Surface-sensitized luminescence

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excess NBD-Cl, present in the test mixture. The NBD-Cl has absorption bands that overlap the SEF emission of the amine derivatives.

Although the detectabilities demonstrated in this work are very good, improvements are expected with a few minor changes. The large background for the conventional fluorescence measurements was minimized by using both a monochromator and a sharp cutoff filter to isolate the emission. However, it was not possible to operate at the emission maxima for the derivatized amines due to the unavailability of a suitable cutoff filter. In addition, the bandpass filter arrangement used for SEF and TPEF detection transmitted only 20% at its maximum. Improvements in signal recovery would be expected with the employment of better filter arrangements. Fluorescent impurities on the thin-layer plates also adversely affected detectability, by increasing optical background and noise levels. These impurities, which appeared as small fluorescent specks when illuminated with a UV lamp, are thought to be carry-over contamination from the manufacturing of fluorophor-coated thin-layer plates.

The cross sectional area of a focused laser beam has a quadratic dependence on the focal length of the lens employed (8). As previously mentioned, SEF and TPEF signals depend inversely on the cross sectional area (A) of the focused laser beam. Consequently, decreasing the focal length of the focusing lens should increase SEF and TPEF signal levels. This expected improvement was not observed. Only a 10% improvement in signal was observed when chromatograms were scanned with a 25-mm focal length lens ($A \approx 2.0 \times 10^{-3}$ mm^2) instead of the 200 mm focal length lens ($A \approx 0.12$ mm^2), presumably due to surface or heating effects. More significant was the appearance of a large number of noise glitches. These glitches increase in number and magnitude as the cross sectional area of the focused laser beam is made to approach the size of the fluorophor impurity specks.

Calibration plots of NBD derivatized methylamine exhibited linear response from its limit of detection to 0.50 ng for conventional fluorescence, linear regression constant of 0.9997, and to 2.0 ng for SEF, linear regression constant of 0.9999. A TPEF calibration plot for the oxadiazole PBD exhibited a linear response from its limit of detection to 20 ng, with a linear regression constant of 0.9948.

Detector saturation, during the intense laser pulse, and thermal problems limit the linear dynamic range for these detectors. Detector saturation can result from the large signal levels for the more concentrated samples. The large background from specular scatter in conventional fluorescence detection also contributes to the detector saturation problem. This is evidenced by an earlier "roll-off" for the conventional fluorescence plot relative to the SEF plot. In both conventional fluorescence and SEF detection, the linear dynamic range was further limited by thermal effects, which occur when a large amount of absorbed radiation is nonradiatively dissipated. The TPEF technique involves a very inefficient absorption process and, therefore, is not expected to be limited by these thermal effects. The linear dynamic range observed for the TPEF detection is more than an order of magnitude greater than that observed for the other techniques.

The laser fluorometric modes of detection investigated in this work yielded reproducible signals at low solute concentrations. However, at high concentrations small reductions in signal and slight charring of the thin-layer plate were noticed during successive scans. These reductions in signal were absent for TPEF detection.

In summary we have demonstrated the feasibility of utilizing different modes of laser fluorometric detection in thin-layer chromatography. The excellent limits of detection observed for these techniques and the unique focusing properties of the laser should result in future applications of laser fluorometry in HPTLC detection.

Registry No. NBD-Cl, 10199-89-0; β-NPD, 967-72-6; PPD, 725-12-2; BBO, 2083-09-2; PBD, 852-38-0; dimethylamine, 124-40-3; isobutylamine, 78-81-9; isoamylamine, 107-85-7; methylamine, 74-89-5.

LITERATURE CITED

(1) Zlatkis, A., Kaiser, R., Eds. "HPTLC High Performance Thin-Layer Chromatography", Elsevier: Amsterdam, 1977.
(2) Fried, B.; Sherma, J. "Thin-Layer Chromatography"; Marcel Dekker: New York, 1982.
(3) Berman, M. R.; Zare, R. N. *Anal. Chem.* **1975**, *47*, 1200–1202.
(4) Bicking, M. K. L.; Knisley, R. N.; Svec, H. J. *Anal. Chem.* **1983**, *55*, 200–203.
(5) Bertsch, W.; Hara, S.; Kaiser, R.; Zlatkis, A. "Instrumental HPTLC"; Proceedings of the First International Symposium on Instrumentalized High Performance Thin-Layer Chromatography (HPTLC), Bad Dürkheim, West Germany, Alfred Hüthig; Verlag: New York, 1980.
(6) Sepaniak, M. J.; Yeung, E. S. *Anal. Chem.* **1977**, *49*, 1554–1556.
(7) Huff, P. B.; Tromberg, B. J.; Sepaniak, M. J. *Anal. Chem.* **1982**, *54*, 946–950.
(8) Wirth, M. J.; Kaskelo, A.; Sanders, M. J. *Appl. Spectrosc.* **1981**, *35*, 14–22.
(9) Lin, H. B.; Topp, M. R. *Chem. Phys. Let.* **1977**, *47*, 442–447.
(10) Tromberg, B. J.; Eastham, J. F.; Sepaniak, M. J. *Appl. Spectrosc.*, in press.

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Surface-Sensitized Luminescence

Sir: Luminescence techniques have found a secure place among analytical methods in part because of their high sensitivity (*1*, *2*). Even this sensitivity is, however, limited. The fluorescence emission signal F of an analyte is given by the formula

$$F = kA(c,\lambda)I(\lambda)\phi_f \qquad (1)$$

where k is an instrumental factor, $A(c,\lambda)$ is the fraction of incident light absorbed by the analyte at concentration c and exciting wavelength λ, $I(\lambda)$ is the input light intensity at the exciting wavelength, and ϕ_f is the fluorescence quantum yield of the analyte. Heretofore almost all attempts to improve sensitivity have focused on either improving instrumental details (k) or increasing the input light intensity (I). Both approaches suffer the disadvantage that noise and signal are often amplified in unison, and high input intensities can lead to additional complications of heating and photochemistry. The key restraint is that *at low concentrations analytes emit very little light because they absorb very little light*: $A(c,\lambda)$ approaches zero at low concentrations.

Here we describe a simple technique that transcends the above limitation. In the surface-sensitized luminescence (SSL)

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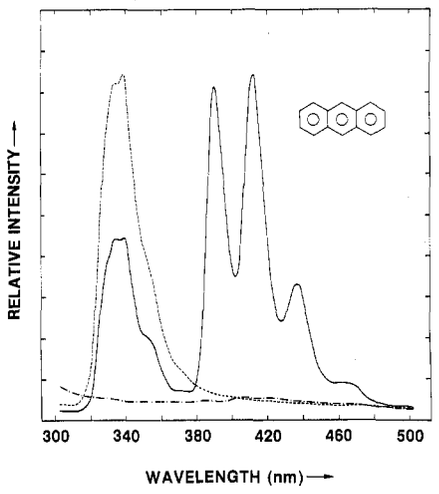

Figure 1. Fluorescence of naphthalene and anthracene on filter paper: (---) naphthalene (9 μmol) alone; (-•-•-) anthracene (0.5 nmol) alone; (—) anthracene and naphthalene on same surface.

analysis method a second, sensitizing substance is employed in a matrix to absorb incoming light and transfer excitation energy to a luminescent analyte. In effect, this technique replaces the term $A(c,\lambda)$ in eq 1 by a new term $A'(c',\lambda')\phi_x$, where $A'(c',\lambda')$ is the fractional light absorption by the sensitizer at concentration c' and new exciting wavelength λ', and ϕ_x is the quantum efficiency of energy transfer from sensitizer to analyte. Here ϕ_x contains the dependence on analyte concentration. Since direct absorption by the analyte is usually negligible at low concentrations, the new analyte signal becomes

$$F' = kA'(c',\lambda')\phi_x I(\lambda')\phi_f \qquad (2)$$

For a sensitizer present in high concentration $A' \gg A$, and for a properly chosen sensitizer in close contact with the analyte ϕ_x may in some cases approach unity. Also, within limits the wavelength λ' can be chosen to make best use of the sensitizer absorbance and light source spectrum. In this way one can obtain $A'\phi_x \gg A$, and use of a sensitizer can, in principle, considerably amplify the luminescent signal. The SSL method thereby takes advantage of the high energy transfer efficiencies typically found in solids. It has much in common with the energy transfer process in photosynthesis wherein accessory pigments and antenna chlorophyll molecules funnel energy to a reaction center.

The practical use of the method is illustrated by the example of naphthalene (sensitizer) and anthracene (analyte) on a filter paper surface. Experimental techniques used are similar to those previously used to observe room-temperature phosphorescence (*3*, *4*). Five-microliter samples of anthracene and naphthalene in acetone were applied to $^1/_4$ in. circles of Whatman No. 42 filter paper and dried in air for 3 min. The fluorescence signal from a 10^{-4} M solution (0.5 nmol) of anthracene alone was barely detectable above the background emission of the paper; when applied in solution with 1.8 M naphthalene a strong, clean anthracene emission signal was obtained (Figure 1). At this concentration the anthracene signal was amplified approximately 40-fold, with a wavelength shift of about 10 nm for the peaks implying formation of mixed microcrystals. At the same time the naphthalene fluorescence signal was significantly diminished, indicating that energy transfer to anthracene competes successfully with naphthalene emission under these conditions. Sensitization extends the limit of detection of anthracene approximately 3 orders of magnitude: Figure 2 shows an emission from 1 pmol of anthracene sensitized by naphthalene, in which the anthracene emission lines at 388, 412, and 436 nm appear on the tail of the strong naphthalene fluorescence. At this concentration

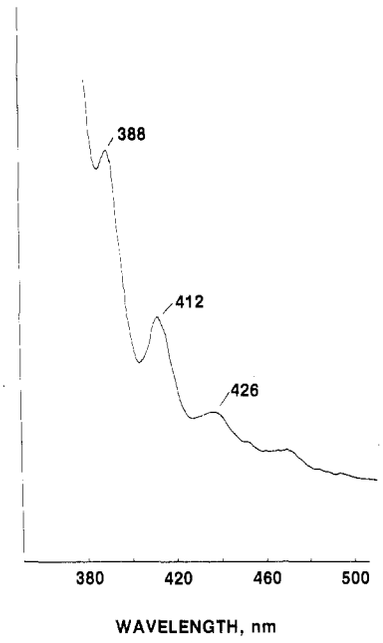

Figure 2. Fluorescence signal from 1 pmol of anthracene on filter paper, sensitized by naphthalene.

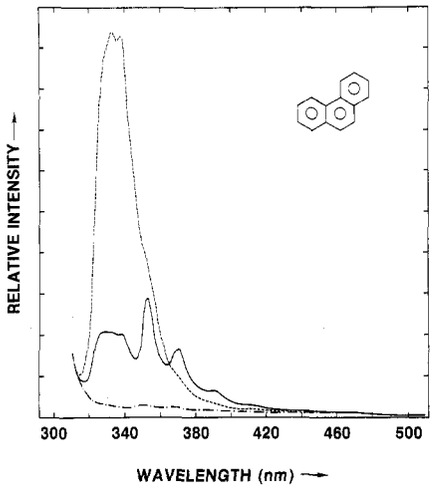

Figure 3. Fluorescence of naphthalene and phenanthrene on filter paper: (---) naphthalene (9 μmol) alone; (-•-•-) phenanthrene (0.5 nmol) alone; (—) phenanthrene and naphthalene on surface.

Table I. Comparison of Premixed and Separately Applied Solutions[a]

	relative intensities		
peak	anthr. alone	premixed	sep appl
390 nm	0.9 ± 0.2 (402 nm)[b]	30.5 ± 4.2	23.5 ± 6.1
410 nm	1.1 ± 0.3 (420 nm)[b]	31.8 ± 4.2	22.5 ± 5.2

[a] For 5 nmol of anthracene and 9 μmol of naphthalene on Whatman No. 42 filter paper; averages for five runs ± absolute standard deviation. [b] Corresponding peak.

the ratio of anthracene to naphthalene molecules on the surface is 1:9 × 10^6.

The two compounds could be applied either in common solution or separately, with the former tending to yield higher signals and slightly better reproducibility, as shown in Table I. The latter method may, however, be more practical for most analytical applications. For separate applications, 3 min of drying time was allowed between application of the sensitizer and analyte solutions. There was a tendency for the second solution to wash the sensitizer to the edges of the filter paper circles. Application of acetone solution alone diminished

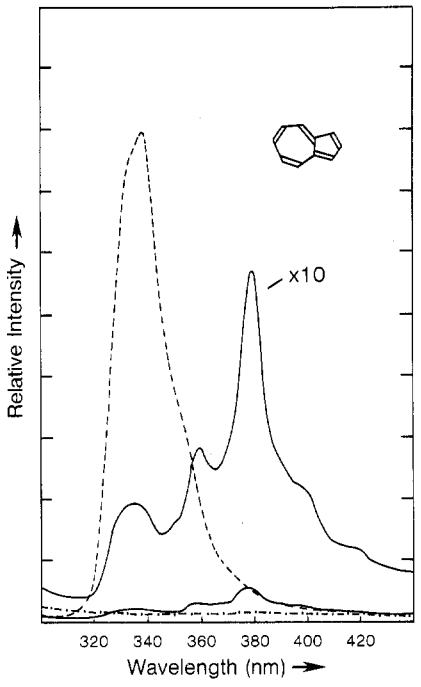

Figure 4. Fluorescence of naphthalene and azulene on filter paper: (---) naphthalene (9 μmol) alone; (-·-·) azulene (5 nmol) alone; (—) azulene and naphthalene on surface.

the naphthalene signal by about 30%.

Naphthalene-sensitized fluorescences from phenanthrene and azulene on filter paper are illustrated in Figures 3 and 4, respectively. The azulene example is of interest since it represents emission from a second excited singlet state, the well-known anomalous fluorescence of this compound (*5*, *6*). The strong quenching of the naphthalene emission in this example indicates that energy transfer is exceptionally efficient, as might be expected from the similarities of the molecular structures. Our attempts to sensitize fluorescence from the lowest excited singlet state of azulene have thus far not been successful. This latter state is known to be only very weakly fluorescent ($\phi_f \approx 10^{-7}$) (*7*).

Several earlier studies employing similar systems have been reported but have received little attention. Hornyak (*8*, *9*) adsorbed compounds from solution onto filter paper: tetracene fluorescence was sensitized by anthracene (*8*) and by benz[*a*]anthracene (*9*), and a good linear relation between analyte concentration and fluorescence signal was obtained. Hornyak also reported the sensitized emission of pentacene (*10*). A qualitative spot test procedure for detecting polycyclic aromatic hydrocarbons in the field has been described by Smith and Levins (*11*). Kreps et al. (*12*) reported the use of colloidal dispersions to detect tetracene in anthracene via energy transfer.

Surface-sensitized luminescence offers a relatively simple means for amplifying luminescence and detecting trace amounts of substances. A more detailed examination of this technique is in preparation.

Registry No. Naphthalene, 91-20-3; anthracene, 120-12-7; phenanthrene, 85-01-8; azulene, 275-51-4.

LITERATURE CITED

(1) Schulman, S. G. "Fluorescence and Phosphorescence Spectroscopy: Physiochemical Principles and Practice"; Pergamon Press: New York, 1977.
(2) Wehry, E. L. "Modern Fluorescence Spectroscopy"; Plenum Press: New York, 1976; Vols. 1 and 2.
(3) Meyers, M. L.; Seybold, P. G. *Anal. Chem.* **1979**, *51*, 1609-1612.
(4) McHale, J. L.; Seybold, P. G. *J. Chem. Educ.* **1976**, *53*, 654-656.
(5) Beer, M.; Longuet-Higgins, H. C. *J. Chem. Phys.* **1955**, *23*, 1390-1391.
(6) Viswanath, G.; Kasha, M. *J. Chem. Phys.* **1956**, *24*, 574-577.
(7) Rentzepis, P. M., *Chem. Phys. Lett.* **1969**, *3*, 717-720.
(8) Hornyak, I.; Lendvay, E. *J. Lumin.* **1971**, *3*, 369-377.
(9) Hornyak, I. *Microchim. Acta* **1978**, *II*, 23-29.
(10) Hornyak, *J. Lumines.* **1975/76**, *11*, 241-248.
(11) Smith E. M.; Levins, P. L. In "Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects"; Bjorseth, A., Dennis, A. J., Eds.; 4th Int. Symp.; Battelle Press: Columbus, OH, 1980; pp 973-982.
(12) Kreps, S. I.; Druin, M.; Czorny, B. *Anal. Chem.* **1965**, *37*, 586-588.

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Comparison of Reflectance and Photoacoustic Photometry for Determination of Elemental Carbon in Aerosols

Sir: Several methods have been developed for the determination of elemental carbon (EC) in atmospheric aerosol samples collected on filters. They include such methods as γ-ray analysis for the light elements (*1*), proton elastic scattering (*2*), Raman spectrometry (*3*), combustion (*4*, *5*), transmittance (*6*), and reflectance (*7*). The first three techniques are relatively expensive and involve instruments not common in most laboratories; the combustion method is destructive; transmittance requires optically thin filters and samples; and reflectance can vary with surface characteristics of the filter, requiring measurement of the reflectance prior to loading. The latter two techniques are, however, inexpensive and can be assembled from readily available components. We have investigated the use of photoacoustic spectroscopy (PAS) for the analysis of the elemental carbon content of atmospheric fine aerosols. For this study a low-cost system was constructed from available components. Details of the construction of the system and preliminary results of the comparison of PAS with reflectance are the subjects of this paper.

Photoacoustic spectroscopy has been reviewed previously (*8*-*10*) and will therefore be discussed here only briefly. PAS involves two discrete steps—absorption of energy from a modulated light source and detection of the thermal modulations from the sample as pressure waves in the gas surrounding the sample (*9*). A photoacoustic signal is generated only when the wavelength of the incident light corresponds to an absorption band of the sample; thus PAS spectra are

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