*Journal of Chromatography*, 455 (1988) 332–335
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 922

## Note

# High-sensitivity laser-induced fluorescence–high-performance liquid chromatography studies of polyaromatic hydrocarbons using the argon ion laser–Yeung detector cell configuration

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(First received December 15th, 1987; revised manuscript received August 2nd, 1988)

The outstanding properties of lasers (*e.g.*, monochromaticity, temporal and spatial coherence, high photon flux) have made them increasingly attractive sources for fluorescence detection of low concentrations of high-performance liquid chromatographic (HPLC) effluents[1–10]. In order to take the fullest advantage of laser sources a number of detector cell designs have been developed and reported in the literature[3]. We recently reported[10] excellent laser-induced fluorescence (LIF)–HPLC results for tagged amino acids (at less than femtomole levels) using the detection cell configuration reported by Sepaniak and Yeung[5] in conjunction with the 458-nm line of the CW argon ion laser. In order to demonstrate further the applicability of this LIF detection configuration for other HPLC effluents, we have used an essentially identical system with the UV laser lines from the argon ion laser to detect polyaromatic hydrocarbons (PAHs) at low levels. There is, of course, no universal laser source, but the CW argon ion laser is attractive because it has a number of useful laser lines in both the near UV and visible spectral ranges. Thus the principal goal of this short communicaton is to demonstrate the versatility of the argon ion–Yeung cell configuration by operating with UV excitation. We have also utilized the tripled output of a pulsed Nd-YAG laser to provide a qualitative comparison with another commonly available laboratory laser.

## EXPERIMENTAL

### *Reagents and chemicals*

All the polyaromatic hydrocarbons used in this study were purchased from Aldrich (Milwaukee, WI, U.S.A.) and were used without further purification. The PAHs investigated include anthracene (An), fluoranthene (Fl), pyrene (Py), benz[*a*]-anthracene (BaA), chrysene (Chr), benz[*b*]fluoranthene (BbF), benz[*k*]fluoranthene (BkF), benz[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (ah) and benz[*g,h,i*]perylene (ghi). Reagent-grade hexane, toluene and chloroform and HPLC-grade acetonitrile were

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0021-9673/88/$03.50 

purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.). Deionized, distilled water was used for all experiments. Solvents were degassed using an ultrasonic bath and further degassing was effected by flushing with helium gas throughout the experiment. Stock solutions of the various PAHs were prepared in a solvent mixture of hexane–chloroform–toluene–acetonitrile (81:11:2:6) to ensure solubility of all the PAHs and also compatibility with the mobile phase in the HPLC system.

*Apparatus*

All separations were performed on a 250 × 2.6 mm I.D. reversed-phase column (Perkin Elmer HC-ODS 0089-0716) using either a Milton Roy Model 396 single piston pump or an LKB 2150 HPLC pump, and a Rheodyne 7125 injector fitted with a 20-$\mu$l loop. The detector cell, irradiation configuration and fiber optics for fluorescence collection were identical to the system described in our earlier work[10]. For the argon ion laser (Spectra Physics Model 171-17) experiments the total UV output (334, 351 and 363 nm) was utilized after removing superradiance with a Schott UG-11 filter. Fluorescence from the PAHs was detected by a photomultiplier tube after bandpass (10 nm) filtering at 430 nm, and signals were processed by conventional photon counting methods. The comparison Nd-YAG pulsed laser (Quantel YG-481) experiment used the tripled output at 355 nm after filtering with the same Schott UG-11 filter to remove residual 532-nm radiation. Photomultiplier tube detected fluorescence at 430 nm was processed at the 10-Hz laser repetition rate using a boxcar averager (PARC M162/164). Chromatographic data were either output directly to a strip chart recorder or were processed with a microcomputer data acquisition system before plotting.

## RESULTS

Fig. 1 presents a chromatogram obtained under isocratic conditions for a mixture of ten PAHs using the argon ion laser system. Note that all but the two PAHs with the longest retention times are readily identified and distinguished although

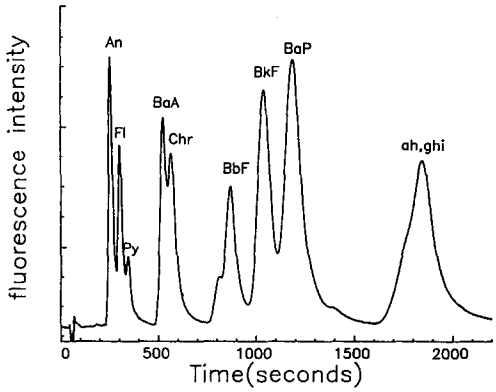


Fig. 1. Chromatogram of a mixture of ten PAHs using isocratic conditions: acetonitrile–water (70:30) at a flow-rate of 1.0 ml/min. A 20-$\mu$l injection contained 200 pg of An, Fl, BkF and BaP; 300 pg BbF; 500 pg ghi; 1 ng BaA and ah; 2 ng Py; and 40 ng Chr.

resolution is certainly not complete. From chromatograms of the pure components, detection limits have been determined for several of the PAHs as summarized in Table I. The calibration curves were linear over more than two decades with $r \geqslant 0.997$. Similar data were also collected for the same PAHs using the pulsed Nd-YAG laser system and the detection limits are presented in Table I.

## DISCUSSION

The detection limits observed for the CW argon ion experiments vary roughly over the range of 0.2 to 1.3 pg (*ca.* 1–6 femtomol), approximately 100 times lower than obtained with a conventional HPLC system with $\lambda_{ex} = 365$ nm and $\lambda_{em} = 455$ nm[11]. On the other hand, conventional HPLC fluorescence detection limits reported for deuterium lamp excitation at 280 nm are within a factor of five of our argon ion results[12], showing the efficacy of irradiating into the more intense shorter wavelength UV band. In any case, the argon ion–Yeung cell system clearly performs excellently in the UV with detection limits comparable to those reported earlier for tagged amino acids irradiated in the visible spectral region[10]. Again, as in our earlier studies[10], we find the Yeung detector cell design and optical geometry to be trouble free and experimentally reliable for routine studies.

When the tripled pulsed YAG laser output at 355 nm is used in place of the argon ion laser lines, the detection limits as presented in Table I are seen to be generally degraded by approximately one order of magnitude. This poorer performance was not unexpected and arises primarily because the YAG laser used in these studies shows pulse-to-pulse power variations of up to 10%, while the CW argon ion laser output has a stability approaching 1%. In addition, the spatial beam quality of the YAG output is substantially poorer than that of the CW argon ion laser which leads to poorer focusing and an increase in scattered light.

For either laser source, the detection limits vary from one compound to the other depending upon factors such as the absorption coefficient at the exciting line wavelength(s) and the fluorescence yields in the filter bandwidth of the detection system. In the case of the pulsed laser, the detection limits may also be importantly effected by the gated integrator aperture delay and duration if not properly matched to the fluorescence lifetimes, which may vary from one compound to the other.

Overall, it is clear from this work and our earlier studies[10] that the CW argon ion

TABLE I
DETECTION LIMITS FOR FIVE PAHs

| *Compound* | *CW argon laser** | *Pulsed YAG laser** |
|---|---|---|
| Anthracene | 0.16 | 0.80 |
| Fluoranthene | 0.38 | 0.38 |
| Benz[*a*]anthracene | 1.3 | 23.0 |
| Benz[*b*]fluoranthene | 0.72 | 15.0 |
| Benz[*a*]pyrene | 0.18 | 0.80 |

* Detection limits in pg based on a signal-to-noise ratio of 2.

laser used with the detector cell configuration of Sepaniak and Yeung[5] yields highly reliable and sensitive chromatographic determinations for compounds whose excitation bands are accessible to the UV or visible lines of the laser.

ACKNOWLEDGEMENTS

Support of this work by the National Science Foundation (Grant CHE 81-08395), and by the Kansas Commisson on Advanced Technology and Oread Laboratories, Inc., is gratefully acknowledged.

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