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Multi-beam polarized photometric detector for high-performance liquid chromatography

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Abstract

A novel multi-beam polarized photometric detector (PPD) for high-performance liquid chromatography (HPLC) is described. By pairing a polarizing prism with a thin quartz plate as a retarder, many linear polarized beams are produced at every 1/2 wavelength of the plate, and the polarizing axes of the adjacent beams intersect each other. The addition of another prism inclining its polarizing axis by $\pi/4$ against the first one enables the simultaneous measurement of optical rotations based on the PPD at many wavelengths. The combination of these optics with a photo-diode array detector can be used to construct a modulated type polarimeter. This detector is designed to measure the optical rotation of an analyte at its absorption band. The spline function connecting the points at 1/4 wavelengths of the plate was used as a baseline to extract the PPD waves. The use of the similarity factor as a noise filter gave high sensitivity. Application of the proposed technique to an analyte carrying the Cotton absorption band provided good results.

Keywords: Multi-beam polarized photometric detection; Detection, LC; Polarized photometric detection

1. Introduction

Several polarimetric detectors based on different principles are presently available for high-performance liquid chromatography (HPLC). Although modulated polarimeters are very effective in measuring small optical rotations, when they are used with low-volume flow-cells, such as those used as HPLC detectors, the light intensity is insufficient to allow optimum performance. Consequently, the sensitivity of these detectors is not significantly different from that of non-modulated polarimeters with simple optical systems. We have proposed a non-modulated polarimetric detector, called a polarized photometric detector (PPD), which enables measurement of the optical rotation of transparent, optically active analytes [1,2]. The main disadvantage of a conventional polarimetric detector is its limited sensitivity. Solving this problem would result in a much wider use of these detectors. Generally, the optical rotation of a chiral compound is greatly changed at its absorption band [3], so utilization of this band might enhance the detection sensitivity. But non-modulated type detectors do not permit measurements at the absorp-

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tion band. Similarly, modulated type detectors based on a Faraday rotator use non-diffractive light to compensate for the low light intensity [4], and thus are not suited for this purpose.

Recently, we have developed a multi-beam circular dichroism (CD) detector that utilizes the optical system of a conventional photodiode array (PDA) detector [5]. This detection method is based on extraction of a modulated CD wave superimposed on the absorption spectrum. The CD wave is produced by a multiple-order retardation plate, which creates circularly polarized beams with opposite rotational senses at each adjacent quarter-wave. A chiroptical detector utilizing modulation in the wavelength axial direction, such as a multi-beam CD, can be easily constructed with a conventional optical system. This combination of a polarizer and the retardation plate also creates linear polarized beams at many wavelengths by an intermediate process in the inversion of the rotational sense of circularly polarized beams. Moreover these polarizing planes perpendicularly intersect each other. Setting up a second polarizer on the transmitted light side of a flow-cell with its polarizing axis inclined by $\pi/4$ with respect to the initial plane of polarization would generate the modulated waves created by the optical rotation, i.e. the PPD waves, in the wavelength direction. In the present study, we first made static spectrometric measurements of the PPD waves and then a liquid chromatographic (LC) system was used to identify optically active analytes in the near-ultraviolet region. We focused on the optical rotatory dispersion curve at the Cotton absorption band, because many optically active compounds exhibit the maximum or minimum value at the end part of this absorption band. This is expected to allow the modulated polarimetric detector to attain high sensitivity. Features and limitations of the multi-beam PPD are discussed.

2. Experimental

2.1. Apparatus

The LC system consisted of a Shimadzu (Kyoto, Japan) LC-10AD pump, a Rheodyne (Cotati, CA, USA) 7125 injector, a Tosoh (Tokyo, Japan) CO-8011 column oven and a Shimadzu SPD-M10Avp PDA detector. Two Gran-Taylor prisms (1 cm^3, Sigma Koki, Hidaka, Saitama, Japan) were placed separately in the compartments in the PDA detector; one for the wavelength calibrating filter and the other for the spectral diffraction. The phase angle between the two prisms was set at $\pi/4$. A quartz plate (5 mm^2, 0.371 or 0.455 mm thick) as a multi-order retarder, purchased from Five Lab (Kawasaki, Japan), was placed on the incident light side of the flow-cell, whose principal axis was inclined by $\pi/4$ from the first polarizing axis. The three-dimensional data obtained were converted into ASCII format with the aid of the detector's bundled software, and were processed with a spreadsheet program (Excel, Microsoft Corp., Redmond, WA, USA).

The PPD waves by static method were confirmed with a Shimadzu UV-2200 spectrophotometer in which a quartz plate with 1.133 mm thickness (Five Lab) and two prisms were combined.

2.2. Chromatographic procedures

Analysis of camphor was performed on a 25 cm×4.6 mm I.D. reversed-phase column (Capcell Pak UG120 Å, Shiseido, Tokyo, Japan) maintained at 40°C. Acetonitrile/water (3+1) was used as the mobile phase at a flow-rate of 0.6 ml min^{-1}.

Analysis of tryptophan was performed on a 15 cm×6 mm I.D. reversed-phase column (RSpak DS-613, Shodex, Tokyo, Japan) maintained at 40°C. Subsequently, 2 m*M* each of *o*-phthalaldehyde (OPA) and *N*-acetylcysteine (NAC) in 10 m*M* disodium hydrogen phosphate, 2 m*M* sodium hydroxide/acetonitrile (8+1) was used as the mobile phase at a flow-rate of 1.5 ml min^{-1}.

2.3. Reagents

Single enantiomers of camphor were purchased from Sigma Aldrich Japan (Tokyo, Japan). HPLC-grade acetonitrile and other reagents of guaranteed grade were purchased from Wako Pure Chemicals (Osaka, Japan).

2.4. Spectral similarity factor

To evaluate the match between the spectra ob-

tained and the authentic ones, the concept of similarity factor has received practical application [6,7]. In a spectrum consisting of n data in wavelength, when the absorbance at each wavelength is regarded as the magnitude of a vector, a vector is composed in n-dimensional space from these vectors. The directions of two vectors obtained from an experimental and an authentic spectrum greatly depend on their similarity. The vectorial angle, θ, is calculated from a formula for the scalar product:

$$\cos\theta = \sum_{i=1}^{n}(A(i)\cdot O(i)) \Big/ \left(\left(\sum_{i=1}^{n}A(i)^2\right)^{1/2} \cdot \left(\sum_{i=1}^{n}O(i)^2\right)^{1/2}\right)$$

where $A(i)$ is the value of the absorbance of the authentic component over wavelengths (i), and $O(i)$ is that of the objective one. The value of $\cos\theta$ approaches 1 if both spectra agree well and becomes -1 if they are highly different. This value is called the similarity factor and is a very good indication of similarity of spectra since the values over the whole spectra are compared.

3. Results and discussion

3.1. Principle of multi-beam PPD

PPD is a method for determining the optical rotation of a chiral compound as a change in absorbance by sandwiching the sample cell between two polarizers, largely altering the phase angle between them (see Fig. 1 in Ref. [2]). However, this technique restricts the measurement to the non-absorbing region of an analyte, because the baseline is not defined in the absorption region. To solve this problem, it is necessary to determine the absorbance value as a baseline, and the change in absorbance due to the optical rotation can be calculated by subtracting this value. Although a split-type flow-cell [8] is theoretically able to secure a baseline, the individual difference in signal response between two photodiodes makes the measurement of the absolute change in absorbance impossible. A multi-diode system is desired for the solution of this problem. As shown in Fig. 1, the combination of a prism and a retardation plate acts as a circular polarizer, but it simultaneously produces many linear polarized beams at $n\pm1/2$ wavelengths of the plate. If the second prism is oriented parallel to the plate axis, every linear polarized beam, whose polarizing planes mutually incline on either side at $\pi/4$, causes PPD action in opposite directions alternately. Because there is no difference in the light intensities transmitted through the second prism between the circularly polarized beam and the linearly polarized beam whose plane of polarization is rotated precisely $\pi/4$ from the prism axis, no change occurs in the baseline spectrum without optical rotation. A finite optical rotational signal produces an output wave possessing the maximum and minimum points at $n\pm1/2$ wavelengths of the plate. The amplitude of this PPD wave is proportional to the rotation angle.

In the static mode, measurement of the PPD waves

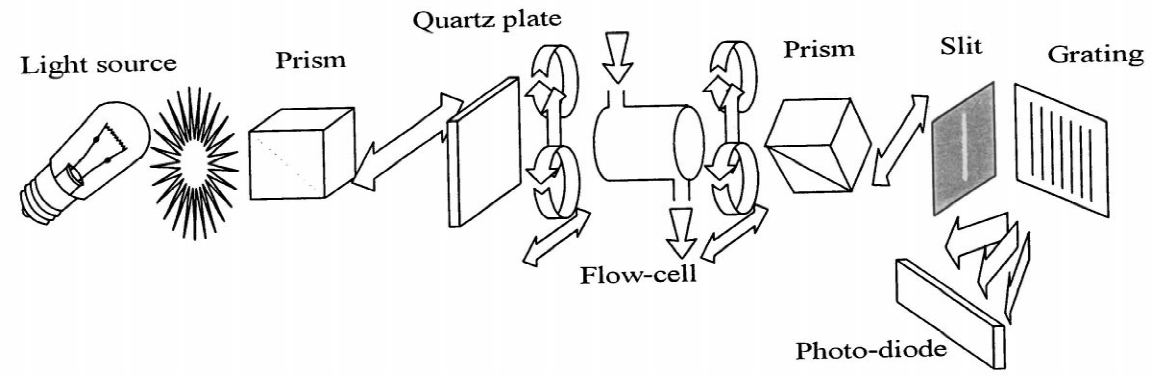

Fig. 1. Schematic diagram of the optical configuration for the multi-beam PPD by based on a conventional PDA detector.

of an optically active sample was attempted by setting the optics across the cell in the spectrophotometer in the same manner as in Fig. 1. To detect the PPD waves accurately, the spectral band width of the transmitted beam must be narrowed less than a quarter-wave interval of the quartz plate. The enhancement in the resolving power of the spectrophotometer results in a noisy spectrum due to the decrease in light intensity. Thus, the resolving power was fixed at 2 nm, and a 1.133-mm-thick quartz plate with a half-wave interval of about 7 nm at 300 nm was employed. Some typical absorption spectra are shown in Fig. 2, and the waves derived from the PPD are clearly noticeable in the spectra. While the change in the amplitude of the PPD wave of sucrose, which has no chromophore, is small, that of (+)-camphor, which has a Cotton band based on a carbonyl group around 290 nm, steeply increases in this region.

3.2. PPD at the absorption band by a PDA detector

Theoretically, the light intensity from the light source is reduced by a half by the first prism, and is reduced by a half again by the second prism. Further, light absorption by the prisms also reduces the intensity. The sensitivity of the PPD greatly depends on the light intensity [9]. There was a marked lowering in the light intensity in the ultraviolet region when two Gran–Taylor prisms were set with a phase difference of $\pi/4$ in the PDA detector, and it was impossible to detect the PPD wave below 300 nm. Unfortunately, the highly sensitive polarimetric detection in the ultraviolet region, where the enhancement of the optical rotation is expected, by this multi-beam PPD would be carried over until the brighter optics becomes available.

The effect of the number of diodes used for data acquisition at a specific wavelength on the noise level was investigated with reference to our previous experiment [5]. Although increasing the number of diodes reduces the noise in regions where the intensity of transmitted light is low, a period of PPD wave is required to be expanded and it makes the detection of the wave difficult, because a period of the baseline wander is not so short. Thus, four diodes, corresponding to a spectral bandwidth of 4 nm, were used for subsequent experiments. To make the 1/2 wavelength interval between 10 and 15 nm, two quartz plates, 0.371 and 0.455 mm, were properly used.

The absorption spectra of (+)- and (−)-camphor are shown in Fig. 3A. The spectra were obtained by injecting 7 μg of each compound in a chromatographic cell and by using a quartz plate with 0.371 mm thickness in the PDA detector. These spectra were differentially processed to selectively detect the analytes as described in our previous paper [5]. All data processing was performed by the Savitzky–Golay polynomial algorithm [10]. The second derivative spectra of (+)- and (−)-camphor twined round each other and the points of intersection of the

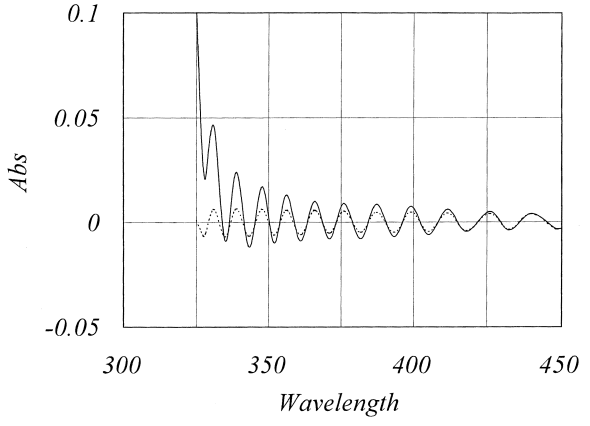

Fig. 2. Absorption spectra of 2% (+)-camphor (—) and sucrose (·····).

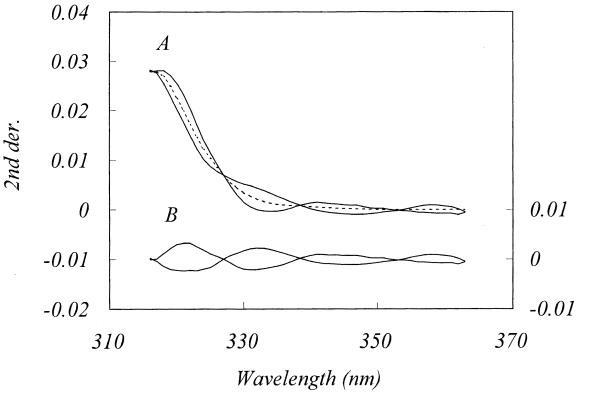

Fig. 3. Extraction of the PPD wave by using a spline function as a baseline.

two lines corresponded to the 1/4 wavelengths of the quartz plate. To extract the CD wave from the absorption spectrum, we previously used the spectrum of a racemate as a baseline [5]. The PPD wave suitable for the proposed method exists on the end part of the absorption band in contrast to the CD wave which exists on the absorption top, and thus its baseline may be a gentle curve. Thereupon, we attempted to extract the PPD wave by using the spline function as a baseline, which passes through the values at 1/4 wavelengths. The dotted line in Fig. 3A represents this function, and the PPD waves obtained from this procedure are shown in Fig. 3B (right axis).

The PPD chromatogram based on this amplitude was prepared. The peak of camphor was buried in the baseline noise even when we used the aggregate of the amplitude values as the vertical axis (Fig. 4A). The similarity factor was used as the noise filter. The value of this factor is localized around 1 (it can never exceeds 1) when the magnitudes of the vectors are always positive such as an absorbance value, whereas it varies from 1 to −1 when their signs are mixed. The PPD wave is the sinusoidal curve having crossover points at 1/4 wavelengths, so this wave seemed to be suitable for the application of the similarity factor. As the spectrum in Fig. 4B indicates, this factor was nearly 1 in the analyte eluting zone and varied widely in the other zones. The peak of camphor clearly appeared from the noise when the square of this factor was multiplied by the original amplitude values (Fig. 4C). Practically, this chromatogram is improved by a smoothing procedure. The detection limit can be seen to be around 1 μg of camphor on the column, and this value is equal to that of the multi-beam CD method [5].

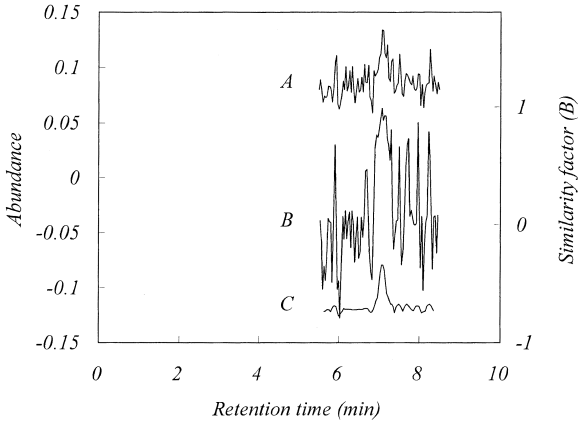

Fig. 4. Similarity factor as a filter to smooth the noise fluctuation. Sample, 7 μg of camphor on column; retardation plate, 0.371-mm-thick; detection wavelength range, 300–370 nm; spectral bandwidth, 3 nm; other chromatographic conditions see Experimental section.

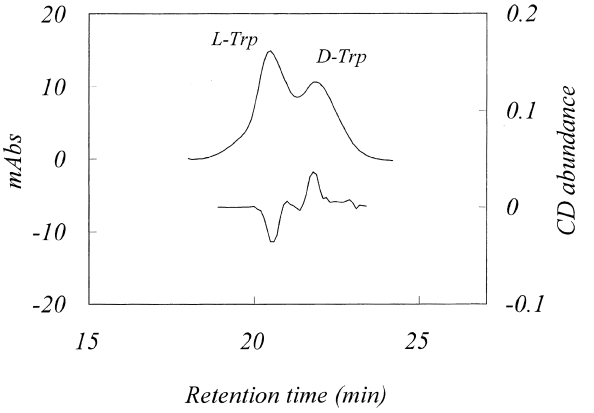

Fig. 5. Chromatograms of OPA-tryptophan. Sample, 10 nmol of rac-Trp on column; retardation plate, 0.455-mm-thick; detection wavelength range, 480–600 nm; spectral bandwidth, 4 nm; other chromatographic conditions see Experimental section.

Fig. 5 shows the ultraviolet (350 nm) and multi-beam PPD chromatograms of a tryptophan derivative. The optical activity of amino acids is generally enhanced by derivatization [11]. We used an on-column derivatization technique in which OPA and NAC reagents were added to the mobile phase [12]. The formation of diastereomers with NAC made D,L-tryptophan separate chiroptically. Although their separation was not complete, the similarity filter removed the influence of the mixed zone of the diastereomers. These results lead to the possibility of highly sensitive polarimetric detection by the proposed method at the end part of the Cotton absorption band.

4. Conclusion

A multi-beam PPD detector, which can measure optical rotation at the absorption band of an analyte, was constructed by inserting two prisms and a thin quartz plate into a PDA detector. In this system, the

shortage of light intensity could not be overcome because the phase angle between the two prisms was set at $\pi/4$. Consequently, the detection limit of transparent sucrose was several micrograms on the column even though the measurement was made in the near-ultraviolet region, and was about an order of magnitude lower than that of the normal PPD [8]. However, the proposed detector gave high sensitivity for an analyte carrying a Cotton absorption band. The detection performance for the proposed technique would clearly be improved by using a brighter optical system such as a lamp of high intensity or prisms suitable for the ultraviolet region.

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