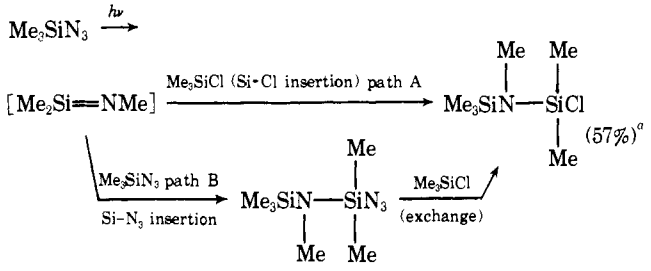
Scheme III



[a] Yield based on recovered starting material.

termediates, ample precedent exists for the reaction of initially formed singlet species as such, without undergoing intersystem crossing (assuming, of course, that the triplet is the ground state). In the present work, a p_π–p_π bonded unsaturated silicon–nitrogen intermediate is also suggested by the well-known analogy of carbon–nitrogen double bond formation from carbon azides. Thus, the results in the present paper appear predictable only on the basis that silicon and carbon azides can behave analogously—an assumption which had to be tested since it is certainly not true in many other comparisons between silicon and carbon analogues.

Work is continuing on the photochemical as well as thermal generation and reactions of this interesting class of reactive intermediates.

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References and Notes

(1) For a recent review see L. E. Gusel'nikov, N. S. Nametkin, and V. M. Vdovin, *Acc. Chem. Res.*, **8**, 18 (1975).
(2) (a) M. C. Flowers and L. E. Gusel'nikov, *J. Chem. Soc. B*, 419 (1968); (b) D. N. Roark and G. J. D. Peddle, *J. Am. Chem. Soc.*, **94**, 5837 (1972); (c) I. M. T. Davidson and J. F. Thompson, *Chem. Commun.*, 251 (1971); (d) C. M. Golino, R. D. Bush, D. N. Roark, and L. H. Sommer, *J. Organomet. Chem.*, **66**, 29 (1974); (e) C. M. Golino, R. D. Bush, and L. H. Sommer, *J. Am. Chem. Soc.*, **96**, 614 (1974); (f) L. H. Sommer and J. McLick, *J. Organomet. Chem.*, **101**, 171 (1975); (g) R. D. Bush, C. M. Golino, G. D. Homer, and L. H. Sommer, *ibid.*, **80**, 37 (1974); (h) P. Boudjouk and L. H. Sommer, *J. Chem. Soc., Chem. Commun.*, 54 (1973).
(3) New compounds were characterized by their ir, NMR, and mass spectra. Satisfactory exact mass determinations were obtained either on the parent molecular ion or the P − 15 *m/e* (Me) ion.
(4) I. Haiduc, "The Chemistry of Inorganic Ring Systems", Part 1, Wiley-Interscience, New York, N.Y., 1970, p 429.
(5) C. M. Golino, R. D. Bush, and L. H. Sommer, Abstracts, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1974, No. ORGN 005.
(6) C. M. Golino, R. D. Bush, P. On, and L. H. Sommer, *J. Am. Chem. Soc.*, **97**, 1957 (1975).
(7) D. W. Klein and J. W. Connolly, *J. Organomet. Chem.*, **33**, 311 (1971).

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Laser Fluorimetry. Sub-Part-per-Trillion Detection of Solutes

Sir:

While conventional fluorimetry[1] has a typical detection limit of part-per-billion (1 part in 10^9) or in some cases sub-part-per-billion, the replacement of blackbody excitation sources (discharge lamps) by pulsed lasers and the use of gated-detection electronics can substantially increase the detection sensitivity.[2] We report here a study on the use of this detection technique applied to solutions of the dye, rhodamine 6G, and the carcinogenic mold metabolite, aflatoxin. Linear analytical curves are obtained which extend from a molar concentration of 10^{-6} to 10^{-13} M. This represents more than three orders of magnitude improvement over conventional means of detection. The analytic detection of dyes is used extensively in studies of pollution and water flow monitoring by fluorescent tracer techniques.[3] The analytic detection of toxins, such as aflatoxin, is of serious concern in the processing of food and feed stocks,[4] as well as in the analysis of tissue and body fluids. However, the significance of this work is thought to transcend the individual substances investigated; rather, laser fluorimetry holds the promise of dramatically extending the sensitivity limits of liquid chromatography as an analytic tool.

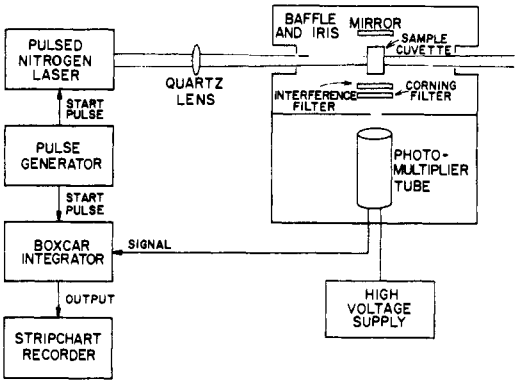

Figure 1. Schematic of laser fluorimeter.

Figure 1 shows a schematic of the experimental setup. The excitation source is a 337.1-nm pulsed nitrogen laser (AVCO-Everett Model C950). The laser power supply is charged from a 1000-W voltage regulator (SOLA). This stabilization is found to be important to the reproducible detection of dye concentrations below 10^{-10} M. The laser is fired 15 pps, each pulse having a 12-ns width, and operated at a power level of about 80 kW.

The laser beam is collimated and focused onto a 2-ml Suprasil quartz cuvette that holds the sample. To ensure signal reproducibility, the cuvette must be rinsed and filled with sample in situ. Baffles at the entrance and exit slits of the sample housing reduce scattering of laser light. A Centronix 4238 S-20 photomultiplier, shielded in a separate housing, views the sample fluorescence at right angles to the laser beam. To detect the dye solutions, an Optics Technology interference filter (6258-450) and a Corning filter (3-66) are placed in front of the photomultiplier to reduce scattered light and to eliminate Raman bands from the solvent, respectively. The interference filter, which cuts off light below 355 nm and transmits higher wavelengths through 700 nm was found to be more effective at diminishing the background of scattered laser light than comparable Corning filters. The latter were also found to fluoresce strongly when exposed to the laser. To detect the aflatoxin solutions, a different Corning filter (4-96) which transmits light from 345 to 650 nm is used. A boxcar integrator (PAR Model 162) receives the pulsed signal from the photomultiplier as well as the start pulse from the pulse generator that triggers the nitrogen laser. The signal-averaged output is displayed by a stripchart recorder (Hewlett-Packard Model 7100B). Rhodamine 6G (mol wt = 479) was obtained from New England Nuclear-Pilot Chemical Division (laser dye grade) and was used without further purification. The molar extinction coefficient at 337.1 nm is 8×10^3 M^{-1} cm^{-1} and the fluorescence quantum yield is 0.95.[5] The fluorescence

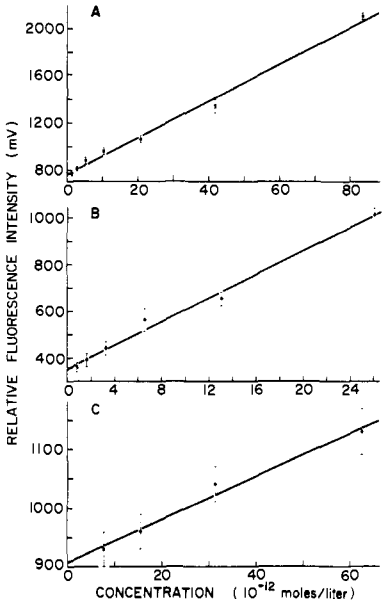

Figure 2. Analytical fluorescence curves of (A) rhodamine 6G in ultrapure water with photomultiplier voltage at 1250 V; (B) rhodamine 6G in purified ethanol at 1200 V; and (C) aflatoxin B_1 and G_1 (1:1) in purified ethanol at 1250 V. The straight lines are best least-squares fits. The error bars on the data points represent estimates based on the fluctuations in the signal level. Note that the slope in (C) is flatter than in (A) although the same photomultiplier voltage setting is used. This is a consequence of the fact that the quantum yield of aflatoxin is less than that of rhodamine 6G.

maximum occurs at 555 nm.[6] Aflatoxin B_1 and G_1 (1:1 mixture) is obtained from Dr. L. M. Seitz (U.S. Grain Marketing Research Center, Manhattan, Kan.).

The sample cell and all glassware were soaked in concentrated nitric acid, rinsed with hydrofluoric acid, and washed amply with solvent and sample. The strong background fluorescence of solvent impurities, when exposed to laser light, was considerably reduced by purification of the solvents. Absolute ethanol was obtained by fractional distillation,[7] and ultrapure water was obtained using four filters, two deionizing tanks, two carbon tanks, followed by all-glass distillation. The dye solutions were not deoxygenated; however, the effect of dissolved oxygen on rhodamine 6G is mainly to depopulate triplet states.[8]

Figure 2 shows the dependence of the fluorescence signal on concentration for rhodamine 6G in water (Figure 2A), rhodamine 6G in ethanol (Figure 2B), and aflatoxin B_1 and G_1 (1:1) in ethanol (Figure 2C). An upper limit to the linearity for the dye solutions occurs at 2×10^{-6} M, where self-quenching and reabsorption of the luminescence causes nonlinearities. For low concentrations, the limit of detection of rhodamine 6G in water is 1.3×10^{-12} M (0.62 parts per trillion) and in ethanol 8.2×10^{-13} M (0.39 parts per trillion). For the aflatoxin solution the limit of detection is 7.8×10^{-12} M (2.5 parts per trillion). Since the laser, when focused on the sample cell, excites molecules only in about 0.6 ml of the total volume, the given detection limit implies that 3×10^8 dye molecules in solution are being detected by the present technique. This limit also corresponds to 1.7 pg of aflatoxin in the volume excited. This appears to be the smallest quantity of aflatoxin yet detected.[4,9] Even so, we have not attempted here to take advantage of the coherence properties of the laser beam, which permit it to be focused to a diffraction-limited spot, facilitating the use of laser fluorimetry in microanalysis.

At present, the major background interference arises from residual light scattered by the sample cuvette and transmitted by the filters. However, this difficulty can be removed by an improvement in the cell design and filter system, allowing the detection limits to be extended still further. These results encourage us to believe that in many diverse situations requiring ultrasensitivity, laser fluorimetry can reduce the limits of detection by several orders of magnitude.

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References and Notes

(1) J. D. Winefordner, S. G. Schulman, and T. C. O'Haver, "Luminescence Spectroscopy in Analytical Chemistry", Wiley-Interscience, New York, N.Y., 1972.
(2) R. N. Zare and P. J. Dagdigian, *Science*, **185**, 739 (1974).
(3) See "Fluorometry Reviews", G.K. Turner, Palo Alto, Calif., 1968, pp 1–19.
(4) L. A. Goldblatt, "Aflatoxin, Scientific Background, Control and Implications", Academic Press, New York, N.Y., 1969.
(5) K. H. Drexhage, "Topics in Applied Physics", Vol. 1, F. P. Schäfer, Ed., Springer-Verlag, Berlin, 1973, pp 144–274.
(6) G. C. Guilbault, "Practical Fluorescence", Marcel Dekker, New York, N.Y., 1973, p 292.
(7) C. A. Parker, "Photoluminescence of Solutions", Elsevier Amsterdam, 1968, p 421.
(8) B. B. Snavely and F. P. Schäfer, *Phys. Lett. A*, **28**, 728–729 (1969).
(9) M. R. Berman and R. N. Zare, *Anal. Chem.*, **47**, 1200–1201 (1975).

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Thermodynamics of Exciplex Formation by Time-Resolved Nanosecond Flash Spectroscopy

Sir:

We have recently proved[1] the intermediacy of singlet exciplexes in several [2 + 2] photocycloadditions to the phenanthrene chromophore. A complete understanding of both exciplex formation and decay is now clearly implicit to an understanding of many bimolecular photochemical reactions. One important aspect of exciplex decay, reversibility of the formation step, is relatively unexplored. Weller[2] has determined thermodynamic parameters[3,4] in the formation of arene–amine exciplexes, where reversion sometimes dominates other processes of exciplex decay. The negative temperature dependence of several photocycloadditions has been attributed to the reversibility of exciplex formation;[5] however, in these cases complete kinetic analysis was precluded by the lack of exciplex emission. We now present the results of a time-resolved spectrofluorimetric study which leads to an essentially complete analysis of photoreactions involving reversibly formed, emitting exciplexes. Photophysical and thermodynamic parameters are similar for reactive and nonreactive[2] exciplexes. The major difference between the two thus seems no more than the presence of reactive exit channels in the former.

We have utilized a pulsed nitrogen laser (3371 Å, full width at half maximum, FWHM, $\simeq$8 ns), a PAR 162 boxcar integrator equipped with a Model 164 processor module (aperture 5 ns), and a Spex 1704 monochromator equipped with either an RCA 1P28 or a Hamamatsu R446 photomultiplier. The time-constant of the Model 164 processor is ca. 9 ns and hence the laser pulse convoluted with the boxcar response has a FWHM of ca. 15 ns. The boxcar aperture delay could be scanned (mode I) leading to a time pro-