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Note

A multichannel photodiode array ultraviolet–visible detector for micro high-performance liquid chromatography

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Chromatographic peaks are usually identified by comparing their retention times with those of standard solutes in high-performance liquid chromatography (HPLC) when using a conventional spectrophotometer or a refractometer as a detector. However, it is sometimes difficult to evaluate the effects of interfering compounds, which leads to erroneous quantitative results. A mass spectrometer has often been employed as a qualitative detector in gas chromatography (GC) since it gives useful information. Many kinds of qualitative detectors have been examined for HPLC, but they cannot compete with a mass spectrometer in GC with respect to universality and conveniency.

Photodiode array multichannel UV–visible detectors have recently been developed and their potential in HPLC has been discussed[1]. These detectors are capable of recording a series of absorption spectra and storing the data in their own memories and/or in external devices for subsequent data handling and plotting. They enable multi-wavelength detection and yield spectral information in a single chromatographic analysis and consequently have many advantages compared with conventional single-wavelength spectrometers: improved identification; ability to check the purity of chromatographic peaks; rapid selection of an optimal wavelength; economy of time, solvents and samples, etc.

We have examined a photodiode array multichannel UV–visible spectrometer for micro HPLC.

EXPERIMENTAL

The apparatus comprised a Micro Feeder (Azumadenkikogyo, Tokyo, Japan) equipped with a gas-tight syringe MS GAN-050 (Terumo, Tokyo, Japan) as a pump, a micro valve injector ML-422 (0.02 μl; JASCO, Japan Spectroscopic, Tokyo, Japan), a micro packed column, a micro gate photodiode array detector MGPD (Union Giken, Osaka, Japan), a micro computer MC-800 (Union Giken), a monitorscope MC-910 (Union Giken) and a plotter MC-920 (Union Giken). The MGPD system is basically the same as the MCPD-350 (Union Giken) except for some modifications as described below. A diagram of the apparatus is shown in Fig. 1.

The flow cell volume should be reduced to less than 0.1 μl when using a micro

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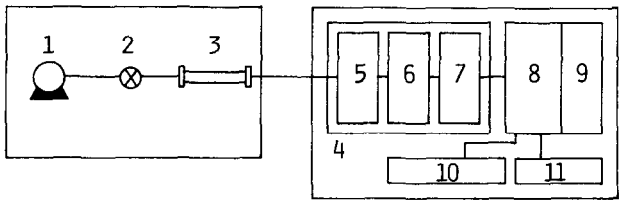

Fig. 1. Block diagram of the apparatus: 1 = pump; 2 = micro valve injector; 3 = separation column; 4 = MGPD system; 5 = micro flow cell; 6 = grating; 7 = photodiode array; 8 = micro computer (MC-800); 9 = magnetic disk; 10 = plotter (MC-920); 11 = monitoscope (MC-910).

packed column of 10–15 cm × 0.2–0.35 mm I.D. Consequently the light pathlength and/or the cross-sectional area of the flow cell is decreased in comparison with conventional flow cells which usually have dimensions of 10 × 1 mm I.D. (volume 8 μl). The structure of the micro parallel flow cell employed is illustrated in Fig. 2. A hole (1 × 0.25 mm I.D.) in a Diflon plate functions as the flow cell. The eluent from the column enters the hole through a ditch (0.2 × 0.2 × 8 mm). The Diflon plate is held between two stainless-steel blocks. The connecting tubing between the cell block and the column is of stainless steel (30 × 0.05 mm I.D.) and fused silica (150 × 0.055 mm I.D.). Thus, the cell volume can be reduced to 0.05 μl. The cross-sectional area of this micro flow cell is sixteen times smaller than that of a conventional one, leading to a decrease in light intensity reaching the detector. This problem can be solved by decreasing the number of optical parts such as lenses and mirrors, and by reducing the distance between the light source and the flow cell and between the flow cell and the grating.

Fused-silica tubing (100–150 × 0.34 mm I.D.; Hewlett-Packard, Amstelveen, The Netherlands) was selected as the column material, and silica ODS SC-01 (5 μm, JASCO) and ODS-Hypersil (3 μm; Shandon, Cheshire, U.K.) were employed as packing materials. The former had irregularly shaped particles and the latter spherical ones. The packing procedure was the same as described previously[2]. Reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) or Tokyo Chemical Industry (Tokyo, Japan).

RESULTS AND DISCUSSION

Extra-column band broadening is sometimes substantial in micro HPLC.

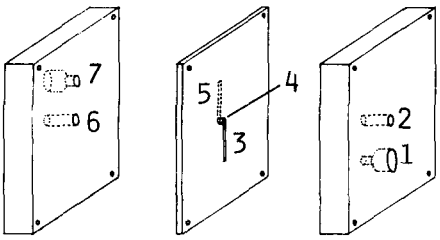

Fig. 2. Schematic diagram of the micro flow cell: 1 = inlet; 2 = quartz glass; 3 = ditch (0.2 × 0.2 × 8 mm); 4 = hole (1 × 0.25 mm I.D.); 5 = ditch (0.25 × 0.25 × 8 mm); 6 = quartz glass; 7 = outlet.

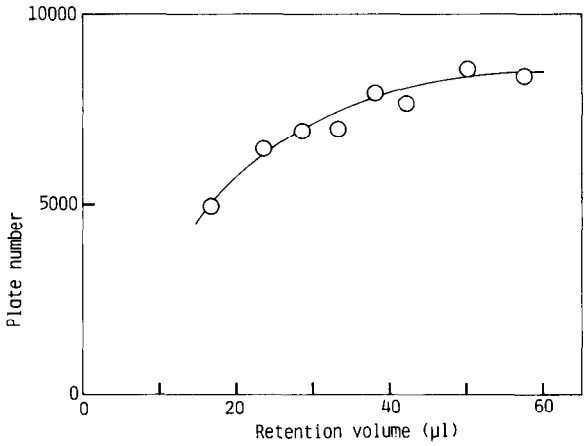

Fig. 3. Relationship between the theoretical plate number and the retention volume. Column: SC-01, 150 × 0.34 mm I.D. Mobile phase: acetonitrile–water (7:3). Flow-rate: 5.6 μl/min. Sample: PAHs.

Thus, the dead volume should be reduced as much as possible. Fig. 3 shows the relationship between the theoretical plate number and the solute retention volume using polynuclear aromatic hydrocarbons (PAHs) as samples. The void volume of the column is *ca.* 8 μl. The theoretical plate number increases with increasing reten-

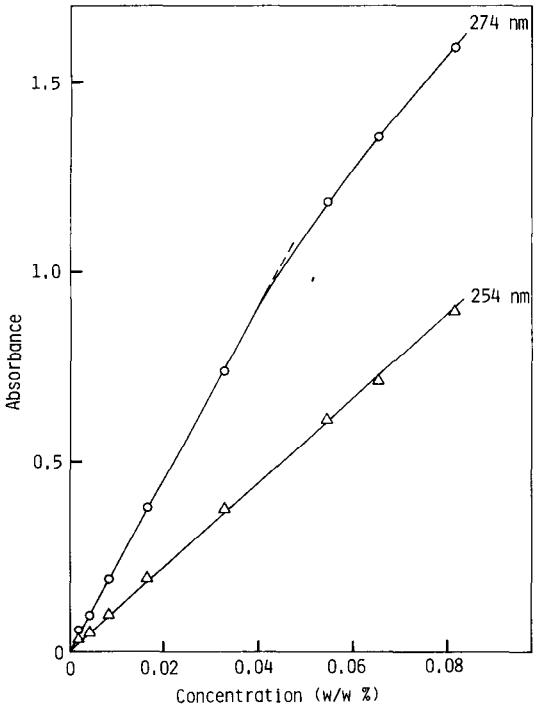

Fig. 4. Relationships between peak heights and the concentration of naphthalene. Sampling time: 25 msec × 8. Resolution: 1.4 nm. Sample: naphthalene dissolved in acetonitrile.

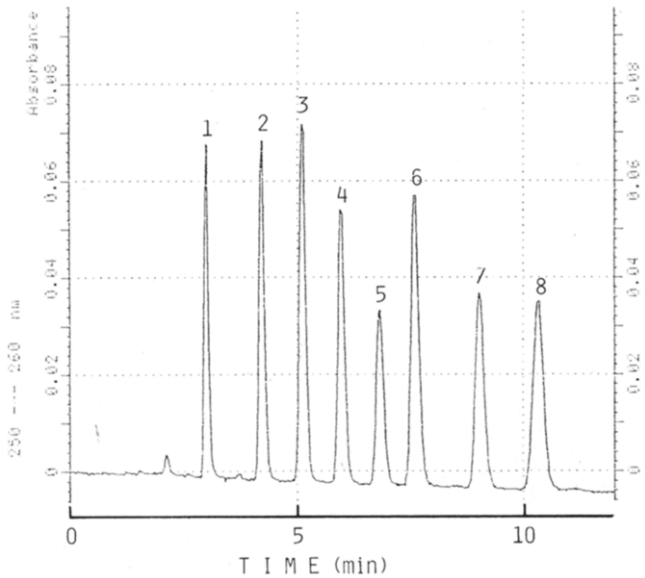

Fig. 5. A typical chromatogram of PAHs. Column: SC-01, 150 × 0.34 mm I.D. Mobile phase: acetonitrile–water (7:3). Flow-rate: 5.6 μl/min. Samples: 1 = benzene (1.9%); 2 = naphthalene (0.14%); 3 = biphenyl (0.037%); 4 = fluorene (0.036%); 5 = phenanthrene (0.0091%); 6 = anthracene (0.0083%); 7 = fluoranthene (0.040%); 8 = pyrene (0.039%). Wavelength: 250–260 nm.

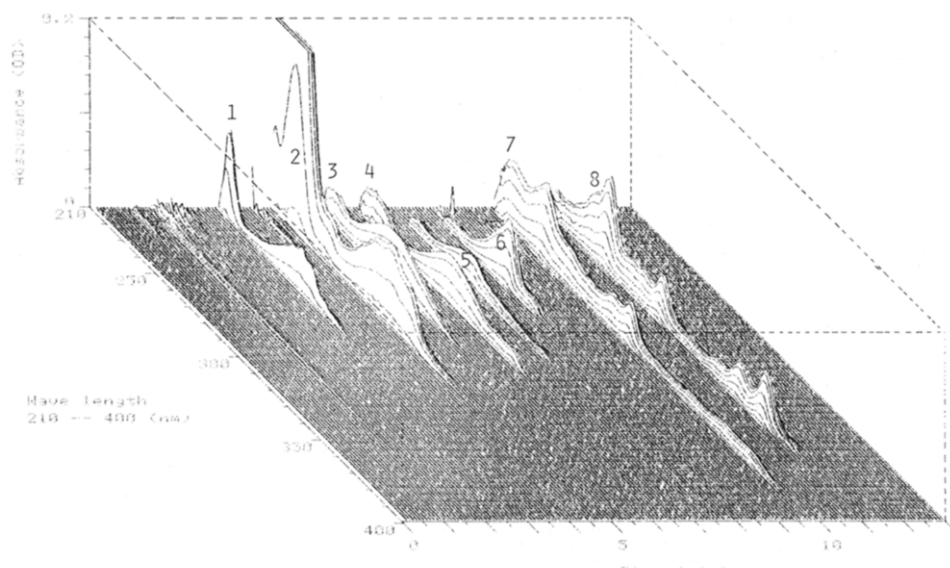

Fig. 6. A three-dimensional chromatogram of PAHs. Column: SC-01, 140 × 0.34 mm I.D. Mobile phase: acetonitrile–water (65:35). Flow-rate: 5.6 μl/min. Samples as in Fig. 5. Sampling time: 25 msec × 8.

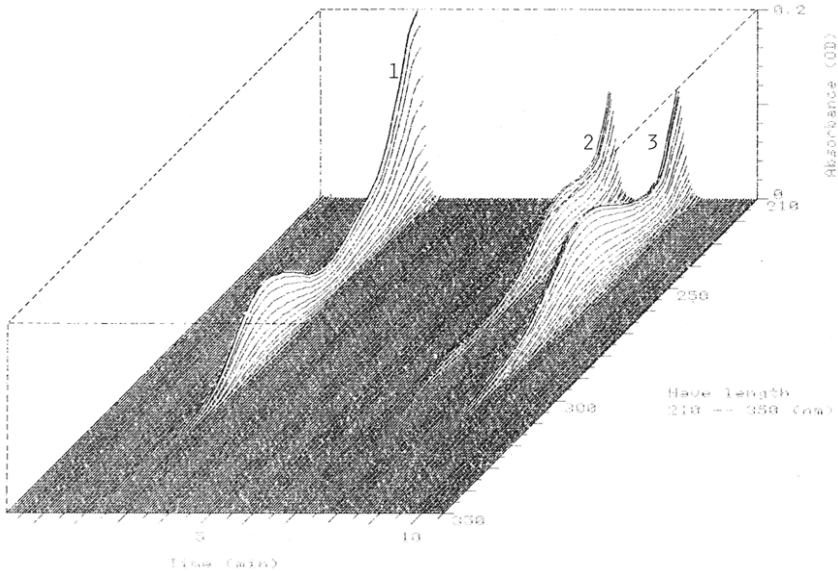

Fig. 7. A spectrochromatogram of typical components in a pharmaceutical preparation. Column: ODS-Hypersil, 100 × 0.34 mm I.D. Mobile phase: acetonitrile–water–orthophosphoric acid (20:79.9:0.1). Flow-rate: 4.2 μl/min. Samples: 1 = caffein (1.0 mg/ml); 2 = aspirin (1.1 mg/ml); 3 = phenacetin (1.0 mg/ml). Sampling time: 25 msec × 8.

tion volume up to *ca.* 50 μl, indicating that extra-column band broadening occurs and is significant for weakly retained solutes. Broadening in the inlet ditch (0.2 × 0.2 × 8 mm) may be substantial and can be minimized by using a cross flow cell which permits small-dead-volume connections. This drawback is unfortunate considering the otherwise improved identification possibilities.

The linearity of the detector response was examined by using naphthalene as a solute. Fig. 4 illustrates the relationships between the absorbance and the concen-

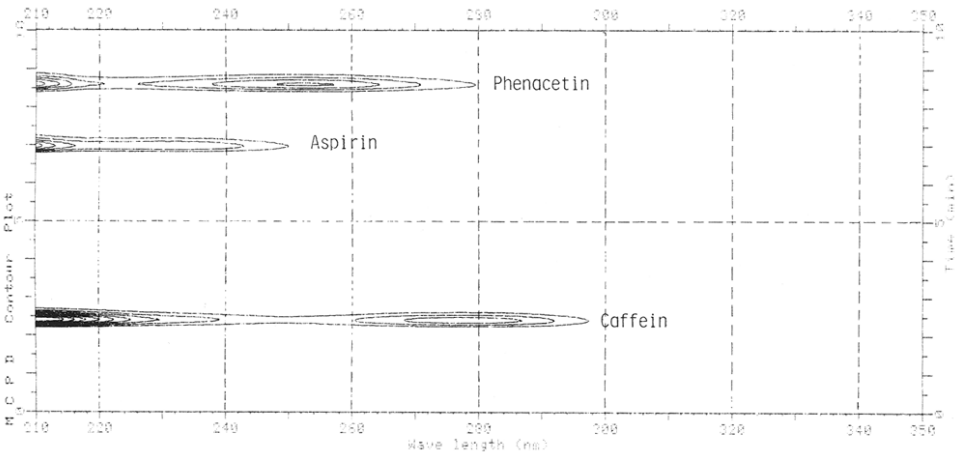

Fig. 8. Contour plot for caffein, aspirin and phenacetin. Operating conditions as in Fig. 7.

tration of naphthalene at the wavelengths of 254 and 274 nm. The absorbance is proportional to the concentration when it is less than 1, but the plots deviate from linearity when the absorbance is >1.

Fig. 5 shows a chromatogram of PAHs obtained with an ODS column (150 $\times$ 0.34 mm I.D.), with mean peak heights at the wavelengths of 250–260 nm.

A three-dimensional chromatogram of PAHs is shown in Fig. 6. A deuterium lamp was used as the light source and thus the system can cover the wavelength range 200–600 nm.

Another example involves the photodiode array detection of typical components in a pharmaceutical preparation. The three-dimensional spectrochromatogram of caffein, aspirin and phenacetin is shown in Fig. 7. Fig. 8 shows a contour plot of caffein, aspirin and phenacetin at intervals of 0.02 O.D. over the optical density range 0.02 to 0.2. This plot is useful to select the optimal conditions for single-wavelength detection.

CONCLUSIONS

A multichannel photodiode array detector can be coupled with micro HPLC after some modifications. This system permits the recording of three-dimensional spectrochromatograms and other new methods in micro and conventional HPLC. It will be of use in the analysis of real samples, and applications will be reported elsewhere.

ACKNOWLEDGEMENTS

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