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BETA-INDUCED FLUORESCENCE AS A DETECTION TECHNIQUE FOR LIQUID CHROMATOGRAPHY

I. PRELIMINARY EXPERIMENTS

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SUMMARY

A fluorescence detector in which ^{63}Ni β^- decay radiation is used as exciting source has been constructed and attached to a commercial liquid chromatograph. Pulses of fluorescence emission induced by the β^- decays were counted for a number of fluorescent materials in solvents of hexane, toluene and methanol. The detector response was found to be linear over low concentration ranges, although in one instance a marked deviation from linearity was observed at higher concentrations.

INTRODUCTION

The last decade has seen a dramatic increase in the use of high-performance liquid chromatography (HPLC)[1]. Continuous improvements in technology have resulted in the widespread availability of excellent pumping systems and associated hardware, and in the modern, small-particle column-packing materials. However, comparatively little change has occurred in the third major component of liquid chromatography systems—the detector. The most commonly used detectors are based on UV absorption, refractive index or fluorescence, although a great variety of other detecting systems have also been described[2].

For many applications, it is desirable that a detection system for liquid chromatography should combine high sensitivity for the compounds of interest with a high degree of stability. Fluorimetric detectors have gone some way to meeting the former requirement, although stability is still a major problem. The source of exciting radiation is usually a mercury line or xenon arc lamp, both of which are susceptible to variations in intensity, so that expensive power supplies or other electronic systems for compensating for intensity fluctuations are required. Further, since the emitted fluorescent radiation must be detected at right angles to the incident exciting radiation, a substantial amount of the total fluorescence is generally lost. The

use of filters or monochromators to reduce interference results in a further reduction in the amount of fluorescent radiation detected.

We have been investigating the practicality of using a novel source of excitation energy for the detection of fluorescent materials in the eluent from a liquid chromatograph. Preliminary results for a number of test materials are given below, and these demonstrate that this new technique has considerable potential to meet both the sensitivity and stability requirements for a wide range of liquid chromatography applications.

CHOICE OF EXCITING SOURCE

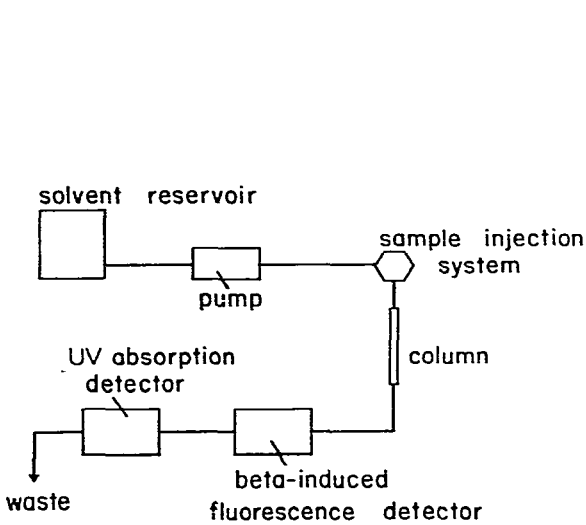
We set out to utilise energy released during the natural decay of a radionuclide as the source of excitation energy for the induction of fluorescence from components in the eluent from a liquid chromatograph. Several reports have been published on the fluorescece of organic compounds in solution under the influence of inosing radiation[3], although most recent work has been directed towards the phenomena involved in liquid scintillation counting[4]. Of the three major classes of radioactivity involving the emission of radiation, the most attractive for work in which the radiation should penetrate a liquid for distances of the order of fractions of 1 mm is β^- decay. In this paper, we confine our attention to one particular radionuclide that decays by β^- emission.

Of the commonly available β^- decay radionuclides, most emit β^- particles with an energy above the Cerenkov threshold[5] in normal liquid chromatography solvents (*e.g.*, 263 keV in water). Of the nuclides emitting low-energy β^- particles[6], ^{3}H, ^{14}C, ^{35}S and ^{63}Ni are readily available, but ^{14}C and ^{35}S were deemed unsuitable for use in these experiments; ^{14}C has a half-life of 5730 years and is consequently difficult to obtain as a surface deposit with high specific activity. On the other hand, ^{35}S has a half-life of 87 days and so could not be used in a detector system without regular replacement and frequent corrections for its activity level. Tritium would probably be a good β^- particle source, since its half-life (12.5 years) allows fairly high specific activities to be obtained and is sufficiently long to ensure that correcting for its decay would not become tedious, However, the average energy of the β^- particles emitted by tritium is only *ca.* 6 keV, and we considered that, for these preliminary experiments, in which relatively crude apparatus was to be used, a somewhat more energetic β^- decay was desirable. For these reasons, we have concentrated on the radionuclide ^{33}Ni (half-life = 100 years; β^- decay E_{max} *ca.* 67 keV). This isotope also had the advantage that it could be electro-plated on to a conductor of virtually any shape, so that high specific activity could be obtained on a surface, which in turn allows a significant number of the β^- particles to escape from the radioactive source.

EXPERIMENTAL

The apparatus used for these experiments is shown schematically in Fig. 1. A conventional reciprocating pumping system (Anachem Limited) was used to move the solvent at a constant flow-rate of *ca.* 1 ml min^{-1}. Samples were introduced into the solvent stream by means of an Altex Model 905 sample-injection

valve fitted with a 20-μl sample loop. For the experiments reported in this paper the "column" consisted of a 25-cm length of empty stainless-steel tubing (I.D. 0.03 in), used to spread samples and produce a more realistic peak shape than was obtained when the sample-injection valve was connected directly to the detector. The eluent from this column passed into the β^--induced fluorescence detector shown in Fig. 2. The flow-cell of this detector consisted of a length of PTFE tubing (I.D. 0.075 cm) that contained a 5-cm length of wire (0.05 cm diam.) on to which 15 mCi of ^{63}Ni had been electro-deposited. The PTFE tubing was formed into an almost complete single loop (diam. *ca.* 2 cm); the shape of the loop was maintained by recessing the PTFE tubing into a former that was covered with bright aluminium foil acting as a reflector.



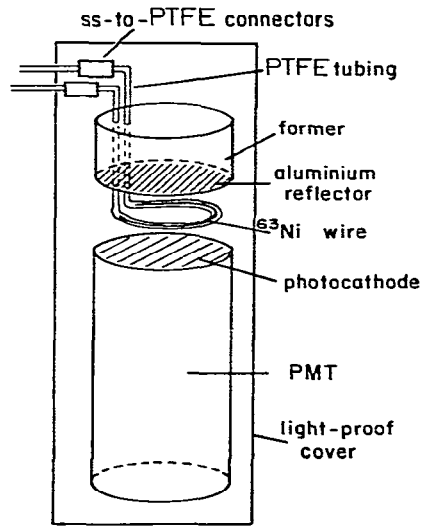


Fig. 1. Schematic diagram of apparatus, showing the connection of the BIF flow-cell to the chromatograph.

Fig. 2. Schematic diagram of BIF flow-cell. Electronic connections to the PMT are not shown.

The flow-cell containing the radioactive source was viewed by a photomultiplier tube (PMT), the whole being enclosed in a light-proof box. The β^- decay of the ^{63}Ni source excited fluorescence from materials passing through the flow-cell, and the detected scintillations were counted on an SR5 scaler–ratemeter (Nuclear Enterprises), the count-rate being continuously monitored on a chart recorder. The PMT used was an EMI type S9514, with a soda-glass end window. For this reason, only materials emitting fluorescence at wavelengths longer than 370 nm could be detected. On the other hand, the use of a soda-glass PMT allowed us to use ordinary HPLC-grade solvent without the problems of an inconveniently high background from solvent fluorescence. (Results of experiments performed using a quartz-window PMT will be reported in a subsequent paper.)

After passing through the β^--induced-fluorescence (BIF) detector flow-cell, the eluent was monitored for UV absorption (254 nm) using an Altex model 150 biochemical monitor. The conventional detector enabled us to cross-check the

quantity of sample injected and provided a monitor for ensuring that material was not being retarded by adsorption on the wire in the BIF flow-cell.

Low-concentration solutions of anthracene, naphthalene, 2,5-diphenyloxazole (PPO), 1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP), *p*-benzoquinone and 9-aminoacridine were prepared and injected into the appropriate flowing solvent (see below). As pure solvent flowed through the system, counts were registered at a reasonably constant rate (typically, *ca.* 6×10^4 cpm with our sample of hexane). When samples of the above materials passed through the flow-cell, the count rate increased to a maximum and then returned to the level characteristic of the pure solvent, thus the recorder trace showed a peak of the kind generally associated with liquid-chromatograph detectors.

Materials

Radioactive source. This consisted of ^{63}Ni (15 mCi) electro-plated on copper wire (5.0 × 0.05 cm) and was supplied by the Radiochemical Centre (Amersham, Great Britain).

Fluorescent compounds. Naphthalene, anthracene, PPO and POPOP (Scintillation Reagents) and *p*-benzoquinone (SLR grade) were obtained from Fisons (Loughborough, Great Britain), and 9-aminoacridine (Grade II) from Sigma (London, Great Britain).

Solvents. Hexane and methanol (HPLC grade), were obtained from Fisons, and toluene (A.R. grade) from BDH (Poole, Dorset, Great Britain).

RESULTS AND DISCUSSION

The operating conditions of the detection electronics were optimised in the usual way, *i.e.*, by seeking the maximum value of $(S—B)^2/B$, where S is the count-rate with solvent plus fluorescent solute flowing through the cell and B is the count-rate from the solvent alone.

By using the dummy column as described above, 20-μl samples of dilute solutions of the fluorescent materials were passed through the apparatus; the UV absorption traces obtained were similar to those that would be produced following reasonable chromatographic separations. The count-rate variations recorded from the BIF detector for a number of samples in hexane are shown in Fig. 3, where the UV absorption records are also given. The similarity in peak shape strongly suggests that the BIF detector responds to the instantaneous concentration of fluorescent material within the flow-cell. No tailing was observed, and the count-rate in pure solvent after a peak returned cleanly to the value before the peak.

For all samples passed through the flow-cell, a "fluorescent count" was obtained by noting the integrated count under each peak and correcting for the equivalent count obtained in the same time interval with pure solvent alone passing through the cell. The fluorescent counts obtained from a number of dilute solutions eluted in hexane are collected in Table I, in which the results are means of several experiments and the errors are standard errors.

Since the linearity of response of a BIF detector would be of considerable importance if the technique were to find analytical application, we determined

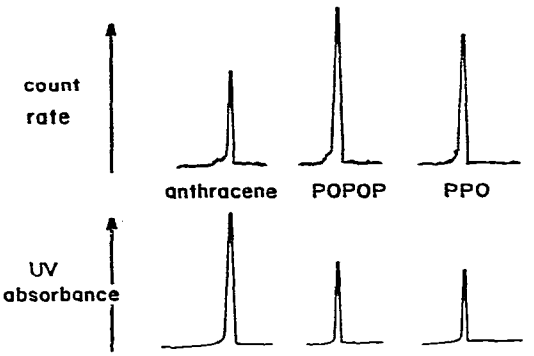


Fig. 3. Count-rate recorded from BIF detector as 0.2-μg samples of fluorescent materials passed through the flow-cell; the UV absorption traces are also shown, corrected for the lag between the two detectors. The axes are of arbitrary units.

TABLE I

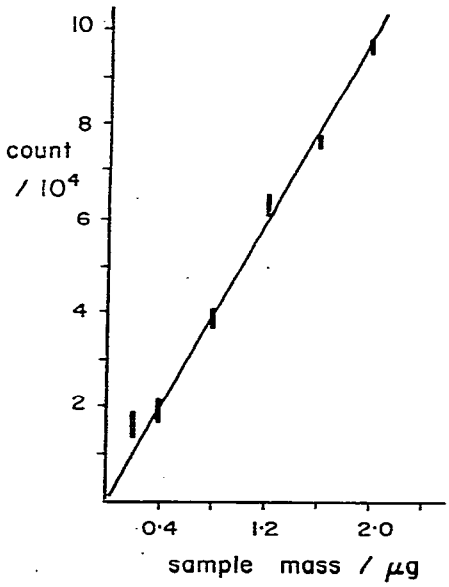
FLUORESCENCE COUNT FROM DILUTE SOLUTIONS ELUTED IN HEXANE

The concentration of each solute was 1 μg/ml.

Solute	*Count from 20 ng of sample*
Anthracene	1520 ± 120
p-Benzoquinone	1110 ± 100
Naphthalene	1350 ± 150
POPOP	7710 ± 200
PPO	5080 ± 150

the fluorescent count from solutions over a range of concentrations. The results for 9-aminoacridine eluted in methanol are given in Fig. 4, where the abscissa shows the mass of compound contained in the 20-μl sample. Clearly, detector response is linear over the range of concentrations studied (10–100 μg ml^{-1}). Results for anthracene eluted in toluene are shown in Fig. 5; again, detector response is linear over the concentration range shown (1–10 μg ml^{-1}). However, results obtained at higher concentrations of anthracene showed a marked deviation from linearity, suggesting that a BIF detector of the present design may have limitations for the quantitative analysis of some solutes at high concentrations. The results of anthracene shown in Fig. 6 are for the same concentration range as those for 9-aminoacridine shown in Fig. 4. (10–100 μg ml^{-1}).

The ^{63}Ni-coated wire appeared to be quite stable in the presence of hexane and toluene, although methanol did cause significant amounts of activity to be dissolved in the flowing solvent. During our experiments with 9-aminoacridine in methanol, approximately 6 μCi of ^{63}Ni were eluted in *ca.* 3 l of methanol. For this reason, we have not carried our further experiments in alcoholic or aqueous solutions with the present apparatus. One early experiment involved the passage of Rhodamine B through the flow-cell; a dramatic increase in count-rate was obtained, but was not cleared when the flow reverted to pure solvent. It became clear that almost every salt passed through the cell became adsorbed on the wire and could



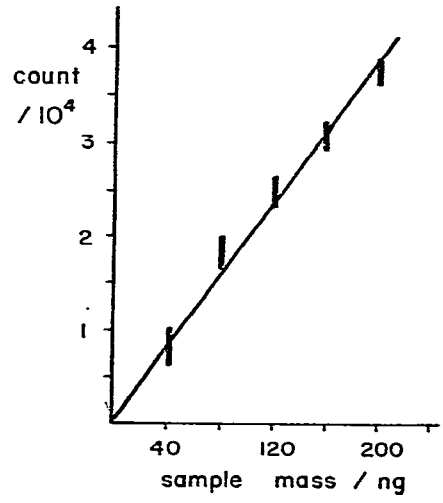


Fig. 4. Fluorescent count obtained as function of mass of 9-aminoacridine passed through the BIF detector. Solvent: methanol.

Fig. 5. Fluorescent count obtained as function of mass of anthracene passed through the BIF detector. Solvent: toluene.

only be released by the passage through the cell of methanol–water (1:1)—a procedure that took toll of the ^{63}Ni activity.

While it is clear that many improvements can be made to this prototype detector, there seems good reason to hope that the inherent stability in the rate of

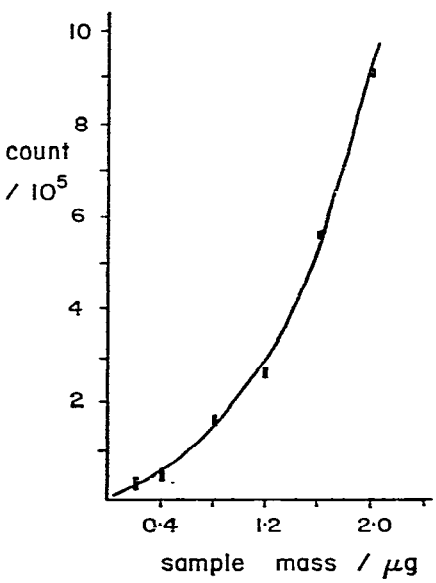


Fig. 6. As Fig. 5, but for higher concentrations of anthracene.

β^- decay characteristic of a high-activity source will offer considerable advantages for a fluorescence detection system. A BIF detector can be operated for very long periods without deterioration and without short-term variations in source intensity. The relatively robust nature of the detector may provide an additional advantage for mobile equipment, since conventional lamp sources often require long stabilisation periods before use.The system also lends itself to miniaturisation with simple battery-operated electronics, so that relatively compact and portable detection systems would be feasible.

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REFERENCES

1 J. J. Kirkland, *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971.
2 R. P. W. Scott, *Liquid Chromatography Detectors*, Elsevier, Amsterdam, Oxford, New York, 1977.
3 H. Kallmann and M. Furst, *Phys. Rev.*, 79 (1950) 857; 81 (1951) 853; and 85 (1952) 816.
4 D. L. Horrocks, in D. L. Horrocks (Editor), *Applications of Liquid Scintillation Counting*, Academic Press, New York, 1974, p. 12 and references therein.
5 G. Friedlander, J. W. Kennedy and J. M. Miller, *Nuclear and Radiochemistry*, Wiley, Chichester, 1955, p. 154.
6 C. M. Lederer, J. M. Hollander and I. Perlman, *Table of Isotopes*, Wiley, Chichester, 1967.