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MICROBORE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A MOVING-WIRE FLAME IONIZATION DETECTOR

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SUMMARY

The use of a Pye LCM 2 moving-wire flame ionization detector with 25 cm × 1 mm I.D. packed, reversed-phase microbore high-performance liquid chromatographic columns is reported. It was found that with flow-rates of 60–100 μl/min, all the column effluent was transported by the moving wire. The method was evaluated for coating efficiency, band broadening, and reproducibility. Applications to the analysis of fatty acids, glycerol esters, and carbohydrates are presented. The limits of detection ranged from 160 ng for xylose to 400 ng for lactose. Transport-type detectors, based on a moving wire, appear to be compatible with the low flow-rates of microbore high-performance liquid chromatographic columns.

INTRODUCTION

Considerable efforts have been devoted during the late sixties and early seventies to develop transport systems in combination with flame ionization detection (FID) for use with high-performance liquid chromatography (HPLC). The use of a conveyor in the form of a "chain" for HPLC was first reported in 1963 by Haahti and Nikkari[1]. The first moving-wire detectors were reported by James *et al.*[2] and by Karmen[3]. A newer, improved moving-wire detector was reported by Scott and Lawrence in 1970[4]. In this detector, the wire passes through an evaporator to remove the mobile phase and then to an oxidizer, in which the sample undergoes combustion in oxygen to carbon dioxide and water. The carbon dioxide is then reduced in the presence of hydrogen and a nickel catalyst to methane, which is detected by FID. In 1972, van Dijk[5] enhanced the sensitivity of moving-wire FID by a factor of 20–50 by spraying (instead of coating) the column effluent on the wire.

Other modifications, such as the use of a spiral[6] or coated[7] wire, as well as the use of a "disc"[8], have also been reported. An endless, perforated stainless-steel belt was used in combination with FID by Privett and Erdahl[9] to determine albumin, glucose and lipids. Tsuda *et al.*[10] used microbore HPLC columns, fitted with a glass microcapillary applicator to coat a twisted steel wire. Very recently, thermospray

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deposition of the sample onto a moving stainless-steel belt with subsequent vaporization or pyrolysis and detection by photoionization and/or electron capture, was reported by Yang *et al.*[11]. The unique feature of this approach is that the solvent is vaporized and removed without being deposited as a liquid on the belt.

However, since the early seventies, transport-type detectors for HPLC have received limited attention, because with the relatively high flow-rates used in conventional HPLC columns, only a very small fraction of the column effluent is coated on the moving surface, thus seriously impairing the limit of detection. The lower flow-rates used with microbore columns mean that a larger fraction of the effluent can be coated on the wire. This does not improve the detection limit in concentration, as the amount of material conveyed to the detector proper at a given concentration level is independent of the column radius. However, the detection limit in terms of mass will be considerably better when microbore columns, are used.

In this investigation, we report the use of a Pye LCM 2 moving-wire flame ionization detector with microbore HPLC columns. The method was evaluated for coating efficiency, reproducibility, and detection limits. Applications to the separation and determination of fatty acids, glycerol esters, and carbohydrates are presented.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a DMP 1515 Orlita pump (Dossiertechnik, Giessen, F.R.G.); a Model 7410 injector with an internal 5-μl loop (Rheodyne, Berkeley, CA, U.S.A.); two 250 × 1.2 mm I.D. microbore HPLC columns, one packed with 7-μm Zorbax BP C_{18} silica, the other with 7-μm Zorbax BP-NH_2. The column outlet was connected with a low-dead-volume fitting to a 10-cm section of fused-silica capillary tubing (100 μm I.D., 170 μm O.D.). The outlet end of the capillary was secured in a Swagelok fitting, which was in turn fastened to a movable micrometer screw, permitting the capillary tip to be positioned laterally with respect to the moving wire at the coating block position. This assembly was supported on a movable jack, enabling vertical adjustment of the capillary tip. The detector was a Pye Unicam LCM 2 moving-wire flame ionization detector (Pye Unicam, Cambridge, U.K.). This instrument was modified so that it could accept a 0.40-mm stainless-steel wire rather than the standard 0.12-mm wire supplied by the manufacturer. The fused-silica capillary that served as applicator was positioned at 90° to the wire for some of the experiments and at 30° for others (with the flow of liquid in the same direction as the motion of the wire). The applicator configuration is shown in Fig. 1. The moving wire in the Pye Unicam detector is supplied from a rotating spool and initially passes through a cleaner oven, operated at 850°C, where impurities are removed by oxidation in a stream of air. The wire then moves past the coating block, where the column effluent is deposited, and then through an evaporator (180°–200°C), which selectively removes the solvent in a stream of air. Finally, the wire passes through the oxidizer tube, where sample components are oxidized at 850°C to carbon dioxide and steam in a stream of air. The resultant carbon dioxide is mixed with a stream of nitrogen and hydrogen, reduced to methane in the reactor (385°C) in the presence of a nickel catalyst and finally detected by FID, at 205°C. A diagram is shown in Fig. 2. The moving-wire detector gas-flows were set as follows: cleaner air,

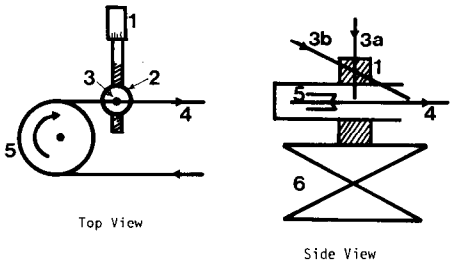


Fig. 1. Interface between the column and the moving wire. 1 = Micrometer screw for horizontal adjustment; 2 = Swagelok fitting; 3a = fused-silica capillary (100 μm I.D., 170 μm O.D.), 90° angle; 3b = fused-silica capillary (30° angle); 4 = moving wire; 5 = coating block pulley; 6 = vertical adjustment platform.

90 ml/min; evaporator air, 78 ml/min; FID air, 590 ml/min; nitrogen, 32 ml/min; and hydrogen, 35 ml/min. The moving wire was operated at speeds of 6.25 cm/s and 8.0 cm/s.

Chemicals and reagents

HPLC-grade methanol, acetone and acetonitrile were obtained from Merck (Darmstadt, F.R.G.). Formic, acetic, propanoic, butyric, pentanoic and hexanoic acids were purchased from BDH (Poole, U.K.). Triacetin, tricaproin, tributyrin and tripelargonin were from Fluka, Buchs, Switzerland); the remaining triglycerides esters were from Applied Science Labs. (State College, PA, U.S.A.). Xylose (D+), maltose and lactose (D+) were obtained from Merck; glucose and sucrose were from BDH.

RESULTS AND DISCUSSION

Theory of liquid transport on a moving wire

It is very difficult to predict the film thicknesss of liquid transported on a moving wire. The only equations currently available are those that have been published for dynamic coating procedures used in capillary gas chromatography[12–14]. These equastions are given below. If $10^{-3} < (u\eta\sigma^{-1}) < 0.09$, then

$$d_f = 0.005rc(u\eta\sigma^{-1})^{1/2} \quad (1)$$

and when $(u\eta\sigma^{-1}) < 10^{-3}$, then

$$d_f = 0.0134rc(u\eta\sigma^{-1})^{2/3} \quad (2)$$

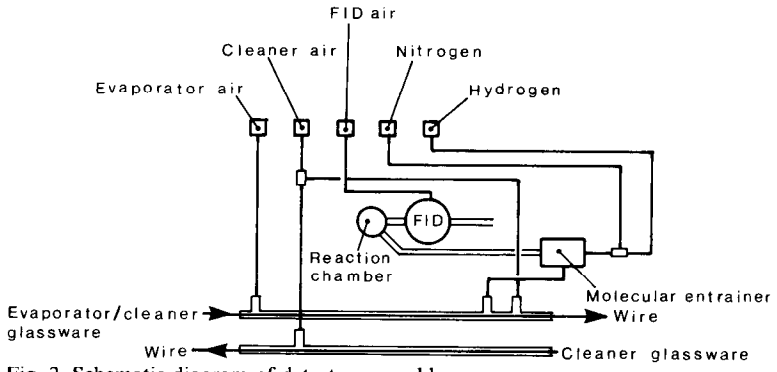


Fig. 2. Schematic diagram of detector assembly.

where u = wire velocity (m/s), η = viscosity of the liquid (Pa s), σ = surface tension (N m^{-1}), r = radius of the wire (m), and c = concentration of the stationary phase in the coating solvent. For a pure solvent $c = 100$.

The quantity of the liquid (Q, in m^3/s) that can be transported per second by a moving wire is

$$Q = 2\pi r d_f u \tag{3}$$

If eqn. 3 is in fact valid, then the amount of liquid transported would increase linearly with the square of the wire radius.

Although it has not been shown that these equations apply to a moving wire, we decided to evaluate the possibility of increasing the wire diameter from the standard value of 0.12 mm (supplied by the manufacturer of the moving-wire detector) to 0.40 mm, the thickest wire compatible with the mechanical components of the instrument.

Effluent transport and quantitative studies

Two different configurations of the fused-silica capillary with respect to the moving wire (a 90° angle and a 30° angle) were used for the coating process (see Fig. 1). In the 90° configuration, it was found that elution with mobile phase for *ca.* 0.5 h at the start of daily experimental work was necessary before efficient coating of the wire was achieved. Also, it was noted that small droplets of liquid crept up outside the wall of the capillary. This effect tended to cause occasional spikes in the baseline. In the 30° position, the coating process commenced immediately at the start of experimentation, but because the wire vibrated somewhat, there was occasional loss of liquid. In either position, it was found that the best results were obtained when the capillary was placed slightly to the side of and in contact with the wire.

Effluent coating on the wire was studied by steady-state introduction of sample, dissolved in mobile phase from a 3-ml sample loop, placed between the column and the detector. Continuous introduction of a 98-ppm solution of triacetin with 95% aqueous acetone showed that, even though the wire transports all of the effluent, the coating process was not uniform, as evidenced by a relatively high noise level, which was 2% of full scale at an attenuation of 320. This was in contrast to the process of coating by mobile phase alone.

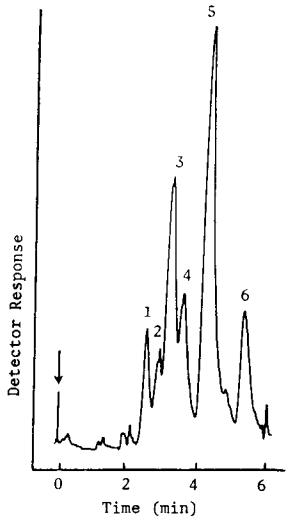
Repeated injections of 2.2 μg of lactose from a 5-μl sample loop placed between the column and the detector showed a satisfactory response to a variety of carbohydrates, but the reproducibility of peak sizes was not entirely satisfactory. A linear

TABLE I

MINIMUM DETECTION LIMITS FOR CARBOHYDRATES

Compound	*Minimum detection limit* (ng)*	*Minimum detection limit (ng carbon)*
Xylose	160	60
Glucose	110	40
Sucrose	120	50
Maltose	240	100
Lactose	400	160

* Based on a signal-to-noise ratio of 2.



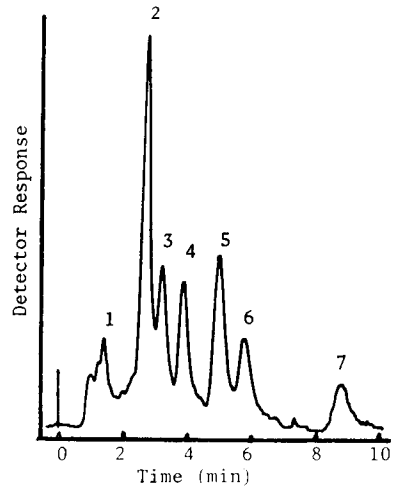


Fig. 3. Chromatogram of aliphatic acids (*ca.* 1 μg each). Mobile phase, methanol–water (70:30); column, Zorbax BP C_{18}; flow-rate, 67 μl/min; wire speed, 6.25 cm/s; attenuation, 1280. Peaks: 1 = formic acid, 2 = acetic acid, 3 = propanoic acid, 4 = butanoic acid, 5 = pentanoic acid and 6 = hexanoic acid.

Fig. 4. Chromatogram of triglyceride esters (*ca.* 1 μg each). Mobile phase, methanol–water (70:30); column, Zorbax BP C_{18}; flow-rate, 67 μl/min; wire speed, 6.25 cm/s; attenuation, 256. Peaks: 1 = tripalmitin, 2 = trielaidin, 3 = trimargarin, 4 = tristearin, 5 = trierucidin, 6 = triarachidin and 7 = tribehenin.

calibration curve for lactose was obtained between 0.3 and 13 μg. Similar results were obtained for other sugars (xylose, glucose, sucrose, and maltose). The minimum detection limits for the carbohydrates tested are listed in Table I, and were found to range from 110 ng for glucose to 400 ng for lactose. This is a considerable improvement (by a factor of *ca.* 90) over the conventional (UV) method for detecting carbohydrates[15].

Separations

A number of separations were successfully achieved with this system. Fig. 3 shows a chromatogram for several low-molecular-weight carboxylic acids obtained with methanol–water (70:30) as the mobile phase. A separation of several triglyceride esters with the same mobile phase is shown in Fig. 4, and a 27-min separation of five carbohydrates (xylose, glucose, sucrose, maltose, and lactose), achieved on a 25-cm Zorbax NH_2 bonded-phase microbore column with acetonitrile–water (70:30) is shown in Fig. 5. Evidently, this detection system is potentially useful for the monitoring of classes of compounds that are difficult to detect by conventional means, such as UV, fluorescence, or electrochemical detectors.

CONCLUSION

It has been shown that a moving-wire flame ionization detector can be used successfully with microbore HPLC columns, operated at flow-rates between 60 and 100 μl/min. The total column effluent can be transported on a 0.4-mm stainless-steel wire, at speeds of 6–8 cm/s. Coating the wire with volatile mobile phases produces essentially no noise. However, coating the wire with mobile phase and dissolved sample components produces a non-uniform film, causing some fluctuations in the

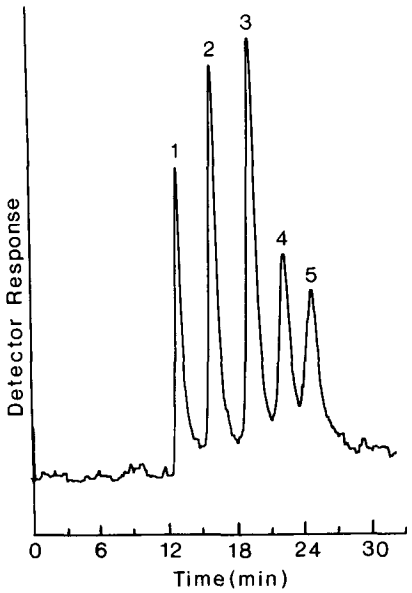


Fig. 5. Chromatograms of carbohydrates (*ca.* 2 μg each). Column, Zorbax BP NH_2, 7 μm; mobile phase, acetonitrile–water (70:30); flow-rate, 42.5 μl/min; evaporator temperature, 500°C; wire speed, 8.0 cm/s; attenuation, 32. Peaks: 1 = xylose, 2 = glucose, 3 = sucrose, 4 = maltose and 5 = lactose.

signal. The detector can successfully monitor some compounds, such as fatty acids, triglyceride esters, asnd carbohydrates, that are difficult to detect with conventional detectors. Minumum detection limits ranged from 100 ng for glucose to 400 ng for lactose. Experiments are underway to improve the reproducibility and reliability of the coating procedure.

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