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Development of an analytical method of nitropolycyclic aromatic hydrocarbons using ultraviolet-excitation micro-photothermal heterodyne-interferometer

紫外励起光熱変換ヘテロダイン干渉法を利用したニトロ多環芳香族炭化水素の分析法開発

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1.Introduction

An ultraviolet-excitation micro-photothermal heterodyne-interferometer (PHI) combined with semi-micro high performance liquid chromatography (HPLC) has been developed for label-free detection of nitropolycyclic aromatic hydrocarbons (NPAHs) in a micro liter amount of sample. NPAHs are known carcinogenic and/or mutagenic. However, they are barely present in the environment, therefore, a highly sensitive method has been required.[1)] In addition, a label-free direct detection with no derivatization steps is preferred because most of the target chemicals are nonfluorescent or weak fluorescent. Representative spectrofluorometry as conventional analysis methods can detect fluorescent substances of a low concentration but the method can detect only fluorescent substances and versatility is lacking. A complicate pretreatment process, chemical modification of fluorophor, could partly give the versatility but it needs much time.

Photothermal spectroscopy has been focused to develop unique analytical methods.[2)3)] One of the applications is ultrasensitive detection of nonfluorescent or weak fluoroscent substances. The point in the present study is combination of ultraviolet-excitation PHI and semi-micro HPLC. Performance of the newly developed equipment is checked and optimized.

2. Experimental

A schematic diagram of the newly developed analyzing equipment is shown in **Fig.1**. It consists of mainly two parts: semi-micro HPLC as separation part and PHI as detection part. Two lasers are used in PHI. One is excitation light (semiconductor laser, wavelength 375 nm, intensity 70 mW, TC20-0375, NEOARK Corp.) and the other is probe light (helium-neon laser, wavelength 632.8 nm, No.32734, Research Electro-Optics, Inc.) for the heterodyne-interferometer. The excitation wavelength was selected because most of NPAHs have an absorption bands at 375 nm and the liquid solvent does not absorb the wavelength. The excitation laser is set up to generate 50 Hz intensity-modulated light by function generator synchronizing with the lock-in-amplifier. The probe beam is split into two and frequency-shifted using a beam splitter and two different acousto-optic modulators, respectively. One of the beams passes a flow cell of the sample. The two beams are combined again and interfered each other with another beam splitter to detect beat signals just at 30 MHz of the differential frequency between the two different operating-frequencies of the acousto-optic modulators. The interferometer detects change in the phase of the probe light caused by the change in refractive index of the photoexcited and heat-generating liquid solution in the flow cell. The liquid solution expands and refractive index changes by this heat. Magnitude of the change in phase depends on the amount of generated heat, thus the sample concentration was quantitatively evaluated.

The semi-micro HPLC system (a pump Jasco PU-4180HG, Column Oven Jasco CO-4060) coupled with an UV-detector (multiple wavelength Jasco UV-4075) was used. A flow cell (Cat.No.6001-70174, GL Sciences) with 5 mm of the optical path length and 250 nL of the volume was used.

Both the solvent of the sample and the mobile phase optimized are 80 v/v% acetonitrile in pure water. The injected volume was 2 μL. Liquid chromatography was operated using InterSustain C18 (150 × 2.1 mm; i.d. 2 μm particle size, GL Sciences). The injected sample concentration was 10 μmol/L. The most prominent modification in the present study was the use of the f_1 lens in Fig. 1, which was placed in front of the flow cell for focusing the probe light and the excitation light into the flow cell.

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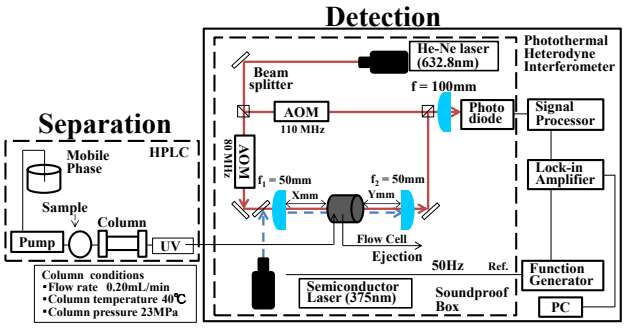

Figure 1 Schimatic diagram of a semi-micro HPLC combined to PHI and UV

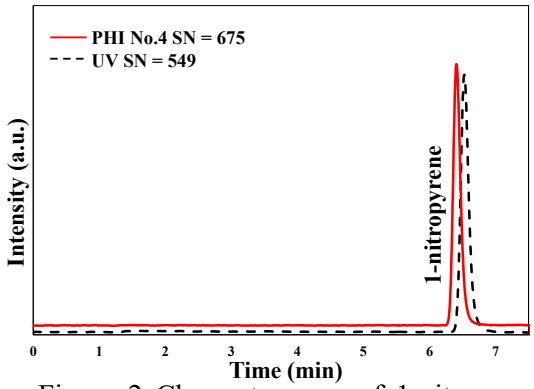

Figure 2 Chromatograms of 1-nitropyrene obtained with UV and PHI detectors under the optimized condition of No.4.

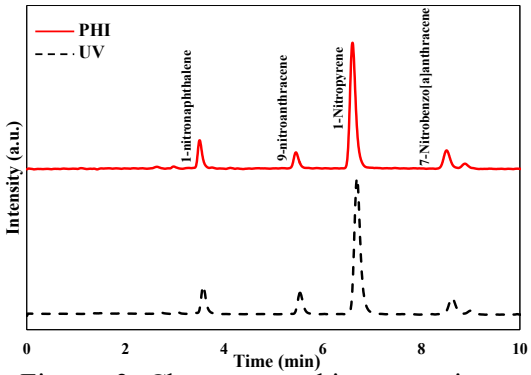

Figure 3 Chromatographic separation of NPAHs by UV and PHI and detectors under the ptimized condition of No.4.

3. Results and Discussion

Table 1 shows dependence of S/N ratio on the distance of the flow cell from lenses: f_1 in front of the flow cell (X) and f_2 in the rear of the cell (Y), respectively. High S/N ratio was achieved, arranging lenses position, namely, changing X and Y distance. Arrangement condition No.4 showed the highest S/N ratio. UV detection's S/N ratio was comparable to PHI detection one under this condition No.4. A chromatogram of 1-nitropyrene (a kind of NPAHs) by PHI (solid line) and UV detector (dotted line, detection wavelength 375 nm) is shown in **Fig. 2**. The retention time of the sample was approximately 6.5 min. The S/N ration of the PHI detection achieved was higher than UV detectio. A chromatogram of NPAHs' mix solution (1-nitronaphthalene, 9-nitroanthracene, 1-nitropyrene, and 7-nitrobenzo[a]anthracene) by PHI (solid line) and UV detector (dotted line) is shown in **Fig. 3**. As shown, chromatograms comparable to UV detector were successfully obtained for PHI detection the condition No.4.

The apparent dependence was observed by changing lenses. One of points for achieving highly sensitive PHI is to decrease background noises of baseline. A choice of lenses and the best positioning are needed for achieving a better result. A shorter focal length lens was tried to tightly focusing probe and excitation beams. However, baseline became unsteady. This is because the lights may scatter in the flow cell by irradiating on flow cell walls before they passed though. The sensitivity of PHI strongly depends on the length (convergence) and the focus position in flow cell.

Table 1. Dependence of S/N ratio on the distance of the flow cell from f_1 lens in front of it (X) and f_2 lens in the rear of it (Y), respectively

Condition No.	Distance of lenses from the cell (mm)		S/N ratio
	X	Y	
1	68	81	320
2	73	81	413
3	78	74	669
4	83	70	675

4. Conclusion

A ultraviolet-excitation micro-photothermal heterodyne-interferometer combined with a semi-micro HPLC is developed for label-free detection of NPAHs. Separation and label-free detection were successfully demonstrated with the ultraviolet laser and the HPLC column selected. Good separation and highly sensitive detection are demonstrated. PHI has a higher S/N ratio than UV after the optimization of the optical elements. There still remains some ways of improvement in sensitivity, especially by optimization of the flow cell design.

Acknowledgment

A method for the heterodyne-interferometric detection of photothermal signal originally developed by Kobe Steel, Ltd, was used with modification.

References

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