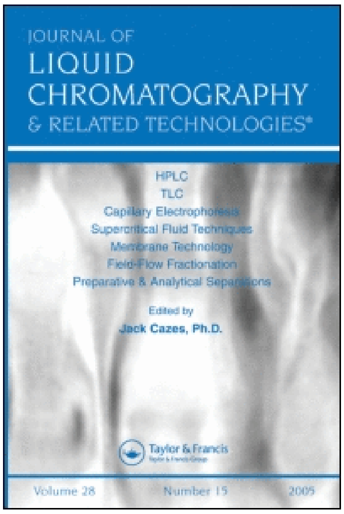

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A Crystalline Quartz, Laser-Excited Fluorescence, Photoacoustic, and Two-Photon Photoionization Detector for HPLC

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A CRYSTALLINE QUARTZ, LASER-EXCITED FLUORESCENCE, PHOTOACOUSTIC, AND TWO-PHOTON PHOTOIONIZATION DETECTOR FOR HPLC

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ABSTRACT

A crystalline quartz flow cell has been developed for simultaneous fluorescence, photoacoustic effect, and two-photon photoionization detection of aromatic compounds in HPLC eluents. Excimer laser-excited three mode detection of naphthalene, 7,8-benzoflavone, N-ethylcarbazole, and anthracene in 70/30 v/v acetonitrile/water is exhibited. The flow cell is suitable for use with deoxygenated solvent systems and is orientation independent.

INTRODUCTION

Recently, we demonstrated the feasibility of a laser-excited windowless flow cell which allows the simultaneous and independent detection of HPLC eluents by their fluorescence (FL), photoacoustic effect (PA), and two-photon photoionization (PI) [1]. The analytical figures of merit (detection limits) previously reported, though good, are far from optimal since any one of the three techniques may be greatly improved by re-designing the flow cell to eliminate the other two techniques. See, for example, Yamada et al. [2] for a windowless flow cell design which results in greatly improved photoionization detection power at the expense of elimin-

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ating the FL and PA detection modes. We have deliberately chosen to emphasize the development and optimization of three mode detectors since they afford the possibility of monitoring the three most important photophysical energy loss pathways available to excited molecular species. This should be especially advantageous in HPLC applications, where the mixture to be separated is complex and/or the number of analyte species is only a fuzzy estimate, since more information is available per analyte. This also means that the laser excitation system is better utilized, i.e., the bother and expense of a laser-based technique is traded off for greater detection power and selectivity per laser pulse.

The single greatest disadvantage of our windowless three mode flow cell is the relative insensitivity of the PA mode: it is about 100× worse than a cuvette cell (static) PA detection system [3,4]. This is presumably due to inefficient acoustic coupling between the transducer and the region where the acoustic waves originate. Other (minor) disadvantages include the need to operate the flow cell in an upright position and the inability, without inert gas sheathing modifications, to handle de-oxygenated solvent systems.

Since the cuvette cell was much better, in the PA detection mode, than the windowless flow cell, it was used as the starting point in a new three mode flow cell design intended to eliminate the afore-mentioned disadvantages. The cuvette cell detector consisted of a lead zirconate-titanate (PZT) ceramic piezoelectric transducer glued to a thick fused quartz disc (1.25 cm diameter and thickness). A Suprasil fluorescence cuvette (1 cm × 1 cm) is placed on the disc with a drop of glycerol (an acoustic coupling fluid) between the cuvette bottom and disc surface. The preamplifier enclosure shields the transducer since the quartz disc passes through a rubber grommet in the enclosure wall.

Elimination of the fused quartz disc and glycerol by gluing the PZT transducer directly to the cuvette bottom improves the acoustic coupling efficiency, but complicates the shielding of the transducer and cleaning of the cuvette. It also increases the likelihood of false signal production by the PZT transducer via the pyroelectric effect. This effect is strong in barium titanate and very strong in PZT [5]. It can be eliminated by using a crystalline quartz piezoelectric transducer since quartz has neither a primary pyroelectric effect (transient polarization change due to uniform temperature change at constant strain) nor a secondary pyroelectric effect (transient polarization change due to uniform-temperature-induced strains and consequent direct piezoelectric effect)[6]. The acoustic coupling efficiency also improves in going from a fused quartz/PZT interface to a fused quartz/crystalline quartz interface. It takes no great leap of the imagination to simplify the fused quartz cuvette and attached crystalline quartz transducer combination to a simple hollowed out quartz crystal which serves as both the solution container and transducer. For an HPLC flow cell, it is convenient to use a drilled quartz crystal as the integrated detector. Our design, which also incorporates the FL and PI detection modes, is shown in Fig. 1. Note that this cell may also be considered as being related to the cylindrical

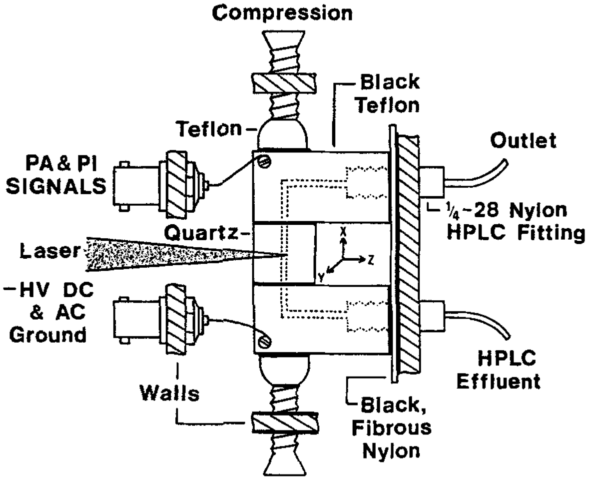

Figure 1: Schematic of the crystalline quartz cube HPLC flow cell.

piezoelectric ceramic transducer designs of Lahmann et al. [7] and Oda et al. [8] because they also integrate the transducer and vessel functions. The major difference with the present design is that laser excitation occurs through the transducer itself. It is therefore unnecessary to employ adhesives to attach windows to the cylindrical PZT transducer. It is also easier to contact the signal electrode in the present design.

Crystalline quartz was selected as the cell material because it is doubtful whether any other material would be suitable in such a design. Since the laser beam passes through the transducer, it is imperative that the transducer material not exhibit primary or secondary pyroelectric behavior. It must also have extremely low absorbance in the ultraviolet (UV) and visible spectral regions to avoid solarization, filtering of FL emission, and the tertiary pyroelectric effect (transient polarization change due to non-uniform-temperature-induced strains and direct piezoelectric effect)[6,9]. Crystalline quartz is well-suited in this regard since it is the material of choice for the intracavity end windows of high power argon ion laser tubes characterized for UV operation. It is also chemically inert, is relatively inexpensive and easily fabricated, and offers high sensitivity, i.e., the piezoelectric g coefficient (generated electric field per unit pressure input) is 5.8×10^{-2} V $m^{-1}Pa^{-1}$ for quartz and only $\sim 10^{-2}$ V $m^{-1}Pa^{-1}$ for barium titanate and PZT [5]. It also has low thermal expansion coefficient and high volume resistivity ($\sim 10^{12}$ Ω m) at ordinary temperatures [5]. The latter property is essential for PI mode operation with non-polar, i.e., non-shunting, solvent systems in order to avoid 'shorting' the bias and signal electrodes together.

Disadvantages include low dielectric constant (which necessitates placement of the preamplifier as close to the transducer as possible) and lack of a volume expander piezoelectric mode. The latter causes quartz to be useless in 'hydrostatic' pressure sensing applications, i.e., it cannot respond to isotropic pressure changes. Since volume expander piezoelectric behavior may be exhibited only by members of the polar crystal classes, and the same applies for primary and secondary pyroelectric behavior [6], the trade-off of volume expander mode for freedom from pyroelectric effect is generally unavoidable.

To overcome lack of sensitivity to isotropic pressure changes, the hole drilled in the quartz cube of Fig. 1 is through the centers of the X faces, i.e., the cube faces normal to the X piezoelectric axis (electric, compression, or dihedral axis). The laser beam is incident along the Z axis (optic, crystallographic 'c' axis) for two reasons. First, the very small absorbances of the solvent and eluent species cause a negligible transient pressure gradient along the piezoelectrically inactive Z axis. Second, the unpolarized incident laser beam is uneffected by the relatively high UV optical activity (circular birefringence) of quartz along the optic axis [10]. Thus, the 'cylindrical' acoustic waves spread out radially, from the illuminated fluid region, in the X,Y plane, but effectively only stimulate (the transducer) along the Y axis.

Estimation of the PA preamplifier output voltage due to weak absorption by eluting analyte species is difficult for several reasons. First, both the excitation and detection geometries are quite different from the usual cases of coaxial laser beam excitation and fluid flow considered by others [11-17]. These geometric effects cannot properly be ignored since, for example, the generated pressure is strongly dependent on laser spot size (w) when the laser pulse duration (τ_p) is much less than the acoustic transit time ($\equiv w/v_a$ where v_a is the speed of sound in the liquid) of the pressure disturbance across the illuminated region [16]. This condition is satisfied in the present case since $\tau_p \simeq 10$ ns, $w \approx 100$ µm, and $v_a \approx 10^3$ m s^{-1}. A second difficulty arises because of the critical dependence of the output signal on the relative frequency responses of the generated pressure pulse, *semi-clamped* transducer, and preamplifier, and on the acoustic impedance match between the eluent liquid and the transducer [16].

Since we have previously shown that 10 ns laser pulses are only slightly more efficient in PA signal generation than 1 μs pulses of the same pulse energy [3], the expected pressure may be roughly estimated as ∿ 1 Pa (which is the calculated value given by Patel and Tam [15] for typical conditions such as 10^{-3} cm^{-1} absorptivity and 1 mJ pulse energy). Since the molar absorptivity of anthracene at 308 nm is ∿3200 M^{-1} cm^{-1}, the anthracene concentration corresponding to 10^{-3} cm^{-1} is ∿60 ppb. Thus, the transducer output can be no larger than ∿580 μV for a 1 cm cube cell. This value will be greatly reduced, despite the preamplifier gain, due to various coupling losses (acoustic and electrical), bandwidth restrictions in the preamplifier, and wiring and preamplifier input capacitance.

EXPERIMENTAL

A 1 inch cube of oriented, synthetic quartz crystal (Sawyer Research Products, Eastlake, OH) was fabricated into several 1 cm oriented, polished cubes (B. Collins, Hyde Park, NY) with the cube faces normal to the X, Y, and Z piezoelectric axes. A hole of 0.027 inch diameter was drilled through the center of the cube normal to the X faces (Wilmad Glass, Buena, NJ). The drilled hole was successively polished with diamond powder paste (15, 6, 1, & ¼ μm particle sizes, Abrasive Sales, Rindge, NH) and waxed dental floss. For clarity, the quartz cube in Fig. 1 is shown rotated 90° about the X axis (so that the unabsorbed portion of the excitation beam is shown impinging on the black background rather than passing out of the electrically shielding cell housing).

The bias and signal electrodes were fabricated fron conductive black PTFE (30% by weight graphite-filled PTFE, Fluorocarbon, Anaheim, CA). This material was convenient because it is easily machined, chemically inert, has low reflectivity, and deforms slightly under pressure, thereby conforming to the polished quartz cube surface (for efficient charge collection) and providing a fluid-tight seal.

Solutions were prepared by four successive five-fold dilutions in ethanol of a freshly prepared stock solution of naphthalene (200. µg/mL), 7,8-benzoflavone (200. µg/mL), N-ethylcarbazole (200. µg/mL), and anthracene (82.0 µg/mL). Solvents were de-oxygenated by bubbling with He for $\sim$1 hr prior to the HPLC injections of 20 µL samples. The HPLC system, column, and flow rate (1.5 mL/min) were the same as before [1]. Solvent programming was used to prepare the 70/30 v/v acetonitrile/water mobile phase.

The cube cell was operated simultaneously with and downstream from a conventional 254 nm UV absorbance detector with connections to the HPLC as shown in Fig. 1. Excitation pulse energy was kept low ($\sim$0.1 mJ, see below) and 308 nm scatter was removed on the FL emission side by a 400 nm long pass filter. No artifact signals were observed, in any mode, upon illumination of neat solvent flowing through the cell. However, damage to the drilled hole *was* observed when focussed beams of $\gtrsim$1 mJ per pulse were incident at the quartz/liquid interface. Microscopic examination revealed conchoidal fracturing, but no evidence of melting. No damage occurred when the cube cell was empty.

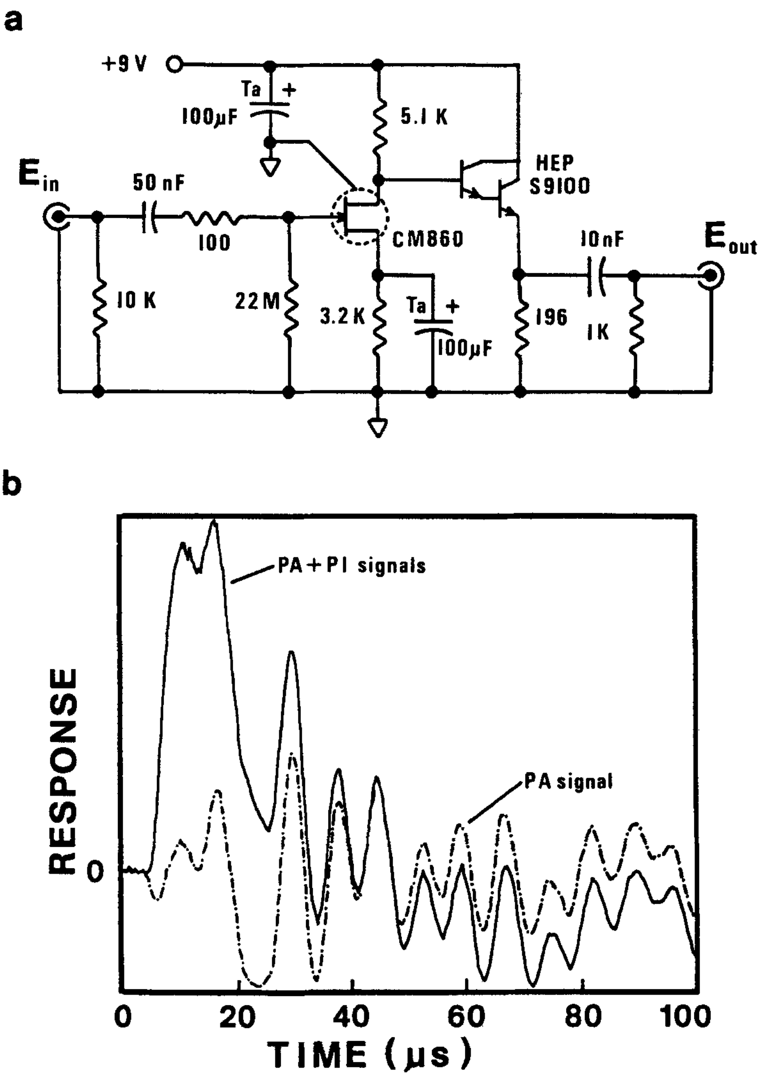

Figure 2: (a) Low noise preamplifier with R-C input coupling for polar mobile phases. The CM860 JFET is the four lead version of the 2N6550 JFET (Teledyne Crystalonics, Cambridge, MA).

(b) Typical preamplifier output waveforms for an (unseparated) five-fold stock solution dilution. Full scale is ∿13 μV.

The detection scheme is almost identical to that previously employed [1] except in one regard. Note in Fig. 1 that the DC bias voltage (V_b) of -1 kV was applied to the bottom PTFE electrode. This electrode was capacitively bypassed to ground (50 nF, 1600 WVDC, silver-mica). The other electrode is both the piezoelectric and photoionization signal pick-up. The joint signals were amplified with the preamplifier shown in Fig. 2a and typical waveforms with V_b = 0 V (PA effect only) and V_b = -1 kV (PA and PI effects) are shown in Fig. 2b. Photoionization chromatograms may be obtained with negligible PA signal addition by gating the boxcar averager so that it is near the peak of the PI waveform and overlapping a zero-crossing of the PA waveform. It is also possible to utilize the sum of the two non-radiative effects as a single analytical measure. Both effects are proportional to analyte concentration, but it should be remembered that the PA technique readily measures small solvent absorbances [18] while the PI technique is more nearly 'zero-baseline' with respect to solvent signals.

RESULTS AND DISCUSSION

Three mode chromatograms of naphthalene, 7,8-benzoflavone, N-ethylcarbazole, and anthracene in 70/30 v/v acetonitrile/water are shown in Figs. 3 and 4 together with a companion UV absorbance chromatogram. Detection limits, calculated as before [1], are presented in Table 1. The UV absorbance and PI results are essentially unchanged from the windowless flow cell values. The FL results are $\sim 2\times$ worse while the PA results are $\sim 4\times$ worse.

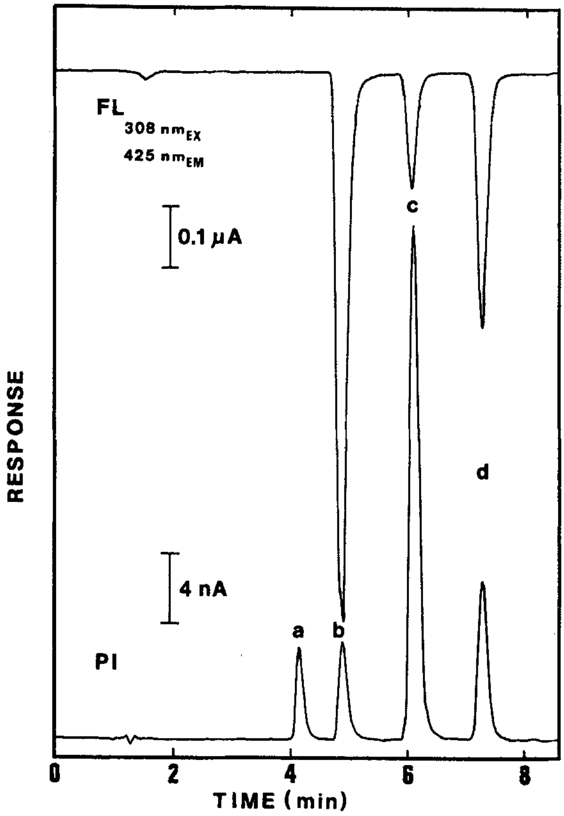

Figure 3: Fluorescence (FL) and Photoionization (PI) chromatograms for a five-fold dilution of stock solution of (a) naphthalene, (b) 7,8-benzoflavone, (c) N-ethylcarbazole, and (d) anthracene.

As stated earlier, none of the three flow cell modes is optimized. Even so, both the FL and PI modes exhibit the high detection power needed for HPLC applications. Both modes can be improved greatly (>100×) with relatively minor changes in the construction and operation of the cube cell. For example, the PI mode can be

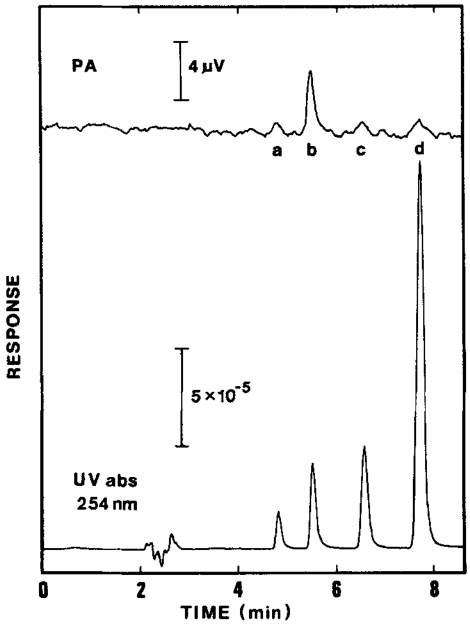

Figure 4: Photoacoustic (PA) and UV absorbance chromatograms for the same solution as in Fig. 3.

TABLE 1

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

	Naphthalene	7,8-benzoflavone	N-ethyl carbazole	Anthracene
UV absorbance	0.4	0.2	0.2	0.02
FL emission	---	0.1	0.5	0.09
PA	30.	5.	30.	10.
PI	0.5	0.5	0.08	0.1

improved by using higher bias voltage (-5 kV), higher pulse energy (∿1 mJ), and closer electrode spacing (∿2 mm) [2,19,20]. This means using MHV connectors rather than BNC connectors and using a 2 mm quartz cube.

Unfortunately, the PA mode performance is not so readily improved. Although the cell meets the goal of gas-tight operation in any orientation, it is ∿100 × worse than a conventional (254 nm UV absorbance) detector. Even taking into account the disparity in excitation wavelengths, the low laser pulse energy, and the use of polar solvents (which 'short' the transducer faces), it probably cannot be improved more than a hundred-fold at best. This is not the case with PA mode detection using PZT detectors since, for example, the first PA mode HPLC detector (Oda and Sawada [21]) achieved detection limits for several azobenzene isomers which were ∿25 × better than UV absorbance results (7.9×10^{-6} cm^{-1} at S/N=2). They used an acousto-optically modulated Ar ion laser, PZT disc, and lock-in amplifier detection and expected to greatly improve their PA mode results by using pulsed laser excitation. Lai et al. [22], using both nitrogen and XeCl excimer laser (pulsed) excitation, a non-contacting PZT cylindrical transducer, and boxcar averager signal processing, achieved LODs which were ∿10 × worse than the conventional detector. The disparity in PA mode HPLC flow cell detection powers for the pulsed versus CW laser excitation cases is curious and precisely the opposite from the observed behavior in static cells [15]. Consequently, future efforts will be concentrated on resolving the anomaly and in developing a three mode cell which is at least 10× better than UV absorbance detectors in all modes.

ACKNOWLEDGMENTS

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REFERENCES

1. Voigtman, E. and Winefordner, J.D., A Simultaneous, Laser-Excited Fluorescence, Photoacoustic, and Two-Photon Photoionization Detector for Liquid Chromatography, J. Liq. Chromatogr., 5, 2113, 1982.

2. Yamada, S., Kano, K., and Ogawa, T., Laser Two-Photon Ionization Technique for a High Sensitive [SIC] Detection of Pyrene, Bunseki Kagaku, 31, E247, 1982.

3. Voigtman, E., Jurgensen, A., and Winefordner, J.D., Condensed Phase Photoacoustic Spectroscopic Detection of Porphyrins and Dyes, Anal. Chem., 53, 1442, 1981.

4. Voigtman, E., Jurgensen, A., and Winefordner, J.D., Comparison of Laser Excited Fluorescence and Photoacoustic Limits of Detection for Static and Flow Cells, Anal. Chem., 53, 1921, 1981.

5. Neubert, H.K.P., Instrument Transducers, Oxford at the Clarendon Press, 1963.

6. Nye, J.F., Physical Properties of Crystals, Oxford at the Clarendon Press, 1957.

7. Lahmann, W., Ludewig, H.J., and Welling, H., Opto-Acoustic Trace Analysis in Liquids with the Frequency-Modulated Beam of an Argon Ion Laser, Anal. Chem., 49, 549, 1977.

8. Oda, S., Sawada, T., and Kamada, H., Determination of Ultra Trace Cadmium by Laser-Induced Photoacoustic Absorption Spectroscopy, Anal. Chem., 50, 865, 1978.

9. Miers, H.A., Minerology, Macmillan and Co., Ltd., London, 1902, pp. 115-116.

10. Nussbaum, A. and Philips, R.A., Contemporary Optics for Scientists and Engineers, Prentice Hall, Englewood Cliffs, NJ, 1976.

11. Kohanzadeh, Y., Whinnery, J.R., and Carroll, M.M., Thermoelastic Waves Generated by Laser Beams of Low Power, J. Ac. Soc. Am., 57, 67, 1975.

12. Hordvik, A. and Schlossberg, H., Photoacoustic Technique for Determining Optical Absorption Coefficients in Solids, Appl. Optics, 16, 101, 1977.

13. Burt, J.A., Extension of the Theory of the Fluid-filled Optoacoustic Cell, J. Phys. D: Appl. Phys., 13, 1985, 1980.

14. Atalar, A., Photoacoustic Effect as a Liquid Absorbance Detector, Appl. Optics, 19, 3204, 1980.

15. Patel, C.K.N. and Tam, A.C., Pulsed Optoacoustic Spectroscopy of Condensed Matter, Rev. Mod. Phys., 53, 517, 1981.

16. Nelson, E.T. and Patel, C.K.N., Response of Piezoelectric Transducers used in Pulsed Optoacoustic Spectroscopy, Optics Lett., 6, 354, 1981.

17. Liu, G., Theory of the Photoacoustic Effect in Condensed Matter, Appl. Optics, 21, 955, 1982.

18. Tam, A.C., Patel, C.K.N., and Kerl, R.J., Measurement of Small Absorptions in Liquids, Optics Lett., 4, 81, 1979.

19. Voigtman, E. and Winefordner, J.D., Two-Photon Photoionization Detection of Polycyclic Aromatic Hydrocarbons and Drugs in a Windowless Flow Cell, Anal. Chem., 54, 1834, 1982.

20. Voigtman, E. and Winefordner, J.D., Simultaneous Fluorescence, Photoacoustic, and Two-Photon Photoionization Detection for Liquids in a Cuvette Cell, Talanta, in press.

21. Oda, S. and Sawada, T., Laser-Induced Photoacoustic Detector for High-Performance Liquid Chromatography, Anal. Chem., 53, 471, 1981.

22. Lai, E.P.C., Su, S.Y., Voigtman, E., and Winefordner, J.D., A Simple, Corrosion-Resistant Flow Cell for Laser-Induced Photoacoustic Spectroscopic Detection of High-Performance Liquid Chromatography Effluents, Chromatographia, 15, 645, 1982.