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Laser-excited fluorescence detection of gas-phase chromatography eluates

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ABSTRACT

A high-spectral-resolution laser-excited molecular fluorescence gas chromatographic (GC) detection system is evaluated. It utilizes a pulsed supersonic jet expansion to yield very-narrow-bandwidth (*e.g.* $\leq$ 0.1 nm) fluorescence excitation spectra that may be rapidly scanned via a tunable dye laser. A microcomputer synchronizes the entire system, collects data, performs calculations, and creates visual displays of results. The laser-excited fluorescence detection system was interfaced to a gas chromatograph to exploit both the selectivity of the chromatography and the excellent spectral selectivity of the detector. Fluorescence excitation chromatograms were acquired by monitoring fluorescence emission from selected transition wavelengths characteristic of the GC eluates. The excitation wavelengths were also programmed to change at appropriate retention times to provide greatest selectivities for individual analytes as they eluted, and to allow multiple analytes to be determined in a single elution. Response factors for the system varied appreciably from run to run, which precluded the use of external standard quantitation procedures. However, excellent stabilites for within-run relative response factors were sufficient to allow for good quantitative measurements using internal standard techniques.

INTRODUCTION

Aromatic compounds such as polynuclear aromatic compounds (PNAs) typically have strong molecular absorbance bands between 180 and 350 nm. Multi-ring PNAs also tend to exhibit high fluorescence quantum efficiencies, resulting in part from their rigid molecular structures that exhibit few vibrational degrees of freedom, and thus limit non-radiative internal conversion. At room temperatures PNAs tend to exhibit broad-bandwidth fluorescence excitation spectra due to transitions from many overlapping electronic, vibrational, and rotational energy states. Correspondingly, at higher temperatures characteristic of gas chromatographic (GC) eluates, *e.g.*, $>250°C$, absorbance and fluorescence excitation spectra of PNAs lack detail, typically exhibiting three broad bands arising from electronic $\pi \rightarrow \pi^*$ transitions in the UV region [1,2].

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Most gas-phase UV fluorescence detectors have monitored emission from a fixed excitation waveband to produce chromatograms [3–10]. However, other systems allow fluorescence spectra to be acquired [11,12]. Limits of detection have been below nanogram levels, but vary considerably [3,4,8,11]. The fluorescence detectors show great selectivities, allowing PNA eluates to be measured in the presence of other non-fluorescencing, coeluting compounds. Judicious choice of the excitation band further enhances selectivities and sensitivities. The temporal resolution of the GC system supplements the powerful spectral selectivities of those detectors. Some of the detectors have allowed concurrent measurements of high-temperature GC eluates by both absorbance and fluorescence [3,4,11]. One of the systems [11] provides rapid-scanning capabilities and variable-wavelength illumination that may be changed easily as the chromatography separation develops. Several of the gas-phase fluorescence detectors are compact, of simple design, show excellent sensitivities, and exhibit low limits of detection.

Supersonic jet expansions (SJEs) are extremely useful for high-spectral-resolution fluorescence measurements [13], allowing for fluorescence spectra to be observed which are characteristic of very low temperatures, *e.g.*, < 10–100 K, and thereby show little of the vibrational and rotational sequence congestion observed in high-temperature spectra. The high spectral resolution has been exploited for GC eluates using constant-wavelength laser excitation [10,14–17]. Mass *versus* response relations were shown, and low nanogram limits of detection were achieved [15], however, no evaluation of potential quantitative GC analysis utility was done, and difficulties that plague GC calibrations and determinations were not addressed.

In this work, we evaluate a specially constructed GC detection system that exploits the unique spectral qualities of laser-induced fluorescence (LIF) and pulsed-flow SJEs of GC eluates. Herein we show superior selectivities offered by the system and evaluate quantitative GC applications for which it is suited.

EXPERIMENTAL

Reagents

Fluorene ($>98\%$ pure) and 1-methylfluorene ($>99\%$ pure) were purchased from Aldrich. Dichloromethane solvent was pesticide-grade quality, and all other chemicals used were reagent grade. Solutions were maintained in a darkened refrigerator at 0°C in PTFE-capped gas-tight vials between use. Gases used were $>99.999\%$ pure.

Apparatus

A block diagram of the entire system is illustrated in Fig. 1. Samples were introduced to the measurement cell via one of two methods. In one case, a small amount of one of the test compounds was placed in a Pyrex glass thimble inserted into a heated Swagelok Tee. Helium was then allowed to flow through the Tee, carrying the vaporized test compound continuously into the measurement cell. The second method utilized a Hewlett-Packard Model 5710A gas chromatograph for GC separations through a 1 m $\times$ 4 mm I.D. glass column filled with 100–120 mesh Supelcoport solid support coated at 3% with SP-2250 liquid phase. The oven temperature was programmed from 100 to 250°C at 16°C min^{-1} and held at 250°C for 10–30 min.

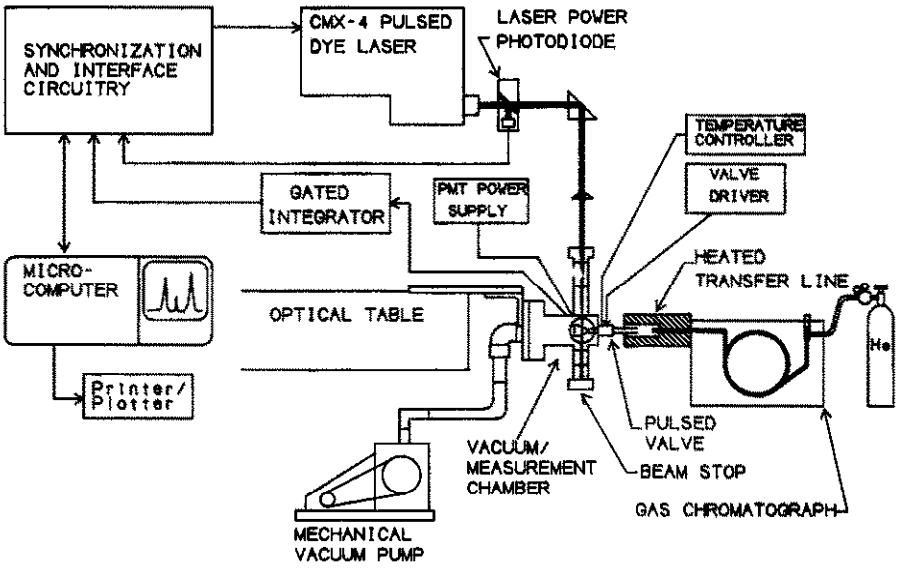

Fig. 1. Block diagram of the gas chromatography–laser-excited fluorescence–supersonic jet expansion experimental system.

Helium carrier gas was used at 30 ml min^{-1} with 40 p.s.i. inlet pressure. Injected volumes ranged from 0.1 to 2 μl into the 300°C injection port. Eluates from the GC system were transported to the SJE–LIF detector via the heated interface of the 5710A gas chromatograph, which had formerly been used with a Hewlett-Packard 5892 gas chromatographic–mass spectrometric (GC–MS) system. An 18-kg lead ingot was placed on top of the GC system and a 5-kg lead block on the transfer line to dampen mechanical vibrations. However, the GC fan remained a principal source of mechanical vibrations and thus fluorescence signal noise in the detector.

The SJE–LIF measurement cell (Fig. 2) was constructed from aluminum and was anodized black to reduce scattering of the laser excitation radiation. All vacuum seals were made with Viton O-rings. The entrance window was a 1-in. diameter, 0.25-in. thick fused-silica disc. Three entrance baffles with 0.196-in. diameter apertures were spaced along the 6-in. entrance arm of the cell to reduce stray and scattered light. The laser beam exited into a 4-in. deep beam stop through two additional baffles. The overall body of the cell was heated by a 2.5-in. I.D., 1.5-in. wide mica band heater, while the flange containing the pulsed valve was maintained at 145°C by an additional temperature-controlled cartridge heater. The measurement cell was evacuated to a pessure of 10^{-3} Torr via an Edwards ED200 200-LPM two-stage mechanical vacuum pump (Edwards High Vacuum, Sussex, UK).

The pulsed-nozzle device was a modified BMW automobile fuel injector with a 0.33-mm diameter axis-symmetric nozzle, controlled by special timing circuitry. The pulsed valve was maintained at 145°C to prevent condensation of the GC eluates, which were pulsed into the cell in 1- or 2-ms bursts at a frequency of 5 or 10 Hz. The Chromatix CMX-4 flashlamp-pumped tunable dye laser (Chromatix, Sunnyvale, CA, USA) was synchronized to fire 0.5–1.5 ms after the beginning of the SJE pulse and was

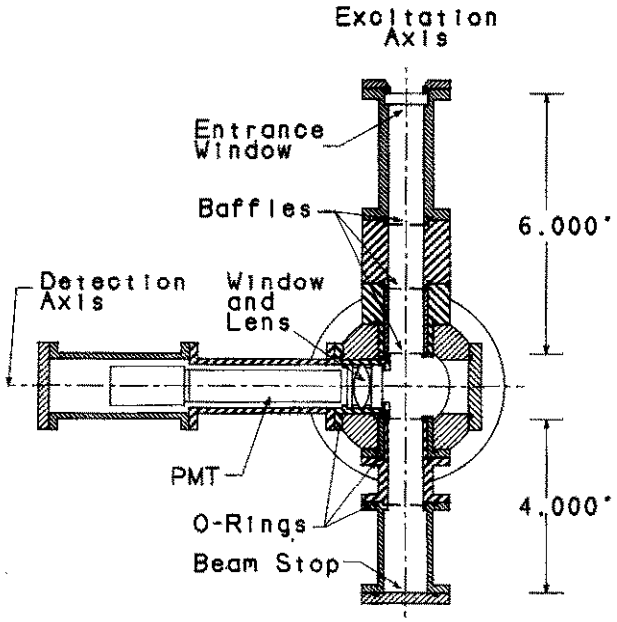

Fig. 2. Front view (looking down the expansion axis) of the supersonic jet expansion measurement cell. The supersonic nozzle and front flange are not shown.

aligned to intersect the central expansion axis 1 mm downstream from the nozzle tip. Total fluorescence emission was collected at right angles to both the expansion and excitation axes through a f/1 fused-silica bi-convex lens, which focused the fluorescence emission onto the photocathode of a Hamamatsu R1464 head-on photomultiplier tube (Hamamatsu, Middlesex, NJ, USA) maintained at −725 V. The PMT photoanodic current arising from the pulse of fluorescence emission was collected by an Evans gated integrator (Model 4120, Evans Assoc., Berkeley, CA, USA) and was digitized by a Metrabyte Dash-8, 12-bit-resolution analog-to-digital converter (ADC) (Metrabyte, Taunton, MA, USA). The ADC system was controlled by a Corona microcomputer (Cordata, Thousand Oaks, CA, USA). The computer served as the central control device for all synchronized parts of the system, and for the data collection, computations, and graphical displays.

The dye laser was wavelength-calibrated over the Rhodamine-590 laser dye tuning range using an optogalvanic technique [18]. In addition, the laser was modified to rapidly scan ultraviolet wavelength between 290 and 300 nm via automated computer control. Wavelength scanning via the birefringent filter tuning element and corrections for pulse-to-pulse variations of laser power were accomplished as has been done by other researchers [18]. The automation of the system allowed the laser to rapidly change from one wavelength to another during the course of a chromatographic run, allowing measurements of multiple analytes.

RESULTS AND DISCUSSION

Initial experiments involved characterization of the system with continuously introduced fluorene and 1-methylfluorene. High-resolution fluorescence excitation spectra were acquired using either argon or helium as a diluent gas. Spectra were acquired by stepwise adjustments of the laser's birefringent filter to achieve 0.01-nm increments. The fluorescence excitation spectra of fluorene and 1-methylfluorene in a helium supersonic expansion using the SJE–LIF system are shown in Fig. 3. The peak widths (full width at half maximum) were approximately 0.1 nm, as compared with bandwidths of 20–30 nm found in the solution and gas-phase spectra. Excitation wavelengths of 296.0 nm for fluorene and 294.7 nm for 1-methylfluorene were used in chromatographic experiments discussed below.

Fixed-wavelength fluorescence excitation chromatograms of a mixture of 100 ng fluorene and 100 ng of 1-methylfluorene taken at 296.0 and 294.7 nm are shown in Fig. 4. Chromatographic peak widths of 10–15 s were typically obtained, with slight tailing of the peaks due to cold trapping of the analyte on the pulsed valve, which could be heated no hotter than 145°C to protect internal plastic parts. The programmed

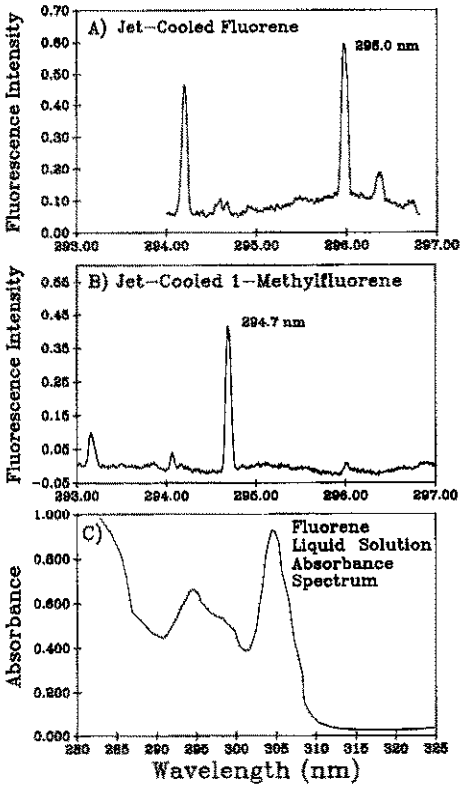

Fig. 3. Fluorescence excitation spectrum of (A) fluorene and (B) 1-methylfluorene acquired using the experimental system described herein, compared with (C) the conventional liquid solution absorbance spectrum of 5 μg/ml fluorene in cyclohexane.

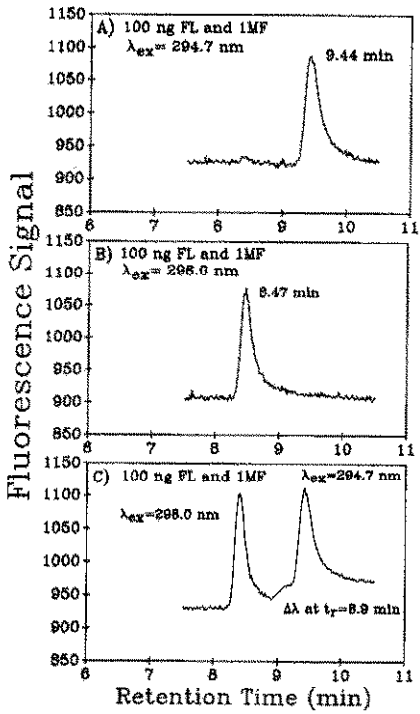

Fig. 4. Fluorescence excitation chromatograms of a mixture of 100 ng each of fluorene (FL) and 1-methylfluorene (1MF) acquired at fixed wavelengths of (A) 294.7 nm and (B) 296.0 nm, and (C) the chromatogram of the same mixture produced by a programmed excitation wavelength scan.

excitation wavelength fluorescence excitation chromatogram of the same mixture is also shown in Fig. 4. In this case, the system was programmed to change from 296.0 to 294.7 nm just after elution of the fluorene.

Good sensitivities were routinely achieved with the system, *e.g.*, 24.4 $\pm$ 0.7 area units ng^{-1} at 95% confidence. Consequent limits of detection approximated 5 ng, even with considerable background noise caused by mechanical vibrations from the GC fan and the vacuum pump. Selectivities were outstanding with little interference between the closely related test compounds, except for a slight response for 1-methylfluorene with 296.0-nm excitation due to minor spectral overlap. Excellent selectivities were also exhibited during the analyses of a very complex mixture. Fig. 5 shows fluorescence excitation chromatograms acquired using selective excitation wavelengths for fluorene and 1-methylfluorene for a hydrothermal oil sample taken from a deep-ocean hydrothermal vent located in the Guaymas Basin of the Gulf of California. This sample was also analyzed by GC–MS and contained a wide variety of PNAs including fluorene and four methylfluorene isomers. The only significant responses observed in these chromatograms were due to fluorene and 1-methylfluorene despite the fact that many other PNAs and the three other methylfluorene isomers were present at similar or greater concentrations.

Because of the complexity of the instrumentation, however, sensitivity of the

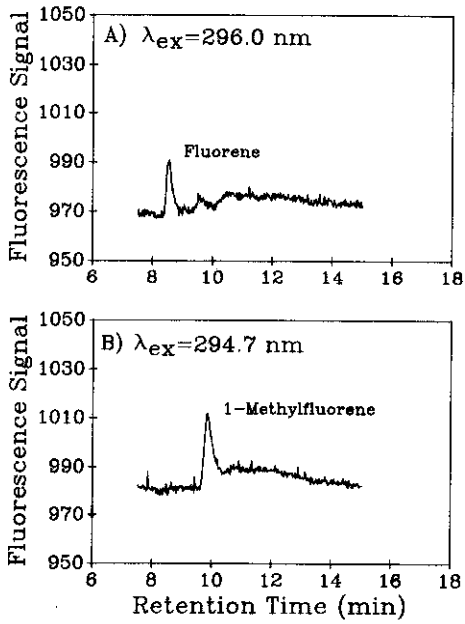

Fig. 5. Fluorescence excitation chromatograms resulting from 2-μl injections of a hydrothermal oil sample taken from the Guaymas Basin seabed in the Gulf of California.

detector system varied appreciably from day to day, and often from run to run, and therefore required repeated optimization to ensure good performance. Because of this variability, precise quantitation would be difficult if the external standard method were used, *e.g.*, for most fixed-excitation-wavelength analyses. However, with the programmed excitation wavelength system developed in this work, multiple analytes can be measured from a single elution, providing the potential for internal standard quantitation to be used. Response factors evaluated for eight replicate measurements of a fluorene and 1-methylfluorene mixture over the course of one day showed a relative standard deviation (R.S.D.) of 18 and 19%, respectively, while the R.S.D. for the relative response factor of 1-methylfluorene to fluorene was 3.5% for the same eight measurements. Thus, the detection system can provide good precisions for determinations via the internal standard technique, but may be unsuitable for the external standard method.

The GC–SJE–LIF system described herein provides programmed excitation wavelength selection and rapid wavelength scanning, thereby allowing multiple analytes to be determined for each chromatographic separation, with great selectivities for each measured analyte. However, irreproducibilities in sensitivities varied greatly from run to run, precluding reliable external standard measurements. Fortunately, relative sensitivities were very reproducible and thus reliable internal standard measurements are feasible with this detector system. Future instrumental improvements should diminish mechanical vibration-induced signal noise to considerably enhance limits of detection. In addition, alternative internal standards, such as deuterated isomers of desired analytes, are being investigated and may help to improve the utility of the technique.

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