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 B1 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 B1 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 B1 and B2 aflatoxins are named for their fluorescence in the blue (425 nm) while G1 and G2 for the former eliminated unwanted substrate fluorescence and phosphorescence signals. 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 photometric 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.

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Paul G. Seybold
Wayne White
Department of Chemistry
Wright State University
Dayton, OH 45431

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CHROMATOGRAM

Figure 1. Schematic of experimental setup

STRIP CHART RECORDER

START PULSE

TRIGGER GENERATOR

PULSED NITROGEN LASER

CHROMATOGRAM

PHOTOMULTIPLIER

QUARTZ LENS

CORNING 0-52 FILTER

START PULSE

BOX CAR INTEGRATOR

OUTPUT

H V POWER SUPPLY

Figure 2. Laser fluorescence chromatogram scans

(a) Separation of B1 and G1 aflatoxin peaks; and (b) detection of aflatoxin B1 at lowest level attempted. Only an expanded portion of the plate is shown. For a solvent development length of 9.5 cm, the B1 and G1 spot maxima are separated by about 1 cm.

AUGER PARAMETER IN ELECTRON SPECTRO SCOPY 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 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.

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Michael R. Berman
Richard N. Zare

Department of Chemistry
Columbia University
New York, NY 10027

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

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