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Note

Non-dispersive atomic fluorescence spectroscopy, a new detector for chromatography

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Metal pollution and related health problems are becoming increasingly common. The incidents involving mercury have shown that total metal data is insufficient and often misleading. Metal compounds must be identified and determined quantitatively. The many types of chromatography provide the most powerful approach to the separation of these compounds. Conventional chromatographic detectors, however, show poor selectivity to the metal compounds of interest.

Gonzalez and Ross[1] and Longbottom[2] described the interfacing of an atomic absorption spectrophotometer with a gas chromatograph for the detection of alkylated mercury compounds. This detector is metal specific and hence will only record peaks for the metal used as the radiation source. A number of researchers have used this system for the study of compounds of lead, mercury and chromium.

In 1973, Manahan and Jones[3] used atomic absorption spectroscopy as a detector for high-pressure liquid chromatography in the study of chromium compounds. As in the case of gas chromatography, the metal specificity of this detector greatly simplifies chromatograms compared to those obtained using less specific, conventional detectors.

Since the above pioneering work, atomic absorption spectroscopy has become a routinely used detector for chromatography in metal speciation studies. In this regard, it has made an invaluable contribution to the study of metal compounds.

It has been found by the present authors that atomic fluorescence spectroscopy can be used as a metal specific detector with column chromatography. If non-dispersive atomic fluorescence spectroscopy is employed, two distinct advantages over atomic absorption can be obtained. Firstly, simultaneous multielement detection can be achieved. Secondly, the detection limits for most elements can be improved by 1 to 3 orders of magnitude compared to atomic absorption.

Non-dispersive atomic fluorescence spectroscopy has been described in detail, as a technique for trace metal analysis by Larkins[4]. As with flame atomic absorption, interfacing of atomic fluorescence as a column chromatographic detector is simple. For liquids, the effluent drain of the column is connected directly to the nebulizer capillary of the burner. When gas chromatography is used, the effluent can be introduced into the flame through a port at the base of the burner.

Non-dispersive atomic fluorescence equipment is inexpensive to assemble.

This is another advantage compared to atomic absorption spectroscopy. If the detection limit advantage mentioned above is to be realized, then high intensity hollow-cathode or electrodeless discharge lamps are essential. The latter can be obtained commercially for many elements at about the price of ordinary hollow-cathode lamps.

The unique capabilities of this detector can be demonstrated in the difficult separation and detection, simultaneously, of three different metal–glycines (amino acids) and in the same sample the three metal–EDTA compounds. This combination occurs in clinical samples obtained during the diagnosis and treatment of metal poisoning.

EXPERIMENTAL

Equipment and reagents

A 3-channel non-dispersive atomic fluorescence spectrometer was assembled as diagrammed. Two AA4 amplifiers, one modified to modulate at 325 Hz and the other capable of modulating at the normal 285 Hz were employed. Two Techtron AA4 lamp power supplies were used with these amplifiers. A home-made amplifier and lamp power supply with a modulation frequency of 80 Hz were used for the third channel. R-166 and R-106 photomultipliers (Hamamatsu TV) were used. The former, a solar blind tube, was used for Ni and Zn and the latter for Cu.

Optics consisted of 50 mm focal length by 4 cm diameter silica lenses, arranged as shown in Fig. 1. A 9.2-mm aperature iris was placed in front of the photomultiplier.

Perkin-Elmer intensitron hollow-cathode lamps were run at 15 mA for all elements. When better detection limits are required, electrodeless discharge lamps or high intensity hollow-cathode lamps can be employed.

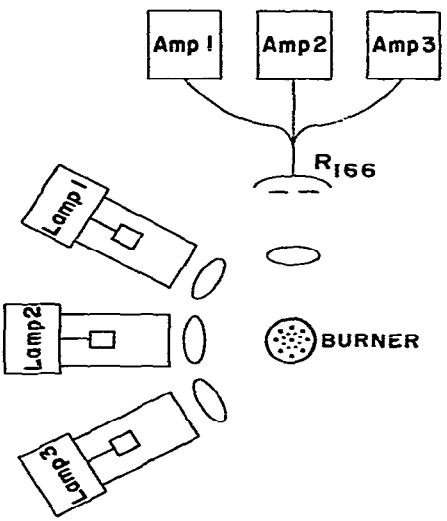

Fig. 1. Schematic of a 3-element non-dispersive atomic fluorescence instrument.

A nitrogen shielded, air–acetylene flame was used. The home-made burner was 15.8 mm in diameter with three concentric rings of 1.09 mm holes. This burner fits inside a SB-E sheathing device (R11C, London, Great Britain) and was used with a Perkin-Elmer Model 303, pre-mix chamber-nebulizer system.

To record the output from the three amplifiers, it was necessary to employ Model 56 double-pen and Model 165 single-pen recorders (Perkin-Elmer). The chart speeds of these were equal allowing superimposition of the output.

A Perkin-Elmer Model 601 high-pressure liquid chromatograph was used. The cation-exchange column was packed with Perkin-Elmer Partisil-10 SCX. The column temperature was maintained at 55°. Interfacing is simple. The outflow from the column is connected directly to the nebulizer capillary of the burner (*cf.* Fig. 2). A column flow-rate of 4 ml/min was maintained to be compatible with the nebulizer flow-rate of the fluorescence burner. A pressure of 100 p.s.i. was used.

The amino acid– and EDTA–metal compounds were prepared by the method of Lau[5].

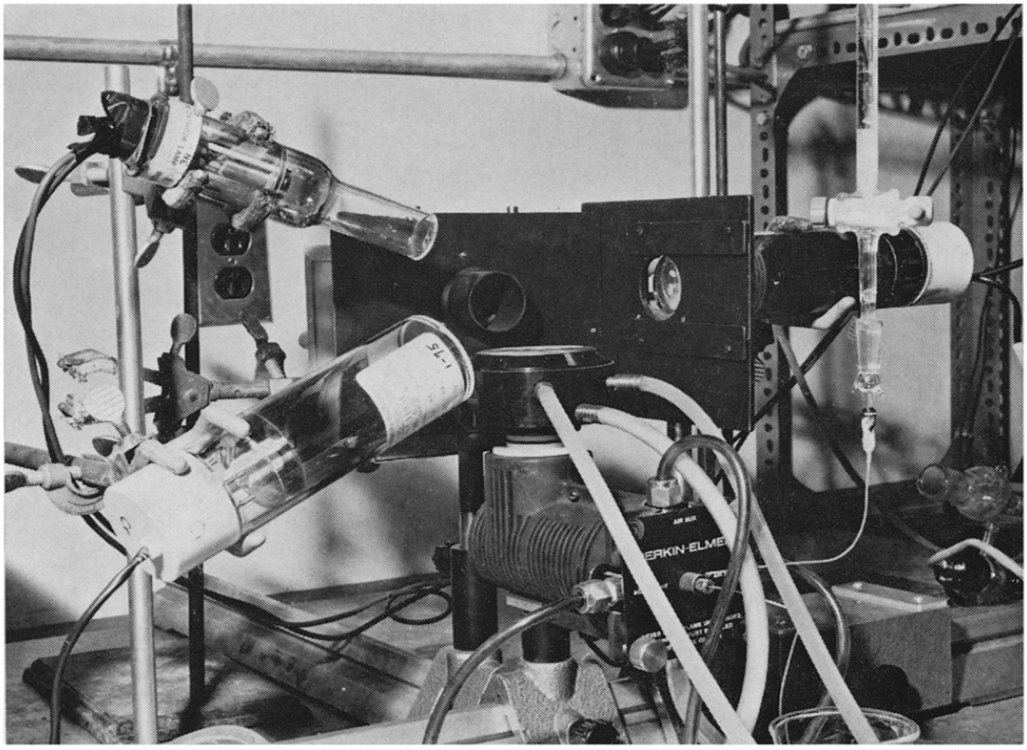

Fig. 2. Non-dispersive atomic fluorescence detector for chromatography.

Procedure

The column is rinsed and equilibrated with water. The column temperature is adjusted to 55°. A 25-μl sample is injected and the column eluted with pure water. After the first peaks (*E* on Fig. 3) come over, a 5-min convex gradient, curvature 999, to 100% 1 *M* NH_4NO_3 is used.

RESULTS AND DISCUSSION

Fig. 3 is a chromatogram of the three elements Zn, Ni and Cu. Peaks designated *E* are metal–EDTA complexes, those marked *G* are metal–glycines, and peak T is Cu-Trien. Because of their physical and chemical similarities, the glycine peaks have almost identical retention times. The same is true for the EDTA peaks. A conventional UV–visible detector would fail to resolve individual glycines or EDTA's. The automatic fluorescence detector, on the other hand, gives excellent resolution of these compounds.

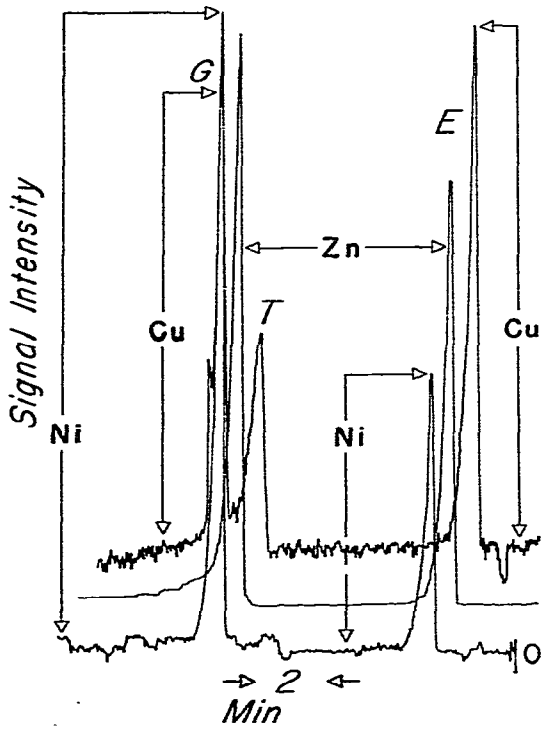

Fig. 3. Chromatogram for Cu–, Zn– and Ni–glycines, –EDTA's and Cu-Trien.

CONCLUSIONS

Until recently, it has been impossible to provide metal speciation data in quantity. This has been due to the difficulty in detecting trace metal compounds in complex samples. Atomic absorption spectroscopy used as detector for chromatography has provided the first breakthrough toward solving this problem. Nondispersive atomic fluorescence spectroscopy has all the advantages of atomic absorption spectroscopy in this application while providing, in addition, better detection limits and a simultaneous multielement capability.

Future work

Now that the technique has been shown to work a better multielement spectrometer, such as that described by Larkins and Willis[6], will be constructed. This instrument will contain 7 channels and will be used with a 7-pen recorder.

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