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HIGH-SENSITIVITY MICRO ULTRAVIOLET ABSORPTION DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A UV absorption detector with a 0.6-μl flow cell for high-performance liquid chromatography (HPLC) was developed. In order to improve the signal-to-noise ratio when the cell volume is reduced, the flow cell has a reflective layer on the internal wall of the optical path. The cell transmittance is hardly affected by changes in solvent conditions, based on a pulse flow from a pump. The flow cell can enhance sensitivity in the low-volume UV detector by making it possible to reduce the baseline shift and noise which are caused by variations of solvent conditions. Extra-column dispersion in the detector flow cell is reduced at high flow-rates of over 0.5 ml/min. The detector is especially effective for fast and micro-scale HPLC.

INTRODUCTION

Recently, high-performance liquid chromatographic (HPLC) methods with packing particles smaller than 5 μm and microbore columns have been developed for rapid analysis. However, it is necessary to decrease the extra-column dispersion that occurs outside the column when these smaller packings and microbore columns are used. A UV detector with a small flow cell and a new flow cell structure developed for this purpose was described in previous papers[1–5]. Our aim is the effective use of columns of length 10–50 mm and I.D. 2–3 mm, packed with 1–3-μm particles. Theoretically, a detector with a cell volume of less than 1 μl is required for such small packings[6].

In the design of the UV detector cell, it is necessary to minimize extra-column dispersion while maintaining sensitivity. It is difficult to minimize the cell volume in a simple structure, as the sensitivity of the UV detector depends on the Lambert–Beer law. In order to maintain sensitivity, the cell diameter should be reduced while keeping a long optical path. However, reducing the cell diameter makes the light flux smaller and results in noisier signals. In fact, our preliminary experiments showed that the signal-to-noise ratio was decreased on reducing the cell diameter and a baseline shift appeared based on changes in light energy due to refractive index (RI) phenomena of the solvent. The RI change occurs as the result of poor flow cell design and changes with the solvent flow, temperature, etc. and it causes light rays to bend in

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the cell. In general, the cell wall is opaque to UV light and therefore part of the incident light to the cell is lost by absorption and scattering on the internal wall. The changes in cell transmittance are caused by variations in the loss on the internal wall due to changes in RI; hence the development of a low-volume UV detector cannot be achieved by simply miniaturizing current commercial instruments, but rather reduction of the light absorption and scattering in the cell must be realized.

In a current commercial instrument, the flow cell has a reflective surface, which is a polished surface of a bright metal[7]. However, the reflectance of the polished surface is worse than that of an evaporated surface[8]. In this paper, we describe a new micro flow cell with a reflective layer formed by an evaporation method to reduce the influence on RI changes.

EXPERIMENTAL

Detector flow cell

Three types of flow cells were designed for the UV detector, which had an I.D. of 0.5 mm for a 3-mm path length. The total volume of each flow cell was 0.6 μl.

The first cell was made by drilling out a quartz block to make the necessary flow channels and fitting quartz windows in the ends of the block. The second was made in the same way, except that the internal wall was polished. The third had a reflective layer on the internal wall of the cell optical path. More specifically, this cell was composed of two plate-shaped flow cell bodies provided with a light path having a reflective layer, inlet and outlet passages and a pair of quartz windows on the side of the cell bodies. The reflective layer along the light path was formed by thermal evaporation or electron beam evaporation before the cell bodies were joined. A schematic diagram of the third cell is shown in Fig. 1.

The performances of the flow cells were compared with those of conventional semimicro flow cells of 1.0 or 1.1 mm I.D. for a 3-mm path length (cell volumes 2.4 or 3.0 μl, respectively) with a slit width of 0.5 mm I.D.

Apparatus

A Hitachi 655A-12 liquid chromatograph was used. The mobile phase was passed by the pump to a sample injector (Tokyorika 5001) and through a column (50 mm × 4 mm I.D.) packed with 2-μm glass beads. The sample injector was connected directly to a modified Hitachi 655-51 UV monitor without a column. The output from the UV detector was fed to an analysing recorder (YEW Model 3655).

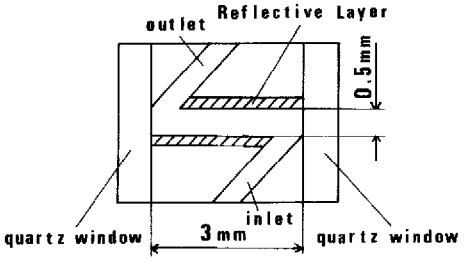


Fig. 1. Schematic diagram of flow cell.

The sensitivity of a UV monitor is dependent on the magnitude of the change in the cell transmittance due to changes in the RI of the solvent in the flow cell. Therefore, we measured the absorbance change that appeared as a baseline shift according to the stepwise change in the flow-rate. The sensitivity of the UV detector was evaluated from the magnitude of the baseline shift due to the change in flow-rate.

Methanol was used as the eluent, into which 0.1 μl of 0.5% (v/v) benzene was injected. Practical measurements of peak dispersion were carried out without a separation column. The problem in the experiment was that the band profiles to be determined were very narrow. As most of the measurements have to be carried out in less than 1 s, we used a system that had an overall time constant of 10 ms for the detector electronics and a sampling rate of 1 ms with a 1-kHz low-pass filter for the recorder. The dispersion was determined by the standard deviation of a concentration profile, taking the width at 60.7% of the maximum height.

RESULTS AND DISCUSSION

The first aim of the study was to obtain a detector cell with a volume of 0.6 μl. We wanted a structure of the micro flow cell for the UV detector that would be suitable for fast or micro HPLC. In order to identify the problems that occur on reducing the cell volume, the first micro flow cell was made by drilling out a quartz block according to the conventional method. This first cell was a Z-type of the same shape as a conventional Hitachi semimicro flow cell (3.0 μl). The magnitude of the baseline shift was measured quantitatively for flow cells of 0.5 and 1.0 mm I.D. diameter by the stepwise changes in the flow-rate. The results are shown in Fig. 2. The slope of the baseline shift for the 0.5-mm I.D. flow cell was about three times that for the 1.0-mm I.D. flow cell. Reduction in the cell diameter resulted in an increase in the ratio of the absorbed and scattered light on the internal wall to the incident light. Therefore, the baseline shift for the smaller diameter flow cell might appear even as a result of a slight change in the solvent conditions. This led to greater noise and drift, resulting in low sensitivity and instability in the analysis.

We then tried to reduce the baseline shift by setting a slit in front of the cell. It was expected that the slit might be effective in preventing the incident light from striking the internal wall, even when RI changes of the solvent would cause the light to bend. The effect of the slit was evaluated first with the 1.0-mm I.D. flow cell with a

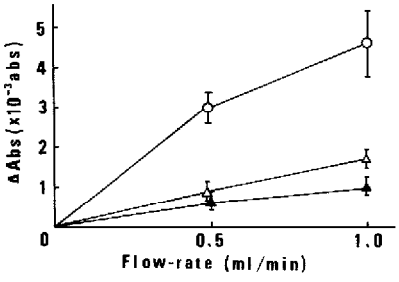


Fig. 2. Effect of flow-rate change on baseline shift. ○, 0.6 μl (0.5-mm I.D., 3-mm path length); △, 2.4 μl (1.0-mm I.D., 3-mm path length), ▲, 2.4 μl (1.0-mm I.D., 3-mm path length, with 0.5-mm I.D. slit).

0.5-mm I.D. slit. The magnitude of the baseline shift for this flow cell decreased to about 60% of that without the slit. However, reducing the cell diameter to 0.5 from 1.0 mm I.D. caused a larger baseline shift. Therefore, application of the slit was inadequate for a flow cell of less than 0.5 mm I.D. In addition, it became increasingly difficult to make straight, narrow-bore holes by drilling into quartz glass or even into plastics when the path length was greater than ten times the diameter. Fabrication by drilling may have limitations with regard to usable dimensions when a parallel-type flow cell is designed. Therefore, it is difficult to make a high-sensitivity flow cell smaller than 0.1 μl by using a conventional manufacturing method.

It is necessary to decrease the baseline shift to maintain the sensitivity when the cell volume is reduced by minimizing the diameter. In general, the cell wall is opaque to UV light and therefore the light strikes the cell wall due to RI changes and is absorbed or scattered. The loss in the cell must be decreased to maintain sensitivity, and consequently a new flow cell was needed with a reflective layer to reduce the changes in cell transmittance. In a current commercial instrument, a flow cell with a polished surface of a highly reflective material is applied. However, the reflectance of the evaporated surface used in this study is higher than that of a polished or electroplated surface in the same material[8]. We used an aluminium or rhodium layer as the reflective layer because of their high reflectance of UV light[9]. When using the aluminium layer, a silcon dioxide layer was formed on the reflective layer by electron beam evaporation to prevent corrosion on the reflective surface. When the flow cell was used under the usual conditions of reversed-phase chromatography, no reflectance change was observed.

The magnitude of the baseline shift with stepwise flow-rate changes is shown in Fig. 3 for different the flow cells. The baseline shift for the flow cell having a polished internal cell wall was about 50% of that of the untreated cell. This meant that the polished cell wall could enhance the sensitivity in the micro flow cell. Roughness of the internal wall seemed to produce a significant enhancement of the effects of RI changes of the solvent, even with only a slight change in flow-rate. The baseline shift with the flow cell having an aluminium or rhodium reflective layer decreased to about 80% of that with the untreated flow cell. In the flow cell having the reflective layer, it seemed that part of the incident light that did not pass straight along the light-path

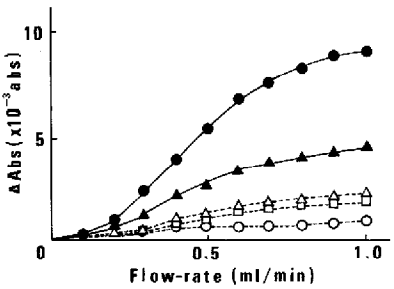
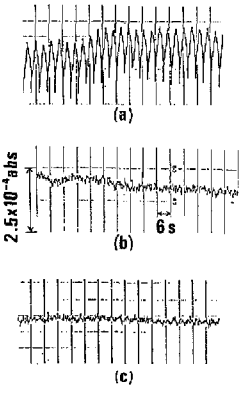


Fig. 3. Effect of flow-rate change on baseline shift. ●, 0.6 μl (0.5-mm I.D., 3-mm path length, untreated); ▲, 0.6 μl (0.5-mm I.D., 3-mm path length, polished); △, 0.6 μl (0.5-mm I.D., 3-mm path length, Rh-coated), □, 0.6 μl (0.5-mm I.D., 3-mm path length, Al-coated); ○, 3.0 μl (1.1-mm I.D., 3-mm path length, with 0.5-mm I.D. slit)



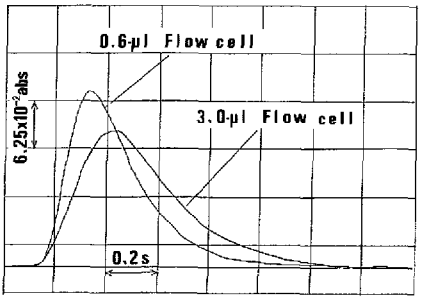


Fig. 4. Noise on chromatograms. (a) 0.6 μl (0.5-mm I.D., 3-mm path length, untreated); (b) 0.6 μl (0.5-mm I.D., 3-mm path length, Al-coated); (c) 3.0 μl (1.1-mm I.D., 3-mm path length, with 0.5-mm I.D. slit). Flow-rate, 1.0 ml/min; eluent, methanol.

Fig. 5. Variance contribution by UV detector cells. Sample volume, 0.1 μl; flow-rate, 1.0 ml/min; solute, benzene; eluent, methanol.

axis was reflected by the internal wall. Therefore, the ratio of the loss by absorption and scattering on the internal wall to the incident light was hardly changed, resulting in a very small baseline shift. Consequently, lower noise and high sensitivity and stability could be obtained with the flow cell having the reflective layer.

The noise levels on some chromatograms are shown in Fig. 4. The noise level on the chromatogram obtained with the flow cell having an aluminium or rhodium layer was as low as that with the conventional semimicro 3.0-μl (1.1 mm I.D. for a 3.0-mm path length) flow cell with a 0.5-mm I.D. slit. Therefore, the new micro flow cell seemed to be effective when applied with micro-bore columns or small particle columns.

The effect of reducing the cell volume on the band dispersion is shown in Fig. 5. The concentration profiles were obtained by detecting benzene eluted at a flow-rate of 1 ml/min. The reduction in cell volume had an effect on the band width. The relationship between band dispersion and flow-rate is shown in Fig. 6. The effect of the reduction in cell volume was clearly apparent at flow-rates above 0.5 ml/min. The qualitative dependence of dispersion on various parameters, especially diffusion coef-

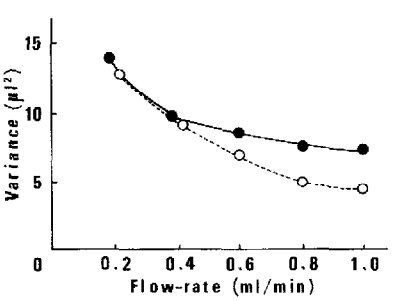


Fig. 6. Effect of flow-rate on variance. ●, 3.0-μl flow cell; ○, 0.6-μl flow cell.

ficient and flow-rate, is given by the Golay equation[10]. However, the present results showed that the dispersion decreased with increasing flow-rate, contrary to the theoretical prediction. The present method did not take into account the band broadening in the connectors and the injector. The theory suggested that such band broadening might be negligible, being much smaller than that in the detector cell. Hupe *et al.*[11] reported the same dispersion behaviour as ours and the major source of the band broadening might be the injection procedure. The shape of the curves in Fig. 6 suggested that a laminar flow profile existed at low flow-rates and might be destroyed by a secondary flow at higher flow-rates. If the flow profile in a cell is similar to that in a open tube, the flow profile in the Z-type flow cell in the main channel should be of the Poiseuille type, and including secondary flow or turbulence in the channel connections. However, there is no theoretical explanation regarding dispersion in a flow cell or even a zig-zag tube, because the relevant Navier–Stokes differential equations for complex geometrical forms have not been solved.

CONCLUSION

The UV detector with a 0.6-μl flow cell having a metallic reflective layer shows high sensitivity and stability. The results showed that a baseline shift was almost completely absent with variations in the solvent conditions and flow-rate. Therefore, the new flow cell should be useful for fast and micro liquid chromatography using small particle packings or narrow-bore columns.

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