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## CERENKOV PHOTON ABSORPTION DETECTOR FOR LIQUID CHROMATOGRAPHY

### I. PRELIMINARY EXPERIMENTS

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#### SUMMARY

An high-performance liquid chromatographic detector system based on the absorption of UV photons produced by the Cerenkov effect is discussed. A simple flow cell constructed around a standard commercial source of strontium-90 is described and a number of example chromatograms, recorded by monitoring the Cerenkov light intensity at 254 or 200 nm, are presented.

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#### INTRODUCTION

During the last decade there has been a dramatic increase in the use of high-performance liquid chromatography (HPLC) as an analytical technique. The technology associated with pumping systems, column packing materials and detectors has improved considerably over this period, and the last few years in particular have seen the birth of a number of ideas which have led to new principles being applied to each of these components of the chromatographic system<sup>1</sup>. In spite of this the most popular form of detection system has remained the UV absorption monitor, generally based on a mercury or deuterium lamp source. While not the most sensitive detection system for some compounds, the UV absorption detector combines the advantages of relative simplicity and good sensitivity for a large number of compounds. However the conventional UV absorption detector does have some disadvantages. For example, lamp sources are prone to both short-term and long-term intensity fluctuations, requiring dual-beam operation and expensive power supplies for stability at moderate sensitivities. Mercury lamps are generally only useful for detectors operating above 254 nm, and, although deuterium lamps are used in detectors which operate down to 190 nm, these lamps do tend to have unpredictable lifetimes.

In this paper we describe some preliminary experiments with a new type of absorption detector which overcomes the difficulties mentioned above, which can be

used over a wide range of wavelengths and which has no lamp to fail. The principle of operation of the detector is discussed in the section below, followed by a description of the experimental detection system constructed at Loughborough. Finally some examples of results obtained using the detection system are given for reversed-phase chromatography.

#### THE PHOTON SOURCE

In the present detector system photons are produced by the Cerenkov effect<sup>2</sup> from energetic electrons resulting from the decay of a radioisotope. The Cerenkov effect is based on the fact that when electrons travel through a medium with a velocity greater than the velocity of light in that medium, the electrons lose energy by generating photons (Cerenkov photons) until the electron velocity is lower than the velocity of light in that medium. The theory of the Cerenkov effect was developed on a classical basis by Frank and Tamm<sup>3</sup>, and has been reviewed by Robin<sup>4</sup>.

A radioactive material may be used to generate Cerenkov photons in a variety of ways. A  $\beta$  decay nuclide which emits electrons with an energy above the Cerenkov threshold (*ca.* 0.26 MeV in water, refractive index 1.33) may be used to generate Cerenkov photons by allowing the  $\beta$  particles to travel into a suitable transparent medium. Each  $\beta$  particle may give rise to a number of Cerenkov photons emitted in rapid succession and covering a range of wave-lengths (for example a 1 MeV  $\beta^-$  particle travelling through water will yield approximately 100 photons). An alternative technique is to use an energetic  $\alpha$  decay nuclide which gives rise to energetic electrons when the  $\alpha$  particles interact with matter. However, for the preliminary experiments reported below we have confined our attention to beta decay sources.

Any high-energy  $\beta$  decay radionuclide may be used for the production of Cerenkov photons, and the higher the  $\beta$  decay energy, the more photons are available. However, to be suitable for use in a liquid chromatography detector other criteria may be applied to the selection of the radioisotope. Firstly it is desirable that the nuclide chosen has a half-life of at least a year—so that frequent replacement of the source is not required—and yet be available at sufficiently high specific activity that a small volume source with a high  $\beta$  particle output may be produced. Secondly it is desirable that the  $\beta$  decay process of the source does not lead to any energetic  $\gamma$ -emitting daughter nuclides—as high energy  $\gamma$  radiation could give rise to problems of  $\gamma$ -induced fluorescence within the optical system and to radiation hazards for personnel operating the detector.

For our preliminary experiments we have chosen strontium-90 as the source of energetic electrons.  $^{90}\text{Sr}$  is a  $\beta^-$  emitting nuclide with a half-life of 28 years and a short-lived  $\beta^-$  emitting daughter ( $^{90}\text{Y}$ ) which decays to a stable nuclide ( $^{90}\text{Z}$ ).  $^{90}\text{Sr}$  is readily available at low cost and in a variety of forms from the Radiochemical Centre, Amersham, Great Britain. The source chosen in the present work was a 1 mCi "point source", mounted on a  $10 \times 2$  mm diameter cylindrical stainless-steel holder and covered by a  $50\text{-}\mu\text{m}$  stainless-steel window, which effectively protected the radioisotope from the solvents which passed over the window during some of the experiments. [The source was obtained from the Radiochemical Centre, Code Number SIF. 32].

The radioactive source was used to generate Cerenkov photons in a variety of

flow cells as described below. Photons which passed through the flow cell window were detected using a quartz window photomultiplier tube.

By placing interference filters between the flow-cell and the photomultiplier tube the Cerenkov photon flux at wavelengths of 210, 254 or 280 nm could be monitored, (bandwidth *ca.* 15 nm in each case).

#### THE EXPERIMENTAL SYSTEM

A schematic diagram of the experimental Cerenkov photon absorption (CPA) detector is shown in Fig. 1, and schematic detail of one of the flow cells is given in Fig. 2. The flow cell was machined from brass, having a  $10 \times 1.5$  mm diameter flow hole closed at one end by the face of the strontium source. The other end of the flow cell was closed by a 20 mm diameter  $\times$  2 mm Spectrosil A window. Inlet and outlet tubes of 0.01-in. bore stainless-steel were soldered into the brass body of the cell.

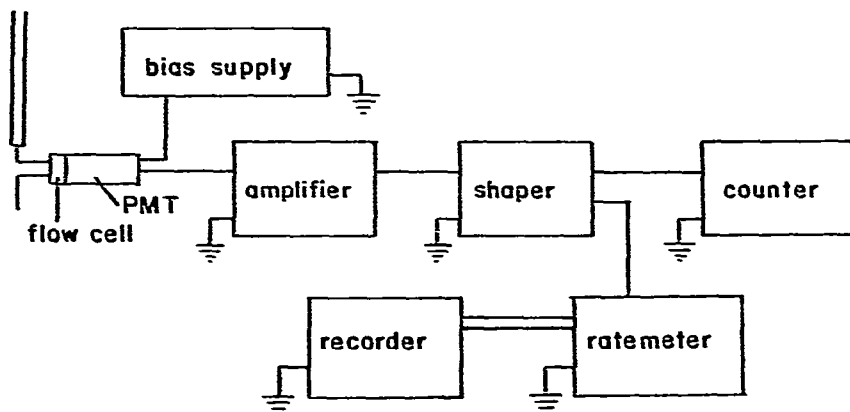


Fig. 1. Block diagram showing major components of CPA detector system. PMT = Photomultiplier tube.

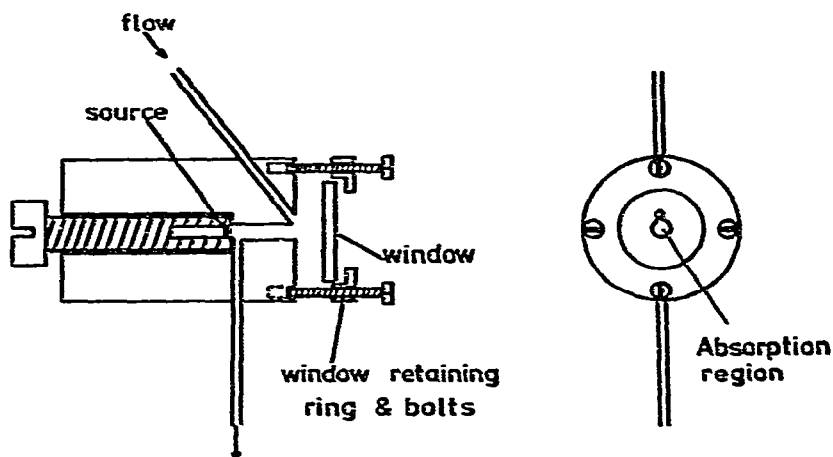


Fig. 2. Detail of CPA flow cell.

The interference filters used to select the wavelength monitored were supplied by Anachem (254-nm and 280-nm Altex filters) or loaned by A.C.S. (210-nm filter).

The quartz windowed photomultiplier tube (EMI type 9804QB) was operated with a grounded photocathode and an anode potential of 1400 V. An emitter follower preamplifier, built onto the tube base, was designed to allow pulses with half-widths of *ca.* 100 nsec to be passed to a fairly conventional nuclear pulse counting system<sup>5</sup> consisting of the following modules: Model 2110, timing filter amplifier; Model 1433, discriminator; Model 1776, scaler counter-timer; and Model 1481L, ratemeter; all obtained from Canberra Instruments. The ratemeter output signal (0–100 mV analogue) was passed through a low-pass filter to the chart recorder, and in most cases the filter was operated with a cut-off frequency of 0.2 Hz.

The technique of pulse counting is probably not the most desirable way of using the Cerenkov photon absorption detector, as many of the pulses detected correspond to multiphoton events. However the technique has been adopted for these preliminary experiments because of the low cost of obtaining precisely quantified data with apparatus already available in our laboratory<sup>5</sup>.

The experimental detector system was connected to the outlet of a conventional liquid chromatography system. Columns of Spherisorb ODS and Spherisorb-amino were used in lengths and particle sizes as described below. Eluting solvents of methanol, acetonitrile (Fisons, HPLC grade), acetic acid and distilled water were used. In most cases the elution rate was adjusted to be 1 ml min<sup>-1</sup>. Samples of a variety of materials were made up in solvents of methanol or water (as appropriate). As our purpose here is limited to demonstrating the detector operation, no specific effort was made to obtain especially purified materials; most of the compounds used were of standard laboratory reagent grade.

Samples were loaded onto the columns using a Rheodyne Model 7125 injection valve fitted with a 20- $\mu$ l injection loop. This loop could be partially filled using a syringe, so that where calibrations of detector response vs. sample loading are presented, the different sample loadings have been calculated from the volume of a single solution injected into the loop.

In operation the prototype CPA detector produces a chart record showing a signal proportional to the number of pulses per second detected by the photomultiplier system. This number is simultaneously displayed on a digital ratemeter.

When photon absorbing material passes through the flow cell the recorded rate is lowered, and the chart record shows a chromatogram of conventional appearance. Quantitative detector responses reported below are given in terms of the difference between the number of pulses per second detected when only eluting solvent is present in the flow cell and the minimum number of pulses per second detected as the absorbing component elutes. This somewhat unorthodox way of presenting the results was chosen for the same reason that pulses per second were used in the early stages of  $\beta$ -induced fluorescence development<sup>5</sup>, namely that this allows direct comparisons to be made between different radioactive sources and different flow cells without the risk of confusion arising through the variation of other instrumental parameters.

## RESULTS

A selection of test materials was passed through the chromatograph fitted with

TABLE I

## RESPONSES OF CPA DETECTOR WITH 254-nm FILTER TO TEST SAMPLES

Flow-rate *ca.* 1 ml min<sup>-1</sup>, solvent methanol.

Sample	Amount ( $\mu\text{g}$ )	Peak response ( $\text{sec}^{-1}$ )
Carbazole	1	4430
Ascorbic acid	2	1300
Salicylic acid	2	650
Indole	2	4900
Aniline	2	1750
9-Amino acridine	2	3350
Chlorobenzene	2	2100
<i>p</i> -Dichlorobenzene	2	2300
4-Chlorotoluene	2	2800

a 15 × 0.5 cm 5- $\mu\text{m}$  Spherisorb ODS column using methanol as solvent. When solvent alone is passing through the CPA detector the pulse count rate recorded through the 254-nm filter was *ca.* 8100 sec<sup>-1</sup>. The detector response obtained for each test material is given in Table I.

Further examples of detector operation were obtained by recording analogue chromatograms for a number of mixed samples. Fig. 3 shows the separation of

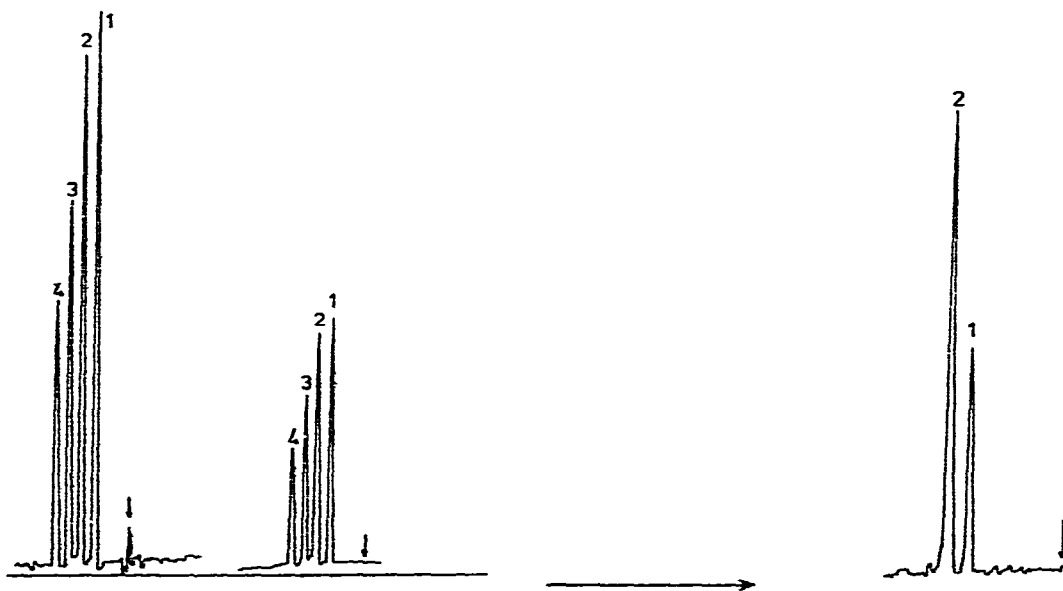


Fig. 3. CPA detection of 20  $\mu\text{g}$  each of four phthalates eluted from a Spherisorb ODS column using acetonitrile-water (60:40) eluent. Peaks: 1 = dimethyl phthalate; 2 = diethyl phthalate; 3 = diallyl phthalate; 4 = diisopropyl phthalate. Left-hand trace is gain  $\times$  2.

Fig. 4. CPA detection of Bendiocarb (1) and Carboryl (2) eluted from a Spherisorb ODS column using acetonitrile-water (35:65) eluent.

dimethyl-, diethyl-, diallyl-, and diisopropyl-phthalates (20  $\mu\text{g}$  of each) using a 25  $\times$  0.5 cm 10- $\mu\text{m}$  Spherisorb ODS column eluted with a acetonitrile-water (60:40) mixture.

Fig. 4 shows the separation of Bendiocarb (13.5  $\mu\text{g}$ ) and Carboryl (8  $\mu\text{g}$ ) using the same column eluted with acetonitrile-water (35:65) solvent.

Fig. 5 shows the separation of anthracene, naphthalene and biphenyl (2  $\mu\text{g}$  of each) using a 15  $\times$  0.5 cm 5- $\mu\text{m}$  Spherisorb ODS column eluted with methanol-water (80:20).

Fig. 6 shows the separation of vitamin A and vitamin D<sub>2</sub> (palmitates) using the 10- $\mu\text{m}$  Spherisorb ODS column eluted with methanol.

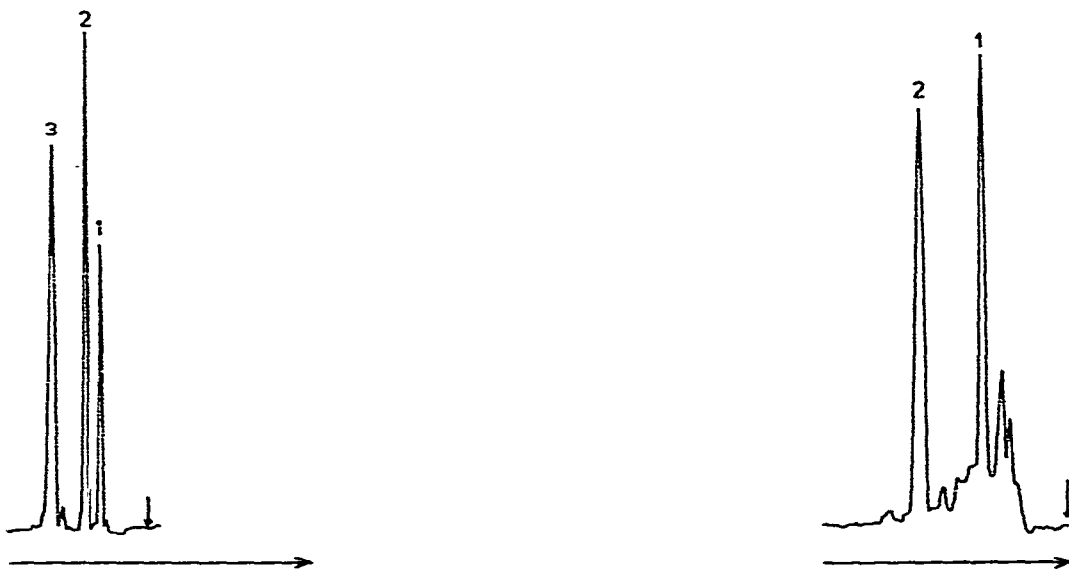


Fig. 5. CPA detection of naphthalene (1), biphenyl (2), and anthracene (3) eluted from a Spherisorb ODS column using methanol-water (80:20) eluent.

Fig. 6. CPA detection of vitamin D<sub>2</sub> palmitate (1) and vitamin A palmitate (2), eluted in methanol from a Spherisorb ODS column.

Fig. 7 shows the variation of detector response (as percentage absorption) with sample size for Carbazole at small loadings (up to 1  $\mu\text{g}$ ), using the 10- $\mu\text{m}$  Spherisorb ODS column eluted with methanol. The chromatographic peaks corresponding to each sample size are shown in Fig. 8.

A limited number of samples which do not absorb strongly at 254 nm were passed through the CPA detector fitted with a 210-nm filter. The detector responses are shown in Table II. In these cases the eluent was water and the photon count-rate recorded with water flowing through the detector was *ca.* 5600  $\text{sec}^{-1}$ . [Note that the 210-nm filter passed only 8% of incident 210 nm radiation, whereas the 254-nm filter passed 17% of the incident 254-nm radiation.]

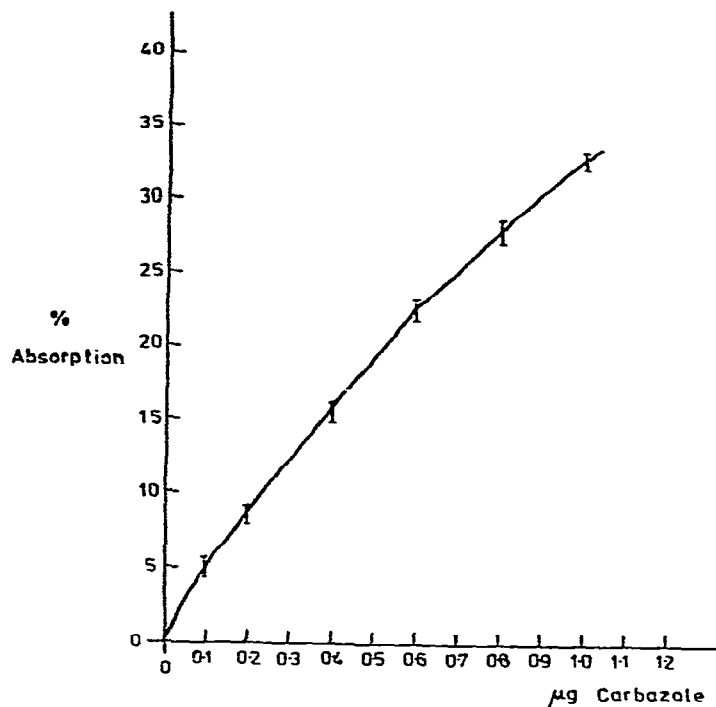


Fig. 7. Variation of CPA detector response (% absorption) with sample size for Carbazole eluted in methanol).

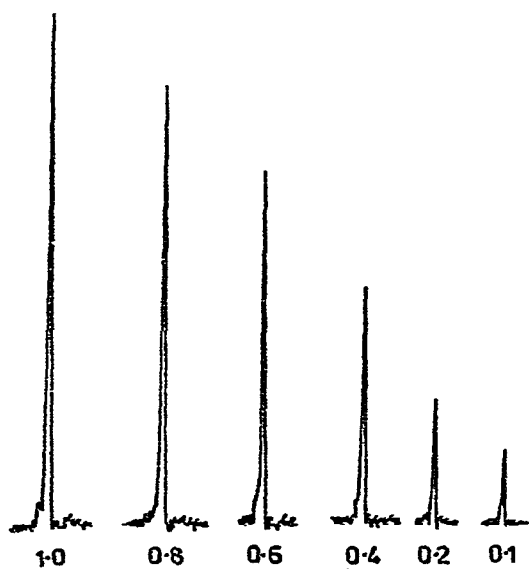


Fig. 8. Chromatographic peaks corresponding to the responses recorded in Fig. 7.

TABLE II  
RESPONSES OF CPA DETECTOR WITH 200-nm FILTER TO TEST SAMPLES

Sample	Amount ( $\mu\text{g}$ )	Peak response ( $\text{sec}^{-1}$ )
Potassium nitrate } }	2 0.2	4220 2600
Sodium nitrite } }	2 0.02	4430 300
Sodium bromide	2	3350

## DISCUSSION

The results presented above clearly demonstrate that the experimental CPA detector may be used for the detection of a variety of materials which absorb UV light—certainly between 210 and 254 nm. The radioactive source used in these experiments appeared to be secure, in that no radioactivity was detected in any of our collected eluents. The system therefore appears to offer an attractive alternative to lamp-operated UV absorption detectors. Clearly there are a number of areas in which improvements still have to be made before the system can be regarded as a practical alternative to conventional UV detectors.

The first of these is the linearity of response, which, as the results of Fig. 7 show, is currently rather poor. We believe that the main reason for the distorted response of the experimental system is the pulse-counting technique chosen for signal detection. Recording the number of photons detected (rather than the number of pulses) should allow the detector to follow Beers' law more precisely, and we are modifying the experimental system accordingly. The second major area where improvement is essential is sensitivity. We anticipate a significant improvement in sensitivity when the detector system has been modified to record photon numbers rather than pulse numbers. However a further improvement should also be obtained by simply using a radioactive source of greater activity, such as 10 mCi rather than the 1 mCi used in this work.

The advantages inherent in the CPA detector compared with conventional UV detectors will become particularly attractive as the improvements outlined above are introduced. For example, the radioactive source decays at a precisely known rate and produces photons without short- or medium-term intensity fluctuations. The system is operational as soon as the electronics are switched on—there is no "warm up" time—and the power requirements are sufficiently small that portable, battery-operated detectors could be readily produced. Cerenkov radiation covers a wide range of wavelengths, certainly from below 200 nm to the visible region, so that a variable-wavelength or variable-filter system has considerable potential as a relatively low-cost detector for a very wide variety of materials.

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