

CHROM. 13,130

BETA-INDUCED FLUORESCENCE AS A DETECTION TECHNIQUE FOR LIQUID CHROMATOGRAPHY

II. PROTOTYPE DETECTOR FOR NORMAL-PHASE OPERATION

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(Received July 3rd, 1980)

SUMMARY

The design of a prototype beta-induced fluorescence detector based on a commercially available radioactive source is described. Results, obtained with the detector attached to an high-performance liquid chromatographic system using normal-phase chromatography, are presented to illustrate (1) the range of materials detected, (2) the linearity of the detector response with sample loading, and (3) the sensitivity of the detector.

INTRODUCTION

Beta-induced fluorescence is the luminescence excited from a compound as a result of the passage of beta particle radiation through the compound or a dilute solution of the compound. A beta-emitting radionuclide positioned in the eluent flow from a liquid chromatography column may be used to excite fluorescence from components eluted from the column. Measurement of the fluorescence emission rate from the eluent thus allows the detection and quantitation of fluorescent materials in liquid chromatography.

In an earlier paper¹ (Part I) the principles of beta-induced fluorescence (BIF) used as a detection technique for liquid chromatography were described, the potential advantages of using a radioisotope as an exciting source were discussed, and some preliminary results obtained using a ⁶³Ni source were presented. We have now constructed a prototype low volume BIF detector system which overcomes many of the problems we observed during our preliminary experiments —the most important of which was the loss of radioactivity from the source into the eluent. The prototype detector has been in use in our laboratory for about a year, and the present paper describes the system and some of the results we have obtained with it during that period.

The prototype detector

Although ⁶³Ni does have many attractions as an excitation source for a BIF detector, one disadvantage which influenced our choice of source for the prototype

detector was the cost of obtaining an adequately protected, high activity, small surface area source. For an experimental system a suitable alternative is ^{147}Pm , which, although having a half-life (2.6 years)² rather short for a commercial instrument is conveniently available in a variety of packagings and protected by silver foil. Our prototype detector has been constructed around a 1 mCi "point source" of ^{147}Pm (code PHC. 32, available from the Radiochemical Centre, Amersham, Great Britain), the elements of which are shown schematically in Fig. 1. The beta-decay energy of ^{147}Pm ($\approx 225 \text{ keV}$)² is somewhat greater than that of ^{63}Ni ($\approx 67 \text{ keV}$), and it would seem likely that this should result in a greater fluorescent photon output per Pm beta decay than from Ni.

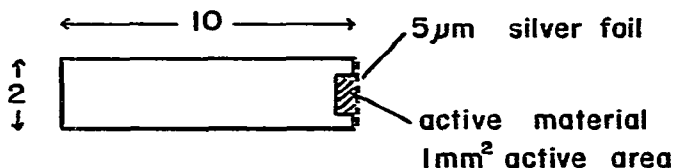


Fig. 1. Structure of the ^{147}Pm source used in prototype BIF detector system. Cylindrical body is of stainless steel and the dimensions shown are mm. Source is available from the Radiochemical Centre, code PHC.32.

The source was used in a flow cell fabricated from stainless steel and shown schematically in Fig. 2. The inlet and outlet connection tubes which carried the chromatographic eluent were braised into the body of the cell. The front face of the cell body was polished to allow a leak-proof seal with the $2 \times 19 \text{ mm}$ (dia Spectrosil A window. The active volume of this cell (*i.e.* the volume in which the beta particles may cause excitation and from which emitted photons may be detected) may be calculated from the 2 mm diameter of the hole containing the β^- particle source and the distance between the face of the β^- source and the cell window. This latter distance could be varied, using the source positioning screw, between 0.5 mm and 3.0 mm, so that active volumes between $1.5 \mu\text{l}$ and $10 \mu\text{l}$ could be used.

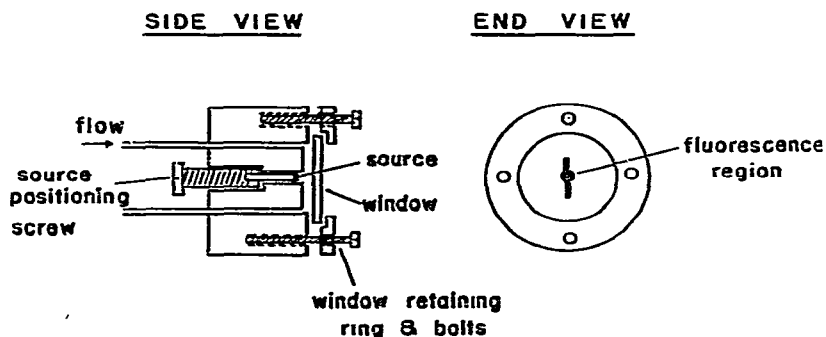


Fig. 2. Schematic detail of construction of prototype BIF flow-cell. Body of cell was of stainless steel, window of Spectrosil A.

While the design of the present flow-cell is not necessarily "ideal", it does offer a number of advantages over the system described in our earlier paper. It is relatively straightforward to fabricate and allows UV as well as visible light photons to be detected; it has a small but adjustable active volume, and yet the distance between the source and the window can be controlled (thus allowing beta-induced fluorescence from the window to be minimised); finally, the photon collection efficiency is high, with the silver foil covering of the source acting as a reflector within the cell. The overall dimensions of the prototype cell were approximately 40×30 mm (dia), neglecting the connecting tubing. The dead volume contributed by the inlet tube was relatively small ($\approx 2 \mu\text{l}$), although the inlet channel on the face of the cell (see Fig. 2) was larger than we would have wished ($\approx 50 \mu\text{l}$) and undoubtedly represents a deficiency in this prototype. The window of the flow-cell was separated from the photomultiplier detector window by an air gap of approximately 1 mm (due to the presence of the retaining ring). A better optical contact would clearly improve photon transmission, but this was not attempted for the work described below. In some of our experiments optical filters were placed between the flow cell and the photomultiplier tube and details are given below at the relevant points.

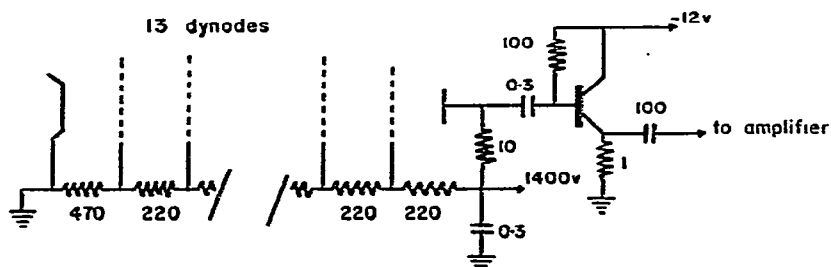


Fig. 3. Circuit of photomultiplier dynode chain and impedance converting preamplifier, both constructed on tube base. Resistance are in $k\Omega$ and capacitances in nF.

The flow cell was connected to an high-performance liquid chromatography (HPLC) instrument as described in Part I, and eluent from the column outlet passed over the β^- particle source, viewed by a quartz window photomultiplier tube (EMI type 9804QB, alkali photocathode, 13 stage). The photomultiplier tube (PMT) was operated at 1400 V and was connected to a dynode resistance chain and simple emitter follower circuit constructed on the tube base according to the circuit diagram shown in Fig. 3. The electronic pulse width produced at the low impedance output was < 100 nsec, which is approximately two orders of magnitude shorter than the pulse width used in our preliminary experiments. The pulses were counted using a fast (30 MHz) nuclear counting system which consisted of the following Nucleas Instrument Modules supplied by Canberra Ltd. (Farringdon, Great Britain) and connected as shown in Fig. 4:

(1) amplifier, Model 2110 timing filter amplifier; (2) shaper, Model 1433 discriminator, producing fast logic pulses; (3) counter, Model 1776 dual scaler/timer, for counting pulses detected under chromatographic peaks; (4) bias supply, model 3102 high voltage unit; and (5) ratemeter; for low pulse rates a NE ratemeter (Nuclear Enterprises, Sighthill, Great Britain) was used to obtain an analogue signal proportional to the instantaneous pulse rate. For high pulse rates ($> 10^4 \text{ sec}^{-1}$) a unit of our

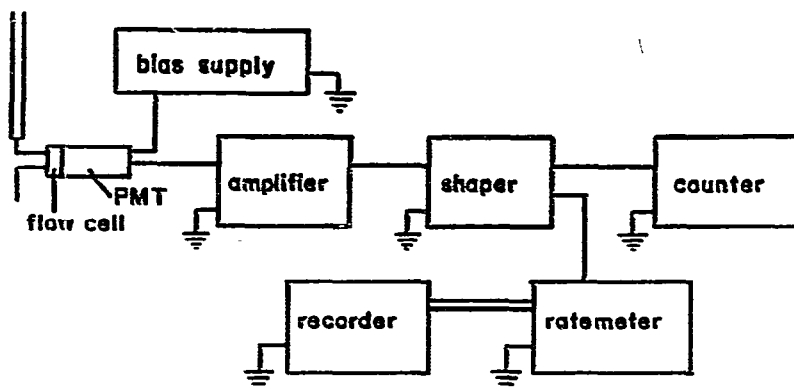


Fig. 4. Block diagram showing units and connections in the prototype BIF detection system. Details of the electronic units are given in text.

own (conventional) design was used to record pulse rates up to 10^7 sec^{-1} . In both cases the analogue output was recorded on a chart recorder to produce a chromatogram of the normal form.

In most of our experiments we have found that it was not desirable to remove statistical fluctuations in the eluent "background" pulse rate—which constitute the noise of the system—by operating the pulse ratemeters with long time constants (>1 sec). Such a procedure distorts the chromatographic peak shape and lowers the apparent resolution of the recorded chromatogram. For these reasons we prefer to operate the ratemeter with a time constant of one second, and remove the statistical noise using a low pass filter connected between the ratemeter and the chart recorder. The procedure and its effects on chromatographic peak shapes have been described recently³. The cut-off frequency on the electronic filter used to obtain the chromatographic records reported below was 0.2 Hz.

EXPERIMENTAL

The BIF prototype detector described above was connected to a conventional reciprocating pump hplc fitted with an Altex 15×0.6 cm I.D. column packed with $5 \mu\text{m}$ LiChrosorb. Samples were loaded onto the column using an Altex Model 905 sample injection valve fitted with a $20\text{-}\mu\text{l}$ sample loop. Elution was with hexane (Fisons, Loughborough, Great Britain, HPLC grade) containing varying amounts of toluene (Fisons, AnalaR grade) as a polar modifier. In our experiments the eluent flow-rate was approximately 1 ml min^{-1} , which required a pumping pressure of between 300 and 500 p.s.i. (depending on the eluent composition). In most cases the eluting solvent was deoxygenated by purging with argon.

RESULTS AND DISCUSSION

The results reported below have been selected to demonstrate three important aspects of the beta-induced fluorescence technique which have become apparent with use: (a) the range of materials which may be detected by BIF; (b) the linearity of

response of the detector with the amount of fluorescent material detected; and (c) the potential sensitivity of the technique.

To simplify comparisons between different experimental arrangements and between different fluorescent materials, the response of our BIF detector to each material tested has been recorded as the number of pulses counted under the chromatographic peak divided by the mass (μg) of the material present. Thus the responses reported below are given in units of counts per microgramme of sample.

To illustrate the range of materials which may be detected using BIF, a variety of compounds in hexane solution were passed through the chromatograph. In most cases the responses reported below were obtained using sample loadings of 100–400 ng. The results, and the fluorescent background from the deoxygenated eluting solvent, are given in Table I. A typical chromatogram showing the BIF signal recorded from the elution of a five-component mixture is shown in Fig. 5.

TABLE I
RESPONSE OF BIF DETECTOR TO ELUTED MATERIALS

<i>Compound</i>	<i>Eluting solvent</i>	<i>Fluorescent background (cps)</i>	<i>Response (counts/μg sample)</i>
<i>p</i> -Terphenyl	Hexane	12,000	813,600
Anthracene			287,000
Fluorene			263,000
Diphenyl hexatriene			520,000
Bis-methyl-styryl-benzene			517,200
Biphenyl			50,300
Tetraphenyl butadiene			251,600
Naphthalene			105,300
Phenanthrene			45,000
Acenaphthene			153,700
Anisole			38,100
Indene			4,550
Benzo[<i>a</i>]pyrene			338,100
3-Methyl cholanthrene			191,800
Fluoranthene	114,600		
Diphenyl oxazole	Toluene	180,000	8,726,800*
Dibenzofuran			759,600*
Bis-methyl-styryl-benzene			5,353,500*
Bis-methyl-styryl-benzene	Hexane-toluene (99:1)	25,200	1,903,000*

* Pyrex slide in front of PMT, see discussion.

The linearity of the detector response over a range of sample loadings was examined for a variety of materials. Typical results are shown for *p*-terphenyl in fig. 6, where the effect of deoxygenation of the eluent may also be seen. The limited solubility of *p*-terphenyl in hexane prevented us extending these results beyond ≈ 100 ppm. However, the results for fluorene in hexane, shown in Fig. 7, demonstrated that the detector response remains linear up to ≈ 1000 ppm in this case. At higher sample loadings the peak count rate could exceed the capability of the counting electronics, so we have not extended our linearity tests further. It would probably be desirable to place a neutral density filter between the flow-cell and the photomultiplier (or to lower the

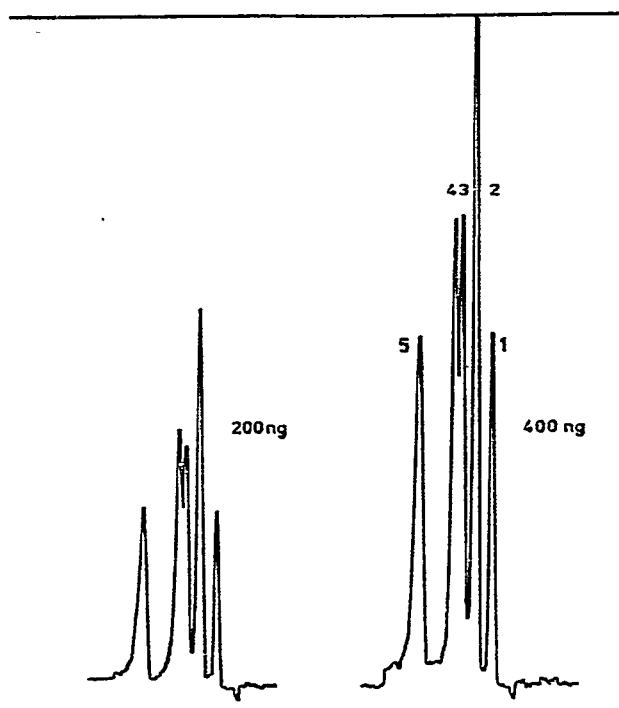


Fig. 5. Records showing BIF detector response to elution of a five-component mixture with deoxygenated hexane. Left-hand trace is for sample containing 200 ng of each component. Right-hand trace is for sample containing 400 ng of each. In each case the solvent peak (negative) is from oxygen in the sample solvent (hexane). Components are 1 = anthracene; 2 = *p*-terphenyl; 3 = perylene; 4 = 1,4-di-(2-methyl-styryl)-benzene; and 5 = 1,6-diphenyl-1,3,5-hexatriene.

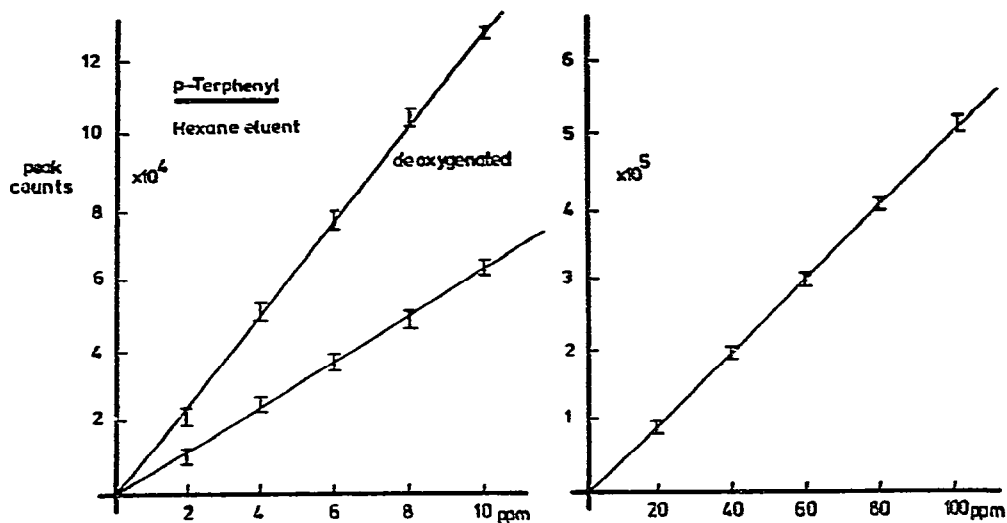


Fig. 6. Integrated peak count from BIF detector vs. sample loading for *p*-terphenyl eluted with hexane. Lower curve in left hand graph is for eluent saturated with air. Other curves are for deoxygenated eluents.

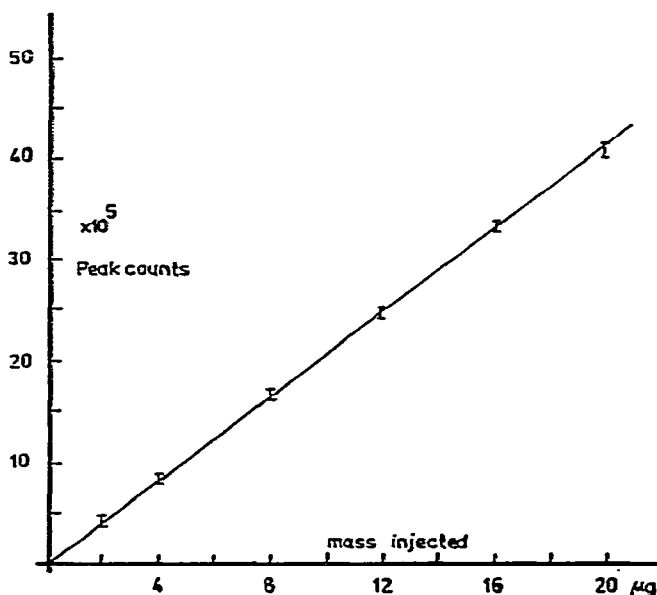


Fig. 7. Integrated peak count from BIF detector vs. sample loading for fluorene eluted with deoxygenated hexane.

TABLE II

EFFECT OF ADDING FLUORESCENT MATERIAL TO ELUTING SOLVENTS

Compound: bis-methyl-styryl-benzene.

<i>Eluting solvent</i>	<i>Background fluorescence (cps)</i>	<i>Response (counts/μg sample)</i>
Hexane	12,000	517,200
Hexane-toluene (99:1)	36,500	1,927,400
Hexane-toluene (95:5)	600,000	1,421,600*
Hexane-toluene (90:10)	650,000	1,465,500*

* Counting system probably in error through dead-time. Approximate corrections could be made and would indicate a response of $(4 \pm 1) \cdot 10^6$, a value confirmed by the use of optical filters (see Table III).

operating voltage of the PMT) for the routine detection of higher levels of fluorescent materials. Our earlier observation of a departure from linearity¹ was in fact caused by pulse overlap in the slower electronics used for our preliminary experiments.

The sensitivity with which an eluted material may be detected using beta induced fluorescence depends largely on the difference between the observed photon emission rates of the eluting solvent and of the solution of the eluted material in that solvent. We have examined two techniques for enhancing this difference in an attempt to improve the sensitivity of our BIF prototype detector. The first technique consists of doping the eluent with a material which improves the efficiency with which excitation energy is transferred to molecules of the eluted fluorescent material⁴. Table II shows the effect of doping hexane eluent with toluene on the eluent count rate and on the integrated peak count recorded for samples of bis-methyl-styryl-benzene. Clearly the addition of toluene to the eluent results in a large increase in the integrated

peak count for bis-MSB, although the enhancement of the sample fluorescence is accompanied by an equally dramatic rise in the fluorescent "background" from the eluting solvent. The use of toluene also allows the polarity of the eluent to be modified so that retention volumes can be varied. As a polar modifier toluene would be inappropriate for use with UV absorption detectors because of its absorption at 254 nm. However, its use in the case of BIF detectors has a number of advantages over more conventional polar modifiers, such as dichloromethane. For example chlorinated materials dramatically lower the BIF emission from fluorescent materials, through the quenching processes which have received much attention in connection with liquid scintillation counting⁴. Toluene's somewhat lower polarity than that of dichloromethane means that retention volumes are less critically dependent on the precise composition of hexane-toluene eluents than on hexane-dichloromethane eluents of similar polarity.

The dramatic rise in the eluent fluorescent emission rate which accompanies the use of doped eluents suggested the second technique which we have used to improve the sensitivity of our BIF detector. Optical cut-off filters, placed between the BIF flow-cell and the photomultiplier tube, may be used to filter out the BIF background from the eluent while allowing the fluorescence from eluted components to be detected normally. The effect of using an L39 cut-off filter ($\lambda_{\text{cut}} \approx 370$ nm) on the "background" count rate from hexane-toluene eluents and on the integrated peak counts recorded from samples of bis-methyl-styryl-benzene are shown in Table III. Clearly the signal to background ratio is improved by more than two orders of magnitude by filtering out the short wavelength fluorescence from the eluent.

TABLE III
EFFECT OF FILTERING FLUORESCENCE ON RESPONSE OF BIF DETECTOR
Compound: bis-methyl-styryl-benzene.

<i>Solvent</i>	<i>Filter</i>	<i>Background fluorescence (cps)</i>	<i>Response (counts/μg sample)</i>
Hexane-toluene (99:1)	—	36,500	1,927,400
	L39	3,050	2,360,100
Hexane-toluene (95:5)	—	1,421,620*	
	L39	3,800	4,017,200
Hexane-toluene (90:10)	—	650,000	1,466,500*
	L39	4,200	4,896,900

* See footnote to Table II.

A similar, although lesser, improvement may be achieved by using a plane pyrex slide positioned between the flow-cell and the photomultiplier tube, or by the use of a Pyrex window tube. Fig. 8 shows chromatogram records of the separation of fluoranthene, benzo[*a*]pyrene and 3-methyl cholanthrene using 1% toluene in hexane as the eluent. The right hand trace shows the BIF signal recorded when a pyrex slide was used to filter the eluent fluorescence, and in this case the "background" count rate was 27,500 sec⁻¹. The negative peak corresponds to the elution of the sample solvent (hexane). The left hand trace shows the BIF signal recorded using the L39 optical filter in place of the pyrex slide, resulting in a background count rate of

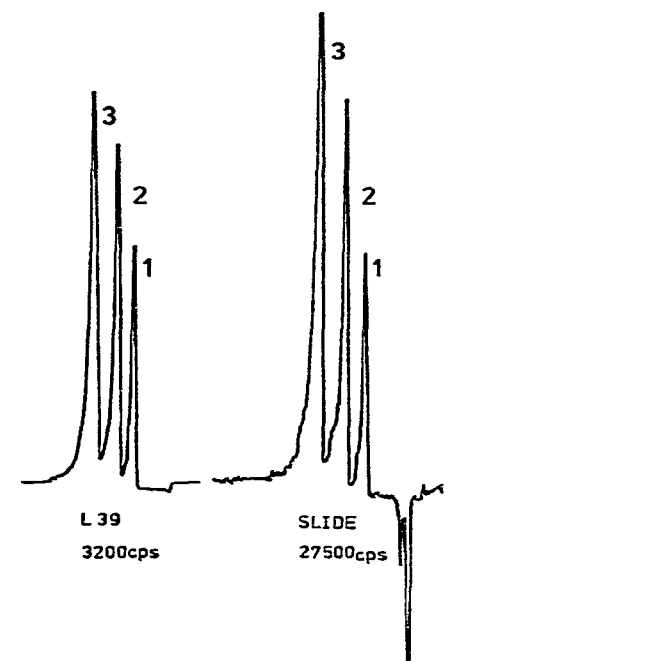


Fig. 8. Records show BIF chromatograms obtained from a three-component mixture eluted with deoxygenated hexane containing 1% toluene. Right hand trace obtained with pyrex slide filter before PMT. Left hand trace obtained with L39 optical filter. Background count rates from eluent alone are shown. Components were 1 = fluoranthene, 2 = benzo[*a*]pyrene and 3 = 3-methyl cholanthrene. Sample contained 300 ng of each component.

$\approx 3200 \text{ sec}^{-1}$. As the optical filter removes most of the toluene fluorescence, the negative solvent peak is now hardly visible.

The combination of a doped eluent together with optical filtration of the eluent's fluorescence results in a significant improvement in the sensitivity of the beta-induced fluorescence detector system. Fig. 9 shows the signal recorded from a 20 ng sample of benzo[*a*]pyrene eluted in toluene-hexane, the right hand trace having been obtained with a recorder gain of three and indicating the statistical noise present on the baseline. From signals such as these we estimate the limit of detection for most of the materials used above to lie between 10^2 – 10^3 pg — that for benzo[*a*]pyrene being ≈ 500 pg.

Our prototype BIF detector was not designed to achieve the maximum sensitivity from the BIF technique, and undoubtedly many improvements could be made in the search for lower detection limits. A higher activity source would, of course, provide a greater signal (our earlier system used 15 mCi of ^{63}Ni), and a smaller dead volume in the flow cell should improve the sensitivity by reducing the peak broadening. The use of interference filters or a grating monochromator to select particular wavelengths of fluorescence would be expected to improve the sensitivity of the detector as well as introducing selectivity of the kind generally associated with

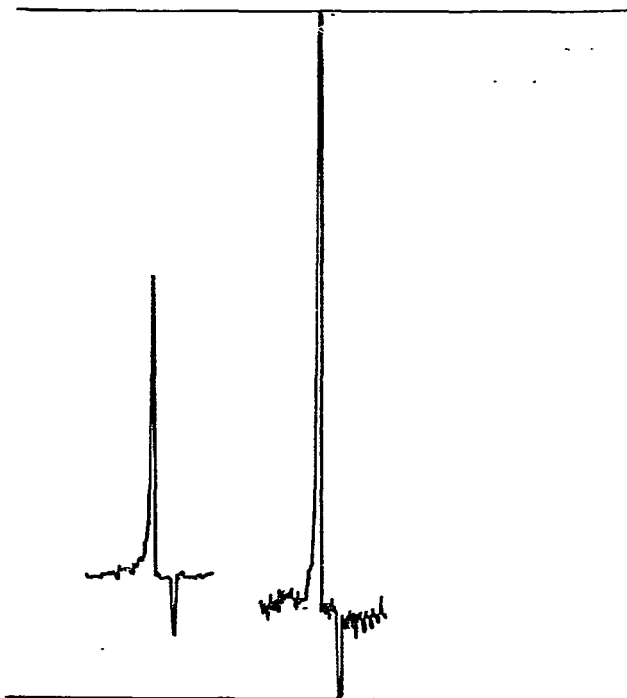


Fig. 9. Records of BIF detector response to 20 ng samples of benzo[a]pyrene eluted with deoxygenated hexane containing 10% toluene. L39 filter was used to suppress detection of eluent fluorescence. Right hand trace obtained with recorder gain $\times 3$.

fluorimetric detectors. However, even in its basic form as described above the BIF detector compares favourably with some commercially available fluorimetric detectors.

ACKNOWLEDGEMENTS

This work was supported by the National Research Development Corporation, who have filed patent applications for the protection of some aspects of beta-induced fluorescence detectors. One of us (P.W.) thanks the Nottinghamshire Education Department for leave of absence during which this work was performed. We also thank the Laboratory of the Government Chemist for providing fluorescent materials for our work and Mr. Anthony Warwick for fabricating the flow-cell.

REFERENCES

- 1 D. J. Malcolme-Lawes, P. Warwick and L. A. Gifford, *J. Chromatogr.*, 176 (1979) 157-163.
- 2 C. M. Lederer, J. M. Hollander and I. Perlman, *Table of Isotopes*, Wiley, Chichester, 1967.
- 3 D. J. Malcolme-Lawes, S. Massey and P. Warwick, *J. Radioanal. Chem.*, 57 (1980) 355-361.
- 4 J. B. Birks, *Theory and Practice of Scintillation Counting*, Pergamon, London, 1964.