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Optimization of a polarized photometric detector equipped with a split-type flow cell and its analytical application to oligo-saccharides[1]

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Abstract

A novel, non-modulated polarimeter called a polarized photometric detector (PPD) was previously described by the authors. The PPD enables the measurement of the optical rotation of chiral compounds as a change in absorbance by placing two linear polarizers on either side of a flow cell of a conventional photometric detector. The present study describes the optimization of the conditions of PPD for highly sensitive detection of saccharides.

To maximize the light intensity, the light balancing filter and slit were removed from the detector (Shimadzu model SPD-10AV). These modifications resulted in an approximately 15-fold increase in the incident light intensity when the maximum current was applied to the lamp. When this intense light was transmitted through the polarizers, the signal intensity followed the theoretical equation for phase angles up to around 1 rad. If the energy of the transmitted light was less than 700 mV, however, the baseline noise was too great to determine the chiral analyte accurately. Setting the phase angle between two polarizers at 50° and the detection wavelength at 400 nm provided the most suitable conditions. This detector was applicable for the determations of oligosaccharides in foodstuffs separated by HPLC using gradient elution. © 1997 Elsevier Science B.V.

Keywords: Foodstuff; Gradient elution; Oligosaccharide; Optimization; Polarized photometric detector

1. Introduction

In previous work, a novel, non-modulated polarimetric detector, called a polarized photometric detector (PPD) [1,2] has been proposed; this detector was made by placing two linear polarizers on either side of a flow cell of a conventional

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UV-visible photometric detector. The PPD enables the measurement of the optical rotation of chiral compounds as a change in absorbance. To improve its detection performance and sensitivity, a split-type flow cell has been developed in which the column effluent passes through both the sample and reference sides [3]. This cell offers some advantages over the normal type. One advantage is that the opposite phase angles in the two cells doubles the pathlength. In addition, it is possible to offset the light absorbance of the analyte and/or the refractive index change that occurs during the gradient elution.

In respect of the sensitivity, it has been stated that the detection limit of the conventional polarimetric detector equipped with a Faraday rotator is $10^{-5°}$ [4]. The signal intensity of the PPD can be magnified without limitation to increase the phase angle between two polarizers [2]. However, increasing the angle brings about a decrease in intensity of the transmitted light through the polarizers so that the baseline noise becomes greater. Altogether a high power of light source is required to magnify the signal intensity without damaging the signal-to-noise (S/N) ratio, the increase in intensity of the incident light on the polarizer reduces the quality of the plane polarized light that is produced by the polarizer, and the signal intensity becomes smaller than the theoretical value. The greatest advantage of the PPD is that it can be easily constructed from an optical system that is designed for absorbance measurement. However, there is no room to incorporate a bulky part such as a polarizing prism in a conventional photometric detector so that the use of the polarizing filters is essential. In the present work, an attempt was made to establish a suitable PPD system by pairing the filters with the photometric detector for highly sensitive determination of saccharides; this system was applied as an on-line detector of oligosaccharides in foodstuffs in an HPLC gradient elution system.

2. Experimental

The gradient elution system comprised two Shimadzu (Kyoto, Japan) LC-10AD pumps, a mixer and an SLC-10A system controller. Separation of saccharides was performed on a 250 × 4.6 mm i.d. amino bonded column (Asahipak NH2P-50, Shodex, Tokyo, Japan) kept at 40°C in a Jasco (Tokyo, Japan) 860-CO air circulating oven by using isocratic and gradient elution with acetonitrile-water. All eluents were degassed using ultrasonic agitation to minimize air bubbling formation in the cells. Sample injection was via a Rheodyne (Cotati, CA, USA) 7125 injector. The optical activity detector was constructed by inserting the split cell assembly [3] in a Shimadzu SPD-10AV spectrophotometric detector. Construction of the optical system of PPD is shown in Fig. 1. Slight modifications of the cell assembly were made. The outlet of the effluent was divided into each cell so that it was easy to adjust the flow balance between two cells. Heat exchange of the effluents between flowing in and out was performed due to the restraint of the baseline disorder by the change in heat. Nippon Polaroid (Tokyo, Japan) HN32 linear polarizers (laminated in plastic, 0.01 in thick) were set at $+50°$ for the sample side cell and $-50°$ for the reference side cell (see Fig. 1). The detection wavelength was 400 nm. Chromatograms were recorded on a Shimadzu Chromatopac C-R7A data processor. A polarimetric detector equipped with a Faraday

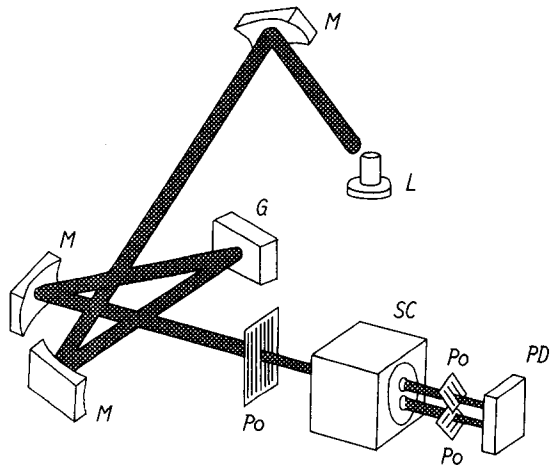

Fig. 1. Schematic diagram of PPD optical system. L, light source; M, mirror; G, grating; Po, polarizer; SC, split-type flow cell; PD, photodiode.

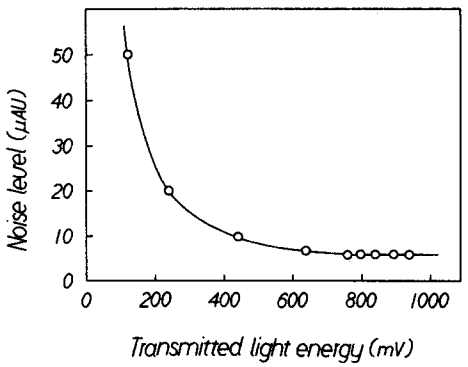

Fig. 2. Relationship between noise level and transmitted light energy.

rotator, (Jasco OR-990) was used to evaluate the performance of the PPD.

HPLC-grade acetonitrile was purchased from Wako Pure Chemical (Osaka, Japan) and was used as received. D-fructose, sucrose, maltose monohydrate, purchased from Nacalai tesque (Kyoto, Japan), and D-glucose, from Wako, were of guaranteed grade. Maltotriose, isomaltooligosaccharides, fructooligosaccharides, purchased from Wako, and isomaltose, from Tokyo Kasei (Tokyo, Japan), were of extra pure grade. The glucose syrup was purchased from Nihon Shokuhin Kakou (Fuji, Japan).

3. Results and discussion

The relationship between the transmitted light energy and the noise level at the SPD-20AV detector used in this work was considered first. Fig. 2 indicates the effect of the light intensity on the noise level where the detector response was set at 1 (fast). This detector showed a constant noise level at transmitted light energies above 700 mV; the noise rapidly increased below this level. This result suggests that a transmitted light energy of 700 mV or above will allow high sensitivity measurements to be made.

The next step was to modify the photometric detector somewhat to obtain a light intensity that was as large as possible. Two filters, one for heat absorption and one for light balancing, are located just in front of the tungsten-halogen lamp in this photometric detector. The latter was removed because there is no great need for optical activity detection in the visible region. Generally, the light from the lamp passes through a slit and is then dispersed by a diffraction grating before reaching a photo-diode. The slit was also removed because the optical rotatory dispersion of saccharides shows a plain curve. Applying the maximum current to the lamp under this instrumental condition increased the intensity of incident light by about 15 times greater then that of the normal intensity in the range 450–600 nm. Because the light balancing filter, which becomes cloudy below 400 nm, had been removed, the increase in intensity was even greater at wavelengths below 400 nm. Using very powerful incident light, the effect of phase angle between the polarizers on the signal intensities was investigated. In PPD, theoretically, the signal intensity is proportional to a tangent of the phase angle (see Eq. 6 in [2]). The relationship between the phase angle and the peak height of a 10 μl injection of 1% sucrose is shown in Fig. 3. The ordinate expresses values relative to the peak height at a phase angle of 50°. Measurements were peformed by suppressing the light intensity according to the phase angle to avoid excessive irradiation on to the photo-diode. When the

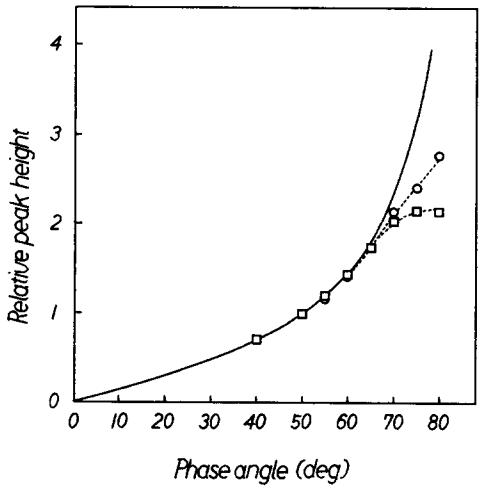

Fig. 3. Deviation of signal intensity from the theoretical values by altering the phase angle between two polarizers. A solid line indicates the theoretical value and circles and squares are the experimental values at 550 and 450 nm, respectively.

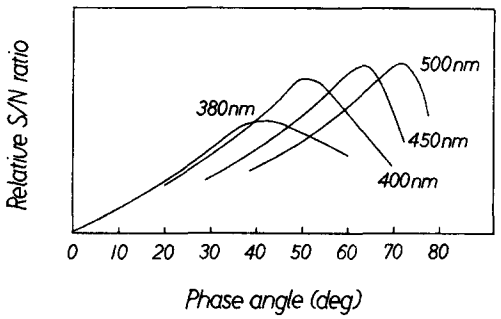

Fig. 4. Changes in the S/N ratio with the phase angle at various wavelengths.

phase angle exceeded 65°, the difference between the experimental and theoretical values became large. The large differences at 450 nm are derived from the extinction ratio of the polarizer which showed almost a constant value above 500 nm but deteriorated below 450 nm. Thus, the difference in both values may increase further as the measuring wavelength becomes shorter.

The phase angle between the two polarizers that optimizes the S/N ratio would be an angle at which the light energy barely exceeded 700 mV. The spectral energy distribution of the light after passing through the polarizers shows a downward curve below 600 nm so that the optimum phase angle would become larger as the measuring wavelength is increased. However, measurements at a long wavelength with a large phase angle can never offer the highest sensitivity because the optical rotation of saccharide generally becomes small in that region. Moreover and contrary to the theory, setting the phase angle above 65° brought about only a small increase in signal intensity (Fig. 3). The changes in the relative S/N ratio with the phase angle calculated at various measuring wavelengths are compared in Fig. 4. Although the maximum values of the S/N ratio increased as the wavelength became longer, the rate of increase above 400 nm was slight. On the basis of the empirical result that the smaller the phase angle between the two polarizers, the smaller the baseline drift, subsequent experiments were performed at a wavelength of 400 nm and a phase angle of 50°

The detection limit under these conditions was compared with that of the OR-990, which is specially designed for optical activity detection. The two chromatograms (both obtained by isocratic elution with acetonitrile-water (5:3, v/v) at a flow-rate of 0.6 ml min^{-1} with an injection of 1 μg sucrose) are shown in Fig. 5. The size of the flow cell in the OR-990 (2.5 cm path length and a 44 μl volume) does not differ much from that in the proposed split cell (2.4 cm length and 48 μl volume). The response scale of 0.02 mAU in the PPD chromatogram corresponds to about 0.65 mdeg. It appeared that the sensitivities of both the PPD and the commericially available polarimetric detector were nearly equal.

An important advantage of the PPD equipped with a split cell is the ease of analysis of components in the eluent gradient whereas the conventional polarimetric detector responds to refractive index changes and requires careful choice of eluents [4,5]. Oligosaccharides, which shown low cariogenicity and lead to the proliferation of the beneficial enterobacterium, have recently attached interest as sweeteners. Therefore, an attempt was made to determine simultaneously the oligosaccharides in foodstuffs with the PPD. Fig. 6 shows typical PPD chromatograms using the gradient elution system. Monosaccharides were eluted under isocratic elution with acetonitrile-water (4:1, v/v) for 5 min. A linear solvent gradient was then applied (5–20 min) to acetonitrile-water (1:1, v/v) for elution of oligosacchardes. The flow-rate of

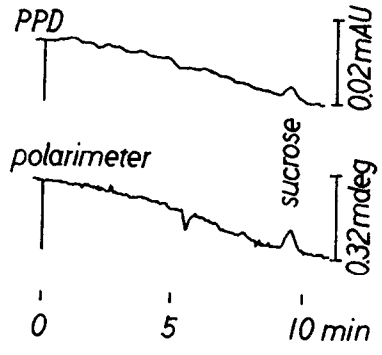

Fig. 5. Comparison of the signal intensity of the proposed instrument with that of the polarimetric detector equipped with a Faraday rotator.

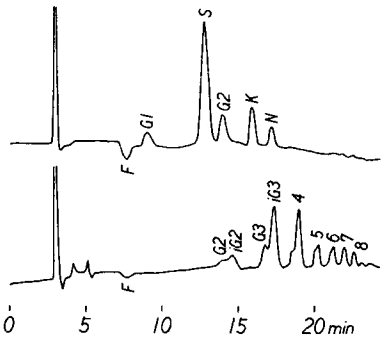

Fig. 6. Gradient elution chromatograms of various samples obtained with the PPD. Peaks: F, fructose; G1, glucose; S, sucrose; G2, maltose; iG2, isomaltose; K, 1-kestose; N, nystose; G3, maltotriose; iG3, isomaltotriose; and each subsequent peak is a mixture of isomalto- and maltooligosaccharides. Numbers at the top of peaks represent the degrees of polymerization in terms of glucose residues.

the eluent was 1 ml min^{-1}. The upper trace illustrates the separation of saccharides in a candy sample containing fructo-oligosacchardies. The candy was dissolved in water at a concentration of 20% (w/v) followed by filtration (0.45 μm) before injection. The chromatographic peaks from fructose, whose degree of polymerization (DP) is 1, to nystose (DP = 4) were successfully separated. The lower trace is from a visibly opaque beer sample, which was concentrated to one-fourth its volume under reduced pressure. The fine separation provided by the proposed system removed the influence of light-absorbing interfering substances. The peaks for polyglucose compounds were split into two peaks, the former being maltooligosaccharides having a α-(1,4) glucosidic bond and the latter being isomalto isomers having a α-(1,6) bond. Although the separation between malto- and isomalto-isomers became unsatisfactory as their DP increased under the present gradient elution conditions, the beer sample seemed to contain isomalto-oligosaccharides as the main polyglucose.

4. Conclusions

An attempt was made to make the PPD equipped with a split cell suitable for the determination of saccharides. In respect of sensitivity, the PPD system was equal to the polarimetric detector based on Faraday effect. In addition, this system was applicable to gradient elution chromatography without being influenced by light-absorbing impurities and thus was suitable for the simultaneous determination of oligosacchalides.

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