Journal of Chromatography, 389 (1987) 236–239
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 302

Note

Analysis of radius variations in microcapillaries using the velocity of a viscous polymer plug and fluorescence photobleaching velocity detection

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(Received November 25th, 1986)

Narrow-bore capillary tubing has been increasingly used for open-tubular liquid chromatography applications[1–3]. Such tubing is commercially available with inner diameters ranging down to 5 μm. In order to determine the exact radius of these tubes, one can measure the transit velocity of a non-retained species. Then, from the velocity, pressure drop, capillary length and fluid viscosity, the capillary radius can be calculated. This determination yields only an average radius. For some applications, however, it is useful or necessary to know the actual radius variations in the tube. These variations may be sufficiently small that observation by light microscopy or mercury intrusion is not practical. In this note, we describe a new technique for measuring these variations by monitoring the velocity of a viscous plug moving through the capillary.

Chromatography systems using small-diameter capillary tubing often contain on-line concentration detectors in order to eliminate peak-spreading arising from connecting tubing and detector flow cells. On-line fluorescence detection, in particular, is an extremely sensitive and selective detection technique[4–6]. If the exciting light in a fluorescence detection system is sufficiently intense (a laser beam is usually used), the fluorescent species to be detected will be irreversibly bleached. When bleaching occurs, the detector measures the flux of the species (velocity times concentration) into the laser beam instead of the concentration of the species within the illuminated volume. If the capillary is filled with a fluorescent species at constant concentration, the detector output will be dependent on velocity only. A complete discussion of the photobleaching effect will be published separately[7].

Using the velocity detector described above, a capillary's radius variations can be measured by injecting a short plug of viscous polymer solution. During flow, most of the pressure drop, ΔP, occurs across this short plug (with viscosity μ_p), and the velocity in the detection volume, V_d, is determined by the velocity of the plug. This velocity will vary according to the capillary radius, R_p, at the location of the plug. Since the fluid is incompressible, the flow-rate, Q, at the plug (p) and the detector (d) can be equated:

$$Q = \pi R_d^2 V_d = \frac{\pi R_p^4 \Delta P}{8 L_p \mu_p}$$

0021-9673/87/$03.50

In addition, the volume of the plug is constant. Thus, the length of the plug, L_p, must vary as its radius varies:

$$L_p \propto 1/R_p^2$$

Then, since R_d, ΔP, and μ_p are constant:

$$V_d \propto R_p^6 \quad \text{and} \quad \frac{dR_p}{R_p} = 1/6\frac{dV_d}{V_d}$$

By measuring the fractional change in the velocity at the detector, the fractional change in the radius at the plug is directly measured. Note that knowledge of the plug's viscosity and length is not necessary. However, the plug must be large enough that the flow resistance is attributed largely to the plug flow. Also, the size of the plug determines the amount of averaging that will take place. For the analysis, it is assumed that the radius is constant for the entire polymer plug.

Note that this analysis is made possible by the fact that the polymer plug remains intact during the flow. This is so because of the non-Newtonian nature of the polymer solution. The stress of the tube flow is not sufficient to appreciably deform and disperse the plug, as would happen with a Newtonian fluid, even one of high viscosity.

EXPERIMENTAL

The capillary flow system, the static splitting injection technique and the on-line fluorescence detector have been described elsewhere[3,4]. The capillary is a 60 cm length of vitreous silica tubing with an inner diameter of 25 μm (SGE). The capillary is filled with a 1-ppm solution of fluorescein sodium salt (Sigma) in water. A small amount of sodium azide (0.002 *M*) is added as a preservative. After adjusting the focussed laser beam (30 mW, 488 nm) for optimum detection, a calibration of photomultiplier tube signal *vs.* pressure drop (proportional to velocity) can be easily performed. Such a calibration is shown in Fig. 1.

The polymer solution, 1% Separan AP30 polyacrylamide (Dow Chemical) in 0.002 *M* aqueous sodium azide, was prepared well in advance in order to assure a homogeneous solution. To perform the radius analysis, the polymer is injected for 4 s at the driving pressure of the flow (44 kPa). Then, the injection tee is extensively rinsed so that pure solvent/dye follows the plug. If rinsing is incomplete, the velocity will decrease monotonically during the analysis as more and more viscous material enters the capillary. The plug is forced through the capillary at constant pressure, and the variations in the fluorescence are recorded on a strip chart recorder. Then, employing the calibration, the fractional velocity changes and the radius fluctuations can be calculated.

RESULTS AND DISCUSSION

An example of the analysis is shown in Fig. 2a, which is the output from the fluorescence detector. The shape of this curve is entirely reproducible, except for the

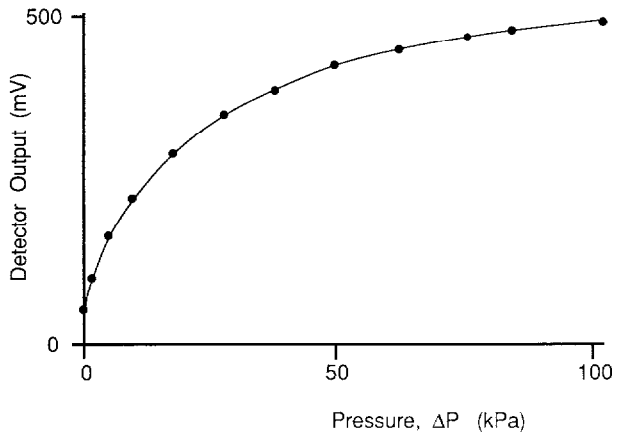

Fig. 1. Calibration curve showing the relationship between fluorescence detector output and pressure drop.

amount of detail, which is determined by the amount of polymer solution injected. On this particular capillary, two types of radius variations become apparent. The first type is a relatively sharp radius change, such as A in Fig. 2a, which represents a radius change of approximately 0.2 μm over an axial distance of 2.5 cm. Smaller radius variations (B in Fig. 2a) comprise the majority of the features detected in the analysis. These small waves are radius variations of approximately 0.04 μm which seem to repeat regularly. These calculations are based on an average capillary radius of 12.5 μm.

In order to verify that the observed effect is due to radius fluctuations and not due to some other flow phenomenon, the capillary was reversed in the flow system and the radius analysis repeated. The fluorescence trace from this second analysis is, as expected, a reverse of the original trace. In Fig. 2b, the trace of the second analysis has been first reversed horizontally and then superimposed on the original trace. Note that essentially all of the features are reproduced.

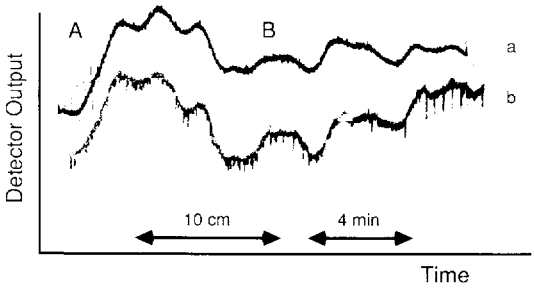

Fig. 2. Fluorescence output during radius analysis. (a) Original trace; feature A represents a variation of 0.2 μm, feature B a variation of 0.04 μm. (b) A trace of the same capillary reversed in the flow system; this trace has been horizontally reversed for direct comparison with (a). The length scale refers to the capillary, the time scale to the analysis.

REFERENCES

1 J. H. Knox and M. T. Gilbert, *J. Chromatogr.*, 186 (1979) 405.
2 G. Guiochon, *Anal. Chem.*, 53 (1981) 1318.
3 J. W. Jorgenson and E. J. Guthrie, *J. Chromatogr.*, 255 (1983) 335.
4 E. J. Guthrie, J. W. Jorgenson and P. R. Dluzneski, *J. Chromatogr. Sci.*, 22 (1984) 171.
5 V. L. McGuffin and R. N. Zare, *Appl. Spectros.*, 39 (1985) 847.
6 S. Folestad, B. Galle and B. Josefsson, *J. Chromatogr. Sci.*, 23 (1985) 273.
7 J. H. Sugarman and R. K. Prud'homme, *Ind. Eng. Chem. Fund.*, submitted for publication.