

# An Optical Rotatory Detector for High-Performance Liquid Chromatography Using Polarization Modulation

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A sensitive and variable-wavelength optical rotatory (OR) detector for high-performance liquid chromatography is presented. This design is entirely different from that of conventional OR detectors consisting of a crossed polarizer pair. By placing a polarizing prism and a retardation plate into a commercial circular dichroism (CD) detector, the OR signal was obtained. The Mueller matrix approach was used to prove the principle of the OR signal appearance. Sugars and 4-androstene-3,17-dione were chosen as test compounds. The limit of detection was below 0.5  $\mu\text{g}$  of injected sucrose at 260 nm, which was superior to that obtained with a conventional OR detector. For 4-androstene-3,17-dione, which is CD active, and shows a large anomalous OR dispersion curve, our detector gave a large OR signal with approximately half the intensity of the CD signal at 340 nm.

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## Introduction

There are various demands for chiral purity determination. The largest comes from the pharmaceutical industry, because two enantiomers may have widely differing biological activity. Direct enantioseparation using chiral columns is a reliable method for this purpose, but it requires much effort to establish the separation conditions, because there is no chiral column that is applicable to all enantiomers. On the contrary, the achiral HPLC method can check the optical purity by using a chiroptical detector based on optical rotatory (OR) or circular dichroism (CD) without separating the enantiomers. However, this method is inferior to the direct separation method in accuracy due to the low sensitivities of these detectors. We first developed a non-modulated OR detector.<sup>1,2</sup> We then developed a more sensitive CD detector<sup>3</sup> and a fluorescence-detected CD (FDCD) spectrometer,<sup>4</sup> which used a modulated beam in the wavelength axial direction. As is obvious from comparisons between OR, CD, and FDCD, the sensitivities are in the order  $\text{OR} < \text{CD} < \text{FDCD}$  whereas the number of compounds that can be analyzed is in the reverse order. Recently, highly sensitive chiral detection methods have been reported, *i.e.*, dissociative photoionization mass spectrometry<sup>5</sup> and thermal-lens CD.<sup>6,7</sup> Both methods are based on the unequal responses from the chiral compounds excited by circularly polarized beams. However, both methods are difficult to apply to actual samples.

Thus, it is important to enhance the sensitivity of the OR detector.

Generally, the optical system of an OR detector uses a Faraday rotator, in which the optical axes of the polarizer and the analyzer intersect at right angles. Therefore, not enough of the probe light reaches the photodetector, and so the Faraday-type OR detector does not use a monochromator to make up for the deficiency of the sensitivity. Theoretically, a modulated OR detector is independent of the light absorption of analytes, but a small OR signal is concealed by a large artifact peak derived from the absorption, as a matter of fact. Thus, a Faraday-type OR detector can not make measurements in the UV region possible where an analyte may have an excellent OR signal. Shindo and his coworkers<sup>8</sup> constructed a polarization modulation spectrometer for obtaining an OR dispersion curve, in which the optical axes of the polarizer and analyzer made an angle of 45°. However, in this instrument, the OR wave with a period of  $2f$  is mixed with a CD wave of  $f$  in the absorption band of chiral analytes, where  $f$  is the frequency of photoelastic modulator (PEM). To solve this problem, we propose a new optical system in which a retardation plate is put in front of the flow-cell in Shindo's system. In this new optical system, an OR wave with a period of  $f$  does not interfere with a CD wave of  $2f$ , and this system makes the signal response double compared with that of Shindo's system, theoretically. The objective of this work is to develop and evaluate a variable-wavelength OR detector with high sensitivity.

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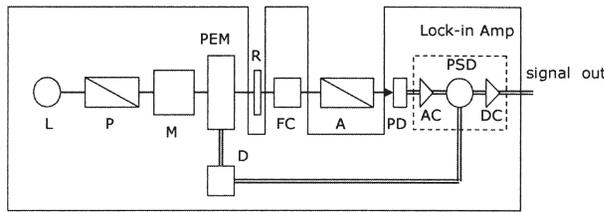


Fig. 1 Block diagram of the proposed OR detector. L, Lamp; P, polarizer; M, monochromator; PEM, photoelastic modulator; R, 1/4 retardation plate; FC, flow-cell; A, analyzer; PD, photodiode; D, PEM driver; AC, ac amplifier; PSD, phase-sensitive detector; DC, dc amplifier. The part within the solid line represents a conventional CD detector.

### Theoretical Development with the Mueller Matrix Approach

Our new OR detector is shown in Fig. 1. It is easy to construct the detector if there is enough space to place a retardation plate and polarizing optics on both sides of the flow-cell assembly of a conventional CD detector. We present the essentials of how to evaluate the performance of our detector with the Mueller matrix approach. To simplify the calculations, all coefficients are normalized. The intensities of the Stokes vector components of an monochromatized incident beam are given as  $S_0 = 1, S_1 = 0, S_2 = 0,$  and  $S_3 = 0$  are expressed as

$$\begin{pmatrix} 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} \quad (1)$$

The Mueller matrix of the polarizer, whose optical axis is horizontal, is given as

$$\begin{pmatrix} 1 & 1 & 0 & 0 \\ 1 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \quad (2)$$

The intensity of light transmitted through the polarizer can be calculated from the matrix product of Eqs. (1) and (2):

$$\begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \end{pmatrix} \quad (3)$$

The Stokes vector expressed in Eq. (3) exhibits a linear polarized beam. When the periodic phase difference of the photoelastic modulator (PEM) is  $\pm\delta$  at a frequency of  $f$  Hz, the modulator retardation,  $\gamma$  is expressed as

$$\gamma = \delta \sin(2\pi ft)$$

where  $t$  is the elapsed time. The Mueller matrix expression for the PEM is given by

$$\begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & \cos \gamma & 0 & -\sin \gamma \\ 0 & 0 & 1 & 0 \\ 0 & \sin \gamma & 0 & \cos \gamma \end{pmatrix} \quad (4)$$

The modulation wave generated by transmitting through the PEM is expressed as the matrix product of Eqs. (3) and (4):

$$\begin{pmatrix} 1 \\ \cos \gamma \\ 0 \\ \sin \gamma \end{pmatrix} \quad (5)$$

Considering a quarter-wave retardation plate as the PEM at  $\gamma = \pi/4$ , the modulation wave transmitted through the plate is given as

$$\begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \end{pmatrix} \begin{pmatrix} 1 \\ \cos \gamma \\ 0 \\ \sin \gamma \end{pmatrix} = \begin{pmatrix} 1 \\ -\sin \gamma \\ 0 \\ \cos \gamma \end{pmatrix} \quad (6)$$

In general, the value of  $\delta$  in the CD spectrometer is set at  $\pi/4$ , and thus the second vector in Eq. (6) oscillates between  $-1$  (meaning that the optical axis of the linearly polarized beam is vertical) and  $1$  (meaning that the axis is horizontal) at a frequency of  $f$  Hz, and the fourth vector oscillates between  $0$  (corresponding to a non-polarized beam) and  $1$  (corresponding to a circularly polarized beam) at a frequency of  $2f$  Hz. In other words, the PEM modulation wave is converted by the quarter-wave retardation plate to a beam that switches between linearly polarized states, at the modulator frequency, alternatively polarized along  $\pi/4$  and  $-\pi/4$  relative to the PEM axis.

The components of linearly and circularly polarized beams intersect at right angles on the Poincaré sphere with radius  $S_0$ , if the assumption is made that  $S_1, S_2,$  and  $S_3$  are rectangular vectors. An OR of  $\Delta$  represents the rotation of the plane inclusive of the  $S_1$  and  $S_3$  axes, and a CD of  $\Psi$  is the rotation of the plane of the  $S_2$  axis. The Mueller matrix for this optical component is given as

$$\begin{pmatrix} 1 & 0 & 0 & -\cos 2\Psi \\ 0 & \sin 2\Psi \cos \Delta & \sin 2\Psi \sin \Delta & 0 \\ 0 & -\sin 2\Psi \sin \Delta & \sin 2\Psi \cos \Delta & 0 \\ -\cos 2\Psi & 0 & 0 & 1 \end{pmatrix} \quad (7)$$

The analyzer is placed on the transmitting light side of the flow-cell, and is oriented with its polarizing axis rotated  $45^\circ$  from that of the polarizer. The Mueller matrix of this optics system is expressed as

$$\begin{pmatrix} 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix} \quad (8)$$

The final light intensity at the photo-detector is calculated from the matrix product of Eqs. (6) – (8):

$$\begin{pmatrix} 1 - \cos \gamma \cos 2\Psi + \sin \gamma \sin 2\Psi \sin \Delta \\ 0 \\ 1 - \cos \gamma \cos 2\Psi + \sin \gamma \sin 2\Psi \sin \Delta \\ 0 \end{pmatrix} \quad (9)$$

The Stokes vector component,  $S_0$ , indicates the energy of the light, and thus we can expand this term in a Fourier series as follows:

$$1 - \cos \gamma \cos 2\Psi + \sin \gamma \sin 2\Psi \sin \Delta = 1 - J_0(\delta) \cos 2\Psi - 2J_1(\delta) \sin 2\Psi \sin \Delta \sin(2\pi ft) - 2J_2(\delta) \cos 2\Psi \cos(4\pi ft) - 2J_3(\delta) \sin 2\Psi \sin \Delta \sin(6\pi ft) - 2J_4(\delta) \cos 2\Psi \cos(8\pi ft) - 2J_5 \dots, \quad (10)$$

where  $J_n(x)$  are Bessel functions of the  $n$ th order. It can be seen

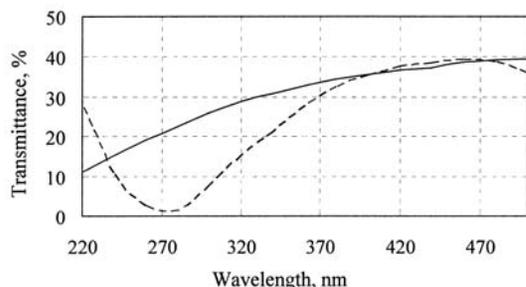


Fig. 2 Light transmittance curves for quartz plates with about 6  $\mu\text{m}$  (solid line) and 26  $\mu\text{m}$  (broken line) thickness, sandwiched between two prisms being at parallel axes.

that the CD wave does not interfere with the OR modulation wave when the latter has an amplitude of  $2\sin\Delta$  and a frequency of  $f$  Hz. Because the CD on the Poincaré sphere is observed as a rotation of the plane inclusive of the  $S_2$  axis, which exists at the center of the  $S_1$  and  $S_3$  axes,  $\sin 2\Psi$  in the third term of Eq. (10) is considered to be  $\sin(\pi/4 + \text{CD})$ , *i.e.*, to be unity. In a conventional CD spectrometer, the CD signal is the amplitude of the modulation wave at frequency  $f$  Hz. Thus, a remarkable feature of our optical system is that the output of the CD spectrometer is equivalent to the OR value.

The OR modulation wave in Shindo's optical system,<sup>7</sup> obtained by expanding the Stokes vector in the same manner, has a frequency of  $2f$  Hz, and its amplitude is reduced to half that in our optical system. This can cause problems, such as giving rise to a small OR signal on a large CD wave, and exchanging the lock-in amplifier carrier frequency for  $2f$ . Although our proposed system can remove these defects, the retardation plate works at a single wavelength, and thus the measurement of OR dispersion curves may be impossible. Changing the measuring wavelength may require another retardation plate.

## Experimental

The LC system consisted of a Hitachi (Tokyo, Japan) L-6200 pump, a Hitachi degassing unit, a Rheodyne (Cotati, CA, USA) 7125 injector with a 20- $\mu\text{L}$  loop, a Jasco (Tokyo, Japan) CO-2065 column oven, and an OR detector. The OR detection system was constructed by using the optical system of a Jasco CD-2095 Plus detector. A prototype flow-cell assembly, whose light path length and cell volume were 10 mm and 16  $\mu\text{L}$ , respectively, was used. A retardation plate, purchased from Sigma Koki (Saitama, Japan), was placed on the incident beam side of the flow-cell, whose optical axis was inclined by  $45^\circ$  from the polarizing axis (horizontal plane). A Gran-Taylor prism (1 cm square, Sigma Koki) was placed on the transmitted beam side with the same angle to the retarder.

Sugars were analyzed on a 15 cm  $\times$  6 mm i.d. amino column (DC-613, Shodex, Tokyo, Japan) at room temperature. Acetonitrile-water (5 + 3) was used as the mobile phase at a flow rate of 0.6 mL/min. An analysis of 4-androstene-3,17-dione was done on a 25 cm  $\times$  4.6 mm i.d. reversed-phase column (Capcell Pak UG120, Shiseido, Tokyo, Japan) maintained at  $40^\circ\text{C}$ . Acetonitrile-water (3 + 2) was used as the mobile phase at a flow rate of 1.0 mL/min.

UV-visible spectra were obtained with a Varian Technologies Japan (Tokyo, Japan) Cary 50 spectrophotometer.

4-Androstene-3,17-dione was purchased from MP Biomedicals

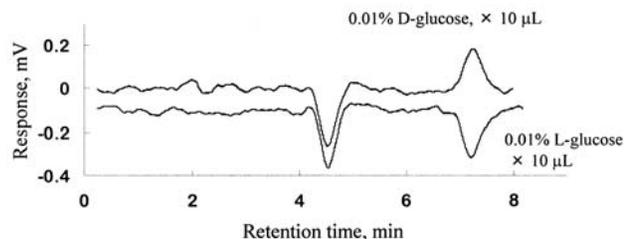


Fig. 3 OR chromatograms of D- and L-glucose at 260 nm.

(Solon, OH, USA). HPLC-grade acetonitrile and other reagents were purchased from Wako Pure Chemicals (Osaka, Japan). Water was purified with a Millipore (Billerica, MA, USA) Elix<sup>®</sup> ultrapure water system.

## Results and Discussion

### Preparation of 1/4 retardation plate

In our system, the spectral resolving power of the CD-2095 detector is sacrificed to ensure high sensitivity. The spectral bandwidth of this detector is fixed at 20 nm. Thus, circularly polarized light with a wide wavelength range is required, *i.e.*, a thin retardation plate is necessary. The thickness of the quartz plate, which is a first-order 1/4 retarder in the UV region, is only a few  $\mu\text{m}$ . We prepared two retardation plates with more practical thicknesses of about 6  $\mu\text{m}$  and about 26  $\mu\text{m}$ , by combining several retardation plates. Figure 2 shows transmittance curves of the plates, which were sandwiched between two prisms inclined by  $45^\circ$  against the optical axis of the plate. The tops of these curves correspond to  $1/2\lambda$  retardation wavelengths, and the polarizing axis of the incident light is rotated by  $90^\circ$ . The latter prism does not transmit light whose polarizing axis is rotated by  $90^\circ$ . The troughs are  $1\lambda$  retardation wavelengths. The 6  $\mu\text{m}$  plate gave a  $1/4\lambda$  retardation around 260 nm, and the 26  $\mu\text{m}$  plate gave a  $3/4\lambda$  retardation around 340 nm. It is clear that the circularly polarized light obtained by these plates was satisfactorily usable, even if the spectral bandwidth was set at 20 nm.

### Analysis of sugars

We analyzed sugars with HPLC-OR detection at 260 nm. The specific rotations of sugars possessing no UV absorption will be about an order of magnitude larger in the UV region than at the Na-D line, due to the OR dispersion effect.<sup>9</sup> Since a commercially available calcite prism, which is needed to be installed in the optical system of our OR detector, becomes less transparent in the UV region, the detection wavelength was set at 260 nm. Glucose was eluted by the normal-phase mode with a water-acetonitrile eluent system. As can be seen in Fig. 3, D- and L-glucose showed entirely the same retention times, but the direction of the peak was inverted. The specific rotation of D-glucose shows a positive value in the UV and visible regions, and thus the direction of the principal axis of the retarder was regulated to obtain a positive peak for the D-isomer. Although the detection limit of a conventional Faraday-type OR detector is about 2  $\mu\text{g}$  sucrose per unit path length (10 mm),<sup>10</sup> that by the proposed detector for sucrose was below 0.5  $\mu\text{g}$  with a signal-to-noise ratio of 3:1. In spite of using opaque optics in the UV region, the proposed OR detector is much superior to the conventional model in sensitivity.

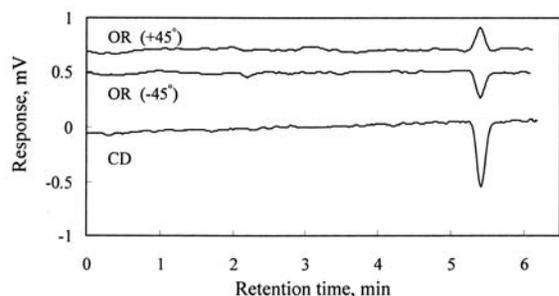


Fig. 4 OR and CD chromatograms of 4-androstene-3,17-dione at the Cotton band. In the upper trace, the axis of the analyzer was inclined by  $+45^\circ$  (clockwise viewed from the light source) from that of the polarizer. The inclination was reversed in the middle trace.

#### OR detection at the Cotton band

Our proposed detector can be highly sensitive by using an anomalous OR dispersion curve at the Cotton absorption band. 4-Androstene-3,17-dione has a small absorption band at 310 nm, derived from a conjugated carbonyl group, and shows a large anomalous OR dispersion curve with a maximum value ( $[\alpha] = 3800$ ) around 320 nm.<sup>11</sup> We analyzed this compound with reversed-phase HPLC with OR and CD detections. As mentioned above, the spectral bandwidth of this detector is fixed at 20 nm and the specific rotation of this chiral dione below 320 nm decreases rapidly. In addition to these facts, its CD spectrum shows a trough extreme at 340 nm ( $\Delta\epsilon = -1.3$ ), so the detection wavelength was set at 340 nm. Its specific rotation at this wavelength is more than  $1000^\circ$ . Figure 4 shows chromatograms of 4-androstene-3,17-dione made with an injection of 3  $\mu\text{g}$  on-column. The lower trace is the CD signal and the upper and middle traces are the OR signals. We made the noise levels of these chromatograms almost uniform to compare the peak intensities. The dione showed a negative CD signal at around 5.4 min. On the contrary, signals with about half intensities of the CD signal were obtained when we installed a retardation plate with 26  $\mu\text{m}$  thickness and a prism (analyzer). The polarizing axis of the analyzer was rotated by  $\pm 45^\circ$  with respect to that of the stationary prism. The rotation of the prism inverted the direction of peaks, and this inversion clearly shows that these peaks were derived from the OR property of the

compound. By installing optics that have better UV light transmittance, the OR sensitivity may be improved in the future.

#### Conclusion

A variable-wavelength OR detector for high-performance liquid chromatography was constructed by inserting a retarder and a prism into a conventional CD detector. In this optical system, the phase angle between the two prisms (one of them is essential for a CD detector) was set at  $\pi/4$ , so there was no shortage of light intensity. When monitoring sugars in the UV region, the proposed detector was inferior to the conventional OR detector in sensitivity. 4-Androstene-3,17-dione showed a large anomalous OR dispersion curve, and its OR signal was almost equal to its CD signal.

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