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## ADVANCED MICROCOLUMN TECHNIQUES IN LIQUID CHROMATOGRAPHY

### USE OF GLASS-LINED STAINLESS-STEEL TUBING COLUMNS

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#### SUMMARY

Micro packed columns made of glass-lined stainless-steel tubing were prepared and their chromatographic performance was examined. The use of glass-lined stainless-steel tubing of 1/16 in. O.D. facilitated operation under high pressures. The system using such columns was applicable to chromatography with carbon dioxide as the mobile phase.

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#### INTRODUCTION

The effect of column materials on the column efficiency in micro high-performance liquid chromatography (micro HPLC) is greater than in conventional HPLC<sup>1</sup>. One of the reasons lies in the difficulty of polishing the inner wall of small-bore tubes. Glass<sup>2,3</sup> and fused-silica tubing of 0.1–0.35 mm I.D.<sup>1,4,5</sup> have consequently been employed as the column material in micro HPLC, as they give reasonable column efficiencies owing to their smooth and inert surface. The latter material was very convenient for long columns owing to its flexibility and good mechanical strength.

Glass-lined stainless-steel tubing of 1/16 in. O.D. with different inner diameters is commercially available. It is expected that this tubing will have a smooth surface like glass tubing and be capable of withstanding high pressures like stainless-steel tubing. Hence micro packed columns made of glass-lined stainless-steel tubing might make it possible to attain high efficiency and withstand high pressures. In addition, glass-lined stainless-steel tubing of 1/16 in. O.D. facilitates the coupling of micro columns with commercially available chromatographs.

This paper describes the performance of glass-lined stainless-steel tubing micro columns and reports some applications.

## EXPERIMENTAL

### *Column preparation*

Glass-lined stainless-steel tubing of 30 cm  $\times$  0.3–0.5 mm I.D. was obtained from Gaskuro Kogyo (Tokyo, Japan) and a suitable length was prepared by cutting the tubing followed by grinding both ends with an Electromotive Baby Cutter (Gaskuro Kogyo). Stainless-steel unions and ferrules were attached to both ends. A 1/16 in. zero dead volume union (Valco, Houston, TX, U.S.A.) was attached to the inlet and a 1/16  $\times$  1/32 in. zero dead volume reducing union (Valco) was attached to the outlet. Octadecylsilane (ODS) packing was slurried in acetonitrile containing 2–4% (v/v) of polyoxyethylene dodecyl ether or in tetrachloromethane containing the same nonionic detergent and packed at 300–350 bar by using a Familic-300S pump [Jasco (Japan Spectroscopic Co.), Tokyo, Japan]. A mixture of acetonitrile and distilled water was selected as the packing solution in the former instance and methanol in the latter. The slurry solution was sucked into the stainless-steel tubing of 1.6 m  $\times$  0.8 mm I.D. The packing materials were prevented from leaking out of the column by using 1/16 in. home-made stainless-steel frits (2  $\mu$ m). Develosil ODS (Nomurachemical, Seto-shi, Japan) and ODS-Hypersil (Shandon, Cheshire, U.K.) were employed as the packing materials in this work.

### *Apparatus*

The liquid chromatograph consisted of a pump, a micro valve injector, a separation column and a detector. A Familic-300S pump, an LKB 2150 HPLC pump (LKB, Bromma, Sweden) and a microMetric metering pump (LDC/Milton Roy, Riviera Beach, FL, U.S.A.) were employed. A 20-nl volume of sample solution was injected with an ML-422 micro valve injector (Jasco). The connecting tubing between the injector and the column was prepared in the laboratory, and consisted of stainless-steel tubing of 0.2 mm O.D.  $\times$  0.08 mm I.D. soldered into stainless-steel tubing of 1/16 in. O.D.  $\times$  0.25 mm I.D. with silver solder. This led to decreased extra-column band broadening. The connecting tubing between the column and the detector consisted of fused-silica tubing of 0.24 mm O.D.  $\times$  0.055 mm I.D. The upstream end of the tubing was fixed into stainless-steel tubing of 0.8 mm O.D.  $\times$  0.25 mm I.D., which allowed the tubing to be connected with the 1/16  $\times$  1/32 in. reducing union attached to the outlet of the column. Uvidec-100 and 100II (Jasco) UV spectrophotometers were employed as detectors. The flow cell was modified in the laboratory and the structure of the flow cell was nearly the same as that in previous work<sup>6</sup>. The cell volume was *ca.* 0.05  $\mu$ l. The time constant of the detector was modified to *ca.* 0.05 sec for rapid separation.

### *Reagents*

Reagents were supplied by Wako (Osaka, Japan) and employed without any treatment, unless indicated otherwise. Sodium 1-hexanesulphonate was supplied by Tokyo Chemical Industry (Tokyo, Japan).

## RESULTS AND DISCUSSION

The performance of the column is strongly affected by the chemical and physical properties of the column tubing. Fused-silica and glass tubing columns give higher

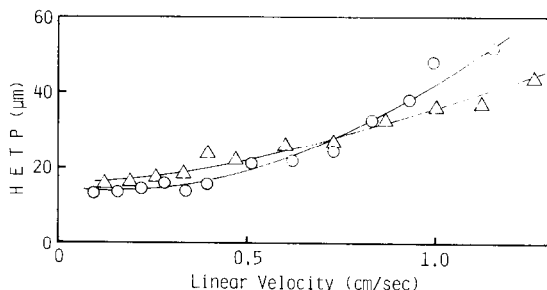


Fig. 1. HETP versus linear velocity. Columns: Develosil ODS-5 ( $5\ \mu\text{m}$ );  $\circ$ , fused silica,  $150 \times 0.34\ \text{mm}$  I.D.;  $\Delta$ , glass-lined stainless-steel,  $141 \times 0.3\ \text{mm}$  I.D. Mobile phase: acetonitrile-water (7:3). Sample: pyrene.

efficiencies in micro HPLC, owing to their smooth surface. The performance of the glass-lined stainless-steel tubing column was compared with that of the fused-silica tubing column using  $5\text{-}\mu\text{m}$  ODS particles. Fig. 1 illustrates the relationships between the theoretical plate height (HETP) and the linear velocity of the mobile phase. No difference in the efficiency of the columns is observed at lower linear velocities, whereas smaller HETP values are attained by the glass-lined stainless-steel tubing column at higher linear velocities. This result may be caused by expansion of the fused-silica tubing at higher pressures, which lead to poorer efficiency of the fused-silica tubing column at higher linear velocities compared with the glass-lined stainless-steel tubing column. The pressure drop across the fused-silica tubing column at a linear velocity of  $1\ \text{cm/sec}$  was *ca.* 180 bar under the conditions shown in Fig. 1, whereas that of the glass-lined stainless-steel tubing column was *ca.* 140 bar.

Fig. 2 shows the separation of polynuclear aromatic hydrocarbons (PAHs) on a glass-lined stainless-steel tubing column of  $141 \times 0.3\ \text{mm}$  I.D. The reproducibility

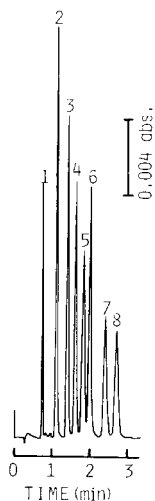


Fig. 2. Separation of PAHs. Column: Develosil ODS-5,  $141 \times 0.3\ \text{mm}$  I.D. Mobile phase: acetonitrile-water (7:3). Flow-rate:  $20\ \mu\text{l/min}$ . Peaks: 1 = benzene; 2 = naphthalene; 3 = biphenyl; 4 = fluorene; 5 = phenanthrene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene. Wavelength of UV detection: 254 nm.

of the retention time and the peak height was examined using an LKB-2150 HPLC pump. The relative standard deviations of both parameters for ten measurements were less than 1%.

Fig. 3 shows the rapid separation of an artificial mixture of L-ascorbic acid, theophylline and caffeine, and indicates that high-speed micro HPLC will be useful in routine analysis.

Fig. 4 demonstrates the separation of components in a commercially available cold medicine. One capsule of the cold medicine was extracted with 10 ml of methanol under ultrasonic vibration, the supernatant solution was filtered with a membrane filter (0.45  $\mu\text{m}$ ) and 20 nl of the prepared solution were injected. Acetaminophen, caffeine and methylephedrine were separated.

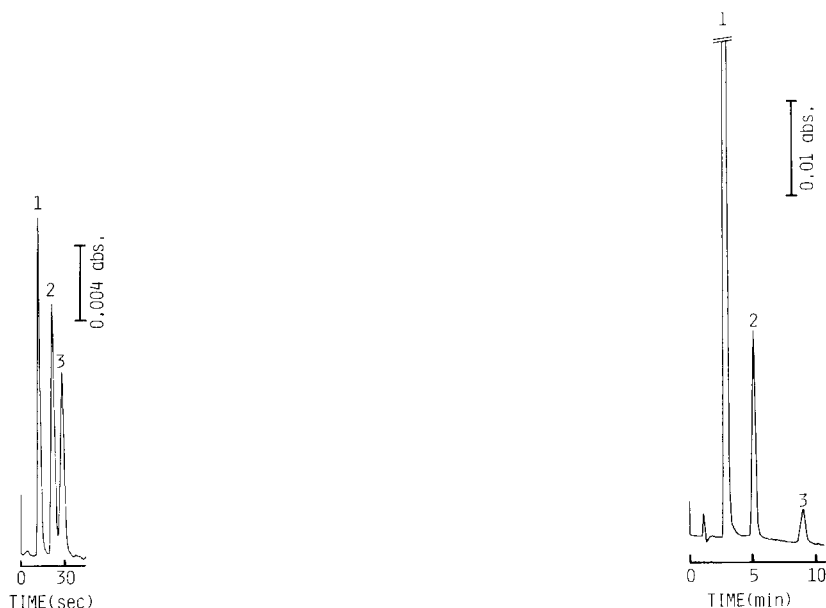


Fig. 3. Rapid separation of an artificial mixture. Column: ODS-Hypersil-3 (3  $\mu\text{m}$ ), 49  $\times$  0.3 mm I.D. Mobile phase: methanol-10 mM acetic acid (25:75). Flow-rate: 20  $\mu\text{l}/\text{min}$ . Peaks: 1 = L-ascorbic acid; 2 = theophylline; 3 = caffeine. Wavelength of UV detection: 265 nm.

Fig. 4. Separation of components in a cold medicine. Column: Develosil ODS-5, 150  $\times$  0.3 mm I.D. Mobile phase: acetonitrile-0.1 M phosphate buffer (pH 3.0) containing 2 mM sodium 1-hexanesulphonate (1:9). Flow-rate: 10  $\mu\text{l}/\text{min}$ . Peaks: 1 = acetaminophen; 2 = caffeine; 3 = methylephedrine. Wavelength of UV detection: 210 nm.

Glass-lined stainless-steel tubing columns facilitate operation at high pressures. The system allowed carbon dioxide to be employed as the mobile phase by keeping the pressure of the whole chromatographic system higher than the vapour pressure of carbon dioxide. The system was nearly the same as that in previous work<sup>6</sup> except for the separation column. A Familic-300S pump was employed in the constant-pressure mode. Fig. 5 demonstrates the rapid separation of PAHs on the ODS column. The mobile phase is liquid as the column temperature is kept lower than the critical temperature of carbon dioxide. Liquid or supercritical carbon dioxide has

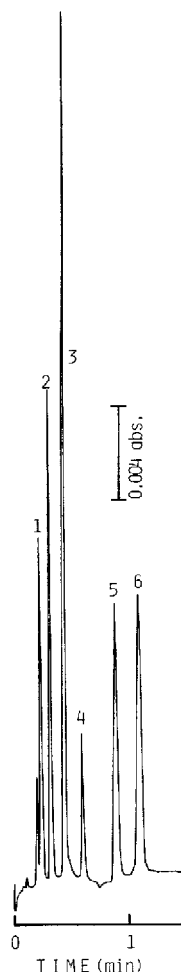


Fig. 5. Rapid separation of PAHs using liquid carbon dioxide as the mobile phase. Column: Develosil ODS-5, 141  $\times$  0.3 mm I.D. Inlet pressure: 150 kg/cm<sup>2</sup>. Temperature: 22.5°C. Wavelength of UV detection: 250 nm. Peaks: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = fluoranthene; 6 = pyrene.

lower a viscosity than common mobile phases in HPLC, which facilitates rapid separations. The system will be applicable to supercritical fluid chromatography with carbon dioxide as the mobile phase.

#### CONCLUSION

Micro packed columns made of glass-lined stainless-steel tubing give reasonable efficiencies, comparable or slightly superior to those of fused-silica tubing columns at higher linear velocities. They facilitate operation at high pressures and are applicable to liquid chromatography with carbon dioxide as the mobile phase.

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