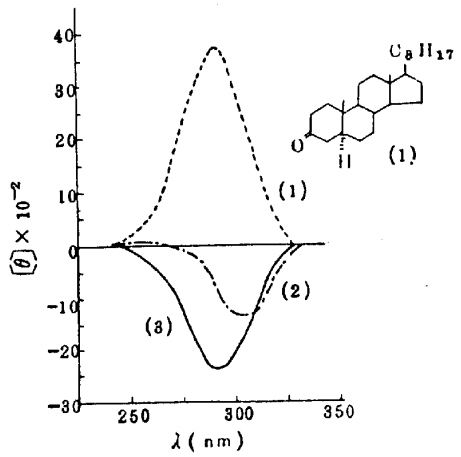


Figure 5.4 CD of steroid ketones

To illustrate the features and advantages of the ORD and CD, typical ORD and CD curves for steroid ketones are given in Fig. 5.3 and Fig. 5.4. The sign of the Cotton effect for cholestan-3-one(1) and -7-one(3) can be determined from either ORD or CD, but those for cholestan-1-one(2) cannot be determined from its ORD given is Fig. 5.3. Here, CD shows clearly the negative Cotton effect with a slight red shift (Fig. 5.4) and clearly reveals the characteristics of the ORD curve. The characteristic ORD curve (2) serves for qualitative detection.

The ORD curves for the derivatives of progesterone and isoprogesterone (4, 5) shown in Fig. 5.5 do not allow the two chromophores to be assigned. However, the CD curves shown in Fig. 5.6 clearly indicate the presence of saturated ketones of different stereochemical environments and of α, β-unsaturated ketones of the identical stereostructure.

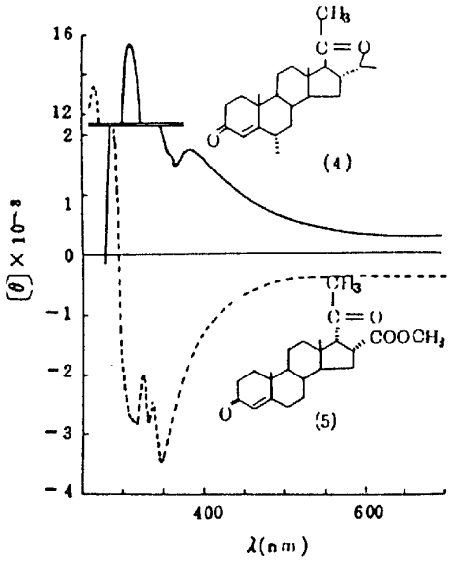


Figure 5.5 ORD of α, β-unsaturated steroid ketones

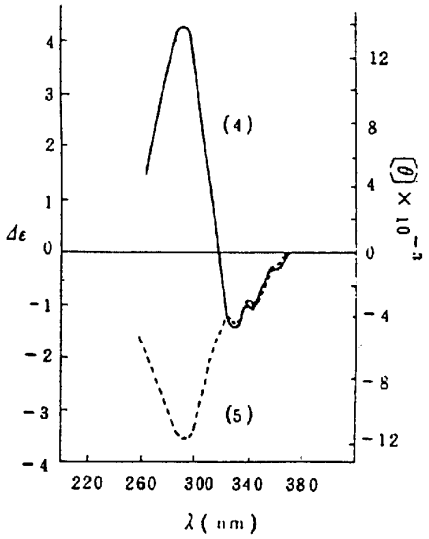


Figure 5.6 CD of α, β-unsaturated steroid ketones

5.2.2 Purity Test and Functional Group Analysis

The determination of specific rotation, $[\alpha]_D$, has been considered an effective means for the purity verification of natural organic compounds. However, in the case of small $[\alpha]_D$ value, the measuring error is frequently large. In this case, ORD can be used effectively for precise determination of purity with a small amount of sample (1 mg unit). On the other hand, ingenious applications of CD to quantitative analysis of mixed steroid drugs have also been reported.

IR and NMR are usually the most powerful tools in the functional group analysis of organic compounds, however, in some cases ORD and CD are also useful. When complicated carbonyl absorption occurs in IR, the Cotton effect can be expected to appear at the wavelength corresponding to the absorption band of optical activity. Therefore, ORD and CD well suit to the identification of carbonyl groups in esters, lactones, ketones, lactams, and others.

When hydrochloric acid is added to the methanol solution of the carbonyl group, hemiketal is formed then and the Cotton effect is diminished. This phenomenon is closely related to the structures near the carbonyl group and often provides important information.

In Table 5.1, Organic chromophores and their absorption maxima at which the cotton effects appear are shown.

Table 5.1 Chromophore

Chromophores	Absorption Maxima (nm)
Ketones	280 ~ 300
α,β-Unsaturated Ketones	330 ~ 360(R) 230 ~ 260(K)
Carboxylic acids	215 ~ 220
α,β-Unsaturated Carboxylic acids	250
Esters	215 ~ 220
Lactones	215 ~ 235
α,β-Unsaturated Lactones	250 ~ 260
Amides, Lactams	220 ~ 235
Conjugated dienes	270
Substituted phenyl	280
Azomethine	235 ~ 250
Oxim	195 ~ 215
Nitro	270
α,β-Unsaturated Nitro	340,260
Sulfoxide	210
Disulfide	280 (6-atom ring) 370 (5-atom ring)

5.2.3 Structural Analysis - Determination of Configuration

One of main application field of ORD and CD is organic stereo-chemistry. ORD and CD are indispensable in the analyses of relative ~ absolute configuration and conformation. In practice, these analyses can be made by comparing the ORD and CD curves with those of reference compounds with similar structure and known absolute configuration or by utilizing empirical rules such as those of octant, helicity, etc.

With optically active substances, the determination of the relative - absolute configuration between the functional groups in an important factor in defining substances. The conformational analysis provides indispensable data for understanding the state and reaction of the substance under given conditions.

(1) Determination by comparative method

The comparative method is based on the following two principles. (1) ORD and CD for an antipode structure give curves with the opposite sign. (2) The figure of the Cotton effect-curve depends on the stereo structure around the chromophore and is less affected by the structure far from the chromophore. A true antipode have the completely reversed absolute configuration around the asymmetric center in molecule and give the positive and negative Cotton effects in ORD and CD, which are mirror image of each other. However, the antipode structures in the comparative method include both the essential antipode around the chromophore as shown in Fig. 5-5 (4,5) and the pair satisfying antipode terminal ring units, as shown in Fig. 5.7. Compounds 6 and 7 in Fig. 5.7 show the ORD curves with very similar positive and negative Cotton effects, respectively.

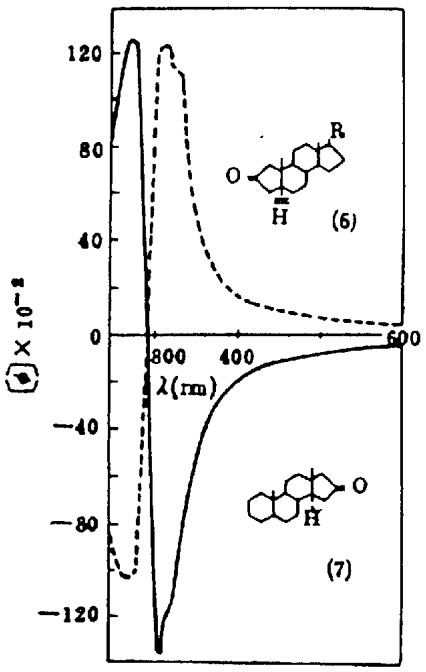


Figure 5.7 Pair ORD showing terminal ring unit in antipodal relation

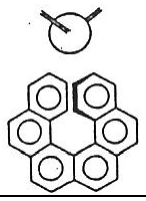
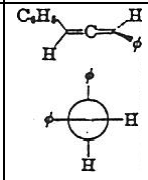
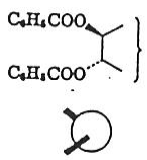
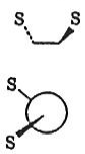
(2) Utilization of empirical rules

The ORD regularity of steroid ketones derived from a number of measurements agreed with the conclusion of Moffit, Moscowitz et al based on physical treatment and had been summarized as the octant rule represented by a cyclohexanone model. The validity of the octant rule signifies not only that ORD and CD measurements are feasible without comparison with a reference substance but also that the rotatory power of a substance under normal condition can be predicted by applying this rule. The idea of the octant rule has been extended to other ring ketones and aliphatic ketones, and became the base for a common rule for unsaturated ketones with essential asymmetry. However, it should be noted that such groups as α-epoxy- and α-cyclopropyl ketones deviate systematically from the octant rule.

(3) Empirical rules for other chromophores

For other chromophores except carbonyl group, ORD and CD have been measured and many empirical rules have been reported. Practically, the Cotton effects of dienes, helicene, diones, spiro-compounds, phenylallenes and disulfides etc. are summarized as the C2 symmetry rule (Table 5.2).

Table 5.2 Absolute configuration and optical activity of molecule with right-helical C_2-point group.

Absorption band	Diene, helicene	Dione	Spiro	Allen	Dibenzoate	Disulfide
						
Longer wavelength	*B(+)*	*B(+)*	*B(+)*	*B(+)*	*B(+)*	*B(+)*
Shorter wavelength	*A(-)*	*A(-)*	*A(-)*	*A(-)*	*A(-)*	*A(-)*

The Cotton effects of α, β- and β, γ-unsaturated ketones, azomethines, thiocarbonates and styrenes are analyzed by chirality rules. For the Cotton effects of lactones and lactams, the combined use of the ring chiralityrule and the axial halo-ketone rule had been proposed (Fig. 5.8 and 5.9).

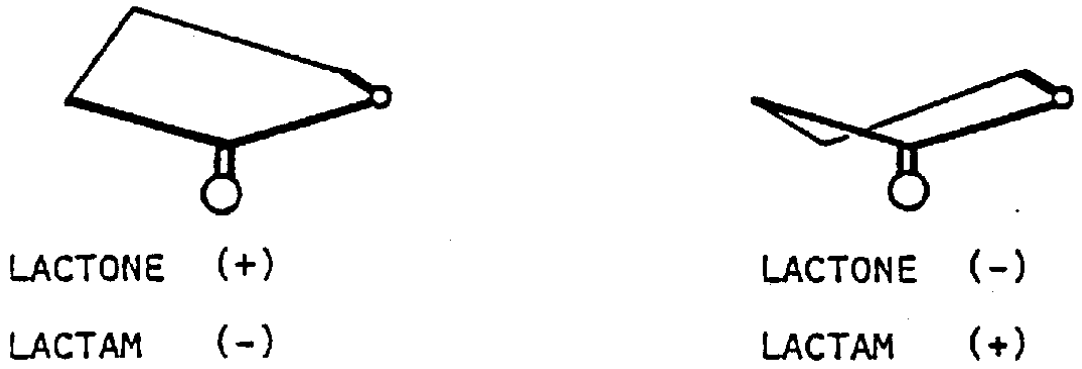


Figure 5.8 Ring chirality rule for five membered Lactone and Lactam

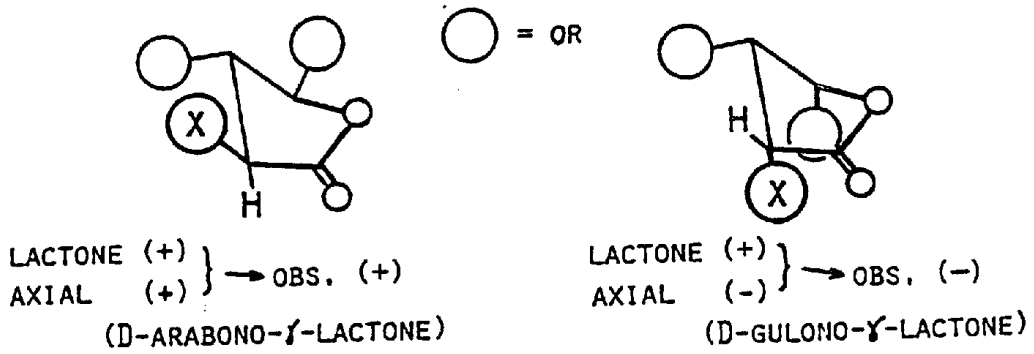


Figure 5.9 Combined use of ring chirality rule and axial halo-ketone rule to γ-lactones

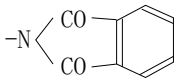
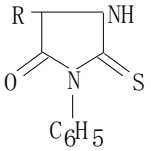
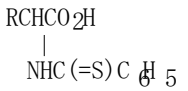
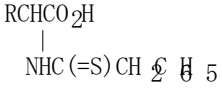
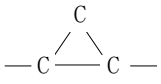
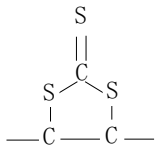
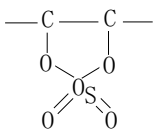
On the other hand, Sector rules or Quadrant rules had been proposed for substituted benzenes, aromatic amines, mono-olefines, episulfides, etc.

(4) Derived chromophores

Hydroxyl and amino groups are familiar to us and constitute biologically important sugars and amino acids respectively, however they show only plain ORD curves due to the instrumental restrictions because their optically active absorption bands are in the far ultra-violet region below 200 nm. Because of the difficulty of analyzing the asymmetric environment from this plain curve, these functional groups are usually derived to the optically active chromophores, which have weak absorption bands in the wavelength region of 250 ~ 500 nm, by mild chemical reaction, and these CD/ORD are measured. Then, these derivatives give clear Cotton effects in their CD/ORD curves and provide important stereo chemical information.

Thus the absolute configurations on α-carbon atoms in amino acids, peptides, organic amines, oxy-acids, alcohols, and olefines etc. that were difficult to analyze can be easily determined by selectively using the various derivatives shown in Table 5.3.

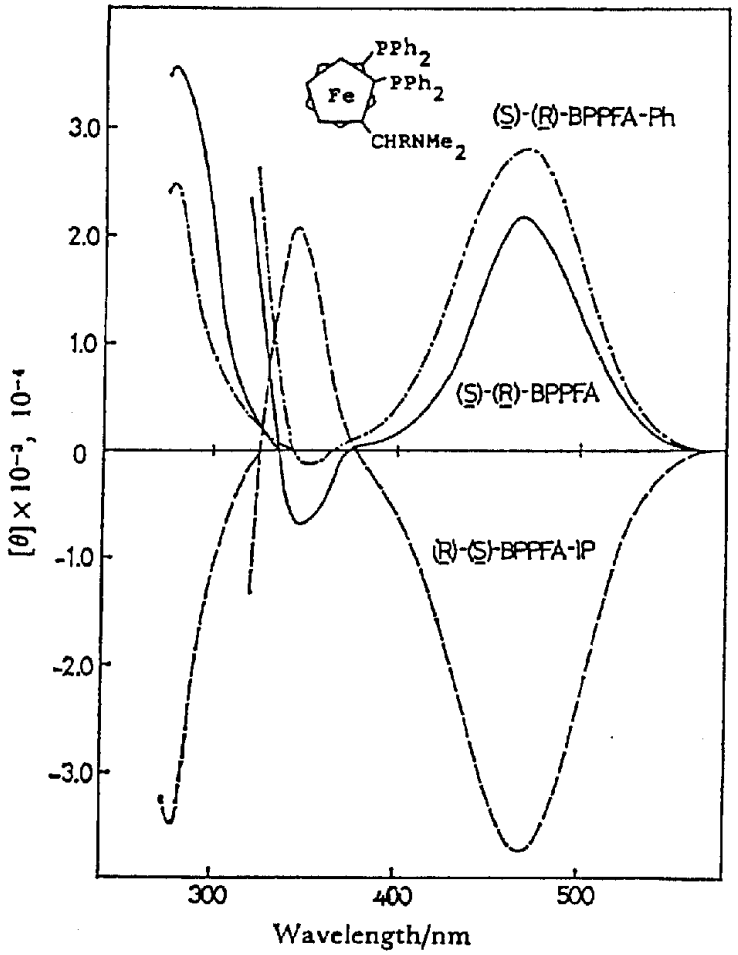
Table 5.3 Chromophore derivatives

Functional Groups	Induced Chromophores	Absorption Maxima(nm)
$-NH_2$	$-NHC(=S)SR$	330
$-NH_2$		300
$>NH$	$>N-NO$	370
$-NHCOR$	$N(NO)COR$	350-450
		310
		380, 290
		335, 270
$-OH$	$-OC(=O)R$	200-230
$-OH$	$-OC(=S)SR$	350
$-OH$	$-OBZ$	220-230
$-OH$	$-ONO$	325-390
$-CO_2H$	$-CONHC(=S)NR_2$	340
$-CO_2H$	$-C(=S)NRR'$	325-360
$-C=C-$		260
$-C=C-$		235, 305, 430
$-C=C-$		450, 550

(5) Non-empirical determination of absolute configuration

When more than two chromophores of π-electron systems are independently involved in an optically active molecule, these correlate strongly each other and form a well-known exciton state.

Then a very strong CD spectrum of couplet type is observed and can be non-empirically determined the absolute configuration from the sign of the Cotton effect (Fig. 5.10). This method had been proposed to determine the absolute configurations of vicinal diols, which were derived to the dibenzoate forms, by Harada-Nakanishi, however, this one has been developed to a CD chirality method by their theoretical and empirical considerations



R=Me ; BPPFA
R=i-Pr; BPPFA-IP (antipode)
R=Ph ; BPPFA-Ph

Figure 5.10 CD and UV spectra (EtOH) of cholest-5-ene-3β, 4β-diyl bis (p-dimethylamino benzoate)

5.2.4 Conformational Analyses by Special Techniques

In general the elucidation of abnormality in CD/ORD accompanying delicate change in the stereostructure requires the detailed consideration by the measurements of the conditionally changing CD spectra.

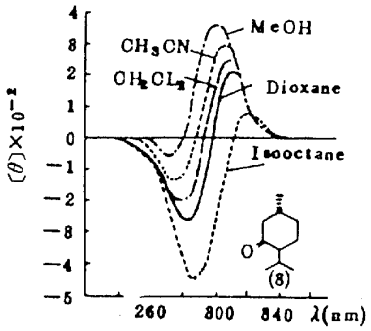


Figure 5.11
CD of (-)-menthone with solvent-change

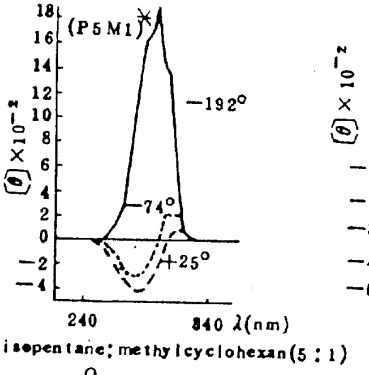


Figure 5.12
CD of (-)-menthone at low temperature

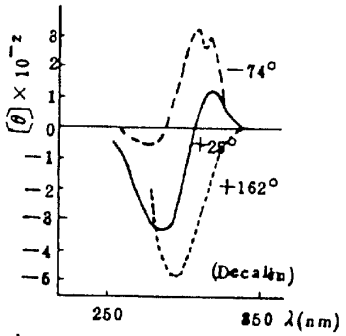
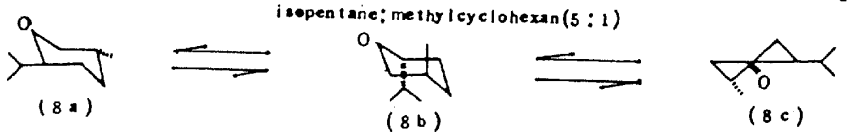


Figure 5.13
CD of (-)-menthone at high temperature



For example, CD of (-)-menthone (8) presents 2 maxima (Fig.5.11), however the Cotton effect changes from negative to positive with an increase of the solvent-polarity from isooctane to methanol. This fact can be interpreted as the phenomenon in which the mole fraction of the most stable conformer increases with increasing the solvent-polarity, if several conformers on one molecule can be expected.

The variable temperature CD spectra (Fig.5.12, 5.13) obtained by using variable temperature devices from low (-192ºC) to high (+162ºC) temperature indicate that the mole fraction of the most stable di-equatorial conformer (8a) increases at -192ºC and that an unstable di-axial -(8b) and twisted conformer (8c), of which negative rotatory contribution is predicted from the octant rule, can exist at +162ºC. Thus, the CD abnormality of (-)-menthone (8) shown in Fig. 5.11 had been explained by conformational equilibration. Such a solvent- and temperature-dependent CD curve can be used very effectively in the dynamic conformational analysis of flexible molecules.

On the other hand the induced CD, which is caused by the interaction between an optically inactive molecule with absorption band and an optically active molecule, is actively measured. For example the induced CD by the formation of inclusion complexes between drugs and β -cyclodextrin or one in cholesteric liquid crystals are representative.

CD in solid state can also be obtained by using the KBr or KCl disk similar to IR and can be used effectively. As special measuring techniques magnetic optical rotatory dispersion (MORD) and magnetic circular dichroism (MCD), which are based on the Faraday effect, should be pointed out. They are useful in explaining the transition mechanism of rare earth metal ions, metal complexes, etc. In the case of the investigation of the complicated transition

mechanism of organic compound and other groups, MCD is more desirable than MORD. Thus, MCD is now used more widely.

From the investigations of the MCD of metal complexes such as hemin and vitamin-B_{12} and of compounds related to nucleic acid it had become clear that MCD had given a much larger signal than CD and reflected sensitively the substituted condition. These facts indicate that MCD is suited to the qualitative and quantitative treatments for trace substances related to biological mechanisms. Moreover its applicability to non-optically active metabolites suggests that its use can be extended to general organic chemistry, biochemistry and clinical chemistry. As discussed above, MORD•MCD can provide characteristic information that completely differs from that by ORD•CD and can be used in a wider application fields by providing an magnetic attachment to the CD/ORD instrument.

5.2.5 Application to Metal Complexes

Various types of optically active metal complexes are well known and have weak absorption bands from the near-infrared to near-ultraviolet wavelength region and have very strong absorption bands in the ultraviolet region. In the neighborhood of these absorption bands, rotatory powers show abnormal dispersions and clear Cotton effects appear in the ORD•CD curves.

Among the optically active complexes, the 6-coordinated complexes (see Fig. 5.14) had been most widely studied. These assignments of each absorption band, vicinal effects, and influences of formation of the ion pair upon rotatory power had been investigated, and spin forbidden bands and charge transfer bands had been also studied.

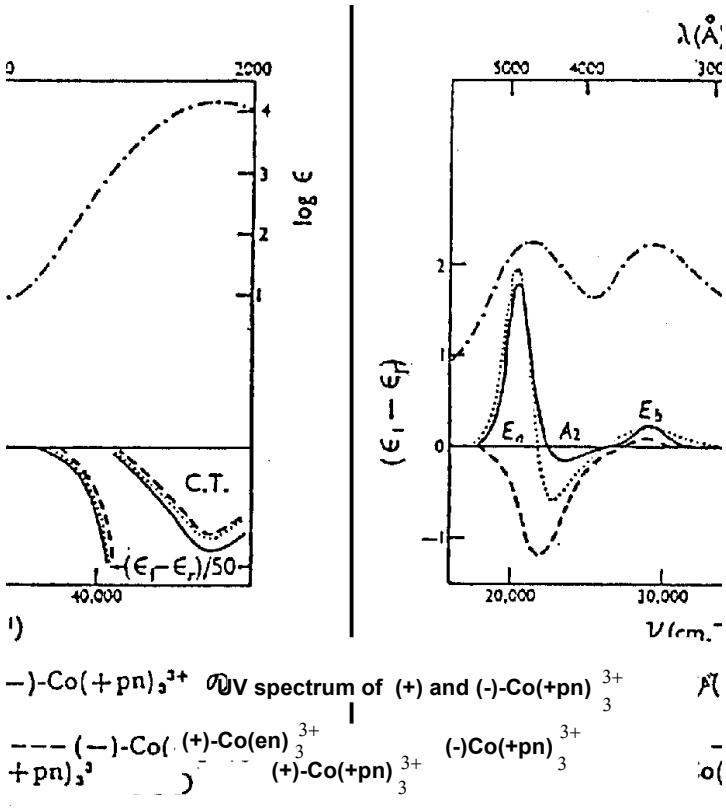
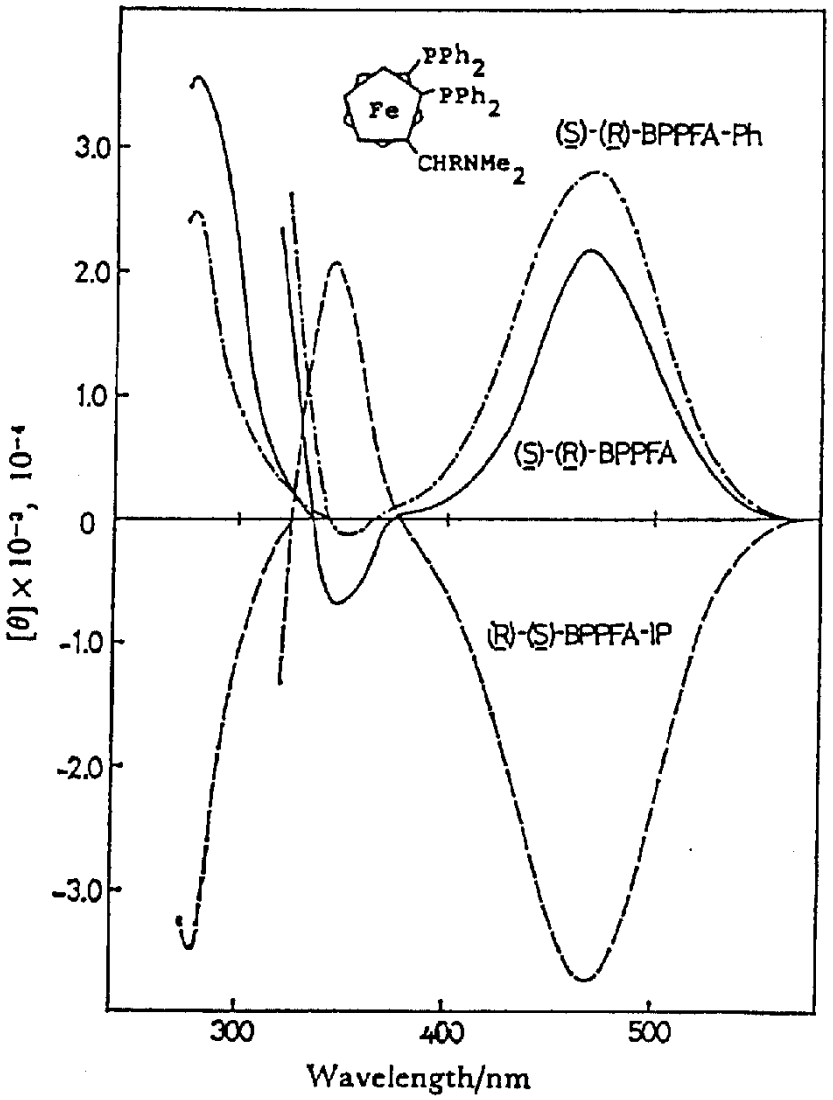


Figure 5.14 CD curve of 6-coordinated complex

The study of optically active complexes, by being combined with the study of the electronic state, is now directed to the study of the absolute configuration, rotational configuration and stereospecificity. A number of new types of isomeric phenomena have been discovered in complexes with such ligands as optically active diamines or amino acids.
Moreover, shift-base complexes and other various complexes are also being studied (Fig. 5.15).



R=Me ; BPPFA
R=i-Pr; BPPFA-IP (antipode)
R=Ph ; BPPFA-Ph

Figure 5.15 Circular dichroism (CD) spectra of BPPFA

5.2.6 Application to Biopolymers

(1) Proteins

A number of measurements have been made since it was found that the ORD of proteins faithfully reflects the higher order structure of protein molecules.

As the correlation between the Cotton effect and the secondary- and tertiary-structures of proteins has become clear, the usefulness of the Cotton effect and the CD/ORD analysis has come to be noticed.

At least three absorption bands correspond to the functional groups of polypeptide and protein:

1) α-asymmetric carbon of amino acid residue: vacuum-ultraviolet region (145 nm),
2) peptide bond: far-ultraviolet region (185 nm),
3) aromatic side chain: ultraviolet region 250 ~ 300 nm.

The Cotton effect occurs when these chromophores are placed in an optically active environment.

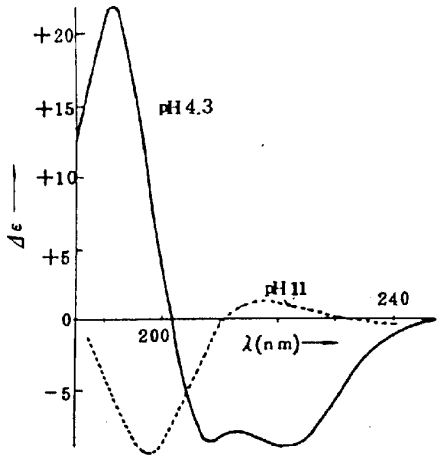


Figure 5.16 CD curve of polypeptide

When a polypeptide such as poly-L-glutamate forms a helical structure, the ORD in the wavelength region of 400 ~ 200 nm shows the negative Cotton effect with a trough at 233 nm and with an inflection point at about 225 nm. The Cotton effect, which can be confirmed by CD (solid line in Fig. 5.16), is almost disappeared by the denaturation of protein. On basis of this phenomenon, both extrema in ORD (233 nm) or CD (222 nm) serves as a rough standard of the α-helix content.

Strong positive Cotton effect occurs in the shorter wavelength region (198 nm in ORD, 191nm in CD). This effect greatly changes and reverses its sign. When the peptide chain form a random coil at pH more than 6, this effect greatly changes and reverses its sign (dotted line in Fig. 5.16). Therefore, this strong Cotton effect in the far-ultraviolet region can also be directly related to the configuration of polypeptide and protein.

Various methods for CD analysis of protein secondary structure have been proposed with their computerized techniques. On the other hand, the CD bands of aromatic transition in protein

are used to understand their behavior and biochemical function in various environments. From behaviors of the CD/ORD of protein molecules under various condition, important biochemical problems shall be being pointed out, and from now, ORD/ CD shall be also actively applied to this field.

(2) Nucleic acid

The ORD of nucleotides was limited within the visible region in the first state, because nucleic acids has a strong absorption band in the ultraviolet region, however the improved spectropolarimeter that became available in 1960 permitted measurements of strongly absorbing samples. By the improvement of the CD/ORD instrument, the Cotton effect of nucleosides and nucleotides in the ultraviolet region has been confirmed and the relationship between the Cotton effect and the higher structure of nucleic acid is being studied. (See Fig. 5.17)

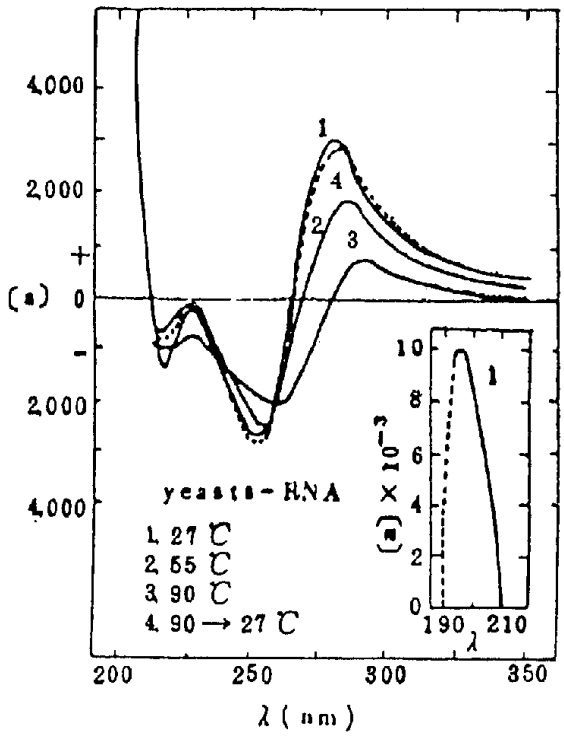


Figure 5.17 ORD curve of DNA

The constituent units of nucleic acid are nucleosides consisting of a purine or pyrimidine base and D-ribose(RNA) or 2'deoxy-D-ribose(DNA), which combine through a phosphate to form polynucleotides.

The following interesting regularity had been found by the application of CD/ORD.

1) Mononucleosides and mononucleotides each show a single Cotton effect at 220 nm to 300 nm.

2) Natural nucleosides and nucleotides are ribosides of β-coordination. These purine and pyrimidine bases show negative and positive Cotton effects respectively. In case of bases of α-coordination, the above relationship is reversed.

3) Derivatives of the same base show the same Cotton effect on the CD/ORD curve regardless of the type of sugar and of the position of the phosphate. The magnitude of the Cotton effect varies characteristically with a kind of the base and with pH-change. The informations on DNA and RNA with ORC/CD are as follows: mononuclectide conformation (mentioned above), tilt of base plane to helical axis, content of base stacking, base sequence of oligonucleotide, confirmation of base-pair (a distinction of single, double, triple strand), a kind of base-pair and its content, and detection of tertiary structural change by using a special base.

Moreover, the quantitative analyses of chromatin structure and left-handed Z-DNA have been studied by use of CD (Fig. 5.18).

Biopolymers include important polysaccaride, in addition to proteins and nucleic acids. CD/ORD is practically applied in this field. It would be no exaggeration to say that ORD and CD are now indispensable means of conformational analysis of optically active polymer.

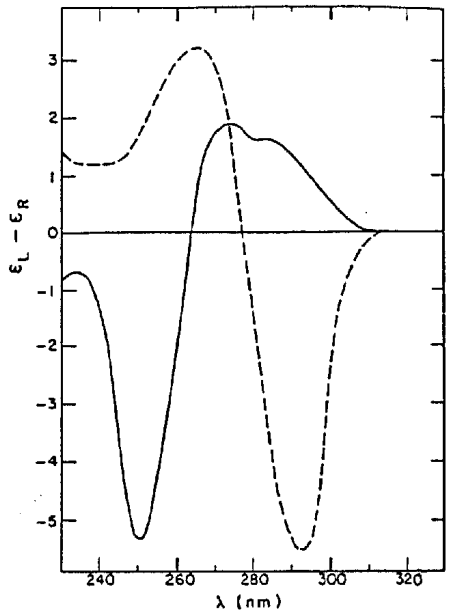


Figure 5.18 CD of poly (dG-dC) • Poly (dG-dC) B form) (—) and Br-Poly (dG-dC) • Poly (dG-dC) (Z form (----) in 15 mM Tris. HCI, pH 7.2/150 mM NaCl/1 mM EDTA.

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2967-5, Ishikawa-machi, Hachioji-shi
TOKYO, JAPAN

Printed in Japan