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Deep ultraviolet-excitation photothermal heterodyne-interferometer combined with micro-HPLC

micro-HPLC/深紫外励起光熱変換ヘテロダイン干渉計

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1. Introduction

Biologically-relevant molecules plays an important role in a variety of disciplines including medical science, molecular biology, etc. Analysis of them gives us valueable information. In order to get much more helpful data, it is demanded developing better analytical method. Remarkable analytical method has not only highly sensitivity but also broad utility and label-free analysis. Conventional method doesn't have all these three factors. For example, absorptiometric analysis and fluorescence analysis have a worse hand on sensitivity and range of application, respectively. Other, fluorescent derivetization method have to follow a troublesome or time-comsuming procedure. Therefore, it has a fault with ease or swiftness.

In this study, photothermal spectroscopy was focused to develop special analytical method.[1) 2)] Particularly, as a nouveaute, general versatility was gotten by using deep ultraviolet pump light because most of biologically-relevent molecules have their absorption bands in the wavelength regions. Photothermal-heterodyne interferometer (PHI) was developed by appplying photothermal method uniquely and combined with micro-HPLC[3)]. This time, alpha amino acids was selected as targets because, in terms of biochemistry, they are important molecures which elaborate protein within an organism. Besides, in physicochemical point of view, most of them are non-fluorescent substances and have only their absorption bands in deep ultraviolet wavelength range. Destination of this study is sepatation and detection of non-label molecules in order of nano molar and sub micro liter.

2. Fundamental

Two lasers are used in photothermal heterodyne-interferometer. One is pump light ($Nd:YVO_4$ laser fifth harmonics, wavelength 213 nm) and the other is probe light (helium- neon laser, wavelength 632.8 nm). The interferometer detects change of refractive index in liquid solution as phase change of probe light. It is main point of this method. In flow cell, samples are photoexcited by pump light and radiate heat. For this heat, liquid solution distends and changes refractive index. For this reason, optical path length and phase of probe light change. This change which hinges upon existence or non-existence of light absorption is detected as phase change of probe light by using lock-in amplifier. According to simulation, it seemed to have a high sensitivity for phase change detecting in order of picometer.

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3. Experimental

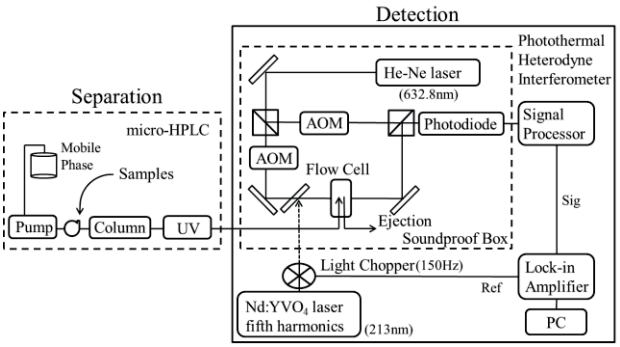

Figure1. Device configuration of PHI combined with micro-HPLC

Fig.1 shows the schematic diagram of new developed analyzing device. It is consisted of micro-HPLC as separation part and PHI as detection part. Separation was performed on a micro- HPLC system (micro21-LC-01, Jasco.co) equipped with an UV-detector (micro21-UV-01, Jasco.co) and an auto sampler (micro21-AS-01, Jasco.co) was used for injection. A separation column of MERCK MILLIPO-RE (Tokyo, Japan) SeQuant ZIC HILIC (150mm×1mm, 5μm particle size) was used for the analysis. In PHI, excitation light (Impress213, Xiton.co) and light chopper combined with lock-in amplifier are used. The volume of flow cell is 250 nanoliter and optical path length is 8 mm. Samples separated by micro-HPLC stream flow cell in the interferometer. Probe light is separated by beam splitter and modulated by acousto-optical modulator. Both of lights interfere and form beat signal. One of the

light passes photodiode via flow cell and the other passes there directly. Beat signal is detected by photodiode and quantitated phase change by signal processor and lock-in amplifier.

4. Result and Discussion

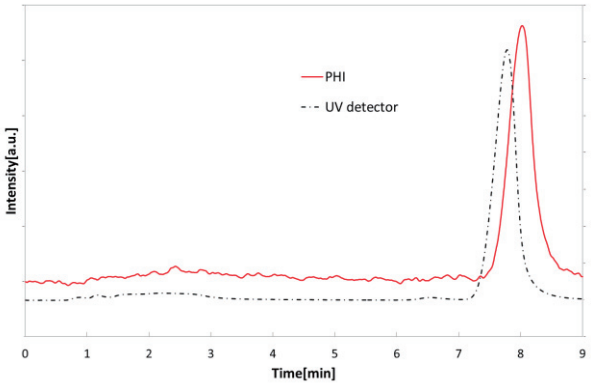

Figure2. Chromatogram of PHI and UV detector

Fig.2 shows chromatogram of, one of alpha amino acids, L-Phenylalanine measured by PHI (solid line) and UV detector (dotted line, detection wavelength 213 nm). As a result, chromatogram comparable to UV detector was obtained successfully. Solvent of the sample and mobile phase of the HPLC system are as follows: mixed solvent consisted of acetonitrile and water 80% (v/v) and 20% (v/v), respectively. Injection volume is 1μL. Column temperature is 30.0°C. Sample concentration is 0.1 milli molar. Retention time of sample is 8.0 minutes.

Table I. Comparison of detection limit

	Detection limit	Cell volume	Absolute quantity
UV	0.8 μM	16 nl	13 fmol
PHI	1.5 μM	250 nl	525 fmol

Table I shows comparison of detection limit between PHI and UV detector. Detection limit in concentration of PHI is 1.5 micro molar. The result of PHI is subequal that of UV detector. Because of difference in cell volume, the result of absolute quantity was different very much.

To evaluate of sensitivity of PHI, background noise had to be decrease and stable. The wavelength 213 nm can be absorbed outside objective substance. So, various substances can be measured by PHI, but foreign substances have to be removed at all points. In addition, solvent which do not have absorption bands in 213 nm is selected accordingly.

5. Conclusion

A photothermal heterodyne-interferometer method combined with micro-HPLC separation is newly developed. Detection of label-free alpha amino acid is successfully demonstrated with deep ultraviolet laser and HILIC column. Good chromatogram coequal for UV detector was measured. Detection limits of the photothermal heterodyne-interferometer in concentration and in injected amount were nearly equal UV detector. Use of shorter wavelength-ultraviolet light for excitation will make detection of almost all of bio-related molecules possible. There still remains some ways of improvement of the sensitivity such as the use of smaller sample cell to suppress influence of diffusion, but this method is promising as a new separation analysis of bio-related substances.

Acknowledgment

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

References

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