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HIGH-PERFORMANCE ULTRAVIOLET ABSORPTION DETECTOR FOR LIQUID CHROMATOGRAPHY

I. PRELIMINARY EXPERIMENTS

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SUMMARY

A novel UV absorption detector for high-performance liquid chromatography is described. The system is based on the UV luminescence of crystals stimulated by energetic electrons, and has the interesting property of exceptionally low noise levels, allowing single-beam operation. A simple experimental flow cell is described and a number of example chromatograms recorded at 215 and 254 nm are presented.

INTRODUCTION

High-performance liquid chromatography (HPLC) 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. Over the last decade the reliability of pumping systems, the efficiency of columns and the sensitivity of detectors has improved considerably, and the wide range of detection systems now available is capable of dealing with the majority of application requirements. However, it is still the case that the most popular HPLC detector is the UV absorption detector[1], operated either with a mercury lamp (for monitoring at wavelengths above 254 nm) or a deuterium lamp (for monitoring above 190 nm). Other detector are generally required because of the limited sensitivity of UV absorption detectors, particularly at the shorter wavelengths.

Conventional lamp sources provide a light output which (perhaps after wavelength selection) may be directed through a flow cell containing the chromatographic eluent absorbance is being monitored. Light leaving the cell is monitored by a suitable detector, which converts the light level into a suitable electrical signal. The limitation on the sensitivity of the detector is determined by the smallest change in this detected light level which can be measured above the noise level in the final electrical signal. Noise in the final signal arise from three sources: fluctuations in the lamp's light output which actually reaches the flow cell, fluctuations in the optical characteristics of the flow cell (apart from absorbance due to the sample), and electronic noise in the light detection system. In a modern high quality instrument the latter two of

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these sources of noise are very small compared with the first, the lamp output level fluctuating by somewhere in the region of 0.1–1% in the time periods acceptable for HPLC (typically 0.5 sec).

The normal solution to this problem is to operate in a dual-beam mode, essentially allowing the output intensity of the lamp to be monitored as well as the intensity transmitted through the flow cell. In practice there are limits to what can be achieved by this technique. For example, monitoring the lamp output generally requires detecting the light output from a different part of the lamp bulb from that producing the light which passes through the flow cell. As the light follows two different pathways an additional source of noise is introduced, although hopefully one considerably smaller than the absolute fluctuation of light output. With techniques such as this, commercially available UV absorption detectors with recorded noise levels of the order of 0.0001 AU have become available. Even so this noise level is still heavily dominated by lamp-derived noise, and improvements of more than an order of magnitude should be possible before the other sources of noise form the limit to sensitivity.

In approaching this problem we have attempted to develop high-stability light sources which have both low short-term noise levels (*i.e.* small output fluctuations over the time scale required), and low drift (*i.e.* long-term changes in light output). In this paper we report some preliminary experiments on the use of one such light source in the fabrication of an experimental HPLC–UV absorption detector, and present some example chromatograms obtained by monitoring an eluents absorbance at 215 and 254 nm.

HIGH-STABILITY LIGHT SOURCE

To obtain a light source emitting in the region of 200–250 nm one needs to bring about an electronic excitation involving an energy change of 5–6 eV. In conventional lamps such excitations are brought about thermally, and the species involved are atoms (or ions) in the gas phase. However, many solid crystalline materials have band gaps[2] in the region of 6–10 eV, and electrons can be excited across these gaps by processes which do not involve the production of high temperatures (a major factor in the noise level of deuterium lamps is the temperature of operation). For example, *x*-ray or electron bombardment of (high purity) sodium chloride crystals can give rise to a substantial luminescence[3] centred on *ca.* 360 nm. A similar effect with thallium doped sodium iodide crystals has been used for many years as the basis of gamma and *x*-ray detector systems.

We have observed that irradiation of several crystalline oxides with energetic electrons provides a luminescence in the 200–280 nm region, and that the stability of the light output may be considerably greater than the stability of the exciting electron flux. The decay of a suitable radioisotope may be used to provide a source of energetic electrons of any desired noise level (*i.e.* short-term fluctuations in the rate of electron emission) and a precisely known drift. Indeed, these properties have be utilised in previous detection systems for gas and liquid chromatography[4,5].

When an energetic electron enters an insulating crystal it loses energy by electronic processes (promotion of electrons to the conduction band) and elastic processes[6]. Above about 0.5 MeV the rate of energy loss with penetration depth is vir-

tually independent of the electron's energy, and energy may be deposited fairly uniformly through relatively thick crystals. We needed an electron source which produced electrons with energies >0.5 MeV, and which had a half-life long enough for incorporation into a laboratory instrument, and yet short enough that a useful rate of electron emission could be obtained from a relatively small mass of material. These considerations led us to select ^{90}Sr as a useful electron source. It is readily available at low cost, has a maximum electron energy of 2 MeV, and a half life of 30 years.

The thickness of a crystal chosen for a light source will be determined primarily by the maximum range of the electrons in the crystalline material. The range of 1 MeV electrons in crystals depend on the nature of the crystal, but for the crystals of relevance here was between 1 and 2 mm.

For the experimental HPLC detector we chose normal spinel ($MgAl_2O_4$) as a suitable luminescent material, as this enabled us to encompass the 200–250 nm range without changing crystals. The luminescence spectrum of the spinel sample is shown in Fig. 1. (Details of the single photon counting spectrometer used to obtain this emission spectrum have been described[7].) It should be noted that a relatively high purity sample is required for a useable light output. Transition metal impurities above a few tens of ppm will degrade the UV luminescence seriously. The sample used in this work had metal impurities of <300 ppm (by plasma emission spectrometry). Each electron entering the crystal does so with an energy taken from the beta decay distribution and produces a large number of photons, a number which we assume is proportional to the energy of the electron on entry. The statistical fluctuations in the photon output are thus proportionately smaller than the statistical fluctuations in the electron emission rate (the latter being the square root of the number of electrons emitted per second[8]).

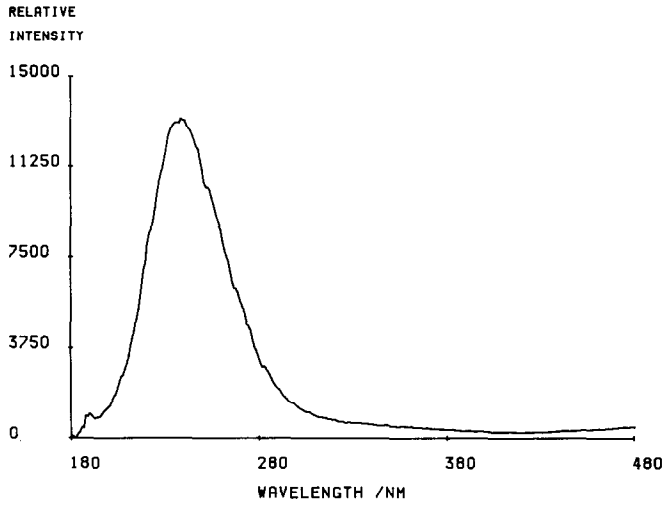

Fig. 1. The luminescence spectrum of normal spinel excited by 2 MeV electrons. Details: slit width, 0.2 mm; photomultiplier tube voltage, 700 V; time interval, 5 sec; error limit set at 0.5%. Readings were taken every 1 nm.

EXPERIMENTAL

The apparatus used in this work is shown schematically in Fig. 2. The chromatograph consisted of an Eldex dual piston 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. The small size of the light source meant that it could be incorporated into the flow cell, and this assembly is shown in Fig. 3. The rear of the flow cavity was closed by a 10-mm (dia) "window" of the spinel, approximately 1 mm thick. Immediately behind this was the ^{90}Sr source, a 10-mCi "point source" housed in a 10 × 2 mm diameter stainless-steel holder (Amersham, source type SIF33). The front of the flow cavity was closed by a 1 × 15 mm diameter spectrosil window. The flow cavity itself measured 5 × 2 mm diameter, giving a cell volume of approximately 15 μl. Other aspects for the Cerenkov photon absorption detector described previously[5].

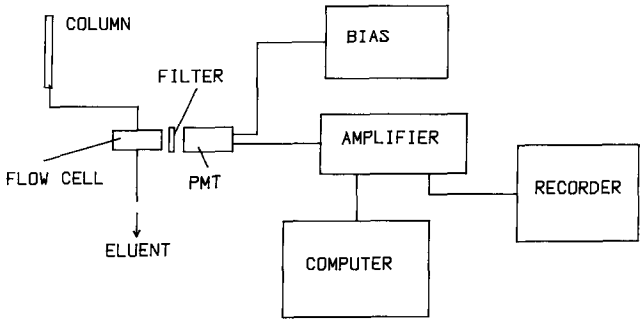

Fig. 2. Schematic diagram of the apparatus used to record chromatograms. PMT = Photomultiplier tube.

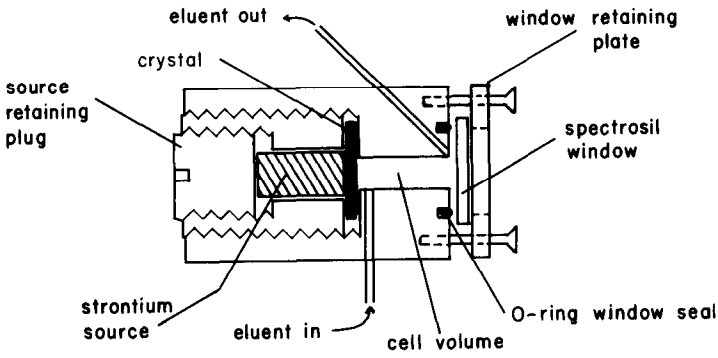

Fig. 3. The design of the detector flow cell based on a standard 10-mCi "point source" of ^{90}Sr.

The photons leaving the cell window were passed through a broad band interference filter, having a maximum transmission of 30% 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 discussion and detected using a bialkali photocathode, quartz window photomultiplier (EMI, type 9789QB).

The chromatographic separations were carried out using Waters Z-module cartridges of C_8 and NH_2, each being 8-mm I.D. and packed with 10-μm packings. These are not particularly suitable for showing off a detector by producing dramatic chromatograms, but they are capable of performing reliably over long periods of detector development.

The eluents used were made from: (a) water, distilled, then purified to a conductivity of 18 Ω^{-1} cm^{-1} using a Water-1 unit (Jencons); (b) methanol, HPLC-grade (Fisons); (c) acetonitrile, spectroscopic purity (Rathburn Chemicals).

The samples used were all standard laboratory reagent grade and were used without further purification.

RESULTS AND DISCUSSION

For a sample component of concentration x and mean molar absorbance a, the Beer-Lambert law predicts

$$I = I_0\exp(-ax)$$

where I_0 is the detected intensity in the absence of any light absorbing species in the flow cell. The system's output voltage signal, V, is proportional to the detected light intensity, *i.e.*

$$V = \mathrm{k}I$$

where k is a constant of proportionality; hence the change in output voltage during a component's recorded peak elution is

$$(V_\mathrm{b} - V_\mathrm{p}) = \mathrm{k}(I_\mathrm{b} - I_\mathrm{p}) = \mathrm{k}'I_\mathrm{b}[1 - \exp(-ax)]$$

where the subscripts b and p refer to values observed for the baseline and peak respectively. It follows that

$$\log(V_\mathrm{b}/V_\mathrm{p}) = \log(V_\mathrm{b}) - \log(V_\mathrm{p}) = ax$$

and a chromatographic recording of $\log(V_\mathrm{p})$ *versus* time should result in chromatograms with peak heights which are linear functions of the component concentration, providing that Beer-Lambert type absorption is occurring.

For this reason the chromatograms presented below were recorded by monitoring the electronics' output signal by the computer, and converting these signals into logarithmic quantities before recording the log value on the chart recorder. Furthermore, as

$$\log(V_\mathrm{b}/V_\mathrm{p}) = \log(I_0/I)$$

the equivalent absorbance of the material in the flow cell could be calculated, and each chromatogram presented below is annotated with an equivalent absorbance range.

Several example chromatograms are shown in Fig. 4 to demonstrate the range of application of the detector system. In each case the sample components are named in the figure caption in the order observed for peak elution. The chromatograms were

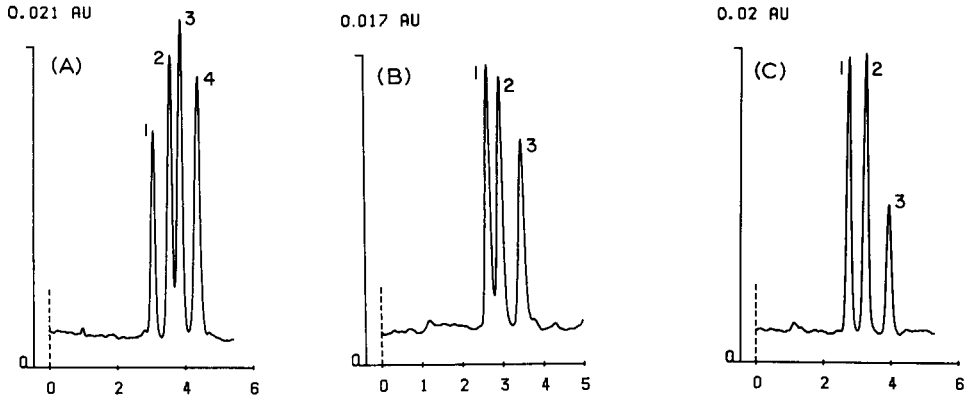

Fig. 4. Chromatograms recorded for samples eluted from a C_8 column with acetonitrile–water (75:25) eluent at a flow-rate of 1.7 ml min^{-1}. (A) naphthalene (1), 50 ng; acenaphthene (2), 76 ng; anthracene (3), 64 ng; pyrene (4), 97 ng. (B) dimethyl (1), 230 ng; diethyl (2), 260 ng; dipropyl (3), 230 ng phthalate esters. (C) naphthol (1), 63 ng; carbazole, (2) 58 ng; biphenyl (3), 69 ng. In each case the small peak at 1 min is a solvent peak.

recorded for 20-μl samples eluted from a 10-μm C_8 column using an eluent of acetonitrile–water (75:25) at a flow-rate of 1.7 ml min^{-1}, and the detector filter was centred on 215 nm. The variation of response with sample size has been examined for each of these systems. As the chromatograms are recorded on a linear scale proportional to absorbance, a plot of peak height (or area) *versus* sample quantity should result in a straight line for materials which obey the Beer-Lambert law. Fig. 5 shows a plot of recorded peak height against sample quantity for biphenyl samples, eluted as described for Fig. 4. Clearly the response is as expected for sample quantities between 1 and 5000 ng.

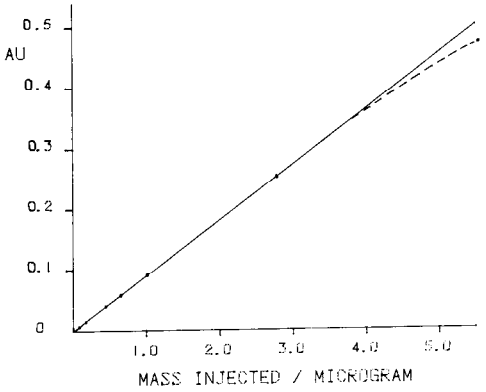

Fig. 5. The variation of the experimental peak heights recorded for biphenyl samples as a function of sample size.

The chromatogram shown in Fig. 6 shows the separation of cytidine (340 ng), guanosine (270 ng), thymidine (560 ng) and adenosine (710 ng), eluted from a C_8 column using an eluent of methanol–aqueous potasssium dihydrogen phosphate solution (0.05 *M*) (15:85) and monitored using a 254-nm interference filter.

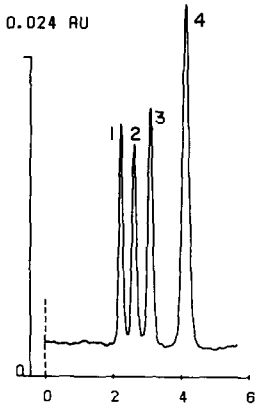

Fig. 6. Separation of cytidine (1), 370 ng; guanosine (2), 270 ng, thymidine (3), 560 ng; adenosine (4), 710 ng, on a C_8 column eluted with methanol–aqueous potassium dihydrogen phosphate (0.05 *M*) (15:85) at 2.6 ml min^{-1}.

The results presented indicate that the detector is capable of responding to a range of materials which have absorbances in the range 200–260 nm. The limit of detection (LOD) for the systems tested vary over the range 3–30 ng, so there is clearly still room for some improvement. One area in which improvement could be made is in the chromatographic system used to test the dfetector, and we anticipate that approximately one order of magnitude reduction in the limit of detection is likely to follow from the use of higher efficiency columns.

As was discussed in ref. 5, the limit of detection remains dependent on the shot noise present in the amplified photocasthode current of the photomultiplier tube, and is given by

$$\mathrm{LOD} = c\, o_I^{-1} - c'\,(e_1 e_2 p S)^{-1/2}$$

where the quantities are as defined previously. With the present system the source strength is 10 mCi, corresponding to $3.7 \cdot 10^8$ electrons per second (assuming only the energetic yttrium daughter electrons to be significant), p is of the order of 100 for the wavelength range 200–230 nm in the case of spinel, and e_2 is of the order of 0.1. The baseline (*i.e.* eluent only) signal level corresponds to a light intensity of approximately 10^6 photons per second, so that e_1 is of the order of 10^{-3}. It is clear that a relatively minor improvement in the absolute value of e_1 could result in a substantial advance in the LOD. For example, the use of a short path length cell (5 mm rather than 10 mm) improves the LOD by a factor of 4 as a direct result of increasing e_1 (and in spite of the reduction in path length for absorption). Improvements in sensitivity would certainly follow from the use of a higher source activity. Modifying the shape of the luminescing crystal and providing it with a reflective coating are just two other improvements currently under investigation.

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