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Note

High-performance ultraviolet absorption detector for liquid chromatography

II.* A prototype detector

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High-performance liquid chromatography (HPLC) followed by component detection using UV absorbance monitors has become one of the most widely used techniques for routine analyses, and this is due in part to the quality and relatively low cost of many of the commercial chromatographic systems available. However, most commercial UV absorbance detectors do have disadvantages associated with their use, particularly detectors which can operate below 250 nm. Their light sources generally consume a considerable amount of power, and as most of this is wasted as heat, this contributes to thermally induced refractive index changes along the light path, and so to the noise level of the detected signal. To limit the effects of this problem, many commercial systems must operate in a dual beam mode, which complicates the structure of the detector and adds to its cost. Furthermore, most systems are only useable at moderate sensitivity once thermal equilibrium has been reached, and this process can take many hours with a typical deuterium lamp system. Conventional light sources are also operated at relatively high light intensities, so that materials passing through the chromatographic flow cell are subjected to high irradiation levels. This in turn can lead to significant amounts of photodecomposition and require frequent cleaning of the cell.

We have been developing a UV absorbance detector based on a radioluminescence light source. In an earlier paper[1] we described the construction and operation of an experimental detector based on a crystalline oxide excited by a standard ^{90}S source. We have shown that different crystalline materials may be used to provided line or broad-band emissions, and have studied the noise levels of emissions from aluminium and mixed oxide crystals[2]. Recently we have been developing the light source to produce higher intensity emissions and have constructed a prototype detector which has been in use since mid-1986. In this paper we report some of the results obtained with the prototype system.

* For Part I, see ref. 1.

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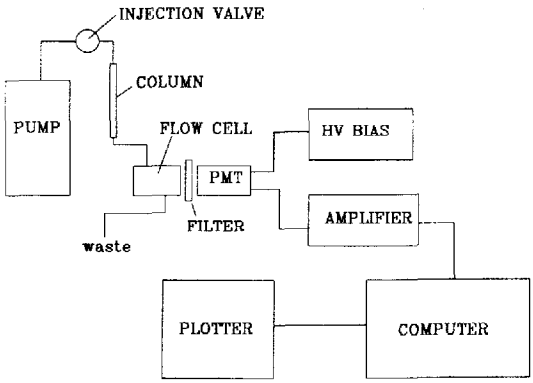

Fig. 1. Schematic diagram of the apparatus used to record chromatograms.

EXPERIMENTAL

The apparatus used in this work is shown schematically in Fig. 1. The chromatograph consisted of a Knauer pump, a Rheodyne Model 7125 injection valve fitted with a 20-μl loop, and a Waters Z-module column system. The eluent from the column was delivered to the detector system flow cell through 0.008-in bore stainless-steel tubing. The small size of the light source meant that it could be incorporated into the flow cell, and this assembly is shown in Fig. 2. The rear of the flow cavity was closed by a 3 mm diameter crystal, approximately 2.5 mm thick, polished on all sides and coated with a thin layer of aluminium by vacuum deposition. Immediately behind this was the ^{90}Sr source, a 10-mCi "point source" housed in a 10 mm × 2 mm diameter stainless-steel holder (Amersham, source type SIF33). The flow cavity itself measured 9 mm × 2 mm diameter and the front of the cavity was closed by a 1 mm × 15 mm diameter Spectrosil window. Other aspects of the cell design and the detector electronics were similar to those described previously[1].

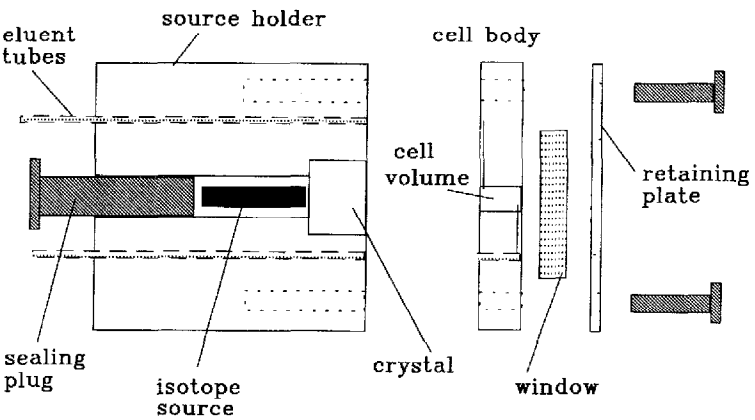

Fig. 2. The design of the detector flow cell based on a standard 10-mCi "point source" of ^{90}Sr.

The photons leaving the cell window were passed through a broad band interference filter, having a maximum transmission of 35% at 215 nm and a bandwidth of 36 nm [Oriel Scientific, type 2.0147; a similar 254-nm interference filter was also used in one case (see results)] and detected using a bialkali photocathode, quartz window photomultiplier (Hamamatsu type R292).

The chromatographic separations were carried out using a Waters Z-module C_{18} cartridge, 100 mm × 5 mm I.D. and packed with 10-μm packings. Chromatograms were recorded by monitoring the detected light intensity as the photomultiplier's anode current using a computer data collection system, and plotting the logarithm of the ratio of intensity detected to (a fixed) maximum intensity on a graphics plotter.

The eluents used were made from distilled water which had been purified to a conductivity of 18 MΩ cm^{-1} using a Water-1 unit (Jencons), and spectroscopic purity acetonitrile (Rathburn). The samples used were all standard laboratory reagent grade and were used without further purification.

RESULTS AND DISCUSSION

One of the potential advantages of the prototype detector over conventional UV absorbance detectors is that it is ready for operation as soon as it is switched on. Fig. 3 shows the detector output recorded directly on a chart recorder for a period of 30 min after switching on [with acetonitrile–water (3:1, v/v) passing through the flow cell at approximately 2 ml min^{-1}]. The rapid fluctuation when power is applied is a consequence of the power supplies, particularly the tube bias, requiring some seconds to reach their stable operating conditions. Also shown in Fig. 3 (broken line) is the analogous signal recorded from our ChiraTech Scientific Instruments absorbance monitor UV-106A1 (cadmium lamp, 214 nm filter) under the same conditions. It can be seen that the conventional lamp source takes a much longer period to stabilise. The prototype detector has been in use for more than a year and there is to date no indication of any change in its operating characteristics.

Several example chromatograms are shown in Figs. 4 and 5 to demonstrate the range of application of the detector system. The chromatograms shown in Fig.

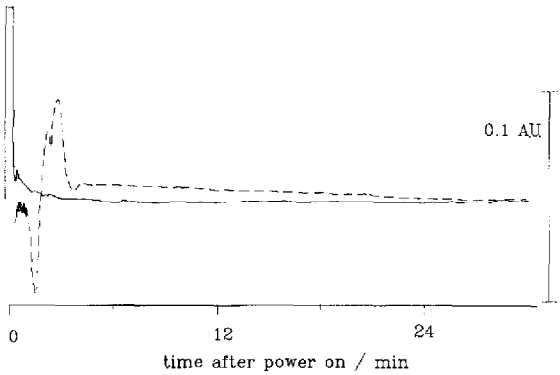

Fig. 3. Detector output variation with time immediately after switch on. The broken line shows the analogous signal recorded form a high-performance commercial detector.

4 use the same components as reported in our earlier paper and are provided to demonstrate the improvement in sensitivity achieved with the prototype system. The chromatograms shown in Fig. 4 were recorded for 20-μl samples eluted from a 10-μm C_{18} column using eluents at flow-rates of approximately 2 ml min^{-1}, and a detector filter centred on 215 nm. Fig. 4A shows a chromatogram recorded from naphthalene (14.3 ng), acenaphthene (11.0 ng), anthracene (14.0 ng) and pyrene (18.3 ng), eluted with acetonitrile–water (75:25). Fig. 4B shows the eluent of four phthalate esters, dimethyl (51.4 ng), diethyl (51.4 ng), dipropyl (103 ng) and dibutyl phthalate

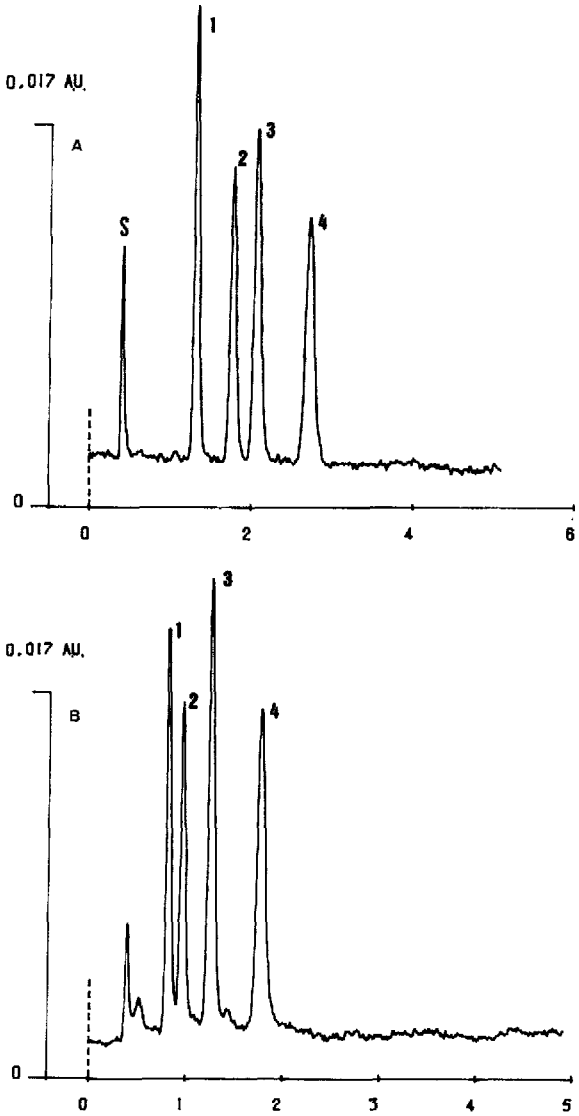

Fig. 4.

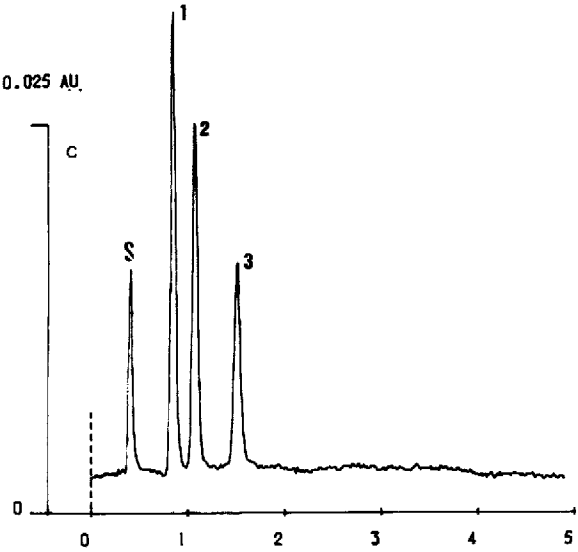

Fig. 4. Chromatograms recorded for samples used in earlier tests of the experimental system. In general the sensitivity improvement is approximately 5-fold. See text for chromatographic details. Time-scale in min. (A) Naphthalene (1); acenaphthene (2); anthracene (3); pyrene (4); S = solvent. (B) Dimethyl (1); diethyl (2); dipropyl (3); dibutyl (4) phthalate ester. (C) Naphthol (1); carbazole (2); biphenyl (3).

ester (103 ng), while Fig. 4C shows naphthol (18.2 ng), carbazole (14.2 ng) and biphenyl (24.2 ng), in both case using the same eluent as in Fig. 4A. The improvement in sensitivity over the chromatograms recorded earlier is approximately 5-fold.

Fig. 5A shows the separation of acetophenone (31.2 ng), acetonaphthone (17.6 ng) and benzophenone using an eluent of acetonitrile–water (50:50) and monitored at 254 nm. Fig. 6B shows the elution of four *p*-hydroxybenzoic acids (HBA), HBA (44.6 ng), ethyl-HBA (46.1 ng), propyl-HBA (42.9 ng) and butyl-HBA (58.1 ng), eluted with a 1:1 mixture of acetonitrile and water made pH 2 by addition of orthophosphoric acid and monitored at 215 nm. Fig. 5C shows the separation of sulphanilic acid (42.2 ng), sulphanilamide (36.5 ng), sulphadiazine (36.4 ng) and sulphamerazine (43.8 ng), using an eluent of acetonitrile–aqueous 50 m*M* potassium dihydrogenphosphate (25:75), also monitored at 215 nm.

It should be noted that all the chromatograms shown above were recorded without any need to change the position of the baseline, even though the chromatograms were obtained weeks apart in time. The only time any baseline adjustment is required for the direct chart-recorder output is when an eluent is replaced by one with a different transmittance at the monitored wavelength. (The computer system does not require baseline adjustment for different eluents.)

The results presented indicate that the prototype detector is capable of responding to a range of materials which have absorbance in the range 200–260 nm. The linearity of response with sample mass injected has been recorded for a number of the compounds used above. In each case the response was linear within experi-

mental error down to the limit of detection and up to several microgrammes. The limit of detection, for the systems we have tested vary over the range 0.5–5 ng, which represents a considerable improvement over the experimental system described earlier, although we continue to seek for further improvements.

In conclusions we believe that the high stability light source detectors offer numerous advantages over concentional lamp sources, including zero-power requirements, negligible thermal and photodecomposition effects, no warm-up time and excellent long-term stability.

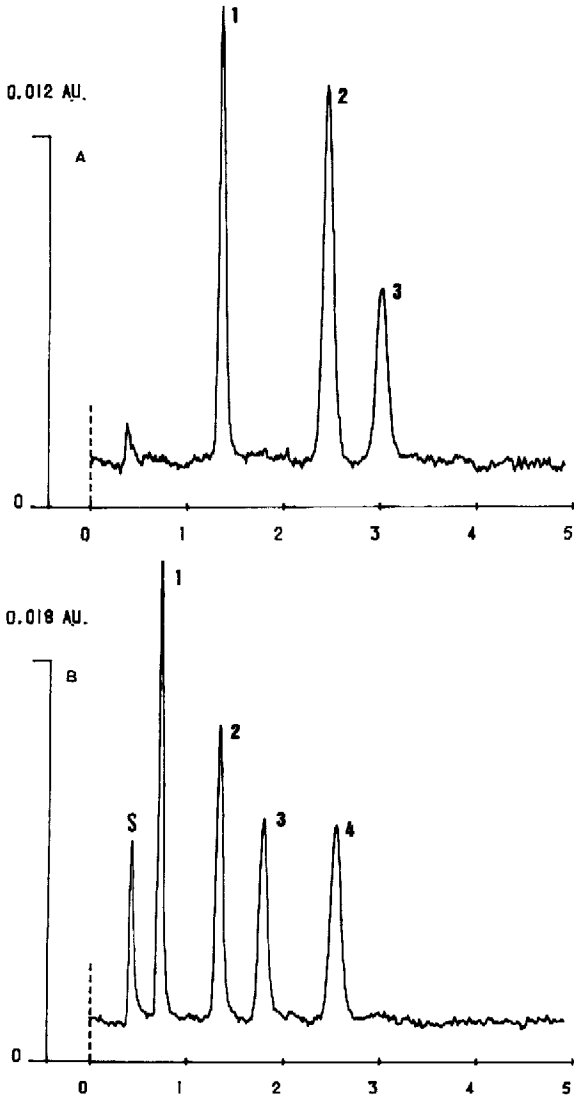

Fig. 5.

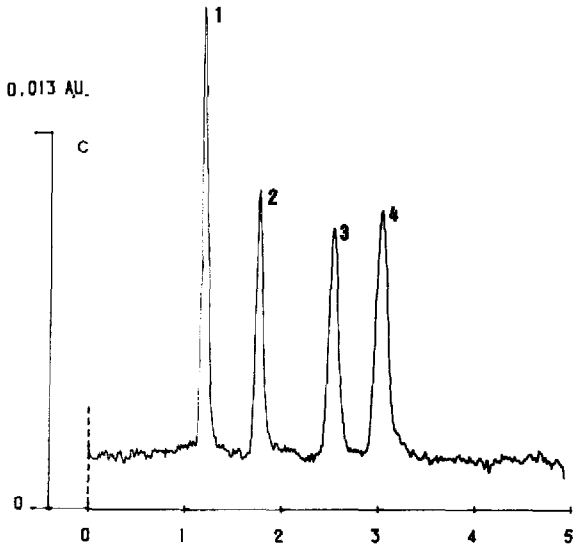

Fig. 5. Examples of chromatograms recorded with the prototype system. Time-scale in min. (A) Acetophenone (1); acetonaphthone (2); benzophenone (3). (B) HBA (1); ethyl-HBA (2); propyl-HBA (3); butyl-HBA (4); S = Solvent. (C) Sulphanilic acid (1); sulphanilamide (2); sulphadiazine (3); sulphamerazine (4).

ACKNOWLEDGEMENTS

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