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Use of a focusing cylindrical lens for increasing sensitivity in the optical detector of a capillary flow-through cell

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ABSTRACT

In capillary column liquid chromatography, a cylindrical flow-through cell is often used in absorbance detection. The cross-sectional shape of the cylindrical flow-through cell perpendicular to the light beam is rectangular. Therefore, the optimum shape of the focused light beam is also rectangular. The use of a cylindrical lens to obtain a rectangular light beam, thus increasing the sensitivity of the UV detector more than 10-fold, is described.

INTRODUCTION

For optimum results, the cell volume of a UV absorbance detector should be extremely small in capillary column liquid chromatography and capillary electrophoresis. This cell volume is much smaller than the volume of conventional liquid chromatographic cells. It is very important to achieve high sensitivity when using such a small cell for detecting trace components separated in the capillary column. Preservation of flow dynamics and separation efficiency in capillary high-performance liquid chromatography or capillary electrophoresis demands a cylindrical detector flow-through cell (CF cell)[1–4]. In most experiments with capillary columns, a cylindrical cell has been used. Theoretical and geometrical treatments[3] and refractive index of CF cells[5] have been extensively discussed. A cylindrical cell is usually oriented perpendicular to the light beam and its path length is therefore equal to the capillary inner diameter (*e.g.*, 0.1–0.01 mm) and its cross-section perpendicular to the light beam is rectangular. For optimum sensitivity, the cell length perpendicular to the light beam should be increased until the decrease in separation efficiency becomes *ca.* 5%. Both the light path length and cross-sectional area of the CF cell are considerably smaller than those used in conventional liquid chromatography (*ca.* 1% of the size). The stability of the photocell and photomultiplier in the UV detector depend on the amount of light reaching the photocell and the area illuminated. Both of these factors for CF cells used with capillary columns are extremely small in comparison with cells for conventional columns. As a result, CF cells for capillary columns exhibit baseline instability.

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As mentioned above, the cross-sectional shape of the cylindrical flow-through cell is rectangular. Therefore, for optimum results the shape of the focused light beam should also be rectangular. To produce a rectangular shape, we used a cylindrial lens for focusing the light. We examined the effects of a cylindrical lens both on sensitivity and the signal-to-noise ratio, and describe the use of relatively wide slits in conjunction with the cylindrical lens.

EXPERIMENTAL

The liquid chromatographic system consisted of a pump (Microfeeder MF-2; Azuma Denki Kogyo, Tokyo, Japan), and injector (Model 7520, 0.02 μl; Rheodyne, Cotati, CA, U.S.A.), an open-tubular capillary column (60 cm × 50 μm I.D.) of fused-silica capillary tubing with an unmodified surface (Shinwa Kakou, Kyoto, Japan) and either a UV double-beam detector at 254 nm (light source, deuterium lamp; arrangement of light path, two mirrors and a grating; type, SPD-1; Shimadzu, Kyoto, Japan) or a fluorescence detector with excitation at 345 nm and emission at 520 nm (RF-535; Shimadzu). Methanol at a flow-rate of 4.19 μl/min was used as the eluent and dansylated alanine in methanol solution (10^{-3} M) as a sample. One end of the column itself was used as the cylindrical flow-through cell. The polyimide coating at the end of the column was removed by using a micro-flame. A focusing cylindrical plano-convex lens (focal length 15 mm, size 10 × 10 mm, made of synthetic quartz, Model CLSQ-1010-15P; Sigma Kohki, Irima-gun, Saitama, Japan) was connected to the cell housing, shown in Fig. 1, at the UV absorption detector. The lens was moved in the

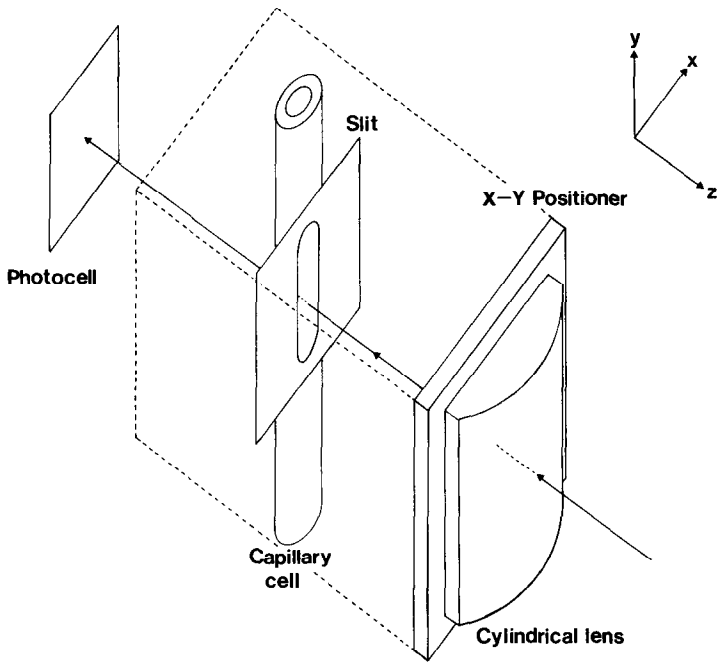

Fig. 1. Schematic diagram of the device for UV absorption detection.

X and Y directions by means of a manual positioner (Sigma Kohki). A slit was positioned in one of two locations. In one configuration, the slit was attached to the outside wall of the CF cell, as shown in Fig. 1. The slit was 50 μm wide and 0.8 mm long (kindly donated by Yokogawa Denki, Tokyo, Japan). This size is commonly used in capillary electrophoresis experiments.

In a second configuration, a slit (0.8 mm wide) was attached to the surface of the cylindrical lens (In Fig. 1, the slit on the CF cell was removed and the second slit was attached to the cylindrical lens). With this configuration, we could use a wider slit owing to the magnification of the CF cell by the cylindrical lens.

With fluorescence detection using an instrument with a xenon lamp, made for liquid chromatography, a long CF cell (5 mm) was used to provide sufficient sensitivity. The positioning of the CF cell and the slit were checked with a visible laser beam. The response of the detector was recorded with a data handling instrument (CR-4A; Shimadzu). All reagents used were of analytical-reagent grade.

RESULTS AND DISCUSSION

The focusing cylindrical lens attached to the cell housing was adjusted by an x–y positioner until the response of the photocell was maximized. The distance between the lens and the CF cell (z axis) was set approximately equal to the focal length of the lens. The cell image had a width of 0.8 mm when the cell inner diameter and the magnification of the lens were 50 μm and 16, respectively. A slit was positioned either on the outside wall of the CF cell or on the surface of the lens. The positioning of the lens in front of the CF cell offers several advantages. First, by using a cylindrical lens, we are able to focus most of the light beam on the capillary flow-through cell. Therefore, by focusing, the beam is 16 times more intense than it would be without using the lens. Second, the light beam is focused on a line drawn vertically through the centre of the capillary cell by the cylindrical lens. Third, we can use a wider slit, *e.g.*, 0.8 mm, for the CF cell of 50 μm I.D. if we position the slit on the surface of the lens. This slit is less expensive to produce and easier to position than the slit mounted on the CF cell.

The schematic arrangement for UV detection is shown in Fig. 1. The slit, which is a rectangle 50 μm wide and 0.8 mm long, is attached to the outside wall of a capillary tube by epoxy adhesive. The positioning of the slit is carefully accomplished under a microscope. With the slit in place, chromatograms obtained with and without the cylindrical lens are shown in Fig. 2, and count numbers of peaks are shown in Table I. From these we conclude that we have obtained peak heights with the lens which are eleven times larger than those obtained without the lens under the same conditions. By using a cylindrical lens to focus the light beam we can obtain a higher sensitivity as a result of the first two advantages above. The use of a cylindrical lens instead of a simple focusing plano-convex lens for focusing the ligh beam is considerably superior for the CF cell because the rectangular shape of the focused light beam by a cylindrical lens corresponds to the rectangular shape of the CF cell. Therefore, we can focus a much greater portion of the light beam on the cell by using a cylindrical lens than we can by using a simple focusing plano-convex lens.

Using a UV detector we examined the effect on the response and signal-to-noise ratio of a slit positioned on the surface of the cylindrical lens. The peak heights with the

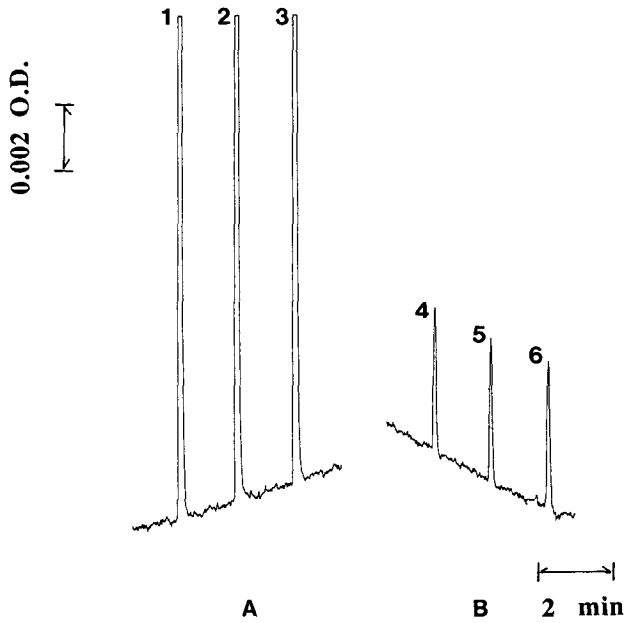

Fig. 2. Comparison of peaks obtained with and without a cylindrical lens (0.02 a.u.f.s.). Peaks 1–3 were obtained with and peaks 4–6 without a cylindrical lens.

slit in place were more than ten times greater than those when no slit was used. As the coherence of the light beam from the deuterium lamp is not good, stray light is a problem when we position the slit away from the cell, such as on the lens surface. Although a slit which is positioned directly on the outside wall of the cell is very effective for excluding stray light, it is much more difficult to position than a slit of large width located on the lens.

In summary, improved detection sensitivity can be achieved by using a cylindrical lens and a slit positioned either on the capillary flow-through cell or directly on the surface of the lens. The slit is easier to make (wider) and position on the surface of

TABLE I

PEAK HEIGHT AND PEAK AREA (COUNT NUMBER)

The count numbers were obtained by a data handling instrument.

Arrangement	*Peak No.*[a]	*Peak height*	*Peak area*
With	1	4869	24632
cylindrical	2	4922	25376
lens	3	4947	24746
Without	4	425	2246
cylindrical	5	425	2109
lens	6	435	2331

[a] Peak numbers correspond to Fig. 2.

the lens than a slit that is positioned on the cell. On the other hand, the slit positioned directly on the cell eliminates more stray light.

The use of a cylindrical lens in conjunction with a slit is also effective for improving fluorescence detection. In this instance, we could place a cylindrical lens in front of the cell housing and a second cylindrical lens, for collecting the fluorescent light, mounted on the flow-through cell, but oriented 90° from the first lens. This second lens would have a slit placed on the lens and a cut filter (passing wavelengths longer than 440 nm) placed in front of the photomultiplier. The sample was 0.2 μl of 10^{-6} *M* dansylated alanine in methanol. By using this device, the noise level decreased by one third (the height of the noise on the strip chart improved from 9 to 3 mm). The peak heights with this device were 14.5 cm and without it 17.5 cm. Therefore the signal-to-noise ratio was improved by a factor of about 2.5. The present device is useful for both capillary liquid chromatography and capillary electrophoresis.

In ordinary commercial instruments made for liquid chromatography, the focusing area of the light beam is about 1 mm^2. Therefore, it is necessary to focus the light beam when we use a capillary flow-through cell of 50 μm I.D. For this purpose we can use either a plano-convex lens or a cylindrical lens. There are certain advantages in using a cylindrical instead of a plano-convex lens. First, the shape of the light beam is rectangular. Second, intense light does not destroy the fluorophor owing to the smaller amount of light intensity per unit area if the total amount of light is equal for both a plano-convex and a cylindrical cell device. Third, saturation of fluorescence might be less likely when using a cylindrical lens. There are also disadvantages in using a cylindrical lens. The cell length is longer than that when a plano-convex lens is used. In this study we used a CF cell 0.8 mm in length. In ordinary capillary zone electrophoresis and micro-column liquid chromatography, the decrease in theoretical plate number might be less than 5% under the above conditions[6].

Hence the most favorable arrangement would utilize both a cylindrical and a plano-convex lens for focusing a light beam on a capillary flow-through cell.

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