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MASS DETECTION LIMITS ACHIEVED WITH A COMMERCIALLY AVAILABLE FLUORIMETER IN MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAHPY

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SUMMARY

The effects of the cell structure on the mass detection limits in fluorimetric detection in micro high-performance liquid chromatography were examined. Scatter of the light by reflection or refraction from the cylindrical quartz cell wall increased the noise level. This type of scattered light could effectively be prevented from entering the photomultiplier by either tilting the flow cell or using appropriate cut-off filters. In fluorimetric detection in the presence of a packing material, scattered light from the surface of the packing material could be shut out by the latter method.

INTRODUCTION

Mass detection limits as low as the sub-femtogram level have been demonstrated by laser-induced fluorimetric detection in high-performance liquid chromatography (HPLC)[1–3]. This is due to the high intensity of the laser, its better focusing properties and miniaturization of the column dimensions. In order to achieve such low mass detection limits, various types of flow cell structures have been proposed[1–3], most effort having been devoted to the elimination of the scattering of light from the cell wall. For example, the photomultiplier was tilted 30° from the plane of scattered laser light in order to reduce the collection of excitation light[1]. Another approach to eliminating scattering involved a free-falling jet[4]. In spite of the excellent detectability of laser-induced fluorimetric detection, it is not popular in routine analysis because of its inconvenience in operation and the limited choice of excitation wavelengths in the ultraviolet region. There are many commercially available fluorimeters for HPLC that already achieve respectable detectability levels.

This paper reports the mass detection limits of a commercially available fluorimeter with a laboratory-made flow cell for micro HPLC.

EXPERIMENTAL

The liquid chromatograph was assembled from a Microfeeder pump (Azumadenki Kogyo, Tokyo, Japan) equipped with an MS-GAN 050 gas-tight syringe (0.5

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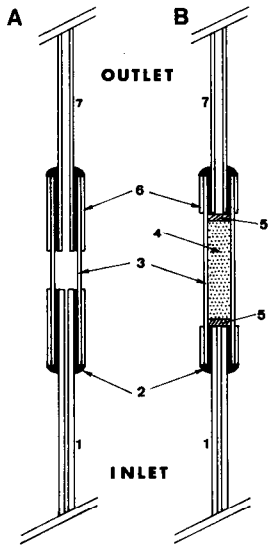


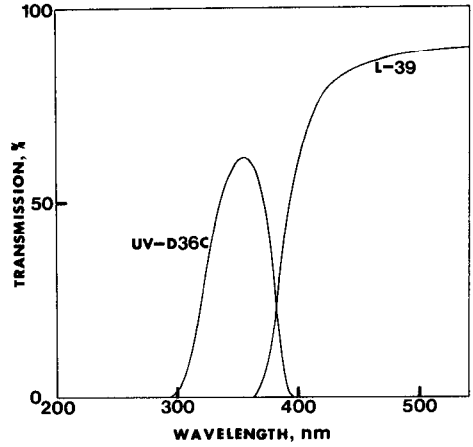


Fig. 1. Schematic diagram of the flow cell. (A) Empty cell; (B) packed cell. 1 = Stainless-steel tubing (0.05 mm I.D. × 0.30 mm O.D.); 2 = adhesive; 3 = fused-silica tubing (0.32 mm I.D.); 4 = packing material; 5 = quartz-wool; 6 = polyimide resin; 7 = stainless-steel tubing (0.13 mm I.D. × 0.31 mm O.D.).

Fig. 2. Optical data for the cut-off filters employed.

ml) (Ito, Fuji, Japan), an ML-422 micro valve injector with an injection volume of 20 nl (JASCO, Tokyo, Japan), a laboratory-made micropacked separation column, an 820-FP spectrofluorimeter with a xenon lamp (JASCO) and a chart recorder. The separation column was composed of fused-silica tubing of 0.35 mm I.D. (Gasukuro Kogyo, Tokyo, Japan) packed with Develosil ODS-3K (3-μm particle diameter) (Nomura Chemical, Seto, Japan).

Flow cells for the fluorimeter were prepared in the laboratory by using fused-silica tubing of 0.32 mm I.D. (SGE, Ringwood, Victoria, Australia) and stainless-steel tubing of 0.13 mm I.D. × 0.31 mm O.D. (Hakkoshoji, Tokyo, Japan) and 0.05 mm I.D. × 0.30 mm O.D. (Nomura Chemical). These capillary tubes were glued with and epoxy-resin adhesive. The structures of the flow cells are illustrated in Fig. 1. For the packed flow cell, the same stationary phase as used for the separation column was packed in the fused-silica tubing, and the packing was prevented from leakage by quartz-wool (1–6 μm) (Wako, Osaka, Japan). The time constant of the detector was kept at 1.5 s in this investigation. The outlet of the flow cell was attached to an MS-GAN 100 gas-tight syringe (1 ml) (Ito) to apply a back-pressure to the flow cell so that the formation of air bubbles in it could be repressed.

UV-D36C and L-39 (JASCO) cut-off filters were employed to reduce the scattering of light or to prevent the scattered light from reaching the photomultiplier of the detector. The former cut-off filter was used for the excitation side and the latter for the emission side. The optical data of these filters are shown in Fig. 2. The transmission is plotted against the wavelength.

All the reagents employed, except for HPLC-grade distilled water (Wako), were of analytical-reagent grade from Wako or Tokyo Chemical Industry (Tokyo, Japan) and were used as received.

RESULTS AND DISCUSSION

Cylindrical quartz tubing is commonly employed as the flow cell in micro HPLC because it is convenient for minimizing the dead volume. However, such cylindrical tubing strongly scatters the incident light by reflection or refraction, which leads to an increase in the noise level. Collection of such scattered light is simply reduced by tilting the photomultiplier from the plane of the scattered light[1]. In laser-induced fluorimetric detection, various types of flow cell such as a sheath flow cell, an optical fibrecapillary tube cell and a free-falling jet have been evaluated for eliminating the scatter of light[2]. When a fluorimeter with a common lamp as a light source was employed as the detector, problems caused by the scattering of light were still encountered. The detector employed in this work allowed the measurement of emission spectra by the stopped flow method. In order to compare the mass detection limits achieved by the fluorimeter with those of a medium-pressure mercury lamp[5], the excitation wavelength of the present fluorimeter was kept at 365 nm, that is, the strongest line of the former detector. The flow cell was fixed on the cell block either in the normal position or a tilted position, as illustrated in Fig. 3.

Fig. 4A shows a spectrum of the scattered light emitted from the empty fused-silica capillary cell fixed in the normal position. Three peaks are observed, at 425, 505 and 665 nm. The wavelengths of the emission spectra were independent of the flow cell material, which indicates that they originated from the incident light. The pattern of the spectrum varied with the excitation wavelength.

Fig. 5 shows noise levels observed at various wavelengths under the same operating conditions as in Fig. 4A. It is found that the stronger the scattered light, the higher is the noise level.

Collection of this scattered light is simply reduced by tilting the flow cell by *ca.* 30°, as illustrated in Fig. 3B. Fig. 4B shows the emission spectrum for this tilted empty flow cell. The intensity of the scattered light is considerably reduced in Fig. 4B compared with that in Fig. 4A. In addition, the noise level of the baseline did not vary with the emission wavelength and it was extremely reduced, which is different from the results in Fig. 5. These results indicate that the noise observed in Fig.5 is mostly caused by the scattered light.

The scattered light from the cell wall can also be eliminated by using appropriate cut-off filters. UV-D36C and L-39 cut-off filters were selected, considering their optical properties and the wavelengths of both the excitation and scattered light. Fig. 4C shows the intensity of the scattered light for the flow cell with these cut-off filters

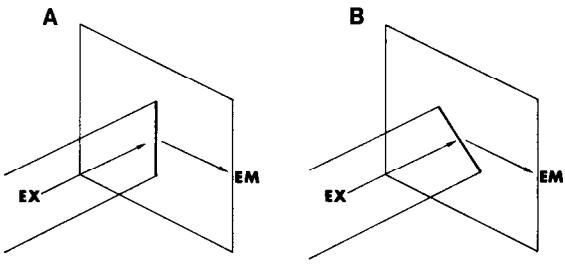


Fig. 3. Schematic diagram of the flow cell positions. (A) Normal position; (B) tilted position.

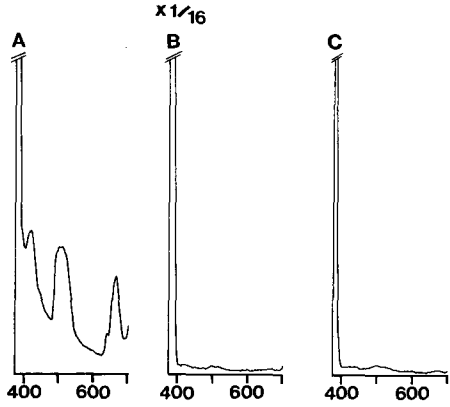


Fig. 4. Emission spectra of scattered light for the empty flow cells. (A) Normal position without cut-off filters; (B) tilted position without cut-off filters; (C) normal position with cut-off filters.

Fig. 5. Noise levels at various emission wavelengths for the empty flow cell set in the normal position without cut-off filters. Excitation wavelength, 365 nm.

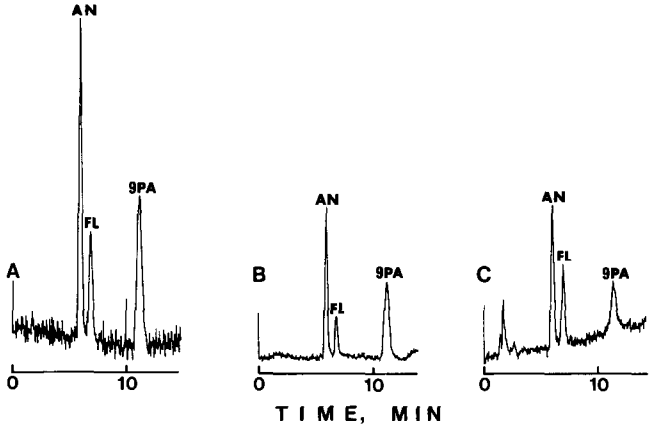


Fig. 6. Chromatograms of aromatic hydrocarbons obtained with the empty flow cells. Column, Develosil ODS-3K (100 × 0.35 mm I.D.); mobile phase, acetonitrile–water (80:20); flow-rate, 4.2 μl/min; excitation wavelength, 365 nm; emission wavelength, 430 nm. Flow cells: (A) normal position without cut-off filters; (B) tilted position without cut-off filters; (C) normal position with cut-off filters. Samples: AN = anthracene; FL = fluoranthene; 9PA = 9-phenylanthracene. Sample amounts: (A) AN = 110 pg, FL = 130 pg, 9PA = 22 pg; (B) and (C) AN = 28 pg, FL = 32 pg, 9PA = 5.5 pg.

set in the normal position. When the excitation wavelength is changed, the cut-off filters must be re-optimized. This is not a convenient situation.

Chromatograms obtained by using the above three types of flow cells are compared in Fig. 6. The amounts of sample injected in Fig. 6A are four times larger than those in Fig. 6B and C. The mass detection limits at a signal-to-noise ratio (S/N) of 2 are given in Table I. With the empty flow cell, the mass detection limits achieved by the tilted flow cell were improved by a factor of *ca.* 10 for the analytes examined, whereas those achieved with the cut-off filters were improved by a factor of *ca.* 5 for anthracene and 9-phenylanthracene and *ca.* 10 for fluoranthene. It is uncertain why the improvement factors differ.

On-column detection, which relates to the detection of analytes on a separation column, improves the mass detection limits owing to the focussing effect of the stationary phase[5–8]. This advantage is also realized by the use of a packed flow cell[9]. When the flow cell packed with the same stationary phase as for the separation column is used, the effect of the stationary phase is essentially same as in on-column detection.

Fig. 7A shows the spectrum of the scattered light for the tilted packed flow cell. Although the flow cell tubing was tilted, the scattered light still reached the photomultiplier and the same profile of the emission spectrum was observed as in Fig. 4A. This is because the scattered light from the surface of the packing material was propagated in all directions. Collection of this scattered light could also be reduced by using the same cut-off filters as in Fig. 4C. The spectrum of the scattered light for the tilted packed flow cell with the cut-off filters is shown in Fig. 7B. It is observed that the cut-off filters effectively reduced the collection of the scattered light.

The chromatogram obtained with the packed flow cell with the cut-off filters is shown in Fig. 8. A difference in the profile of the chromatograms is observed between the empty and the packed flow cells, which is due to the focussing effect of the stationary phase for the latter flow cell. Detection in the presence of the stationary phase has the potential to improve the mass detection limits of analytes.

Table I also gives the mass detection limits for the tilted packed flow cell. The use of the cut-off filters improved the mass detection limits for the tilted packed flow cell by a factor of *ca.* 5 for anthracene and 9-phenylanthracene and 8 for fluoran-

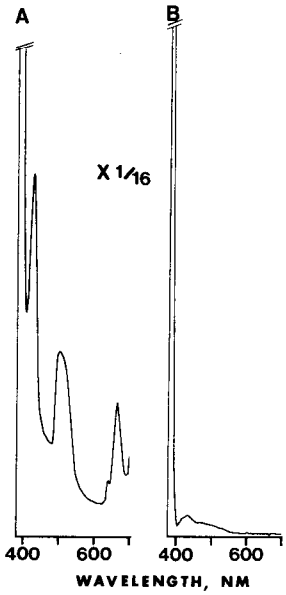
TABLE I

MASS DETECTION LIMITS AT S/N = 2

Column, Develosil ODS-3K, 100 × 0.35 mm I.D.; mobile phase, acetonitrile–water (80:20); flow-rate, 4.2 μl/min; excitation wavelength, 365 nm; emission wavelength, 430 nm.

Flow cell			*Mass detection limit (pg)*		
Stationary phase	*Position*	*Filters*[a]	*Anthracene*	*Fluoranthene*	*9-Phenylanthracene*
Empty	Normal	–	13	45	5.3
Empty	Tilted	–	1.3	4.5	0.48
Empty	Normal	+	2.1	4.1	1.0
Packed	Tilted	–	6.8	20	1.4
Packed	Tilted	+	1.5	2.4	0.25

[a] –, detection without filters; +, detection with filters.



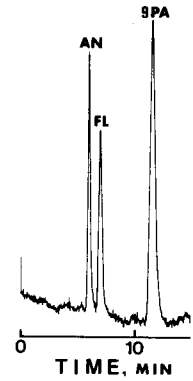


Fig. 7. Emission spectra of scattered light for the packed flow cells. (A) Tilted position without cut-off filters; (B) tilted position with cut-off filters.

Fig. 8. Chromatograms of aromatic hydrocarbons obtained with the tilted packed flow cell with the cut-off filters. Sample amounts: AN = 28 pg, FL = 32 pg, 9PA = 5.5 pg. Other conditions as in Fig. 6.

thene. The lowest mass detection limits were achieved with the tilted packed flow cell with the cut-off filters. In addition, the mass detection limits achieved with a medium-pressure mercury lamp[5] were comparable to those achieved with the tilted packed flow cell with the cut-off filters.

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