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SCANNING FLUORESCENCE DETECTION IN CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

The design and performance of a scanning fluorescence detector for capillary supercritical fluid chromatography are presented. On-the-fly spectra are obtained and stored using a microcomputer. A novel fiber optic-aided detector design eliminates most background noise, and quantities as low as 1 ng give rise to full spectra. Detector dead volume is also minimized when this configuration is used.

INTRODUCTION

The need for detectors that provide qualitative as well as quantitative information about an eluting solute is felt in all areas of chromatography. Detectors based on fluorescence emission are attractive in this respect, because of their inherent high sensitivity and selectivity for compounds that fluoresce under UV light. Scanning fluorescence was first introduced in gas chromatography (GC) and liquid chromatography (LC) in the early 1970's[1,2], although this technique has found limited application in capillary chromatography. By considering necessary detector volumes and optical requirements for capillary supercritical fluid chromatography (SFC), it has been possible to construct a fluorescence detector capable of continuously acquiring either excitation or emission spectra while operating with a cell volume as small as 100 nl.

DESIGN

The scanning fluorescence detector consists of two parts. The first element is the flow cell, which is a variation of the capillary column high-performance liquid chromatographic (HPLC) on-column UV-absorption detector described by Yang[3]. Because of the large background signal due to light scattering from the capillary wall, direct on-column detection was found to be acceptable only for single-wavelength detection[4]. When the excitation or emission wavelengths were scanned, large variations in the background signal were observed. The detector was, therefore, reconstructed as shown in Fig. 1 to minimize background light. A short piece (approx. 30 cm) of 0.3-mm I.D. fused-silica tubing was used to construct a capillary flow cell. Into one end, the analytical column was inserted, and into the other end, a 145-μm

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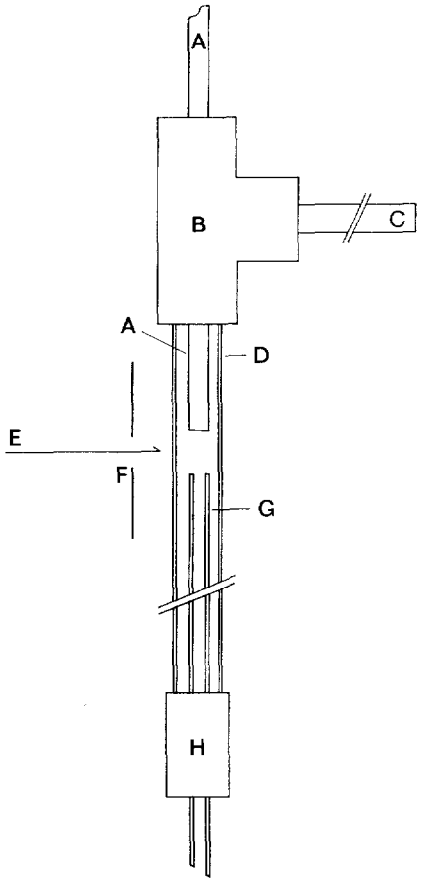

Fig. 1. Diagram of the detector configuration. A = 145-μm O.D. optical fiber; B = 1/16-in. stainless-steel "Tee" connector; C = 10-μm I.D. fused-silica capillary restrictor; D = 300-μm I.D. fused-silica capillary; E = excitation light; F = slit for reduction of stray light; G = analytical column; H = stainless-steel connecting union.

optical fiber (General Fiber Optics) was positioned. The polyimide coating was removed from a short section of the 0.3-mm I.D. capillary so that the volume between the column and the optical fiber could be illuminated by the excitation light. The distance from the column to the optical fiber could be varied to minimize dead volume, but was typically 2.5 mm, corresponding to a volume of 180 nl. Passing the optical fiber axially into the capillary flow cell[5] virtually eliminated all background induced by the curvature of the capillary wall. The narrow acceptance angle of the fiber aided in eliminating stray excitation light from finding its way to the detector and provided a light pipe from the cell to the entrance optics of the emission monochromator.

The second element of the scanning fluorimeter is the wavelength control and data acquisition base. The excitation or emission wavelength of a Perkin-Elmer 204-A spectrofluorimeter was controlled by replacing the slow synchronous motor with a suitable stepper motor (Sigma Instruments). The stepper motor was driven by approp-

riate circuitry, and controlled by an Apple 11+ microcomputer. Making use of existing gearing in the fluorimeter, each step corresponded to 1.2 nm. The ±1 step uncertainty in wavelength alignment was within the expected precision of the fluorimeter. With each increment of the monochromator, the microcomputer acquired a corresponding fluorescence intensity via an analogue-to-digital converter. A machine language routine was written to handle stepper motor control and data acquisition. The rate at which the monochromator scanned was limited by electronic and mechanical restrictions to approximately 200 nm sec^{-1}.

EXPERIMENTAL

The supercritical fluid chromatograph, which is described in more detail elsewhere[4], was built around an HP 5700A isothermal GC oven. The injector was replaced with a Valco 0.2-μl internal volume HPLC valve with capillary outlet. Except for the sensitivity studies, injections were split (approximately 3:1) to eliminate tailing of chromatographic peaks. The columns were produced by cross-linking a 0.25-μm film of SE-54 or 50% phenyl polysiloxane in 50-, 75- and 100-μm I.D. fused-silica capillaries (Hewlett-Packard, Avondale, PA, U.S.A.)[6]. The mobile phase (HPLC-grade *n*-pentane) was delivered by a Varian 8500 syringe pump, which was modified to operate under pressure control, as driven by the microcomputer. The mobile phase in the column was maintained at 210°C.

RESULTS AND DISCUSSION

The capillary fluorescence detector was evaluated as both a fixed-wavelength and scanning detector. Excellent results were found in both modes. To evaluate the detection limits of the detector itself when monitoring a fixed wavelength, a direct loop was connected from the injector to the detector in order to minimize dilution. Fig. 2 shows the results of direct injection of a solvent blank, followed by a solution

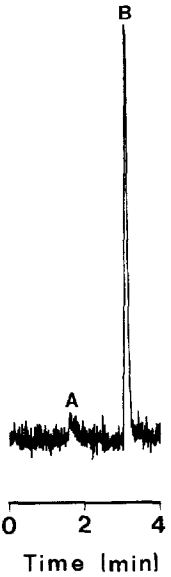

Fig. 2. Detector response for 50 pg of pyrene. Excitation wavelength was 335 nm with 10-nm slit width. Emission wavelength was 385 nm with 20-nm slit width. Detector response time constant was 1 sec. Peaks: A = blank; B = pyrene.

containing 50 pg of pyrene. The standard deviation for the background noise (Fig. 2) for the time interval of 0–1 min was 12 arbitrary intensity units. Assuming a detection limit of 2σ, a minimum detectable amount of 2 pg was obtained. Making the approximation that the peak was Gaussian, a detector sensitivity of the order of 500 fg sec^{-1} was obtained.

In order to illustrate the sensitivity of the detector in the scanning mode, 1 ng of pyrene was injected (without split) and eluted (unretained) on a 12 m × 0.1 mm I.D. SE-54 column. By scanning the excitation light from 260 to 360 nm, the spectrum shown in Fig. 3 was obtained. The elution time was approximately 10 min.

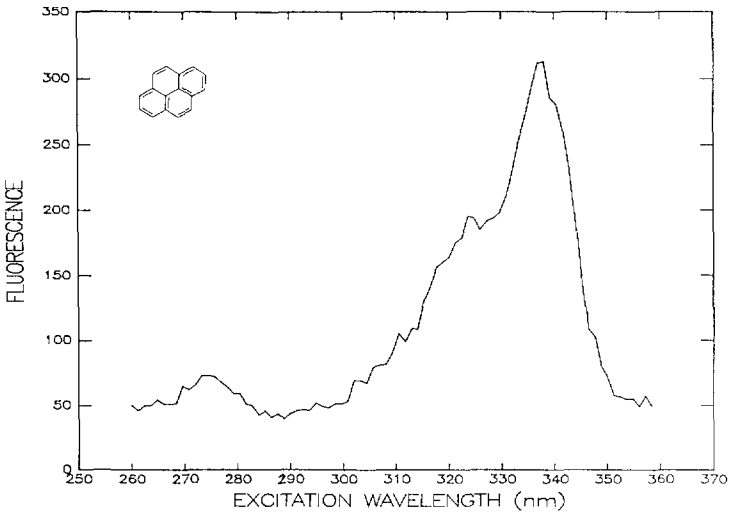

Fig. 3. Excitation spectrum from 1 ng of pyrene. The excitation slit width was 10 nm. The emission wavelength was 390 nm with 20-nm slit width.

The precision of the wavelength drive was evaluated by comparing the spectrum recorded by the analysis of a pure standard and the spectrum of the same compound, eluted from a chromatographic column (see Fig. 4). The shift seen between the two spectra of coronene is due to the inability to calibrate the monochromator absolutely and to remove the slack reproducibly when initially aligning the optical drive train. Furthermore, there is an inherent 1.2-nm step resolution of the stepper motor. To overcome this problem, a known compound can be used as an internal standard in an analysis to calibrate for any wavelength offset.

Good wavelength integrity within a chromatographic analysis is shown in Fig. 5 by the comparison of spectra of two compounds eluted close together in a coal derived product. The first of the two peaks to emerge was identified as pyrene, while several scans later, an alkylated pyrene was eluted, as seen by the bathochromic shift in its spectrum.

To investigate the reliability of a 1-sec scan time, scans from the leading and trailing edge of a benzo[*a*]pyrene peak were compared (Fig. 6). The monochromator was scanned from shorter to longer wavelengths for spectral acquisition. Therefore, the scan on the leading edge of the peak has a slightly higher relative intensity at longer wavelengths than the scan taken from the trailing edge. As is seen from Fig. 6,

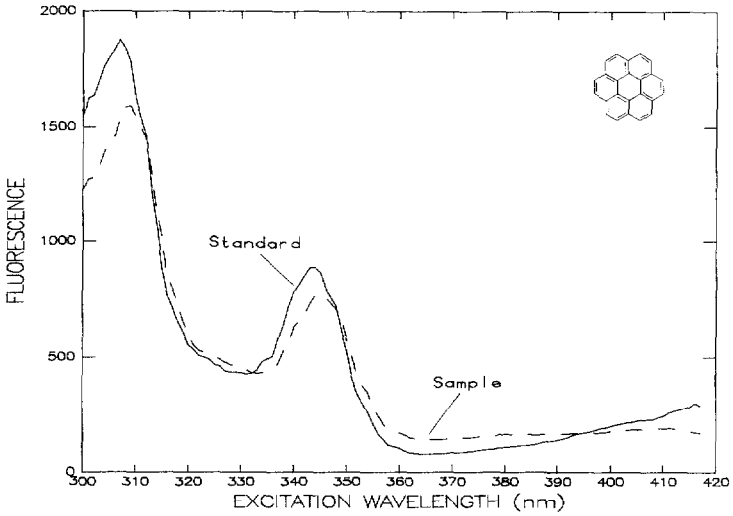

Fig. 4. Excitation spectra of coronene from 300–420 nm with 10-nm slit width.

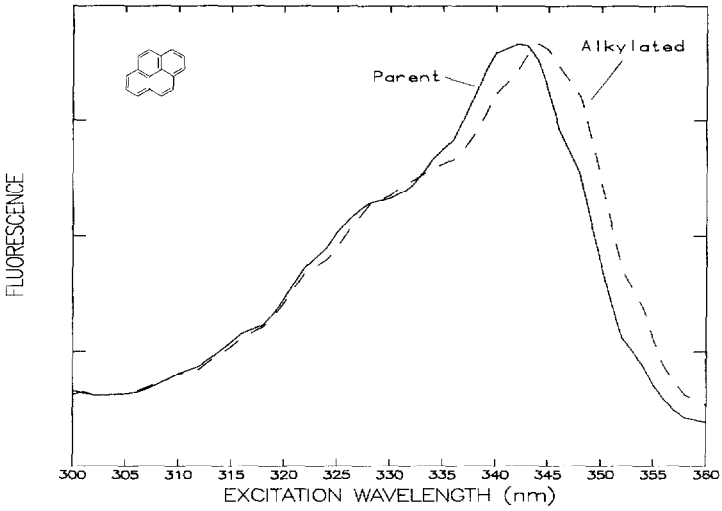

Fig. 5. Excitation spectra of pyrene and alkylated pyrenes from 300–360 nm with 10-nm slit width, vertical attenuation adjusted for comparison.

only a slight skewing effect is noticed with a 1-sec scan time for peaks with reasonable capacity factors (k').

The selective nature of the fluorescence detector can be noted in Fig. 7, in which a portion of a carbon black analysis is shown. Clearly, at 400-nm excitation, no fluorescence is seen from pyrene, whereas perylene and benzo[*ghi*]perylene fluoresce rather strongly. The two benzofluoranthene isomers can also be distinguished by comparing fluorescence at 335- and 400-nm excitation, even though they are not fully resolved.

Finally, to illustrate the ability to analyze complex mixtures, a carbon black

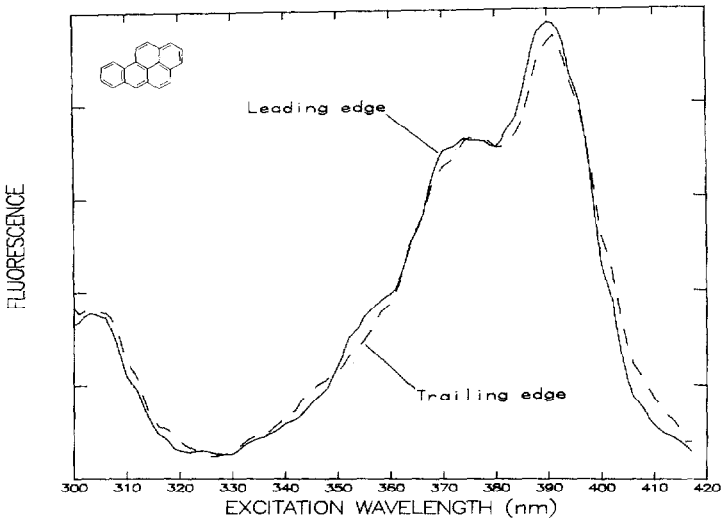

Fig. 6. Excitation spectra of benzo[*a*]pyrene from the leading and trailing edge of the chromatographic peak. Scanned from 300–420 nm (1 sec) with 10-nm slit width, vertical attenuation adjusted for comparison.

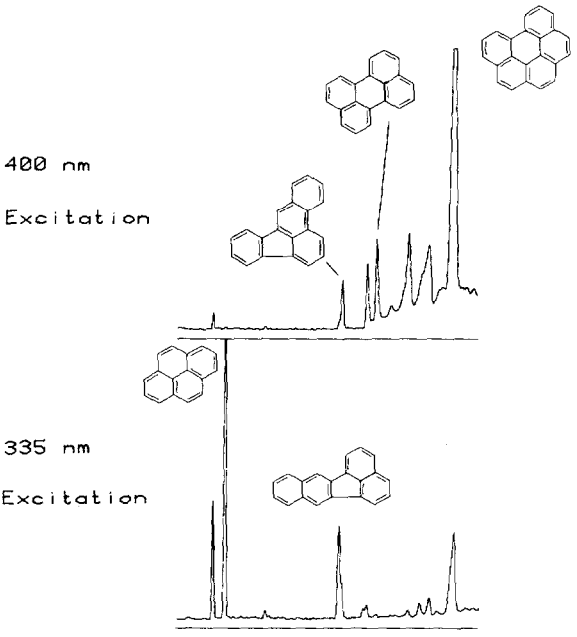

Fig. 7. Selected wavelength reconstructions of a portion of a supercritical fluid chromatogram of a carbon black extract. Conditions: 40 m × 50 μm I.D. column; 50% phenyl polysiloxane stationary phase (0.25-μm film thickness) crosslinked with azo-*tert.*-butane; *n*-pentane mobile phase at 210°C; density-programmed.

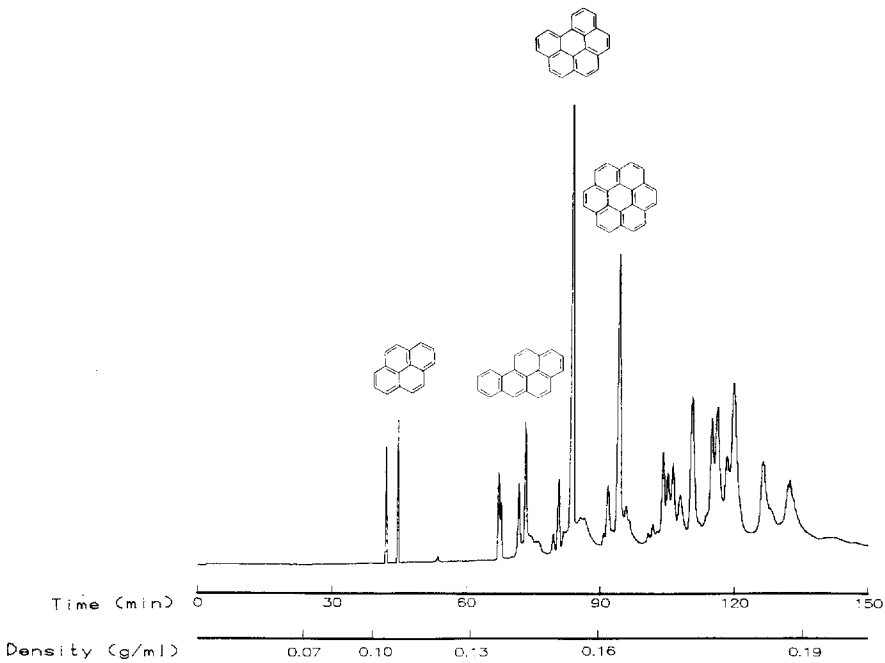

Fig. 8. Total fluorescence reconstruction of a supercritical fluid chromatogram of a carbon black extract. Conditions: 32 m × 75 μm I.D. column; 50% phenyl polysiloxane stationary phase (0.25-μm film thickness) cross-linked with azo-*tert.*-butane; *n*-pentane mobile phase at 210°C; asymptotic density-programmed from 0.07 to 0.19 g ml^{-1} after an initial 24-min isoconfertic (constant density) period.

extract was chromatographed. The excitation wavelength range from 300 to 400 nm was scanned (1-sec scan time) every 5 sec, and each spectrum was stored on a 5-megabyte hard disk which was linked to the Apple II + computer. After the chromatographic run was completed, two types of reconstruction were possible. The data could be recalled to give a selected-wavelength plot (Fig. 7), or the fluorescence of each scan could be integrated to produce a total fluorescence chromatogram (Fig. 8). From either of these two reconstructed chromatograms, scan numbers for various peaks could be selected and representative spectra displayed, thus aiding in compound identification.

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