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CERENKOV PHOTON ABSORPTION DETECTOR FOR LIQUID CHROMATOGRAPHY

II. AN EXPERIMENTAL DETECTOR FOR 215 nm

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SUMMARY

An experimental high-performance liquid chromatographic detector based on the absorption of UV photons produced by the Cerenkov effect is described. The system has been used to detect polyaromatic hydrocarbons, phthalate esters, chlorinated pesticides, nitrate and nitrite in water, and a number of nucleosides by operation at 215 nm.

INTRODUCTION

In a previous paper[1] we described the results of some preliminary experiments in which the absorption of Cerenkov photons[2], produced by allowing energetic beta particles from a strontium-90 source to pass into a chromatographic eluent, could be used for detecting UV-absorbing species eluting from a chromatographic column. The sensitivity achieved was not high, the limit of detection being of the order of 100 ng for a highly absorbing species (carbazole at 254 nm). Furthermore, the linearity of response of the detector as a function of sample size was poor, even though a narrow-band 254 nm interference filter had been used to select the monitored wavelength.

We have now carried out an extensive investigation into ways of improving the Cerenkov Photon Absorption (CPA) detector, and report below on some results obtained with the improved system.

The Cerenkov light source

When energetic beta particles travel through a medium with a velocity greater than the velocity of light in that medium, one of the ways in which the beta particles lose energy is by the production of photons (the Cerenkov effect)[3,4]. The photons are emitted at an angle to the velocity vector of the beta particle, and this angle approaches zero as the beta particle's velocity, V_e, approaches the velocity of light in the medium, V_c. The number of photons emitted per unit path length of the beta

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particle's travel (dN/dl) is related to the difference ($V_e - V_c$), and the spectral distribution of the photons is given classically by[3,4]

$$d(dN/dl) = \frac{2\pi e^2(V_e^2 - V_c^2)}{hcw^2 V_c^2} \cdot dw$$

Consequently the number of photons produced per unit wavelength varies with the inverse of the square of the wavelength over spectral regions in which the refractive index of the medium ($n = c/V_c$) remains approximately constant. In practice most materials display a rapid increase in refractive index and absorb strongly at wavelengths below 200 nm, and the Cerenkov intensity in this spectral region falls sharply.

Fig. 1 shows a theoretical spectrum for a Cerenkov light source based on beta particles from strontium-90 (E_{max} approximately 2 MeV, from the yttrium daughter[5]) passing through water (with the refractive index assumed constant, at $n = 1.3$). The experimentally determined spectrum of Cerenkov radiation produced under these conditions is also shown, although the uncertainty in the monochromator–photomultiplier efficiency at short wavelength (< 250 nm) results in large error bars below 250 nm. However, the spectrum does confirm that the Cerenkov source is expected to have its highest intensity at the short wavelengths (200–250 nm) of considerable interest in high-performance liquid chromatography (HPLC). The short wavelength cut-off is probably an artefact resulting from the rapidly falling efficiency of the monochromator used to record the spectrum and the falling response of the photomultiplier detector, as the onset of rapid refractive index increases in the medium (water in the case of Fig. 1) is expected at somewhat shorter wavelengths. However, few HPLC solvents would be suitable for use at shorter wavelengths.

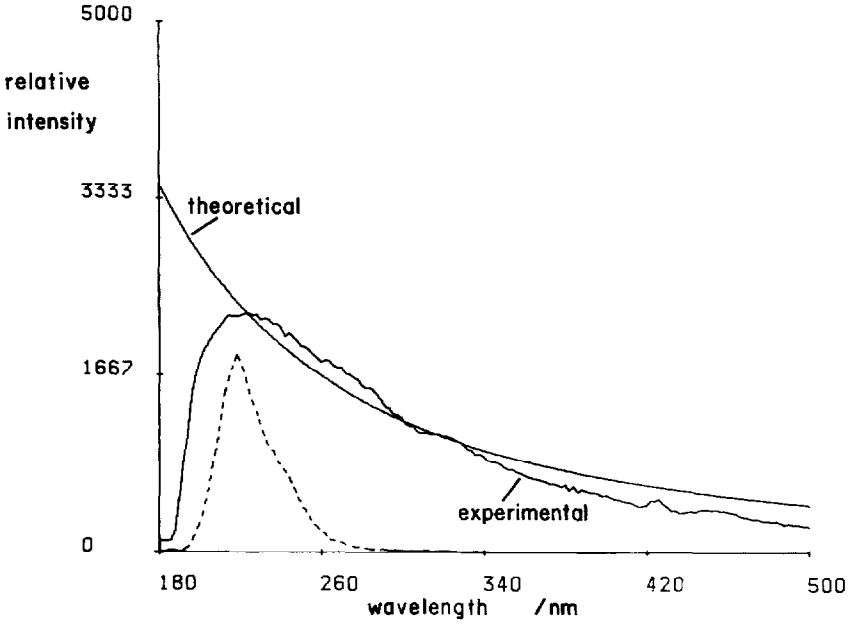

Fig. 1. Theoretical spectral distribution of Cerenkov light produced by 2 MeV beta particles in water and the experimentally recorded Cerenkov spectrum produced by beta particles from Strontium-90 (E_{max} = 2 MeV) passing into water. The broken line shows the spectrum recorded (× 3) from the chromatographic flow cell after passage through the 215 nm interference filter.

In designing the CPA detector described below our objective was to operate at a monitoring wavelength which fulfilled the following conditions: (1) an adequate number of Cerenkov photons would be available to allow intensity measurements with low statistical error; (2) common HPLC solvents could be used without extraordinary purification; and (3) suitable high transmission ($\approx 30\%$) interference filters would be available at reasonable cost. These conditions led us to select 210 nm as a suitable operating wavelength, although (apparently because of manufacturing variations) the filter we have used in this work actually has its peak transmission close to 215 nm. The spectral distribution of Cerenkov light from the strontium-90 source (see below) in water, after passage through our interference filter, is shown as the broken line in Fig. 1, and provides a good indication of the actual spectrum monitored by the CPA detector (the variation of the monochromator efficiency over this wavelength range was not accurately known). We have also recorded spectra using the source in acetonitrile–water (70:30), as this was the eluent most frequently used in our chromatographic testing. However, the differences between the spectra recorded in water and in acetonitrile–water mixtures are very small, showing only a small loss of intensity at short wavelengths in the mixture resulting from the absorbance of the acetonitrile below 210 nm. The acetonitrile used in the work was chosen for its high transmission at short wavelengths. It should be noted that many other samples of acetonitrile for HPLC have much lower transmissions below 210 nm, and would be expected to give rise to rather different sensitivities from those described below.

THE CPA DETECTOR

The CPA detector flow cell is shown in Fig. 2. The cell body was machined from brass and the inside of the cell polished to provide reasonable flow characteristics. The rear of the flow cavity was closed by the strontium-90 source, a 10 mCi point source housed in a 10 mm × 2 mm diameter stainless-steel holder (Amersham International Plc, source type SIF33). The front of the flow cavity was closed by a 1 mm × 15 mm diameter spectrosil window. The flow cavity itself measured 6 mm × 2 mm diameter, giving a cell volume of approximately 18 μl. The eluent inlet and outlet tubes were 1/16 in. O.D. stainless steel, with internal diameters of 0.008 and

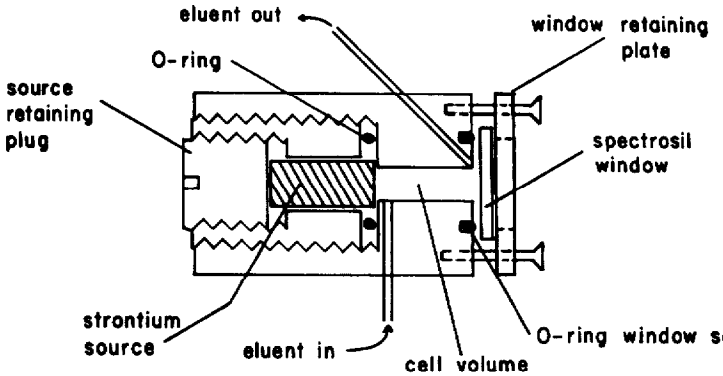

Fig. 2. The design of the CPA detector flow cell based on a standard 10 mCi point source of strontium-90.

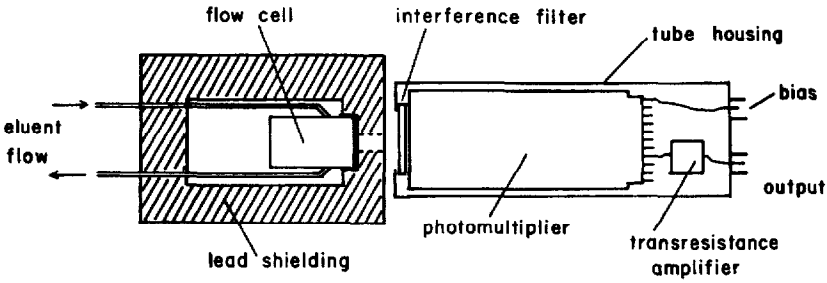

Fig. 3. The CPA detector assembly, showing the flow cell and its lead shielding, the interference filter, and the photomultiplier system and its housing.

0.020 in. respectively, and the entire assembly was housed in a lead pot with 1-in. walls to provide shielding from bremsstrahlung.

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) and detected using a bialkali photocathode. quartz window photomultiplier (EMI type 9789QB). The detector assembly is shown in Fig. 3.

The detecting electronics shown in Fig. 4 were quite straightforward, consisting of a virtually grounded transresistance amplifier connected directly to the photomultiplier tube's anode. Under normal operating conditions (tube voltage 800 V) the output of the transresistance amplifier was about 300 mV, corresponding to an anode current of approx. 1 μA. Unlike the electronic detection system described in our earlier paper, the present system monitors the integrated light intensity at the photocathode (the integration time being determined by the RC product of Fig. 4) rather than counting the number of Cerenkov flashes observed. Many of the Cerenkov radiation-producing events give rise to 50–100 photons within a few nanoseconds, so that even if 90% of these are absorbed within the flow cell, a flash is still recorded by the photomultiplier.

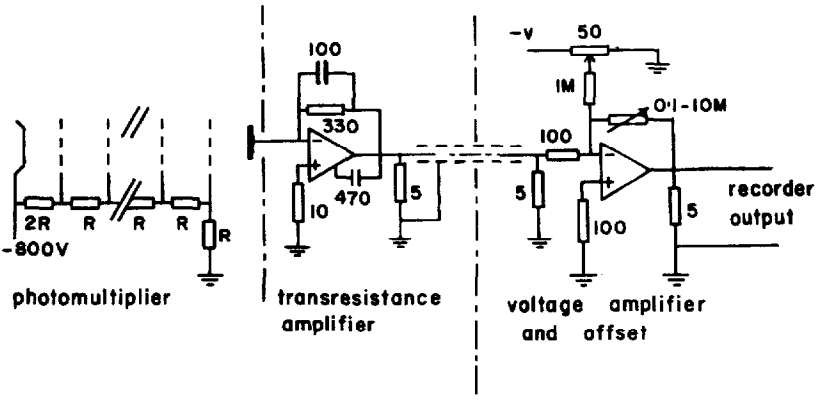

Fig. 4. The electronic system used to monitor the detected Cerenkov light intensity. All resistances in k and capacitances in nF unless otherwise stated. The transresistance amplifier is based on an LM725 and the voltage amplifier on a TL071.

THE CHROMATOGRAPHIC SYSTEM

The detector was connected to a chromatograph consisting 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. Cartridges of C_8 and NH_2 were used, each being 8 mm I.D. and packed with 10-μm packings. The eluents used were made from: (a) water: distilled, then purified to a conductivity of 18 M 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

Several example chromatograms are shown below to demonstrate the range of application of the CPA detector system. In each case the sample components are named in the order observed for peak elution. Fig. 5 shows four chromatograms recorded for 20-μl samples eluted from a 10-μm C_8 column using acetonitrile–water (75:25) at a flow-rate of 1.7 ml min^{-1}. In Fig. 5a the sample consisted of naphthalene (340 ng), acenaphthene (470 ng), anthracene (680 ng) and pyrene (620 ng). Fig. 5b shows the response obtained from three organochlorine pesticides, methoxychlor (1.3 μg), *p,p'*-DDT (2.7 μg) and *p,p*-DDE (1.6 μg), Fig. 5c that from a mixture of phthalate esters, dimethyl (0.96 μg), diethyl (1.2 μg) and dipropyl (1.5 μg), and Fig. 5d that from naphthol (340 ng), carbazole (470 ng) and biphenyl (420 ng).

The variation of response with sample size has been examined for several of the systems. Because our present experimental arrangement is based on linear amplifiers, it is not expected that the peak heights or areas should be directly proportional to the sample quantity. 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.*

$$V = kI$$

hence the height of the component's recorded peak is

$$(V_b - V_p) = k(I_b - I_p) = k'I_b[1 - \exp(-ax)]$$

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

$$\log(V_b/V_p) = ax$$

and a plot of this function should therefore result in a straight line for components

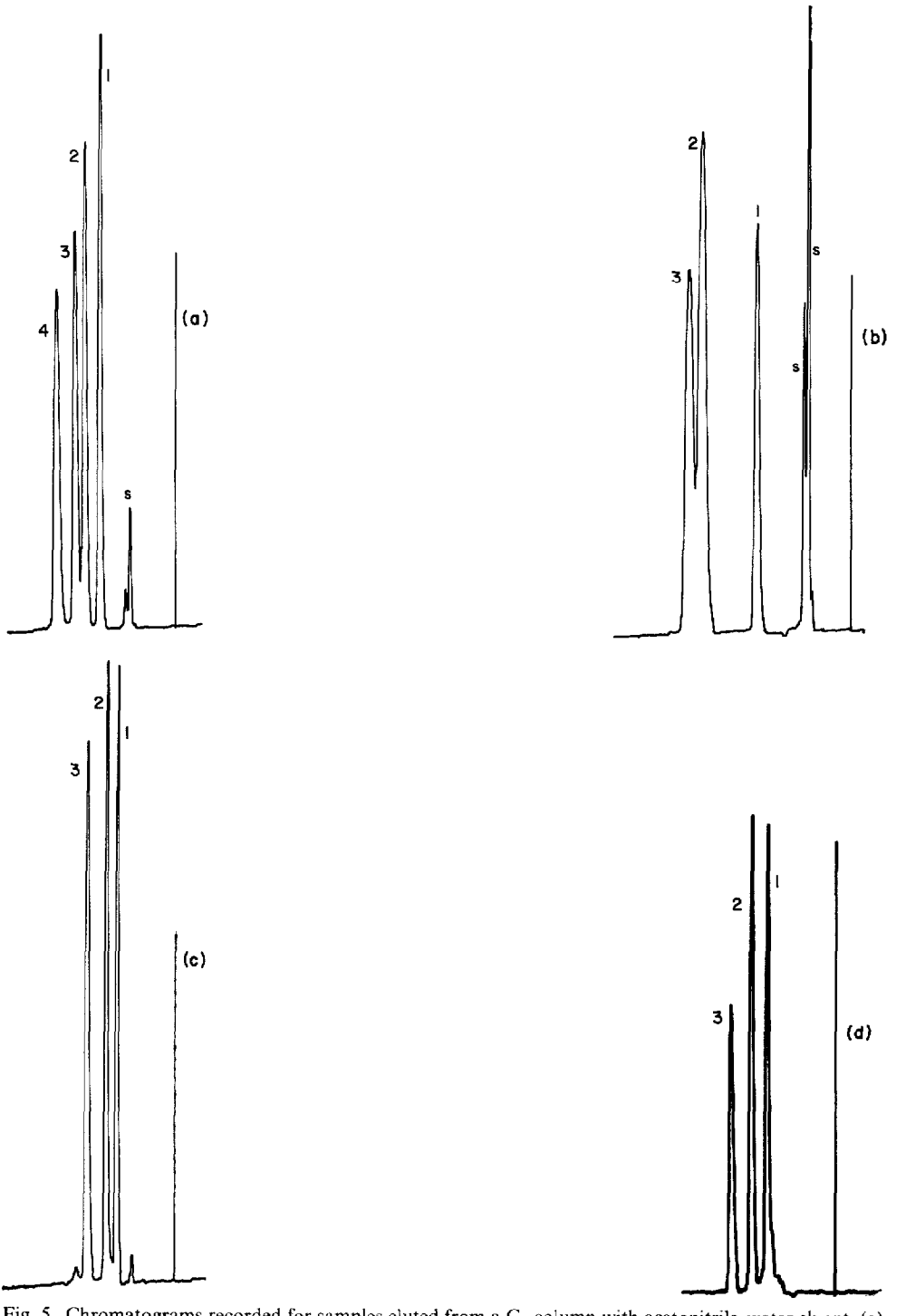

Fig. 5. Chromatograms recorded for samples eluted from a C_8 column with acetonitrile–water eluent. (a) Naphthalene (1), acenaphthene (2), anthracene (3) and pyrene (4). (b) Methoxychlor (1), *p,p'*-DDT (2) and *p,p'*-DDE (3). (c) Dimethyl (1), diethyl (2) and dipropyl phthalate (3) esters. (d) Naphthol (1), carbazole (2) and biphenyl (3). In each case peaks marked "s" are solvent peaks.

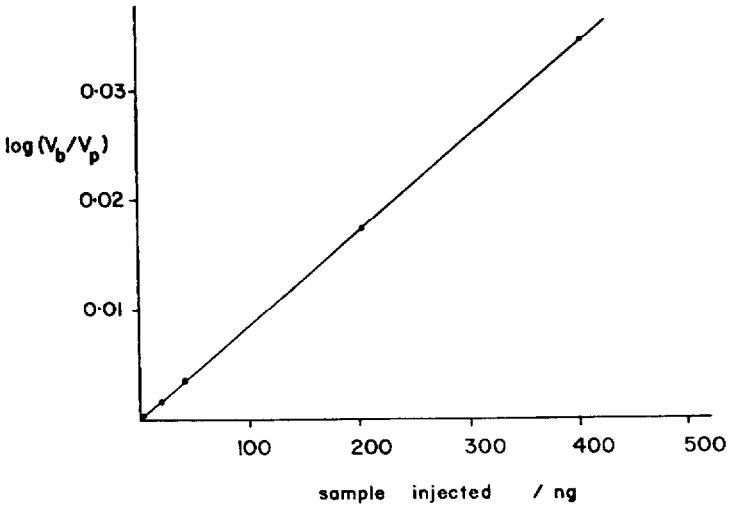

Fig. 6. The variation of the experimental peak heights recorded for biphenyl samples as a function of sample size. The use of base 10 logarithms ensures that the function plotted is directly comparable with absorbance in a.u.

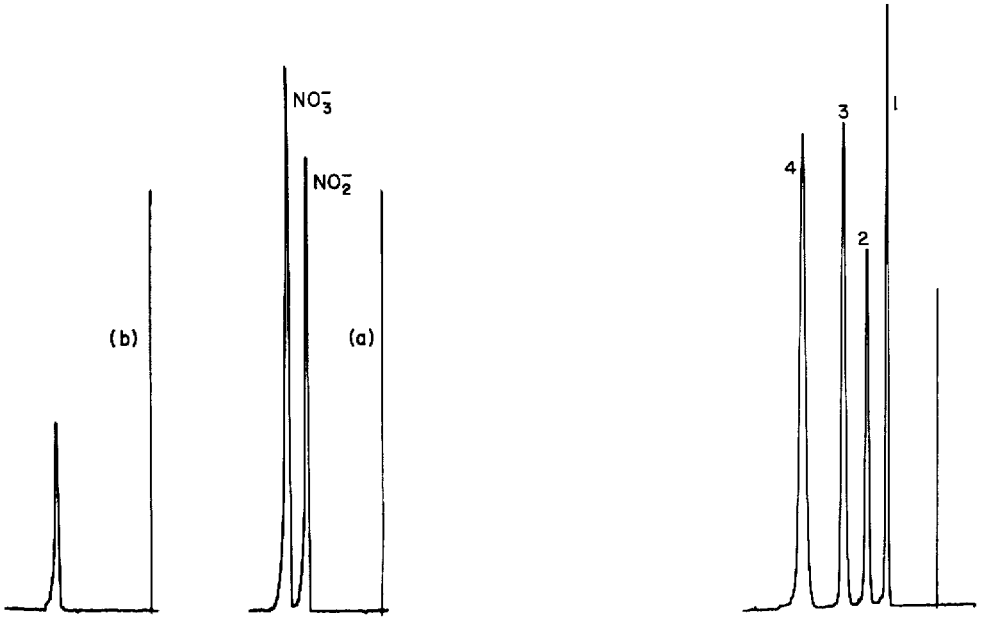

Fig. 7. (a) Separation of nitrite and nitrate ions on an NH_2 column eluted with 0.05 *M* KH_2PO_4 made pH 3.2 with H_3PO_4. (b) A 20-μl sample of tap water, showing a peak corresponding to approximately 37 ppm NO_3^-.

Fig. 8. Separation of cytidine (1), guanosine (2), thymidine (3) and adenosine (4) on a C_8 column eluted with methanol–aqueous phosphate buffer.

providing Beer-Lambert type absorption. Fig. 6 shows a plot of $\log(V_b/V_p)$ against sample quantity for biphenyl samples, eluted as described for Fig. 5. Clearly the response is as expected for sample quantities between 1 and 1000 ng.

Two additional chromatograms serve to illustrate the use of the CPA detector for chromatographic systems in which buffered eluents are present. Fig. 7a shows the separation of nitrite (3.2 μg, as sodium nitrite) and nitrate (2.1 μg, as sodium nitrate) eluted from a NH_2 column using an eluent of phosphate buffer (0.05 *M*, pH 3.2) at a flow-rate of 2.6 ml min^{-1}. For comparison, Fig. 7b shows the result of chromatographing a 20-μl sample of tap water, giving a nitrate peak corresponding to a level of *ca.* 37 ppm. Fig. 8 shows the separation of cytidine (1.4 μg), guanosine (1.5 μg), thymidine (2.6 μg) and adenosine (2.5 μg), eluted from a C_8 column using an eluent of 15% methanol, 85% aqueous KH_2PO_4 solution (0.05 *M*).

The results presented indicate that the CPA detector is capable of responding to a wide range of materials which have absorbances in the range 200–300 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 detector, and we anticipate that approximately one order of magnitude reduction in the limit of detection is likely to follow from the use of better-quality columns.

However, the limit of detection remains dependent on the shot noise present in the amplified photocathode current of the photomultiplier tube. At these low light levels the noise can be taken as proportional to the o_I, the variance in the detected light level expressed in photons per second. (That other noise sources are negligible has been confirmed by placing a single sheet of black paper between the interference filter and the photomultiplier tube.) The limit of detection for a specified sample under specified chromatographic conditions may be taken as

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

where c and c' are constants, e_1 is the efficiency of light utilisation within the cell (*i.e.* the fraction of theoretically produceable photons which actually emerge from a cell filled with eluent only), e_2 is the efficiency of the light detection system (*i.e.* the interference filter and photocathode), p is the number of photons (within the required wavelength range which can be generated per beta decay) and S is the strength of the beta decay source (S is, of course, a function of the nature of the source, but we will assume that only a point source of strontium-90 is under consideration).

With the present system the source strength is 10 mCi, corresponding to $3.7 \cdot 10^8$ beta particles per sec (assuming only the energetic yttrium decay particles to be significant), p is of the order of 30 for the wavelength range 200–230 nm, and e_2 is of the order of 0.1. The baseline (*i.e.* eluent only) signal level corresponds to a light intensity of approx. 10^6 photons per sec, 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 (6 mm rather than 10 mm) improves the LOD by a factor of 3 as a direct result of increasing e_1 (and in spite of the reduction in path length for absorption). We are continuing to investigate ways in which such improvements could be made.

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