ture peculiar to each compound. The advantage of alkali halide salts in this respect is that they are readily available in pure form and highly soluble in aqueous solution.

In summary, use of the external heavy-atom effect in room temperature phosphorimetry can both eliminate the need for an auxiliary phosphorescence assembly in many cases and strongly increase the phosphorescence intensity. In effect, a perturbing heavy atom performs the function of a mechanical chopper by distinguishing between fluorescence and phosphorescence signals. The enhancement of the phosphorescence intensity could push the phosphorimetric detection limit down by one or two orders of magnitude in many cases.

A more detailed analysis of heavy-atoms effects on the luminescence of adsorbed dyes will be published elsewhere.

ACKNOWLEDGMENT

We greatly appreciate the helpful advice of Barry Smith at the beginning of this work.

LITERATURE CITED

(1) E. M. Schulman and C. Walling, *Science*, **178**, 53 (1972).
(2) E. M. Schulman and C. Walling, *J. Phys. Chem.*, **77**, 902 (1973).
(3) P. G. Seybold, R. K. Sorrell, and R. A. Schuffert, Presented at the 165th American Chemical Society National Meeting, Dallas, TX, April 13, 1973.
(4) R. A. Paynter, S. L. Wellons, and J. D. Winefordner, *Anal. Chem.*, **46**, 736 (1974).
(5) D. S. McClure, *J. Chem. Phys.*, **17**, 905 (1949).
(6) M. Kasha, *J. Chem. Phys.*, **20**, 71 (1952).
(7) S. P. McGlynn, T. Azumi, and M. Kinoshita, "Molecular Spectroscopy of the Triplet State", Prentice-Hall, Inc., Englewood Cliffs, NJ 1969.
(8) L. V. S. Hood and J. D. Winefordner, *Anal. Chem.*, **38**, 1922 (1966).
(9) E. Vander Donckt, M. Matagne, and M. Sapir, *Chem. Phys. Lett.*, **20**, 81 (1973).

Paul G. Seybold
Wayne White

Department of Chemistry
Wright State University
Dayton, OH 45431

RECEIVED for review December 17, 1974. Accepted February 18, 1975.

Laser Fluorescence Analysis of Chromatograms: Sub-Nanogram Detection of Aflatoxins

Sir: The presence of carcinogenic mold metabolites, particularly aflatoxins, in food and feed products has become increasingly recognized as a serious health hazard (*1, 2*). However, the extent and severity of this problem is difficult to assess because present means of analysis, employing conventional fluorescence techniques, are normally capable of detecting only nanogram levels of, for example, aflatoxin B_1 although as little as 0.1–0.2 ng has been reported (*3–5*). We describe here a new technique for the analysis of aflatoxins on TLC plates using time-resolved and wavelength-resolved laser-induced fluorescence. We have detected as low as 0.2 ng of aflatoxin B_1 and we believe this simple and general technique is capable of an order of magnitude further improvement in sensitivity.

The aflatoxins are a class of heterocyclic compounds which have an absorption maximum around 360 nm with a molar absorptivity of about 20,000 (*6*). The fluorescence is in the visible; the B_1 and B_2 aflatoxins are named for their fluorescence in the blue (425 nm) while G_1 and G_2 for the blue-green (450 nm). The aflatoxins are separated by thin layer chromatography and analyzed by their characteristic fluorescence on the plate.

We obtained standard samples of (B_1 50%, G_1 50%) aflatoxin. These were separated chromatographically using silica gel G (Merck) on a copper substrate and were developed (*4*) in the dark with chloroform:acetone (85:15 v/v). The copper substrate was chosen over the usual glass backing because the former eliminated unwanted substrate fluorescence. This problem is not normally encountered when less intense sources of UV light are employed.

Figure 1 is a schematic diagram of the experimental setup. The output of a pulsed nitrogen laser (337.1 nm) strikes a chromatogram which may be scanned in position. Whenever the laser beam is incident on a spot containing aflatoxin, this spot fluoresces, and the emission is viewed by a photomultiplier (RCA 7265 S-20 photocathode) through appropriate apertures and wavelength filters (Corning No. 52).

At the heart of this experiment is a boxcar integrator (PAR model 162) which controls when the photmultiplier output is interrogated. The boxcar receives a start impulse from a trigger generator which also controls the firing of the nitrogen laser. The boxcar has a pre-set delay before opening an electronic gate to sample the photomultiplier signal. In addition, the boxcar has a pre-set "window" so that the photomultiplier signal is sampled only for a fixed duration. The laser is fired repetitively, at a typical rate of 10 pps. Each pulse has a duration of about 14 nsec with a power of approximately 100 kW. The boxcar integrator collects and averages the photomultiplier signal; the output of the boxcar integrator drives a stripchart recorder which displays the fluorescence signal as a function of time and, hence, plate displacement.

Figure 2 presents typical chromatogram scans. Here the boxcar window is set at 0.3 μsec and the boxcar gate is set to open with the laser pulse. The chromatographic separation of B_1 and G_1 is illustrated in Figure 2*a*, where the data were recorded for 30 seconds, corresponding to approximately 35 laser pulses at the aflatoxin spots. The plate is scanned manually in the same direction as the chromatographic development; the irregular peak shapes are caused by the variable, uncontrolled scan speed. Figure 2*b* shows the detection of aflatoxin B_1 at the lowest level we attempted. It should be clear from the signal-to-noise obtained and the crudeness of data acquisition employed that further improvement in detection sensitivity can be achieved, probably an order of magnitude with only modest refinement.

Silica gel G also fluoresces in the same spectral region as aflatoxin when the gel is irradiated by the nitrogen laser. Thus, the analysis for aflatoxins requires one to measure the difference between the sample spot and an equivalent background spot. We find that background interference is presently the limiting factor in increasing the sensitivity of this technique. However, the technique of laser fluorescence analysis would seem to enjoy the following advantages compared to fluorescence detection (*7*) using "black light" excitation and spectrofluorimetry: a) the laser light

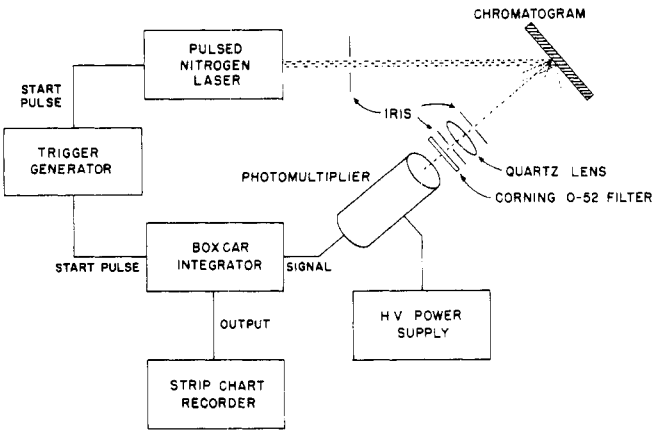

Figure 1. Schematic of experimental setup

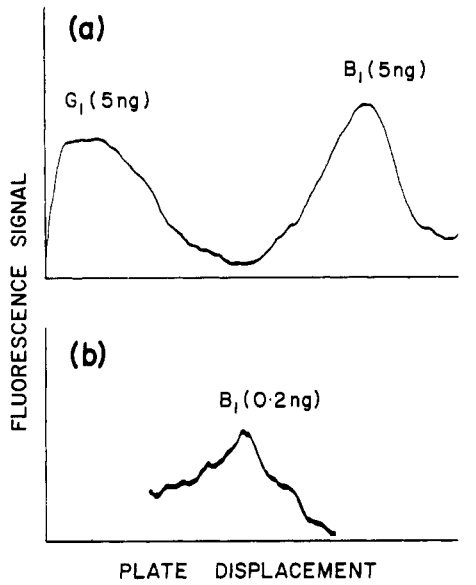

Figure 2. Laser fluorescence chromatogram scans

(*a*) Separation of B_1 and G_1 aflatoxin peaks; and (*b*) detection of aflatoxin B_1 at lowest level attempted. Only an expanded portion of the plate is shown. For a solvent development length of 9.5 cm, the B_1 and G_1 spot maxima are separated by about 1 cm

source is many times brighter than a conventional UV light source during the time the laser is on; b) the laser light source is coherent and, hence, may be focused on a spot rather than illuminating the entire chromatogram; c) gated detection electronics permits the reduction of elimination of unwanted light since the use of a window may exclude or reduce the effect of phosphorescence and scattered laser light; and d) the use of time-resolved as well as wavelength-resolved detection may permit the analysis of a mixture of fluorescent species without the need for their separation. Much work remains, such as the demonstration of linearity with aflatoxin concentration; however, the present preliminary results encourage us to belief that aflatoxins can be detected and quantified at the ultra-trace level with relatively simple equipment. Laser fluorescence analysis has already been used in the gas phase to detect 10^{-18}-gram samples of fluorescent species (*8*) and its use in condensed media, such as high pressure liquid chromatography (*9, 10*), should be expected to grow as convenient UV laser sources are developed.

ACKNOWLEDGMENT

We warmly thank Larry M. Seitz, U.S. Grain Marketing Research Center, U.S. Department of Agriculture, Manhattan, KS, who called this analytical problem to our attention and who provided us with samples of aflatoxins, obtained from USDA–ARS Southern Regional Research Center, New Orleans, LA.

LITERATURE CITED

(1) L. A. Goldblatt, "Aflatoxin, Scientific Background, Control and Implications", Academic Press, New York, 1969.
(2) M. Enomoto and M. Saito, *Ann. Rev. Microbiol.*, **26,** 279 (1972).
(3) J. L. Ayres and R. O. Sinnhuber, *J. Am. Oil Chem. Soc.*, **43,** 423 (1966).
(4) W. A. Pons, Jr., J. A. Robertson, and L. A. Goldblatt, *J. Am. Oil Chem. Soc.*, **43,** 665 (1966).
(5) W. A. Pons, Jr., A. F. Cucullu, A. O. Franz, Jr., and L. A. Goldblatt, *J. Am. Oil Chem. Soc.*, **45,** 694 (1968).
(6) J. A. Robertson, W. A. Pons, Jr., and L. A. Goldblatt, *J. Agric. Food Chem.*, **15,** 798 (1967).
(7) A. J. Pesce, C.-G. Rosén, and T. L. Pasby, "Fluorescence Spectroscopy", Marcel Dekker Inc., New York, 1971.
(8) R. N. Zare and P. J. Dagdigian, *Science*, **185,** 739 (1974).
(9) C. D. Scott, *Science*, **186,** 226 (1974).
(10) "Advances in Chromatography—1974", A. Zlatkis and L. S. Ettre, Ed., Elsevier Scientific Publishing Co., Amsterdam, The Netherlands, 1974.

Michael R. Berman[1]
Richard N. Zare[2]

Department of Chemistry
Columbia University
New York, NY 10027

RECEIVED for review December 31, 1974. Accepted February 26, 1975. This work was supported by the National Science Foundation.

[1] Undergraduate Senior, State University of New York at Stony Brook, Stony Brook, NY 11794.
[2] To whom correspondence should be addressed.

Auger Parameter in Electron Spectroscopy for the Identification of Chemical Species

Sir: X-Ray photoelectron spectroscopy offers two principal kinds of useful information: a semiquantitative estimate of the relative number of atoms of different elements in the layers near the surface, and information on the identity of the chemical species. For the latter, the principal spectral feature used has been the chemical shift in kinetic energy or binding energy.

Early thoughts about the Auger lines led to the conclusion that the chemical shifts should be similar and in the same direction as those of photoelectron lines. Observations on some Auger lines (*1*) led to the contrary conclusion that Auger chemical shifts are abnormally large for metal-oxide pairs with core-type Auger lines (Auger processes with final vacancies in core-type orbitals). Earlier work had also shown that large Auger chemical shifts may also happen between pairs of nonconducting-salts (2). The effect