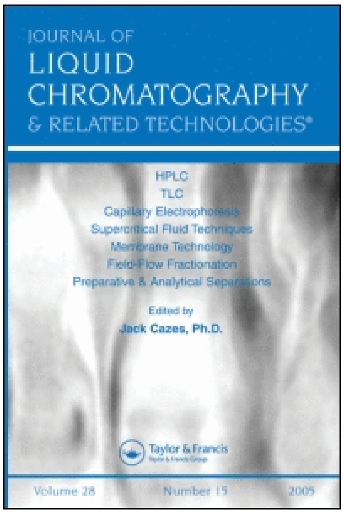

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A Simultaneous, Laser-Excited Fluorescence, Photoacoustic, and Two-Photon Photoionization Detector for Liquid Chromatography

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A SIMULTANEOUS, LASER-EXCITED FLUORESCENCE, PHOTOACOUSTIC, AND TWO-PHOTON PHOTOIONIZATION DETECTOR FOR LIQUID CHROMATOGRAPHY

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ABSTRACT

A laser-excited windowless flow cell has been developed for simultaneous fluorescence, photoacoustic, and two-photon photoionization detection of aromatic compounds in HPLC eluents. Sensitive three-mode detection of acridine, naphthalene, 7,8-benzoflavone, N-ethylcarbazole, and anthracene in 70/30 V/V acetonitrile/water is demonstrated with conservative detection limits in the nanogram range and below.

INTRODUCTION

Although there is still no simple, sensitive, selective, inexpensive, and universal detector for HPLC, significant progress has been made by a number of researchers. Thus we have laser-excited fluorescence (Diebold and Zare (1)), laser-excited photoacoustic detection (Oda and Sawada (2)), (microwave excited) Xe continuum source photoionization detection (Locke et al. (3)), and the laser two-photon excited fluorescence detection of Sepaniak and Yeung (4). In addition, several multi-mode detection schemes have been developed to improve upon the limited selectivity available from any one technique. Thus we have a combined CARS, Raman, fluorescence, and absorbance detection (Boutilier et al. (5)) and Raman/absorbance detection (Yeung (6)). Our efforts to develop an improved multi-mode detector suitable for HPLC applications are detailed below.

In a previous paper (7), we have demonstrated that a N_2 laser-excited, three-mode (fluorescence, photoacoustic, photoionization) windowless flow cell with a simulated liquid chromatograph system may be used to detect a variety of polycyclic aromatic hydrocarbons (PAHs) in n-heptane. The three detection modes were quite sensitive and completely independent. In the paper by Voigtman and Winefordner (8), the primary factors of analytic importance in the operation of the flow cell, i.e., incident laser pulse energy, bias voltage, and solvent effects, are elucidated. The flow cell was used to detect a variety of drugs (important, diversely structured substances) in 50/50 V/V ethanol/water and it was found that excimer laser excitation (308. nm, XeCl) and N_2 laser-pumped tunable dye laser excitation were usable in the two-photon photoionization mode. Doubled dye laser operation was also feasible provided sufficient pulse energies ($\gtrsim 1$ μJ) were available.

The significance of these results is that the three most important photophysical deactivation mechanisms are simultaneously usable for detection purposes. Accordingly, having adequately characterized the analytical performance of the laser-excited windowless flow cell, we have modified the flow cell slightly to allow for direct connection to a commercial HPLC system and have achieved sensitive detection at and below the nanogram level for several model aromatic compounds using acetonitrile/water solvent systems.

MATERIALS

Acridine and N-ethylcarbazole (Aldrich, Milwaukee, WI 53202) and anthracene, naphthalene and 7,8-benzoflavone (Eastman Kodak, Rochester, NY 14603) were used as received. The acetonitrile (Fisher Scientific, Fair Lawn, NY 07410)/water (Barnstead, Sybron Corp., Boston, MA 02109, 15. MΩ cm) solvent mixture was deaerated by bubbling with He.

METHODS

The three-mode HPLC flow cell is shown in Fig. 1. The stainless steel reservoir previously used to simulate an LC system (7,8)

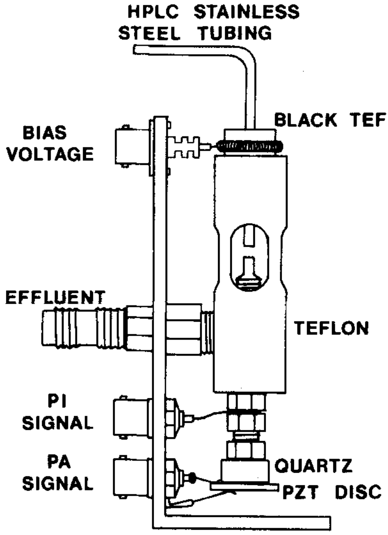

Figure 1: Schematic of the windowless HPLC flow cell.

was replaced with an inert, black, conductive coupling sleeve (1" ong, 1/16" I.D., 5/8-18 outer threading) made of graphite-filled PTFE (Fluorocarbon, Anaheim, CA 92803). The use of this material was convenient, but not essential. Excitation illumination was provided by a Lumonics (Lumonics Research, Ontario, Canada) TE-861S excimer laser (XeCl, 308. nm, ∿1 mJ/pulse, 20 Hz repetition rate) focussed onto the center of the flowing liquid column (∿6 μL suspended volume). The fluorescence (FL), photoacoustic (PA) and two-photon photoionization (PI) signals were acquired by appropriate pre-amplifiers, amplifiers, and gated (boxcar) averager (PAR 160) with chart recorder output. The effluent from the HPLC system (Altex Scientific, Berkeley, CA, Model 312, 1.5 mL/min flow rate, isocratic operation, 4.6 mm x 25 cm column with Sperisorb ODS, 10 μm packing, 20 μL sample injections) passed to a commercial UV absorbance detector (Altex, Model 153, 254. nm) with chart recorder output and then through 0.6 m of narrow bore PTFE tubing to the HPLC stainless steel tubing of the flow cell. The PTFE tubing isolated the bias voltage (-1 kV) on the flow cell from the grounded HPLC system. The DC leakage current that results from the use of the acetonitrile/water system was bypassed to ground through a 10 kΩ metal film resistor while the pulsed PI current was passed to the pre-amplifier by a 50 nF, 1600 WV capacitor as described elsewhere (8).

Several observations concerning the operation of the flow cell are in order. First, bubble formation was avoided by bubbling the solvent mixture with He for at least an hour prior to the experiments. This resulted in shifts in the retention times of the compounds separated by HPLC. Such shifts may be avoided by solvent programming. Second, electrolysis was not observed and electrode corrosion was negligible. Suitable materials for electrode construction include stainless steels, titanium, and graphite-filled PTFE. Third, laser excitation source focussing is important, but not overly critical. The electrode gap was set by screwing the coupling sleeve into the main PTFE body of the flow cell with a measured standard between the electrodes.

RESULTS

The performance of the three-mode flow cell in all three modes is shown in Fig. 2 (FL), Fig. 3 (PA), and Fig. 4 (PI). In each case, the concomitant UV absorbance is included for comparison. Concentrations are given in the figure captions. Note that the excitation

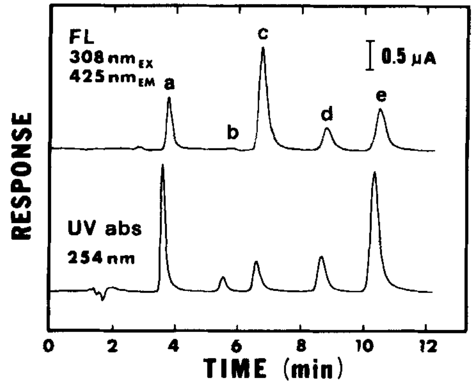

Figure 2: Laser-excited Fluorescence and UV Absorbance Chromatograms. Concentrations (µg/mL) are as follows: (a) acridine (20.), (b) naphthalene (22.), (c) 7,8-benzoflavone (20.), (d) N-ethylcarbazole (22.), and (e) anthracene (8.0). The largest FL peak (c) is 1.7 µA. The largest UV abs peak (a) is 2.0 x 10^{-4} absorbance units.

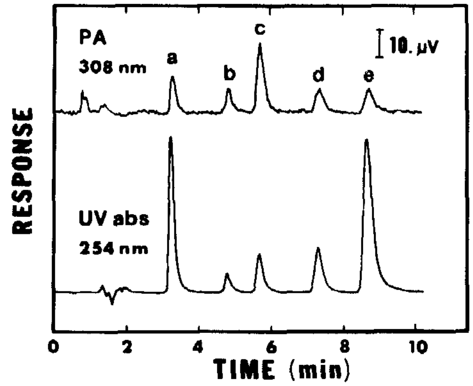

Figure 3: Laser-excited Photoacoustic and UV Absorbance Chromatograms. Concentrations as in Fig. 2. The largest PA peak (c) is 26. µV. The largest UV abs peak (a) is 2.2 x 10^{-4} absorbance units.

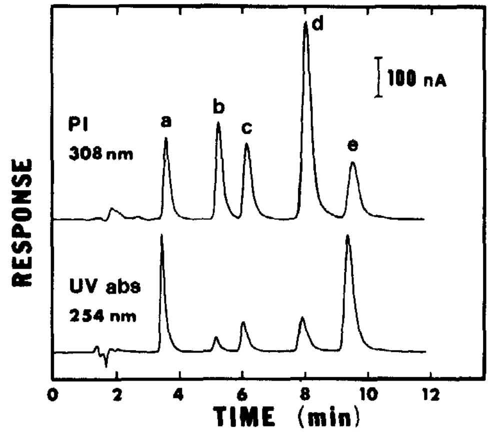

Figure 4: Laser-excited Two-Photon Photoionization and UV Absorbance Chromatograms. Concentrations as in Fig. 2. The largest PI peak (d) is 510 nA. The largest UV abs peak (a) is 1.9 x 10^{-4} absorbance units.

(308. nm) and emission (425. ± 8. nm) wavelengths chosen were non-optimal for the fluorescence mode. The emission wavelength was chosen after examination of the emission spectra (with 308. nm excitation) of the compounds chosen. Note that all three modes provide usable chromatograms although the PA mode is less sensitive in this instantiation as we have previously noted (7).

In order to determine the sensitivity of the three mode flow cell, limiting detectable concentrations were determined by calibration with dilutions from a freshly prepared stock solution of the five compounds indicated. The limit of detection (LOD) is obtained by dividing the peak-to-peak blank noise by the least squares slope of the plot of chromatographic peak height versus concentration of the injected analyte. The results are shown in Table 1.

DISCUSSION

Several conclusions are imediately apparent from the chromatograms and detection limits. First, sensitive three-mode operation is readily achieved despite the fact that none of the three modes is

TABLE 1

Chromatographic LODs (S/N=3) for Five Aromatic Compounds in 70/30 V/V CH_3CN/H_2O. Units are μg/mL.

	Acridine	Naphthalene	7,8-benzoflavone	N-ethylcarbazole	Anthracene
UV absorbance	0.05	0.4	0.2	0.2	0.02
FL emission	0.07	4.	0.04	0.2	0.04
PA	4.	7.	2.	8.	3.
PI	0.4	0.4	0.4	0.2	0.2

optimized. For example, the bias voltage employed in the PI mode was -1 kV while the PI current increases with bias voltage at least to -5 kV, i.e., ∿25 kV/cm field strength. In fact, the PI current is proportional to the bias voltage to the 1.5 power for bias voltages near -5 kV. Hence, considerable improvements are possible.

A second important conclusion is that aqueous solutions do not cause significant detection problems as Locke et al. (3) encountered with their DC liquid-phase photoionization technique. In particular, excitation with two photons at 337.1 nm (7.35 eV) and excitation with two photons at 308. nm (8.05 eV) does not lead to photoionization of water despite the low reported (9) value of the ionization potential (6.05 eV) of water in the liquid phase. The high intrinsic conductivity of water poses no significant problem since the DC leakage current is shunted to ground. This would also apply to leakage currents due to impurities. Note, however, that the shot noise due to the DC leakage current <u>does</u> lead to substantially higher detection limits than can be obtained in solvent systems such as 90/10 V/V n-hexane/ethanol which has a much lower leakage current.

Another important difference between the PI detection mode of the three-mode flow cell and the elegantly simple liquid-phase photoionization scheme employed by Locke et al. (3) concerns the effecttive ionization efficiency, i.e., number of ions collected divided by total molecules injected. Using Faraday's law and estimating the collected ion charge as the chromatographic peak height (in absolute units) times the peak width at half maximum for the five PI mode HPLC peaks in Fig. 4 gives values of 1.3×10^{-2}, 1.2×10^{-2}, 2.2×10^{-2},

4.5×10^{-2}, and 3.5×10^{-2} in order of elution. For comparison, the value obtained by Locke et al. (3) for pyrene is $8. \times 10^{-8}$. Hence the collection efficiency in the present system is much greater and/ or the actual ionization efficiency is far higher. Work performed with a static cuvette cell having adjustable electrode spacing suggests that the reduced electrode spacing in the HPLC flow cell (2 mm) relative to the reported spacing (4 mm) used by Locke et al. (3) accounts for less than a ten-fold increase in our values of effective ionization efficiencies. Thus, the remaining factor of $\sim 10^4$ must be attributed to higher absolute ionization efficiency.

An estimate of the absolute ionization efficiency may be obtained by estimating the number of photons absorbed from the incident laser beam. Assuming an incident pulse energy of 1 mJ at 308. nm, the number of photons is 1.6×10^{15} per pulse. If a 10^{-4} M solution has a molar absorptivity of 2000 $M^{-1}cm^{-1}$ and a 2. mm path length with 1. µL illuminated volume (reasonable values in our system), the number of molecules absorbing radiation is approximately 1.4×10^{14}. The number of molecules in the illuminated volume is $6. \times 10^{13}$. Hence, >50% of the molecules absorb a single photon. This is in agreement with the estimate given by Piciulo and Thomas (10). Therefore, the absolute ionization efficiency can be quite high and, in fact, a molecule can be repeatedly ionized during a single excitation light pulse under the right circumstances. It appears, though, that the effective ionization efficiency may be degraded due to factors such as poor collection geometry, ion recombination, trapping, etc.

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