Applications of ionizing radiation to detection in liquid chromatography

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APPLICATIONS OF IONIZING RADIATION
TO DETECTION IN LIQUID CHROMATOGRAPHY

by

PETER WARWICK, B.A., M.Sc.

A Doctoral Thesis
submitted in partial fulfilment of the requirements
for the award of Doctor of Philosophy of the
Loughborough University of Technology
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Department of Chemistry

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The written word is not powerful enough to express my gratitude to my wife, Margaret, daughter Samantha, and son Andrew, who have endured, patiently, my struggle to remain sane.
This work is dedicated to my father, who
died in April 1981 - the nicest, kindest
man I have ever known.
The author certifies that the work contained in this thesis is his original work, and that it has not been submitted, in full, or in part, to this or any other institution for a higher award.
ABSTRACT

Beta-induced fluorescence (BIF) is the luminescence excited from a compound as a result of the passage of beta-particle radiation through a compound or dilute solution of a compound.

A liquid chromatographic detector, based on the principle of BIF, has been developed which allowed the detection and quantitation of fluorescent materials. A number of flow cells, incorporating a promethium-147 beta particle emitter, were designed and developed with the objective of attaining maximum sensitivity from the detection technique. The response of the detector to eluted materials, the linearity of the detector response with sample loading and the sensitivity of the detector were examined in normal and reversed phase liquid chromatography.

During the development of the BIF detector it became evident that the compounds detected need not be inherently fluorescent. The range of compounds detected could be increased to include those which quench the beta-induced fluorescent emission from the mobile phase. The technique of quenched beta-induced fluorescence was investigated as a detection technique and the response, linearity of response and sensitivity to eluted materials examined.

Cerenkov photons are generated whenever a charged particle, travelling at a velocity greater than the velocity of light in the medium, passes through a transparent medium. Cerenkov photons are emitted from the medium as a continuum of wavelength range between 180 nm and 600 nm. The principle of absorption of Cerenkov photons was investigated as a detection technique in liquid chromatography. Strontium-90 was incorporated into a number of flow cells and the response, linearity of response and sensitivity of eluted materials, examined in normal and reversed phase liquid chromatography.
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CHAPTER 1.

INTRODUCTION

1.1 HIGH PRESSURE LIQUID CHROMATOGRAPHY

Modern liquid chromatography first came into prominence at the Fifth International Symposium on Advances in Chromatography held in Los Vegas in 1969. However, the impetus for this prominence came from workers who were experienced in gas chromatography, and the theoretical ideas which led to its realisation arose mainly from Giddings (1) who in 1964 gave a clear account of the dynamics of chromatography and showed how liquid chromatography could be upgraded so as to have speed, resolution and simplicity of operation associated at that time only with gas chromatography. Prior to this Martin and Synge (2), in 1941, stated that the highest performance in liquid chromatography would be achieved by the use of "very small particles and a high pressure difference across the length of the column". Experimental realisation of this prediction was achieved firstly by Lipsky (3) in 1967 and further developed by Kirkland (4) in 1969 and Huber (5) in 1969, using pressures of up to 3000 psi, columns about one metre in length, particles of approximately 40 x 10^-6 metres diameter and achieving analysis times of a few minutes.

From these beginnings, liquid chromatography has developed into one of the most powerful techniques for the separation, identification and quantification of components within a mixture. Several factors have enabled this technique to achieve such a prominent status, enabling it to challenge gas chromatography (G.C.) in the analytical field. Firstly, in G.C. it is necessary to volatilise the components of the mixture to be analysed prior to analysis. Thus labile molecules cannot be analysed by G.C. No such constraint is imposed on liquid chromatography since the only requirement is
that the components should be soluble in a suitable solvent. Secondly, in G.C., the mobile phase plays little part in the separation of the components. In L.C., the use of a wide variety of solvents of widely different polarities results in another parameter which may be changed so that a successful separation may be achieved.

Gas chromatography has always been a fast analytical technique. Analysis time of a few minutes is common. In contrast, liquid chromatography has been a slow technique taking hours or days to run a sample. The major retarding factor in the development of liquid chromatography as a technique was the non-availability of sensitive, low dead volume, universal detectors. Without highly sensitive detectors, workers have had to use large diameter columns. The larger the diameter of the column, the longer the analysis time and consequently, liquid chromatography in the past has been used primarily for preparative work. Its capability for analysis had not been utilised, with the exception of amino acid analysers.

Since 1969, high sensitivity, low dead-volume detectors have been available resulting in the development of liquid chromatography as an analytical technique. Before surveying the design and performance of detectors used in liquid chromatography, it is appropriate to briefly describe the apparatus used in modern liquid chromatography.
1.2 Apparatus for H.P.L.C.

The solvent to be used as the mobile phase is first degassed and passed to a high pressure pump via a filter. A pressure gauge fitted in line after the pump measures the column inlet pressure. The mobile phase then enters a precolumn (which is often dispensed with if one is attempting adsorption chromatography or is using a bonded liquid stationary phase column), passes through an injection unit and thence to the main column. The detector, with associated electronics and recorder, follows the main column immediately, the objective being to reduce dead-volume to a minimum.

Fig. 1.1 Apparatus used for H.P.L.C.
1.3 H.P.L.C. Detectors

A chromatographic detector is a device which produces an electrical signal proportional to the concentration or weight of solute in the column effluent.

1.4 Characteristics of an "Ideal" Detector

Before surveying the field of currently available detectors it is appropriate to summarise the characteristics of an "ideal" liquid chromatographic detector. The detector should:

(i) have high sensitivity and predictable response,
(ii) respond to all solutes or have predictable specificity,
(iii) be unaffected by changes in temperature and carrier flowrate,
(iv) have a low dead volume — to avoid unnecessary band broadening,
(v) be reliable and convenient,
(vi) respond linearly to concentration of solute and
(vii) be non-destructive.

Unfortunately, no single detector possesses all of the above characteristics and considerable effort has been, and is being expended in the search for the "ideal" detector. The major effort in this search is for a truly universal detector, that is, one which will respond to all solutes. As will be discussed later, a few detectors approach universal response to all solutes but at the expense of sensitivity.
1.5 Performance Criteria of L.C. Detectors

Chromatographic detectors may be classified as concentration sensitive or mass sensitive devices. The detector output of the former is some function of mass of solute per unit volume of mobile phase passing through it, whereas the latter's output is some function of mass of solute passing through it per unit time. L.C. detectors are mostly concentration sensitive devices, and thus the parameter measured by the detector is assumed to be $g \text{ cm}^{-3}$.

1.6 Detector Response

The response of a concentration sensitive detector, as defined by Scott (6), is given by

$$R_c = \frac{A \cdot V}{c \cdot m} \text{ mV/mass/unit volume.}$$

Since the area ($A$, peak height, mV, width, cm, at 0.607 of peak height) under a chromatographic peak is inversely proportional to the flow rate ($V$, cm$^3$ min$^{-1}$) of the mobile phase, the response, as defined above, attempts to produce an absolute measurement for a mass ($m$, grammes) of the solute passing through the detector. $c$ is the chart speed in cm. min$^{-1}$.

1.7 Detector Sensitivity

Scott (7) defines the sensitivity of a concentration sensitive detector as the minimum mass/unit volume of solute passing through the detector that can be discerned from the noise. The size of the signal relative to the noise that can allow the signal to be discerned from the noise is arbitrarily defined and it is accepted that a signal to noise ratio of two will permit unambiguous identification of a signal.
It follows that for a detector responding to the concentration passing through it, the detector sensitivity, $S_c$, is given by (7)

$$S_c = \frac{2N_d}{R_c} \text{ g/cm}^3.$$

where $R_c$ is the response as previously defined and $N_d$ the peak to peak noise measured from the chartpaper.

The equations for response and sensitivity have been defined so that they may be used for comparing different flow cells that have been developed during this research.

1.8 Linearity of Response

Linearity of response is a most desirable property if the detector is to be used for quantitative analysis. A linear detector's output can be described by the following:

$$y = A c$$

where $y$ is the output of the detector, $c$ is the concentration of solute in the mobile phase and $A$ is a constant.

However, no detector is completely linear and to enable a measure of the detector linearity to be made, Fowlis and Scott (8) suggested the following power function

$$y = A c^r$$

where $r$ is the response index of the detector. For a truly linear detector $r$ is equal to 1.

The linear dynamic range of a detector is that range of concentration over which the output of the detector is linear within a given response index range.
1.9 **Specificity**

Specificity may or may not be desirable depending on whether the analytical sample is grossly impure or contains little other than the solutes to be analysed. For the former, high specificity is most desirable whereas for the latter a non-specific detector is preferred, specificity being obtained by improvement in chromatographic technique.

1.10 **Dead Volume**

In liquid chromatography correct design of flow-cell is of particular importance. Svensson (9) has shown that the most correct reading of concentration changes in a liquid stream was obtained when the cross-section of the flow-cell, perpendicular to the direction of flow, was the same as that of the inlet pipeline. This may be achieved in modern L.C. detectors with very small volume flow-cells. In many practical cases, however, this requirement cannot be met.

1.11 **Baseline Stability**

Instability in the recorded baseline signal results from short term noise, long term noise or baseline drift. Short term noise appears as small fluctuations on the baseline and is often eliminated by reducing sensitivity or increasing damping on the recorder. Long term noise has a frequency of the same order as the eluted peaks and is far more difficult to reduce than short term noise. Drift is a change in the detector output which has a frequency significantly smaller than the frequency of the eluted peak. Serious drift can occur if the detector column and mobile
phase is not in thermal equilibrium and can be eliminated by thermostating the detector column and mobile phase supply.

1.12 Liquid Chromatography Detectors Currently in Use

UV Absorption Detector

The UV absorption detector (10) in which the UV absorption of the eluent is measured still remains the most popular detector, generally used in the middle UV range 250-350 nm. The popularity of this detector is partly due to the many important classes of compounds which absorb in the UV, and partly due to the fact that many of the instrumental problems have been solved during the development of commercial spectrophotometers.

UV detectors employ either single or double beam optics, generally employing the latter to compensate for changes in light intensity from a low pressure mercury lamp which delivers about 90% of its radiation at 254 nm.

The UV detector is a sensitive and selective detector which naturally requires mobile phases with no UV absorption. The use of this detector has been extended by producing UV absorbing derivatives before separation on the analytical column. Examples of this technique include the separation of pesticides (11), phenols from sea water (12) and vitamins (13,14).

Multiwavelength detectors have also been described (15).

Refractive Index Monitors

The refractive index monitor (16) measures a general property of the eluate and in common with other non-selective detectors suffers from relative insensitivity, being capable of detecting
1 part of solute in $10^6$ parts of eluate. Even to achieve this sensitivity requires temperature stabilisation to about $10^{-3}$ °C, and even then frictional heat generated by the flow of mobile phase through the detector cell becomes a limitation at the high flow rates used in HPLC.

The use of this detector was reviewed in 1974 (17), and from the above considerations it appears that the future for such detectors is limited.

**Wire or Band Transport Detector** (18)

These detectors work on the principle that the eluate is collected on a moving wire or band which transports it first into a low temperature oven where the mobile phase, which must be volatile, is removed by evaporation. Separated solutes, which must be involatile remain on the band and are carried on to a flame ionization detector which detects and possibly identifies them.

Current models, for example Pye-Unicam, have sensitivities which are slightly better than those of RI monitors, but fall short of UV monitors.

Their greatest weakness, at present, stems from the small portion of eluate picked by the transport mechanism. Since this is generally between 0.1 and 1% of the total, a factor of at least 100 in sensitivity is lost from this alone.

**Fluorescence Detector**

A fluorescence detector (19) provides a highly sensitive and specific method of detection. Fluorescent solutes have been
detected at the low nanogram level using commercially available detectors equipped with small volume flow through cells (10, 21). Since the intensity of fluorescence is proportional to the intensity of the excitation light beam, the use of a laser offers a means of increasing the sensitivity of the detection system. Aflotoxins have been detected at the sub picogram level (22) using laser fluorescence. The use of laser fluorimetric detection in liquid chromatography has been reviewed by Yeung in 1980 (23).

Fluorescence is a rare phenomenon among organic compounds and direct application of this method is restricted to a few groups of interesting compounds (e.g. riboflavines (24), polynuclear aromatics (25, 26, 27), porphyrins (24) and aflotoxins (22). The impetus for the recent development of fluorescence detectors in liquid chromatography comes from post or precolumn derivatization to produce fluorescing species (28, 29). The use of dansyl or fluorescamine derivatives (30, 31, 32) is now well established. A combination absorbance-fluorescence detector (33) has been described in the literature and is now commercially available in the form of a similar instrument, the Du Pont model 836 detector.

Other Detectors

Many other detectors have been cited in the literature, amongst these are detectors based on coulometric and amperometric methods (34, 35, 36, 37, 38, 39, 40, 41), chromogenic reactions (42), (selective and extremely sensitive), radioactivity (43, 44), conductivity (45), heat of adsorption (46, 47), capacitance (48), IR (49), NMR (50), mass spectrometry (51, 52), plasma emission (53, 54, 55, 56) and photoconductivity (57).
The search for a detector which is universal in response to eluted solutes together with high sensitivity continues. The refractive index monitor is considered to be universal but has severe problems associated with its use, as described previously. Several new universal detectors have been recently reported in the literature. The flame aerosol detector (FAD), (58) works on a similar principle to the flame ionization detector that is widely used in gas-liquid chromatography. The detector responds to both organic and inorganic compounds and has a limit of detection comparable to other universal detectors presently available for liquid chromatography. The universal nature of the response, unfortunately, excludes the use of organic solvents, organic/aqueous mixtures, and buffered aqueous solutions as mobile phases. The FAD is apparently restricted to applications in which water can be used as the mobile phase. A universal detector for monitoring organic carbon has been reported (59), and is based on the principle of measuring organic carbon as CO₂ after oxidation. The sensitivity of the detector compares favourably with RI and UV monitors but, owing to its mode of operation, is restricted to an aqueous mobile phase.

From the above considerations it is clear that there is a need for a new detector which has greater sensitivity and stability than that shown by present detectors. The fluorimetric detector has gone part way to solving the former problem, but due to its design, stability and consistency of response are major problems owing to fluctuation of source intensity. A further disadvantage is that a substantial amount of radiation emitted from the fluorescent material is lost, since only that radiation emitted at right angles to the incident radiation is measured.
Beta-induced fluorescence (BIF) is the luminescence excited from a compound as the result of the passage of beta-particle radiation through the compound or a dilute solution of the compound. BIF is widely used in liquid scintillation counting (60) where the objective is to measure the rate of decay of a radioactive isotope by counting the rate at which photons are emitted from a solution of a highly fluorescent material. However, this procedure can be inverted so that with a suitable isotope, decaying at a relatively constant activity, the rate at which photons are emitted from a solution is proportional to the concentration of the fluorescent solute within the solution.

The BIF detector under development during this work does not suffer from the problems associated with a normal fluorimetric detector. Correct design of flow cell should permit the collection of the majority of photons emitted from the fluorescent molecules and because of the detectors inherent simplicity and the absence of a UV excitation source, it should not suffer from the instability associated with current fluorimetric detectors.

1.13 Aims and Objectives of the Work

In earlier work carried out by Malcolm-Lawes, Warwick and Gifford (61), the principles of BIF, applied to liquid chromatography as a detection technique, were investigated, the potential advantages of using a radioisotope as an exciting source were discussed, and some preliminary results using a nickel-63 source presented. However, this detection technique suffered from a serious disadvantage in that the active nickel was eluted from the source when (relatively) polar mobile phases were used to effect chromatographic separations.
The prime objective of this work was to develop a detector, based on the principle of HIF, which is suitable for use in liquid chromatography and is both sensitive and stable in operation. During the development of the HIF detector it became evident that the compounds detected need not be inherently fluorescent and that the range of compounds detected could be increased by quenching the fluorescence emitted from a suitable mobile phase. Furthermore, by using a radioactive isotope which emits beta-particles with an energy above the Cerenkov threshold in a mobile phase, absorption of the resulting Cerenkov photons provides another means of detection which may be exploitable for application to liquid chromatography.

The three detection techniques under investigation are based on relatively new applications of the radiations emitted during radioactive decay. For that reason the rest of this chapter includes a brief description of

(a) absorption of ionizing radiation by matter,

(b) beta-induced fluorescence and quenched beta-induced fluorescence, and

(c) Cerenkov radiation.
1.14 ABSORPTION OF IONIZING RADIATION BY MATTER

1.15 Alpha Particles

Heavy charged particles such as alpha particles interact with matter primarily as a result of coulombic forces between their positive charge and the negative charge of the orbital electrons within the matter atoms. Upon entering the absorbing medium, the charged particle immediately interacts simultaneously with many electrons. In any one such encounter, the electron feels an impulse from the attractive coulomb force as the particle passes its vicinity. Depending upon the proximity of the encounter, this impulse may be sufficient to either raise the electron to a higher energy within the absorber (excitation) or to completely remove the electron from the atom (ionization). The energy that is transferred to the electron must come at the expense of the charged particle, and its velocity is therefore decreased as a result of the encounter. In particularly close encounters, an electron may undergo a large enough impulse so that after having left its parent atom, it still may have sufficient kinetic energy to create further ions by the same mechanism. These energetic electrons are called delta rays and represent an indirect means by which the charged particle energy is transferred to the absorbing medium. For a 5 MeV $\alpha$-particle the maximum energy of the secondary electrons is 2.7 keV.

1.16 Beta Particles

As with an $\alpha$-particle, the passage of a fast electron close to an atom or molecule subjects it to an electric impulse which results in excitation or ionization. The most abundant activations are those of valence electrons to energies of 10–50 eV (62)
(ionization potentials are 10–20 eV). The valence electrons of all atoms and molecules are approximately equally liable to be affected. Fast electrons can also produce electronic transitions involving inner shells. Although there are not many such transitions, the energy given up when one does occur may be very large. However, hardly any of the energy taken up in these transitions is retained in the molecules. Within $10^{-15}$ seconds, X-ray photons are emitted or Auger processes occur, the X-ray photons or Auger electrons carrying away a large part of the energy initially imparted to the molecules.

Absorption of an energy of more than 20 eV may often enable more than one molecule to be excited or ionized. If a molecule were to ionize as a result of the absorption of 40 eV for example, the electron emitted would be expected to carry away a significant fraction of the energy. This electron, although not sufficiently energetic to escape from its positive ion, would still have enough energy to excite or ionize another molecule. Effects of this kind are seen in cloud chambers, where little groups consisting of two or more ionizations can be seen, although single ionizations are more frequent (63).

In condensed phases the groups of events are produced very close together, in clusters or 'spurs' of typical diameter a few nanometers. A very few secondary electrons will have energies of a few hundreds or thousands of electron volts (e.g. the Auger electrons). If their energy is sufficient to enable them to escape far from their site of origin, such electrons may be regarded as distinct particles or 'β-rays' producing in turn large numbers of excitations or ionizations along their tracks. In between the isolated spurs and tracks will be blobs where electrons
of energy 100–500 eV dissipate all their energy in a limited volume (64).

The transitions induced directly by fast electrons are mainly the ones which are optically allowed. The electrons with an energy of less than 100 eV, whether resulting from the deceleration of fast electrons or produced in the medium in spurs, blobs, etc., behave somewhat differently from fast electrons, in that they excite to optically forbidden levels. As electrons slow down still further, their energy drops to a level where they can no longer cause excitation of the principal component in the irradiated medium. In this condition the electrons are called sub-excitation electrons (65). When their energy reaches thermal proportions (≈0.025 eV at room temperature) they may become trapped in the medium or react, for example, with molecules containing electronegative elements or groups, or with positive ions formed during the ionising interactions.

Besides interacting with bound electrons, fast electrons can also interact electrically with the nucleus. When the energy lost from the fast electrons appears exclusively as kinetic energy of the recoil atom or molecule, the interaction is called an elastic collision. The elastic collisions deflect the electrons (Rutherford scattering) but because of the large mass of the nucleus, absorb little of their energy. A fast electron may also interact with the nucleus to produce bremsstrahlung and with matter to produce low energy electromagnetic radiation called Cerenkov radiation. The latter is discussed later in this introduction.

1.17 Gamma Photons

The average specific ionization caused by a gamma photon is one
tenth to one hundredth of that caused by an electron of the same energy. The ionization observed for X-rays is almost entirely secondary in nature and there are three processes by which a gamma photon may lose energy.

**Photoelectric Effect**

At low energies the most important process is the photoelectric effect in which the energy of the photon is totally converted into kinetic energy of an electron. The energy of a gamma photon is typically many thousands of electron volts and since all of this energy, except the binding energy of the ejected electron, is given to the photoelectron it is the latter which cause chemical and physical effects in the medium.

**Compton Scattering**

In Compton Scattering, a gamma photon loses part of its energy by ejecting an electron from an atom, but instead of disappearing it is deflected and continues its path with reduced energy. As in the case of photoelectrons, the ejected electrons (Compton electrons) will usually give energy to the medium, affecting far more atoms or molecules than the original photons. The scattered photons may escape or undergo photoelectric absorption or further Compton Scattering.

**Pair Production**

In this process, the gamma photon, in passing close to the nucleus, becomes converted into a positron-electron pair. For the process to occur, it is necessary for the photon energy to be greater than the energy equivalent of the rest masses of the two
particles, that is, more than 1.022 MeV. The positron and electron produced is then equal to the energy of the photon minus 1.022 MeV. The positron and the electron lose energy in the medium, leading to chemical or physical change, and the positron is ultimately annihilated by combining with an electron, usually to give two photons each of energy 0.511 MeV (annihilation radiation).

1.18 The Choice of Ionizing Radiation

From the previous brief discussion it is apparent that all three types of radiation cause ionization and excitation when absorbed by matter.

Table 1.1 shows the range of ionizing radiation of various energies in air and water together with the linear energy transfer (LET - the energy absorbed in matter per unit path length of a charged particle) values.

**TABLE 1.1 Maximum Range and LET Values**

<table>
<thead>
<tr>
<th>Radiation</th>
<th>Energy (MeV)</th>
<th>MAX RANGE (cm)</th>
<th>LET in Water keV μm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron</td>
<td></td>
<td>AIR</td>
<td>WATER</td>
</tr>
<tr>
<td>1</td>
<td>405</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1400</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4200</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Alpha</td>
<td></td>
<td>0.57</td>
<td>0.00053</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
<td>0.0017</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.5</td>
<td>0.011</td>
<td></td>
</tr>
<tr>
<td>$^{137}$Cs</td>
<td>0.66</td>
<td>$x_1^2 = 8.1$ cm (H₂O)</td>
<td>0.39</td>
</tr>
<tr>
<td>$^{60}$Co</td>
<td>1.20–1.30</td>
<td>$x_1^2 = 11.1$ cm (H₂O)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Alpha particles are intensely ionizing, producing $\sim 10^4$ ion pairs per mm of air for a particle of $\sim 1$ MeV. A beta-particle of
the same energy produces \( \sim 10 \) ion pairs per mm of air whilst a gamma photon causes relatively little ionization. The LET values for alpha particles are comparatively high, demonstrating the high ionizing power of these particles. Although the LET values for electrons and gamma photons appear to be similar, the LET value for the latter refers to Compton electrons produced by the gamma photons and not the photons themselves. An alpha emitter, therefore, appears attractive as an excitation source for inducing fluorescence in a solution.

However, other factors must be considered if the fluorescence is emitted from a small volume (~10 \( \mu l \), path length ~2 mm) flow cell, as used in a liquid chromatographic detector. Table 1.1 shows the maximum range of an electron and an alpha particle of various energies. Alpha particles are easily stopped even by the thickness of a sheet of paper, and therefore would be unable to penetrate into the bulk of the solution within the flow cell. A 1 MeV beta-particle travels approximately 4 mm in water before being totally absorbed, and would traverse the whole of the solution within the flow cell.

Table 1.1 shows that a gamma photon emitted from \(^{137}\)Cs has a value of 8.1 cm for \( x^{\frac{1}{2}} \) (the thickness required to reduce the initial photon intensity by half) in water. Thus a gamma photon of this energy would pass straight through the solution without causing sufficient ionization and excitation within the solution. However, although the range of a gamma photon is greater than a beta-particle, for equivalent energy, a low energy gamma photon may be used to prevent the photon from escaping from solution.

A final consideration must be safety. As stated, alpha-particles are extremely ionizing and if ingested may cause severe damage to the tissue with which they are in contact. Gamma photons
are extremely penetrating and it would therefore be necessary to shield the flow cell from the operator with lead shielding, increasing the weight and bulk of the detector.

It was concluded that a beta-particle emitter would be the most appropriate ionizing source for inducing fluorescence from a solution within a small volume flow cell. The mechanism of beta-induced fluorescence is described in detail below.
1.19 BETA-INDUCED FLUORESCENCE

At energies lower than the Cerenkov threshold (66), beta-particles lose their energy by inelastic collisions with molecules in the liquid resulting in ionization and excitation along the track of each beta-particle. These ionized and electronically excited molecules may lose their excess energy by several involved pathways which result in either photon emission or vibrational and collisional deactivation. It is the deactivation by photon emission which is of interest since it is on this that the beta-induced fluorescence (BIF) detector relies for its operation.

In considering emission of fluorescence photons from a liquid mixture or solution, it is convenient to regard the mixture as though it were composed of two main parts; the solvent (M) and the solute (S). The ultimate result of the scintillation process is the conversion of part of the energy from a nuclear transformation into a number of photons, and the basic mechanism of this process is shown below:

\[ \beta^- + M \rightarrow M^* + S \rightarrow S^* \rightarrow S + h\nu \]

M, M* and S and S* are the ground and excited state of the solvent and solute molecules respectively, \( \beta^- \) is the beta-particle and \( h\nu \) the fluorescence photon.

The number of photons emitted by such a mechanism is related to the energy deposited in the solution and is dependent upon such factors as the amount of scintillator solute present in solution, the presence of certain impurities (quenching), the optical transmission properties (colour quenching) and the scintillation efficiency of the total system.

To understand the mechanism of the beta-induced fluorescence
process, it is necessary to review current understanding of the closely related liquid scintillation process.

1.20 Solvent molecules and Their Excitation

As the beta-particle passes through a scintillator solution, it slows down and is eventually stopped producing many different events. Fig. 1.2 depicts a highly simplified representation of the more important events.

Fig. 1.2 (67)
The largest part of the particle energy is expended in the form of kinetic (thermal) energy of the solution molecules and does not lead to the production of excited solvent molecules. Along the particle track ions, excited molecules, free radicals and secondary electrons are among the different products formed.

\[ \beta^{**} + 1M \rightarrow 1M^{**}, 3M^{**}, 2\sigma^{**}; 2M^+ + e^-; F^+ + F^- \]

**Fig. 1.3 Interaction of Beta-particle with Solvent Molecule**

The secondary electrons (from 4), will produce their own track of events which have been called 'spurs'. These electrons can interact with solvent ions, molecules and free radicals as is shown in Fig. 1.4.

\[ e^- + 2M^+ \rightarrow 6 \rightarrow 1M^{**} \]
\[ + 2M^+ \rightarrow 7 \rightarrow 3M^{**} \]
\[ + 2M^+ \rightarrow 8 \rightarrow \sigma^{**} \]
\[ + 1M \rightarrow 9 \rightarrow 2M^- \]
\[ + F^+ \rightarrow 10 \rightarrow F \]

**Fig. 1.4 Secondary Electron Interaction**

Where
- \( \beta^{**} \) is the beta-particle with initial kinetic energy
- \( \beta^* \) is the beta-particle with kinetic energy after the event
- \( 1M \) is the singlet ground state solvent molecule
- \( 1M^{**} \) is the solvent molecule excited to singlet n state
- \( 3M^{**} \) is solvent molecule excited to triplet n state
- \( 2M^+ \) and \( 2M^- \) are solvent ions
F⁺, F⁻ positive and negative free radicals

σ** solvent molecule excited to σ-electronic states.

Thus excited state solvent molecules are produced along the track of the particle by direct excitation of the solvent molecule and a two-stage ionization and neutralization mechanism. Various studies (68,69) have shown that, in some solvents, 60% of the observed fluorescence is a result of ion recombination as in 6 and 7 above.

1.21 Aromatic Solvents

The most popular solvents used in liquid scintillation are aromatic hydrocarbons which are characterized by benzenoid and/or heterocyclic ring structures containing unsaturated double bonds. The molecular shape is determined mainly by the bonding σ-electrons which link the constituent atoms. Each single bond is formed by a pair of σ-electrons. The second bond of each unsaturated double bond corresponds to a pair of π-electrons. The π-electrons are much less tightly bound to their parent carbon atoms than are the localized σ-electrons. They are mobile within the molecular framework, and they form π-electron molecular orbital systems. These π-electrons are responsible for the near ultraviolet absorption spectra and for the fluorescence properties of aromatic molecules.

The unexcited ground state of the π-electron system in an aromatic molecule is a singlet state, So, in which the electron spins are paired. The system has two series of excited states: the singlet states S₁, S₂, ..., Sₙ, which together with So comprise the singlet manifold; and the triplet states T₁, T₂, ..., Tₙ, which comprise the triplet manifold. Each series of states increases in
energy up to the ionization potential. The triplet states are produced when the spin of the excited electron is reversed, i.e., the total spin of the molecule is no longer zero, the molecule is in a triplet state.

Returning to the excitation of solvent molecules by a beta-particle it should be noted that in an aromatic solvent molecule only about 14 per cent of the electrons are \( \pi \) -electrons so that process 3 is much more probable than processes 1 and 2.

The \( \sigma \) -electronic excited states produced in process 3, dissipate their energy thermally and therefore do not contribute to the fluorescence process.

The largest proportion of the excitation of solvent molecules occurs to higher energy levels. Horrocks (70) proposed that the probability of a given excited state being formed by absorption of energy is proportional to the optical absorption into singlet level (molar absorptivity). Table 1.2 shows the relationship between excitation probabilities and molar absorptivity for a selection of aromatic solvents (for fast electron excitation).

Finally, in any process in which singlet and triplet excited species are alternative products, as in 1 and 2 and 6 and 7, they are produced in the approximate ratio of 1:3 corresponding to the multiplicity weighting factor.
TABLE 1.2  Transition Probabilities for Aromatic Solvents (70)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Transition</th>
<th>Molar Absorptivity</th>
<th>% of Transitions Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>So → S₁</td>
<td>280</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>So → S₂</td>
<td>8800</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>So → S₃</td>
<td>68000</td>
<td>87.1</td>
</tr>
<tr>
<td>Toluene</td>
<td>So → S₁</td>
<td>260</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>So → S₂</td>
<td>7900</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>So → S₃</td>
<td>55000</td>
<td>87.1</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>So → S₁</td>
<td>700</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>So → S₂</td>
<td>8600</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>So → S₃</td>
<td>59000</td>
<td>87.1</td>
</tr>
</tbody>
</table>

1.22 Excimers

In addition to the above processes, excited dimer (excimer) molecules can be formed from molecular ions produced from 4 and 9.

The mechanisms are shown below:- \((71)\)

\[
\begin{align*}
2M^+ + 2M^- & \rightarrow 1_{D^{**}} \\
2M^+ + 2M^- & \rightarrow 3_{D^{**}} \\
2M^+ + 1M & \rightarrow 2_{D^+} \\
2_{D^+} + e^- & \rightarrow 1_{D^{**}} \\
2_{D^+} + e^- & \rightarrow 3_{D^{**}}
\end{align*}
\]

Where \(1_{D^{**}}\) is excimer solvent excited to n singlet state

\(3_{D^{**}}\) is excimer solvent excited to n triplet state

\(2_{D^+}\) is an excimer cation - observed in aromatic hydrocarbons as radiation product in low temperature solutions \((72)\).

The solvent monomer and excimer species discussed above undergo rapid \(\sim 10^{-13}\text{sec}\) internal conversion into \(S_1\) or \(T_1\) the lowest excited singlet or triplet state of the species. Discounting the
presence of solute or quench molecules, the species so formed may undergo any of the following processes:

$$^1M^* \xrightarrow{16} ^1D^*$$

Non-Radiative

$$^1M^* \xrightarrow{17} ^1M$$

$$^3M^* \xrightarrow{18} ^1M$$

$$^1M \xrightarrow{20} ^1M + h\nu_F$$

Radiative

$$^3M^* \xrightarrow{21} ^1M + h\nu_p$$

$$^1D^* \xrightarrow{22} ^3M^* + M$$

Non-Radiative

$$^3D^* \xrightarrow{24} ^2M$$

$$^1D^* \xrightarrow{23} 2M$$

$$^3D^* \xrightarrow{25} ^3M^* + M$$

$$^2M \xrightarrow{26} ^2M$$

$$^1D^* \xrightarrow{27} 2M + h\nu_F$$

Radiative

$$^3D^* \xrightarrow{28} 2M + h\nu_p$$

From the above analysis of solvent molecule interaction with beta-particle energy it is clear that the prime objective is the production of excited singlet state monomer and excimer solvent molecules. Processes producing these states are 1, 2, 6, 11, 13, 14 and 16 and are summarized below:
1.23 Fluorescence of Solvent Molecules

The scintillation emission which originates from $^1M^*$ and $^1D^*$ by the above sequence of events occurs within a few nanoseconds and is known as the fast scintillation component. The scintillation emission which originates from $^3M^*$ - $^3M^*$ collisional interaction yielding $^1M^*$ and $^1D^*$ occurs over a few microseconds and constitutes the delayed or slow scintillation component. The relative intensities of the fast and slow components depend on the specific ionization of the ionizing radiation. With beta-particle excitation, the intensity of the slow component is relatively small, and in the presence of oxygen is quenched.

Under normal beta-irradiation, the scintillation spectrum of toluene is identical with its photo-fluorescence spectrum and includes components of $^1M^*$ and $^1D^*$. Irradiation of pure benzene, toluene and other aromatic liquids at room temperature, with a very intense electron beam (73), produces an emission which originates solely from the excimer $^1D^*$. The monomer ($^1M^{**}$, $^1M^*$) excitation is completely quenched by the high ionization quenching (74) produced by the intense electron beam. These results demonstrate the importance of excimers in the solvent scintillation process, a factor which was overlooked in earlier work on the subject.
The solvents are poor scintillators for several reasons:­

(a) solvent molecules have low probabilities for the emission of photons, i.e. a low quantum efficiency;

(b) the energy (wavelength) distribution for the photons emitted by the solvent molecules is of a range (200-300 nm) where most photomultiplier tubes have low sensitivity;

(c) the probability of re-absorption of emitted photons by the solvent itself is high due in part to the high solvent concentration (this probably does not apply in HeF detectors used in HPLC);

(d) the life of the excited solvent molecule in the fluorescing state is relatively long (~ 30 n sec) which renders it very vulnerable to competitive processes which do not lead to fluorescence, i.e. relaxation quenching or energy transfer.

1.24 Solvent-Solvent Energy Transfer and Migration

Following excitation of a solvent molecule by the beta-particle, the excitation energy, rather than remaining within the molecule, migrates by energy transfer to a neighbouring solvent molecule. Experimental evidence (75,76) indicates that energy transfer occurs predominantly from the first excited singlet energy level, although some energy transfer can occur from upper excited states of the solvent molecule (77,78).

Two theories have been put forward as to the mode of solvent-solvent energy transfer.

(a) Birks-Conte Model (79)

The excited solvent molecules ($^1M^*$) and excimers ($^1D^*$) are in dynamic equilibrium
With such a system in a dilute solution in an inert solvent (e.g., cyclohexane) of viscosity $\eta$, the rate $k_{DM} \left[ {^1 M} \right]$ of the forward reaction is diffusion controlled (80), so that

$$k_{DM} = \frac{8 \cdot p \cdot R \cdot T}{3000 \cdot \eta} \quad (\text{Eq. 1.1})$$

where $p$ is the reaction probability per collision, $R$ is the gas constant and $T$ the absolute temperature. The rate of the backward reaction

$$k_{MD} = \frac{k_{DM}}{K_e}$$

is determined by $k_{DM}$ and by the molar equilibrium constant of the reaction

$$K_e = \frac{\left[ {^1 D^*} \right]}{\left[ {^1 M^*} \right] \left[ {^1 M} \right]} \quad (\text{Eq. 1.2})$$

In an aromatic solvent, where the molar concentration $\left[ {^1 M} \right] \sim 10$ M an excited molecule $^1 M^*$ can interact rapidly with any of its unexcited neighbours $^1 M$ to yield $^1 D^*$. The rate $k_{DM} \left[ {^1 M} \right]$ of excimer formation is determined by the molecular collision rate, which exceeds the rate of a diffusion-controlled process shown in Eq. 1.1. Since $K_e$ (from Eq. 1.2) remains constant, the excimer dissociation rate $k_{MD}$ increases with $k_{DM}$.

Rapid excimer formation and dissociation provide the mechanism proposed by Birks and Conte for the efficient migration of the solvent excitation energy:

$$^1 M_A^* + ^1 M_B \underset{k_{MD}}{\rightleftharpoons} ^1 D_{AB}^* \rightarrow ^1 M_A + ^1 M_B^*, \text{ etc.}$$

where suffixes A and B refer to different solvent molecules. A sequence of processes as above, provides a random walk process by which excitation energy migrates between different solvent molecules.
(b) **Voltz Model**

In this model, Voltz (81) postulates that energy actually jumps to its unexcited neighbour by a non-radiative process.

\[
M_A^* + M_B \leftrightarrow M_A + M_B^*
\]
\[
M_B^* + M_C \leftrightarrow M_B + M_C^*
\]

Where suffixes A, B and C refer to different solvent molecules. The excited solvent molecule may be in the first excited singlet state, which is the process occurring in aromatic solvents (81) or in a higher energy level. Evidence for the latter process was given by Kallmann-Oster (82) and Klein (83) and in aliphatic solvents only, energy transfer occurs from Sn states only and accounts for the relatively high scintillation yield of solutions of fluors in cyclohexane (82) since it has been recognised that no energy transfer occurs when the lowest energy states of this solvent are excited by means of light in the far UV (84).

(c) **Additional Models**

In addition to the model proposed by Voltz, it should be noted that an ionized state \( M^+ \), produced by a beta-particle, may also participate in energy transfer. Such a mechanism, which was proposed by Hamill (85) cannot readily be distinguished from those referred to above.

Furthermore, Voltz (86) and Klein (83) have given a description of energy migration which encompasses diffusion and both the Birk-Conte and Voltz models. Their description, based on an analysis of elementary phenomena involved in diffusion and in excimer formation and dissociation, is as follows:

Once formed on its lowest vibrational level \( D_o \), the excited dimer is submitted to thermal collisions with neighbour molecules
and may then be rapidly raised \((\sim 10^{-11} \text{ sec})\) into one of the higher vibrational levels \(D_{v n}\) whose lifetime is short \((\sim 10^{-12} \text{ sec})\). If the vibronic energy level of \(D_{v n}\) is equal to or greater than the electronic energy of the monomer \(S_1\) state, the excimer can transfer its excitation energy by resonance to a neighbouring molecule because the energy requirement is then achieved. This process is analogous to the Volz model of energy transfer.

Whatever the mechanism of energy transfer is within the solvent, energy transfer occurs between these molecules until contact with a solute molecule is achieved whereupon the excitation energy is transferred from the solvent molecule to the solute molecule.

### 1.25 Solvent-Solute Energy Transfer

Energy transfer from the lowest excited state, \(1_m^*\), of the solvent molecule to a corresponding level \(1_s^*\) of the fluorescent solute molecule may be represented by

\[
1_m^* + 1_s^* \rightarrow 29 \quad 1_s^* + 1_m^*
\]

and similarly from the excimer by

\[
1_d^* + 1_s^* \rightarrow 30 \quad 1_s^* + 2 1_m^*
\]

Survey of the literature presents a certain amount of confusion as to the mechanism of such energy transfer. Three models are presented for the mechanism:

(a) Dipole–dipole interaction (non-radiative transfer),

(b) Radiative energy transfer,

(c) Molecular collision energy transfer.
(a) Dipole-Dipole Interaction

A theory of the interactions has been developed by Forster in several articles (87,88). The strength of the dipole-dipole inter-
action has been shown to be related to the degree of overlap of the
fluorescence spectrum of the donor molecule (solvent) and the
absorption spectrum of the acceptor molecule (solute). Also, the
distance over which the transfer will occur is related to the molar
absorptivity of the acceptor molecules over the region of overlap
of the two spectra (emission of the donor and absorption of the
acceptor).

Forster defined the critical transfer distance $R_0$ as the
distance between an acceptor and a donor molecule such that the
probability of energy transfer is equal to the probability of all
other processes of energy release by the donor molecule (radiative
emission, quenching, radiationless deactivations, etc.) (88).
The rate of energy transfer $k_29$ when the donor and acceptor
molecules are separated by a distance $R$ is given by

$$k_29 = \frac{1}{\tau_0} \left( \frac{R_0}{R} \right)^6$$

(Eq. 1.3)

and $R_0$ can be calculated from the equation

$$R_0^6 = \frac{(9000 \ln 10)K^2}{128\pi n^4 N} \int_0^{\infty} F_D(\nu) \xi_A(\nu) \frac{d\nu}{\nu}$$

(Eq. 1.4)

where $\tau_0$ is the radiative decay time of the donor molecule, $K$
an orientation factor, $n$ the refractive index of the solvent, $N$
Avagadro's number, $F_D(\nu)$ the spectral distribution of the fluores-
cence emission of the donor molecule, and $\xi_A(\nu)$ the molar decadic
absorptivity of the acceptor molecule.

For many molecules the dipole-dipole interaction occurs over
intermolecular distances that are large compared to the collision
distance. The diameter of most organic molecules is about 0.6 nm. Two molecules which just contact each other will have a collision distance the sum of the radius of each molecule, or about 0.6 nm. The Förster radius has been calculated to be 2–6 nm for many organic molecules used in liquid scintillation counting. The Förster radius will vary with the solvent molecule.

(b) **Radiative Energy Transfer**

Transfer of energy from the excited state of the solvent molecule to the solute molecule has been suggested to be by photon absorption (89,90).

Ishikawa (90) showed that the efficiency for radiative energy transfer from solvent to PPO is dependent on the solvent emission spectrum and its quantum yield. Where the solvent has a high quantum yield and an emission spectrum which coincides well with PPO absorption spectrum, the probability of radiation transfer would increase.

(c) **Molecular Collision Energy Transfer**

Birks (91) concluded that energy transfer occurs by a collisional process and not by a long range dipole–dipole transfer process.

He determined the rate parameters of energy transfer to a solute or a quench molecule and found that these values are identical and independent of the nature of the solute.

**Summary of Energy Transfer From Solvent to Solute**

Of the three models proposed above, dipole–dipole interaction is accepted as the predominant mechanism of energy transfer between
solvent-solute molecules in liquid scintillation counting (92). Horrocks has pointed out that the transfer of energy is non-radiative, i.e. no photons are emitted by the solvent molecule and subsequently absorbed by the solute molecules. Energy transfer occurs in $10^{-11}$ s, whereas photon emission occurs with a decay time of $\approx 30 \times 10^{-9}$ s. The energy transfer is also not diffusion controlled which occurs in the order of $10^{-6}$ s at the 3-10 g/l concentrations (93,94).

Birks has shown that the rate parameters of energy transfer to a solute or quench molecule are identical and independent of the nature of the solute. However, Voltz et al. (95) has shown that under UV excitation, where only $S_1$ states can be involved, the trapping rate constant ($k_{29}$) was determined for a number of degassed solutions of different solutes (fluors and quenchers) in aromatic solvents and found to be always greater for a fluor than for a quencher in which case the energy transfer is not collisional, but by long range dipole-dipole transfer to the fluor. Furthermore, for a given solvent and different fluors, the change in the rate constant value is correlated with the variation of $R_o$, the critical distance of dipole-dipole interaction between excited solvent and ground state solute molecules. This latter observation suggests that energy transfer is by a Förster-type of mechanism.

In 1973, Birks (96) stated that the magnitude of the energy transfer rate parameter exceeds that of a collisional diffusion-controlled process since the energy transference usually occurs by Coulombic interaction over the dipole-dipole interaction distance $R_o$, which normally exceeds the molecular dimensions.
Several further points should be noted concerning solvent-solute energy transfer:

(1) The transfer efficiency is dependent on the solute concentration and is related to it by

$$E = \frac{\tau k_{29} [S]}{1 + \tau k_{29} [S]}$$  \hspace{1cm} (Eq. 1.5)

where $E$ is the quantum efficiency of the bimolecular process, $\tau$ the mean lifetime of the $S_1$ state (97).

For liquid scintillators based on aromatic solvents, typical mean values of $\tau$ and $k_{29}$ are $35 \times 10^{-9}$ s and $50 \times 10^9$ M$^{-1}$ s$^{-1}$ respectively. It therefore follows that transfer through $S_1$ states tend towards unity for fluor concentrations as low as $10^{-2}$ M.

(2) Solvent-solute energy transfer can also occur by high energy Mn.

Evidence for this process comes from experimental work performed with aromatic solvents containing chloroform and carbon tetrachloride as energy traps. Chloroform especially is an excellent solute in which to study energy transfer from higher excited levels, since it has been shown (83, 98) not to accept excitation energy from the lower excited states of aromatic molecules but to dissociate when the solution is irradiated by high energy particles, indicating energy trapping and migration of primary solvent higher energy states.

Thus

$$M_n^* + 1S_0 \xrightarrow{k_{31}} 1M_0 + 1S_n^*$$

The transfer efficiency is expressed by a relationship similar to that applying to the lowest excited state.

$$E = \frac{\Theta k_{31} [S]}{1 + \Theta k_{31} [S]}$$  \hspace{1cm} (Eq. 1.6)

Where $\Theta$ is the lifetime of the solvent $M_n$ state, $k_{31}$ is the rate
constant of the transfer reaction and \([S]\) the solute molar concentration. Values for \(k_{31}\) have been determined for chloroform and carbon tetrachloride in aromatic solvents and yielded \(k_{31} \approx 60 \text{ M}^{-1}\) (83). For fluor aliphatic systems such as PPD-cyclohexane solution, the analysis of pulse height versus concentration yields \(k_{31} \approx 100 \text{ M}^{-1}\).

From the HIF spectra, shown in Chapter 3, of various concentrations of anthracene in toluene, the following data may be calculated as shown in the footnotes to Table 1.3.

**TABLE 1.3 Solvent-Solute Energy Transfer**

<table>
<thead>
<tr>
<th>Anthracene (10(^{-3}) M)</th>
<th>Corrected Anthracene Peak (mm(^2))</th>
<th>Corrected Toluene Peak (mm(^2))</th>
<th>(k_{29}) (observed) (10(^{-9}) M(^{-1}) s(^{-1}))</th>
<th>(\epsilon + E) observed</th>
<th>(\epsilon + E) calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1178</td>
<td>44.5</td>
<td>0.304</td>
<td>0.344</td>
</tr>
<tr>
<td>0.280</td>
<td>1092</td>
<td>820</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.561</td>
<td>1299</td>
<td>560</td>
<td>56.2</td>
<td>0.525</td>
<td>0.531</td>
</tr>
<tr>
<td>1.122</td>
<td>1852</td>
<td>327</td>
<td>66.2</td>
<td>0.722</td>
<td>0.788</td>
</tr>
</tbody>
</table>

Footnotes to Table 1.3:

(a) Although unnecessary for this calculation, the anthracene and toluene fluorescence peak areas are corrected for the monochromator transmission (shown in Chapter 3) of 9.5% for the anthracene HIF peak (\(\sim 410\) nm) and 6.6% for the toluene HIF peak (\(\sim 300\) nm).

(b) The energy transfer rate parameter \(k_{29}\) was calculated from

\[
\frac{\dot{\rho}_M}{\dot{\rho}_{SM}} = 1 + K_{SM} [S] \quad (96) \quad (\text{Eq. 1.7})
\]

where \(\dot{\rho}_M\) is the quantum efficiency of toluene in the absence of solute and \(\dot{\rho}_{SM}\) is the quantum efficiency of toluene in the presence of molar concentration of solute. \(K_{SM} = k_{29} \tau M\), the Stern-Volmer coefficient of energy transfer, \(k_{29}\) the energy transfer rate.
parameter and $\tau_M$ the toluene excited state decay time. The value for $\tau_M$ is taken as $35 \times 10^{-9}$ s.

It is assumed that

$$\frac{\phi_M}{\phi_{SM}} = \frac{T_F(o)}{T_{EX}} \frac{T_F(S)}{T_{EX}} = \frac{T_F(o)}{T_F(S)}$$

where $T_F(o)$ and $T_F(S)$ are the number of toluene molecules emitting fluorescent photons in the absence of and presence of anthracene, and $T_{EX}$ is the number of excited toluene molecules.

The observed values for $k_29$ correlate well with the value of $52 \times 10^9$ M$^{-1}$ s$^{-1}$ determined by Birks (71) for anthracene in toluene.

(c) The value of $E + E$ (observed) energy transfer efficiency was calculated from

$$E + E \text{ (observed)} = \frac{\text{Number of excited Toluene molecules transferring energy}}{\text{Number of excited Toluene molecules}} = \frac{T_F(o) - T_F(S)}{T_F(o)}$$

The observed values for $E + E$ correlate well with the values calculated from equations 1.5 and 1.6. In this calculation the value of $\phi k_{31}$ is taken as $80$ M$^{-1}$ and the value of $k_{29}$ as $1750$ M$^{-1}$.

A plot of anthracene and toluene fluorescence peak area as a function of anthracene concentration is shown in Fig. 1.5.
(3) In aromatic solvents where both types (E) and (E) are in evidence, the internal conversion efficiency, of a solvent molecule, $\beta_M$, may be significantly less than unity. In a pure solvent ($[s] = 0$), $\beta_M (0)$ has been reported to be 0.35, 0.50 and 0.65 for benzene, toluene and p-xylene respectively (99). Although values of 0.45, 0.76 and 1.00 respectively have also been reported (100). In the presence of solute, values of $\beta_M$ are lower and given by

$$\beta_M = \frac{\beta_M (0)}{1 + \Theta K M} [S]$$  

(Eq. 1.8)

For polycyclic fluor molecules, however, the internal conversion efficiency, $\beta_s$, is likely to approach unity, as with anthracene and p-terphenyl (101).

At low molar concentrations of solute molecules, $<10^{-3} M$

$$\frac{\beta_M \cdot \epsilon}{E \cdot \beta_s} \approx 5$$  

(Eq. 1.9)

and thus energy transfer occurs via lowest excited M1 solvent states.

At intermediate concentrations $\sim 10^{-2} M$

$$\frac{\beta_M \cdot \epsilon}{E \cdot \beta_s} \approx 0.8$$

and thus both routes, M1 and Mn are possible and are of equal importance.

At higher concentration, $>10^{-1} M$, energy transference occurs predominantly from Mn states.

(4) The energy transfer is exothermic; the excitation energy of the $S_1^*$ excited state of the solute molecule is lower than that of the corresponding $M_1^*$ excited state of the solvent. Thus the energy becomes trapped in the solute molecule and cannot be returned to the solvent species.
1.26 Solute Fluorescence

The result of energy transfer from the excited solvent molecule to the solute molecule is electronic excitation of the latter into higher singlet states \( S_n \). Internal conversion into \( S_1 \) excited state occurs rapidly \( (10^{-13} - 10^{-14} \text{s}) \) and thermal degradation of excess vibrational energy results in the excitation residing in the \( S_{10} \) state, the lowest vibrational state of \( S_1 \).

In the absence of quench molecules, several processes compete for the energy of the excited solute molecule, some of which are:

\[
\begin{align*}
&1S^* \rightarrow 32 \rightarrow S + h\nu \quad \text{Fluorescence} \\
&1S^* \rightarrow 33 \rightarrow S + \text{en} \quad \text{Internal conversion} \\
&1S^* \rightarrow 34 \rightarrow 3S^* + \text{en} \quad \text{Intersystem crossing} \\
&1S^* + S \rightarrow 35 \rightarrow 2S + \text{en} \quad \text{Self quenching} \\
&1S^* + S \rightarrow 36 \rightarrow 1S_2^* + \text{en} \quad \text{Excimer formation}
\end{align*}
\]

Where \( 1S^* \), \( 3S^* \) and \( S \) are the first excited singlet, first triplet and ground states of the solute molecule; \( \text{en} \) is radiationless energy release; \( 1S^*_2 \) is the first excited singlet state of the excimer of the solute \( (102) \).

Intersystem crossing leads to triplet state formation which may result in phosphorescence \( (10^{-5} - 10^{-3} \text{s}) \) or to the solute ground state, i.e.

\[
\begin{align*}
&3S^* \rightarrow 37 \rightarrow S + h\nu \quad \text{Phosphorescence} \\
&3S^* \rightarrow 38 \rightarrow S + \text{en} \quad \text{ISC/IC.}
\end{align*}
\]

However, since phosphorescent emission from solutions at room temperature is relatively uncommon, it is fluorescence of the solute that is of major interest in this study.
The fluorescence spectrum of the solute (and the solvent) is not monoenergetic. The energy of the photons covers a wide band and these correspond to the difference between the zero vibrational level of the $S_1$ state and the many vibrational levels of the ground state. The fluorescence between $S_{10} \rightarrow S_{00}$ corresponds to the maximum energy of a photon emitted by the excited molecule. Other transitions $S_{10} \rightarrow S_{1,2,\ldots, n}$ have less energy associated with the emitted photon. The remaining energy difference $(E_{S_{10} \rightarrow S_{00}} - E_{S_{10} \rightarrow S_{on}})$ is dissipated as excess kinetic energy of the ground-state molecules vibrational energy.

The absence of fluorescence from states higher than $S_1$ is attributed to the efficient and rapid ($\approx 10^{-11}$ s) radiationless internal conversion between adjacent excited states.

It should be noted that since the fluorescence results from transitions from the first excited singlet state, the fluorescence wavelength distribution, following beta excitation is identical to the fluorescence resulting from direct solute UV excitation (103).

1.27 Beta-Induced Fluorescence Quenching

The preceding discussion on beta-induced fluorescence has implicitly assumed that no impurity molecules were present that could reduce the photon yield from a scintillant solution. It is well known that in liquid scintillation counting, the presence of certain types of compounds reduce this yield. These compounds have been described as strong and mild quenchers and diluters (104).

Quenchers are molecules which tend to dissipate the $\pi$-electron excitation energy of any excited solvent or solute


**TABLE 1.4 Classification of Quench Compounds**

<table>
<thead>
<tr>
<th>Strong Quenchers</th>
<th>Mild Quenchers</th>
<th>Diluters</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-Br</td>
<td>RCL</td>
<td>RH</td>
</tr>
<tr>
<td>R-SH</td>
<td>RCOOH</td>
<td>RF</td>
</tr>
<tr>
<td>R-OCOCO-R</td>
<td>RNH₂</td>
<td>R-O-R</td>
</tr>
<tr>
<td>R-CO-R</td>
<td>R-CH = CH-R</td>
<td>(NO₃)PO</td>
</tr>
<tr>
<td>R-COX</td>
<td>R-S-R</td>
<td>R-CN</td>
</tr>
<tr>
<td>R-NH-R</td>
<td></td>
<td>R-OH</td>
</tr>
<tr>
<td>R-CHO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₂N-R</td>
<td></td>
<td>R-COO-R</td>
</tr>
<tr>
<td>RI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-NO₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

molecule with which they collide. The probability of quenching on collision with an excited molecule may be small, in which case the substance is described as a mild quencher, or it may be large so that the substance is described as a strong quencher. Diluters do not reduce the scintillation efficiency dramatically. Their presence dilutes the primary solvent and this reduces the number of primary solvent molecules excited by the ionizing particle, and also reduces the efficiency of energy migration within the solvent.

1.28 *Mechanism of Quenching*

Compounds acting as fluorescence quenchers affect the luminescence yield of the solution by inducing radiationless deactivation of excited molecules of both solvent and solute by a diffusion controlled collisional process:
Where \( {^1}_M^* \), \( {^1}_D^* \) and \( {^1}_S^* \) are the first excited singlet state of the solvent molecule, the excimer solvent molecule and the solute molecule respectively. \( {^3}_S^* \) is the first triplet state of the solute molecule and Q the quench molecule.

Impurity quenching may be divided into two categories:-

(a) Dynamic collisional quenching in which the excited solvent or solute molecules approach impurity or quench molecules to a distance where chemical or molecular interaction may occur, and encounter complexes are formed. These include charge transfer complexes, exciplexes (excited complexes) and molecular complexes. The latter may rapidly dissociate into free radicals to enter chemical reactions. The excitation energy is deactivated by these complexes leading to a reduction of the fluorescence quantum yield of the scintillation system according to the following mechanisms:-

\[
{^1}_M^* + Q \rightarrow {^3}_M + Q
\]
\[
{^1}_D^* + Q \rightarrow 2M + Q
\]
\[
{^1}_S^* + Q \rightarrow S + Q
\]
\[
{^3}_S^* + Q \rightarrow S + Q
\]

where \( {^1}_M^* \), \( {^3}_F \) and F represent the excited state, the triplet state and the ground state of the fluor, respectively, and Q is the quencher.

Klein et al. (178) has provided evidence that transient charge transfer complexes are formed in which aromatic solvents
act as the donor and carbon tetrachloride as the acceptor.

Kallman-Oster (106) showed that the quenching of benzene, naphthalene, anthracene in cyclohexane by CCl$_4$ is by the formation of free radicals. Under similar conditions 2-phenyl-5-(4-biphenyl)-D is not quenched by CCl$_4$. Oxygen and molecules containing heavy atoms reduce the fluorescence yield by complex formation and a consequent increase in the intersystem crossing between the first excited singlet state and the triplet manifold.

In static collisional quenching, the encounter complexes consist of unexcited fluor and quencher molecules and the effect is a decrease of the fluor molecules in the system. The encounter complexes may also cause dynamic quenching of the fluorescence of the solute monomers or excimers (107).

(b) Energy transfer quenching. The excitation energy of the excited molecule can jump from the molecule to a quencher molecule by a dipole - dipole interaction. The jump can occur over several molecular diameters (≈ 50 Å) and the rate of energy transfer by this mechanism is greater than that which can occur by diffusion. The impurity, being non-fluorescent, undergoes radiationless transition from the excited state to the ground state, and converts the excitation energy into molecular translational and vibrational energy.

The requirement for excitation transfer by this mechanism is that the energy level of the acceptor molecule be lower than that of the donor molecule and that there be an overlap of the respective fluorescence and absorption spectra of the donor and acceptor. Many chemical compounds such as aldehydes and ketones may cause quenching by this mechanism.
In 1934 Cerenkov (108) first noticed a very weak visible radiation from pure liquids under the influence of $\gamma$-rays. A study of a variety of liquids led him to the conclusion that this was a phenomenon of a different nature to that of fluorescence.

Using radium as a gamma photon source, Cerenkov measured the relative intensities of the light from sixteen liquids among which were distilled water, paraffin, xylol, toluene, glycerine and various alcohols. He used an optical wedge to measure the point of visual extinction of the light emanating from the liquids. His conclusions were that there was very little difference in the range of relative intensities, that the spectral distribution varied little from one liquid to another, and that the light was concentrated in the blue and violet. On the addition of silver nitrate, potassium iodide and other compounds known to strongly quench fluorescent light no decrease in the intensity of radiated light was found. Furthermore, unlike fluorescence, temperature changes produced no change in light intensity and a small, ~ 20%, polarization effect was observed.

In 1936 Cerenkov (109) investigated the influence of a magnetic field on the exciting radiation using a $\gamma$ source and water. He concluded that the light must be attributed to the secondary electrons produced in the medium by $\gamma$-rays, rather than the $\gamma$-rays themselves.

Two papers, published in 1937 (110,111), showed that the phenomenon could also be produced by beta rays and that there was a marked asymmetry of the light intensity with respect to the direction of the exciting radiation. This latter observation was
predicted by Frank and Tamm in a theoretical paper published in 1937 (112). Cerenkov concluded his work with a group of experiments in 1938 (113) in which he verified the theory of his colleagues Frank and Tamm.

1.30 The Mechanism of Cerenkov Radiation

The electrical field of an electron, moving relatively slowly through a transparent medium, distorts the atoms composing the medium so that the negative charges of the electrons are displaced to one side of the heavier positive charges of the nuclei of these atoms. The medium thus becomes polarized about the vicinity of the moving electron. When the electron moves to another point in the medium the elongated atoms return to their normal shape. While the atoms are distorted they behave like elementary dipoles, with the negative poles pointing away from the track if the passing particle is an electron, or towards the track if the particle is a positron or proton. Thus, as the particle passes through the medium, each elemental region of the medium along the track will in turn receive a very brief electromagnetic pulse. Owing to the complete symmetry surrounding the electron, there will be no resultant field at large distances and therefore no radiation. There is symmetry in all directions when an electron moves relatively slowly through a transparent medium as shown in Fig. 1.6.

If the electron is moving fast, at a speed comparable to that of light in the medium, the polarization field is no longer completely symmetrical as is shown in Fig. 1.7. Symmetry is preserved at all points except that along the axis there is a resultant dipole field which will be apparent even at large
distances from the track of the electron. Such a field will be set up by the electron at each element along the track in turn, each element then radiating a brief electromagnetic pulse.

If the velocity of the particle is lower than the phase velocity of light in the medium, the radiated wavelets from all parts of the track interfere destructively so that, at a distant point, the resultant field intensity is zero. If higher, it is possible for the wavelets from all portions of the track to be in phase with one another so that, at a distant point of observation, there is now a resultant field.

The radiation is only observed at a particular angle, $\theta$, with respect to the track of the particle as shown by a Hygens construction to illustrate coherence in Fig. 1.8.

\[ \beta c \Delta t \]
The angle $\Theta$ is where the wavelets, from arbitrary points such as $P_1$ and $P_2$ on the track $AB$, are coherent and combine to form a plane wavefront $BC$. This coherence takes place when the particle traverses $AB$ in the same time that light travels from $A$ to $C$. If the velocity of the particle is $\beta \cdot c$, where $c$ is the velocity of light in vacuo, then in a time interval $\Delta t$ the particle will travel a distance $AB = \beta c \cdot \Delta t$. Similarly, if $n$ is the refractive index of the medium, the light will travel a distance $AC = \frac{c}{n} \cdot \Delta t$. Thus

$$\cos \Theta = \frac{\frac{c}{n} \cdot \Delta t}{\beta c \cdot \Delta t} = \frac{1}{\beta \cdot n}$$

(Eq. 1.10)

which is known as the "Cerenkov relation".

From the Cerenkov relation it can be seen that:
1. In a medium of refractive index, $n$, defined as

$$n = \frac{\text{velocity of light in a vacuum}}{\text{velocity of light in the medium}} = \frac{c}{v_{LM}}$$

for Cerenkov radiation to be propagated the value $\beta \cdot n$ must be $>1$. Since $\cos \Theta$ must be $<1$ $\beta$ is defined as

$$\beta = \frac{\text{velocity of particle in the medium}}{\text{velocity of light in vacuum}} = \frac{v_{PM}}{c}$$

Thus the value of $n$ sets a lower limit ($\beta_{\text{min}}$) on $\beta$. $\beta_{\text{min}}$ is calculated from

$$\beta_{\text{min}} = \frac{1}{n}$$

The value of $\beta$ is determined by the energy of the beta-particle and in order to calculate it, it is necessary to recall that the kinetic energy of a relativistic beta-particle is given by
\[ KE = \frac{m_0 c^2}{\sqrt{1 - \frac{v^2}{c^2}}} - m_0 c^2 \]  
(Eq. 1.11)

\[ \therefore \quad (1 - \frac{v^2}{c^2})^{\frac{1}{2}} = \frac{m_0 c^2}{E + m_0 c^2} \]

\[ 1 - \frac{v^2}{c^2} = \left[ \frac{m_0 c^2}{E + m_0 c^2} \right]^2 \]

\[ \therefore \quad \frac{v^2}{c^2} = 1 - \left[ \frac{m_0 c^2}{E + m_0 c^2} \right]^2 \]

\[ \frac{v}{c} = \left\{ 1 - \left[ \frac{m_0 c^2}{E + m_0 c^2} \right]^2 \right\}^{\frac{1}{2}} \]

\[ \therefore \quad \frac{v}{c} = \left\{ 1 - \left[ \frac{1}{E/m_0 c^2 + 1} \right]^2 \right\}^{\frac{1}{2}} \]

and therefore the value \( E_{\text{min}} \) the beta-particle threshold energy for Cerenkov radiation, is given by

\[ E_{\text{min}} = m_0 c^2 \left[ \frac{1}{(1 - \beta^2)^{\frac{1}{2}}} - 1 \right] \]

\[ = 511 \left[ \frac{1}{(1 - \beta^2)^{\frac{1}{2}}} - 1 \right] \text{ keV} \]  
(Eq. 1.12)

It has been shown that the refractive index of the medium sets a lower limit on beta-particle energy, below which no Cerenkov photons are produced. This value, \( E_{\text{min}} \), may be calculated for a medium of known refractive index, and is often quoted for water, as 263 keV. However, since Cerenkov radiation is emitted over a range of wavelengths and the velocity of light is given by
where \( \lambda \) is the wavelength and \( \nu \) the frequency of the light, the value of \( n \) depends upon the wavelength of the light. The implication is clear in that if \( n \) varies, \( \beta_{\text{min}} \) and \( E_{\text{min}} \) vary with the wavelength of emitted Cerenkov radiation.

Table 1.5 shows calculated values for \( \beta_{\text{min}} \) and \( E_{\text{min}} \) for the production of different wavelengths of Cerenkov radiation propagated by the passage of a beta-particle through water.

**TABLE 1.5 Relationship between \( \beta_{\text{min}} \) and \( E_{\text{min}} \)**

<table>
<thead>
<tr>
<th>( \lambda ) (nm)</th>
<th>( n(114) )</th>
<th>( \beta_{\text{min}} )</th>
<th>( \nu = \beta \cdot c \times 10^8 \text{ms}^{-1} )</th>
<th>( E_{\text{min}} ) keV</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>1.396</td>
<td>0.7163</td>
<td>2.1474</td>
<td>221.3</td>
</tr>
<tr>
<td>250</td>
<td>1.362</td>
<td>0.7342</td>
<td>2.2010</td>
<td>241.6</td>
</tr>
<tr>
<td>300</td>
<td>1.349</td>
<td>0.7413</td>
<td>2.2223</td>
<td>250.4</td>
</tr>
<tr>
<td>350</td>
<td>1.343</td>
<td>0.7446</td>
<td>2.2322</td>
<td>254.5</td>
</tr>
<tr>
<td>400</td>
<td>1.339</td>
<td>0.7468</td>
<td>2.2389</td>
<td>257.4</td>
</tr>
<tr>
<td>450</td>
<td>1.337</td>
<td>0.7479</td>
<td>2.2421</td>
<td>258.8</td>
</tr>
<tr>
<td>500</td>
<td>1.335</td>
<td>0.7491</td>
<td>2.2457</td>
<td>260.4</td>
</tr>
<tr>
<td>550</td>
<td>1.333</td>
<td>0.7502</td>
<td>2.2490</td>
<td>261.8</td>
</tr>
<tr>
<td>600</td>
<td>1.332</td>
<td>0.7507</td>
<td>2.2505</td>
<td>262.5</td>
</tr>
</tbody>
</table>

The speed of light in a vacuum, \( c \), was taken as \( 2.997 \times 10^8 \text{ms}^{-1} \) for calculating \( \nu \), the velocity of light in water.

Fig. 1.9 shows the calculated beta-particle threshold energy for Cerenkov radiation as a function of refractive index of the medium at a wavelength of \( \sim 600 \text{nm} \). \( E_{\text{min}} \) is calculated from

\[
E_{\text{min}} = 511 \left[ \frac{1}{(1 - \frac{1}{n^2})^{\frac{1}{2}}} - 1 \right] \text{keV.}
\]

(Eq. 1.13)
2. For an ultra relativistic particle $\beta_n \approx 1$, there is a maximum angle of emission, given by

$$\cos \theta = \frac{1}{\beta_n}$$

For example, for $^{90}$Y where $E_{\text{max}} = 2260$ keV

$$\cos \theta = \sqrt{\left[1 - \frac{1}{E_{\text{max}} + 1}\right]^2} = 0.9828$$

and

$$\cos \theta = \frac{1}{0.9828 \times 1.332} = 0.7639.$$
Three points should be noted:

(a) The angle of emission depends upon the energy of the beta-particle and the refractive index of the medium and

(b) The angle of emission decreases to 0° as the beta-particle energy degrades to $\beta_{\text{min}}$ on passing through the medium. This is shown in Fig. 1.10

(c) The radiation emitted occurs mainly in the visible and near UV regions of the spectrum for which \( \beta \cdot n > 1 \). Emission in the X-ray region is impossible for \( n \) is less than unity and

\[
\cos \theta = \frac{1}{\beta \cdot n}
\]

cannot be satisfied.

1.31 Cerenkov Photon Numbers and Wavelength Distribution

From the theory of Frank and Tamm (112), the number of photons per unit path length of an electron over a selected spectral region is given by

\[
\frac{dN}{dx} = 2N \kappa \left( \frac{1}{\lambda_2} - \frac{1}{\lambda_1} \right) \left( 1 - \frac{1}{\beta^2 n^2} \right) \text{ photons cm}^{-1} \quad \text{(Eq. 1.14)}
\]
where
\[ \alpha = \text{the fine structure constant} = \frac{e^2}{\hbar c} = \frac{1}{137} \]

\( \lambda_1 \) is the upper limit of the selected wavelength region (cm) and \( \lambda_2 \) is the lower limit of the selected wavelength region (cm).

To determine the total photon yield from a given Cerenkov event, the above equation would normally be integrated over the limits of \( x \), the particle path length. For electrons in a liquid this simple solution cannot be used since \( x \) is a function of original electron energy. As has been stated, \( \beta \) is also a variable since energy degradation of the electron occurs during its passage through the liquid, and the low energy Cerenkov cut-off must be considered.

Taking these factors into account, the equation shown above was numerically integrated and the results shown in Fig. 1.11 show the Cerenkov photon yield from electrons of various initial energies in water calculated over the range 200-600 nm.

1.32 **Applications of BIF and Cerenkov Radiation as Measurement Tools**

Beta-induced fluorescence is widely used in liquid scintillation counting (60). This technique has been reviewed and no further discussion is required herein.

In 1953, Belcher (115) employed Cerenkov radiation, for the first time, for the measurement of radiation by means of the liquid scintillation technique.

A comprehensive review of the applications of Cerenkov radiation was given by Jelley in 1958 (116).
<table>
<thead>
<tr>
<th>Energy of $\beta$-particle (keV)</th>
<th>Range in Water (cm)</th>
<th>Graph</th>
</tr>
</thead>
<tbody>
<tr>
<td>2270</td>
<td>0.996</td>
<td>1</td>
</tr>
<tr>
<td>1710</td>
<td>0.726</td>
<td>2</td>
</tr>
<tr>
<td>1000</td>
<td>0.384</td>
<td>3</td>
</tr>
<tr>
<td>$\frac{1}{3}(2270)$</td>
<td>0.264</td>
<td>4</td>
</tr>
</tbody>
</table>

Number of Photons per Event per unit wavelength

Wavelength (nm)

Fig 1.11
In 1966, Haberer (117) examined the measurement of beta activities in aqueous samples using Cerenkov radiation and during this investigation found that the addition of impurities such as NaNO₃, FeCl₃ and HNO₃ absorbed Cerenkov light thus reducing the count rate from a K⁴⁰ (Eₘₐₓ ≈ 1.35 MeV) solution. Furthermore, a plot of \( A = \log \frac{I_0}{I} \) versus the log of picric acid concentration showed a linear function over the range 0.02 to 200 mg cm⁻³. Thus the quench effect permitted the determination of coloured substances by "Cerenkov Photometry" after the addition of a known quantity of a hard beta emitter and reference to a previously established calibration curve.

In the same year, Ross (118) demonstrated that the absorption of BIF from a scintillator cocktail affords a quantitative method of analysis for the absorbing species. The technique, when compared to conventional absorption photometry, produces higher precision, increased stability, controlled sensitivity, simplicity, low power requirement and direct digital results. The method uses chlorine-36 (Eₘₐₓ 0.71 MeV) beta-particles to excite fluorescence from a scintillation solution containing p-dioxane, PPO and POPP. The fluorescence from the solution covered the wavelength range 370 to 550 nm, peaking at \( \sim 425 \) nm. Interposing metal-complex solutions of Fe, Ni and Cu in between the light source and the detector absorbed part of the emitted fluorescence.

More recently, an assay for uranium was described that uses the colour quenching by yellow peruranate ion on the Cerenkov emission from zinc-65 (119). Bobrowski (1979) (120) describes the Cerenkov light-self-absorption method which was used to study the kinetics of transient oxygen compound formation.
Kulscar (121) wrote a general article on Cerenkov photometry and investigated the fluorescence from quinine sulphate (122) as a function of concentration. The result shows that a log plot of reduction in Cerenkov light is a linear function of concentration.

The detection of eluted solutes, from an hplc system, by BIF is described in Chapter 2 and by the absorption of Cerenkov photons is described in Chapter 4.
CHAPTER 2. BETA INDUCED FLUORESCENCE

2.1 Preliminary Developments in the BIF Detector

Preliminary experiments (61) on beta-induced fluorescence, as a detection technique for liquid chromatography, showed that the major defect in the performance of the detector was that significant amounts of $^{63}$Ni were washed off of the wire when methanol or methanol/water mobile phases were used. Even when using relatively non-polar mobile phases such as hexane or cyclohexane, detectable amounts of $^{63}$Ni were discovered in the mobile phase. The increasing importance of the safety aspects of laboratory equipment suggests that there may be considerable difficulties associated with the marketing of a detector which could release significant quantities of radioactive material as a result of a minor operator error - such as the use of a mobile phase in which the radionuclide was soluble (61).

The problem may be overcome by

(i) using a physical barrier between the radionuclide and the flowing liquid. The barrier should be non-porous and be as thin as possible so as not to attenuate the electron flux emanating from the radionuclide. The barrier may be polymeric or metallic. A polymer coating has the advantage of being easily applied but suffers the possible disadvantage of not being able to withstand the constant bombardment by ionizing radiation. Furthermore, certain polymers fluoresce (123) under such conditions and this would result in an increase in background signal. Investigations of polymer coatings have been carried out elsewhere (124).

A metallic coating has the advantage that it can be applied
under very strict control, thus enabling a very thin sheath of metal to act as a protection. The protective layer is required to be insoluble in potential chromatographic mobile phases so that silver or gold are obvious candidates.

(ii) employing the correct plating conditions for electroplating $^{63}\text{Ni}$ on to the substrate. Whether a physical barrier is required at all is a debatable point, since the plating conditions of the radioactive wire were unknown. 15 mCi of $^{63}\text{Ni}$ were electroplated on to a 5 cm x 0.05 cm (dia.) copper wire by the Radiochemical Centre, Amersham. The surface of the plated wire appeared grey and heterogeneous. It was decided to investigate the plating of inactive nickel on to support wires to enable the best plating conditions to be determined. The electrodeposited nickel should

a) result from a dilute solution of nickel since, if successful, the active nickel would have to be plated from such a solution,

b) result from a plating bath at room temperature,

c) appear to be homogeneous and "bright" since this would result in a more stable nickel coating, and

d) be stable; i.e. not be washed off the support metal when in the presence of flowing liquids.

Points a, b and c were successfully overcome when electroplating nickel on to nickel or copper support wires. However, when under test in distilled water, the wires showed a loss of nickel, detected by atomic absorption and pulse polarography, from the electroplate. A spokesman for the Radiochemical Centre, Amersham, agreed that it would be
extremely difficult to produce a safe nickel-63 source by this method and so this line of research was abandoned.

(iii) employing an alternative source to $^{63}\text{Ni}$.

A source completely external to the liquid flowcell might be employed since it has the obvious advantage that no radiation could be carried away by the flowing liquid. This excludes the use of a pure alpha or beta emitter since the resultant attenuation of the particle flux would be such as to prevent fluorescence from the liquid. Gamma photons are employed to induce fluorescence from suitable solutions since this type of ionizing radiation is employed as an external standard for quench correction in liquid scintillation counting \((74)\). Although the use of gamma photons was argued against in Chapter 1, it was decided to investigate their use as an excitation source.

2.2. Use of Alternative Excitation Source to $^{63}\text{Ni}$

5 mCi of cobalt-57 (122 keV) and 16 pCi of caesium-137 (662 keV) were used for the investigation in the format shown in Figs. 2.1 and 2.2.

10 cm$^3$ of various concentrations of methyl-styryl benzene and anthracene in hexane and quinine sulphate in 0.01 M sulphuric acid were placed into separate polythene scintillation vials and placed, in turn, into the counting compartment of an Intertechnique SL20 liquid scintillation counter. Each vial was allowed to light adapt for five minutes before taking a series of one minute counts in the preset tritium channel. The results are presented in
Figs. 2.3 to 2.6, where the count rate is the count rate of the solution minus the count rate obtained from the solvent alone.
15 cm$^3$ of each solution shown in Table 2.1 were pipetted into separate polythene scintillation vials. Each bottle was placed, in turn, into the counting compartment and allowed to light adapt for five minutes. A series of one minute counts were taken in the preset tritium channel of the SL20. The tritium isotope was part of a neutron activation target and was of unknown activity but was thought to be a few curies of $^3$H activity. The $^3$H was held on a small copper disc which was placed into a semi micro glass test-tube. As shown in Fig. 2.7, clearly the maximum energy of a beta particle emitted from a $^3$H (0.018 MeV) nucleus prevents beta-particles entering the liquid. However, bremsstrahlung radiation is produced as the beta-particles slow down in the glass walls of the testtube, and it was thought that this radiation might be a suitable excitation source.
The parts per million concentrations of the solutions are shown in brackets. Solution 7 is a 10% (volume/volume) solution of dichloromethane in hexane. Solutions 3, 4, 5 and 6 are solutions in hexane, solution 8 is in 10% dichloromethane in hexane, and solution 10 is an aqueous solution. It should be noted that the counts per minute, values given in Table 2.1 are average values of five separate one minute counts and that the S-B values are calculated by

<table>
<thead>
<tr>
<th>ISOTOPE</th>
<th>$^{137}$Cs Liquid Count (min$^{-1}$)</th>
<th>$^{137}$Cs S-B (min$^{-1}$)</th>
<th>$^{57}$Co Liquid Count (min$^{-1}$)</th>
<th>$^{57}$Co S-B (min$^{-1}$)</th>
<th>$^{3}$H Liquid Count (min$^{-1}$)</th>
<th>$^{3}$H S-B (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMPTY VIAL</td>
<td>12238</td>
<td></td>
<td>14354</td>
<td></td>
<td>37220</td>
<td></td>
</tr>
<tr>
<td>HEXANE</td>
<td>28729</td>
<td>0</td>
<td>18723</td>
<td>0</td>
<td>48429</td>
<td>0</td>
</tr>
<tr>
<td>POP (10)</td>
<td>88846</td>
<td>60117</td>
<td>67463</td>
<td>48740</td>
<td>52903</td>
<td>4474</td>
</tr>
<tr>
<td>NAPHTHALENE (100)</td>
<td>30382</td>
<td>1653</td>
<td>18616</td>
<td>-107</td>
<td>45558</td>
<td>-2874</td>
</tr>
<tr>
<td>AMINO ACRIDINE (10)</td>
<td>24352</td>
<td>-4377</td>
<td>16728</td>
<td>-1995</td>
<td>33322</td>
<td>-15107</td>
</tr>
<tr>
<td>TOluene (100)</td>
<td>28586</td>
<td>-143</td>
<td>17939</td>
<td>-784</td>
<td>41181</td>
<td>-7248</td>
</tr>
<tr>
<td>DCM (10%)</td>
<td>29728</td>
<td>0</td>
<td>18742</td>
<td>0</td>
<td>39923</td>
<td>0</td>
</tr>
<tr>
<td>POP (10)</td>
<td>46820</td>
<td>17092</td>
<td>22294</td>
<td>3552</td>
<td>46820</td>
<td>6897</td>
</tr>
<tr>
<td>WATER</td>
<td>20934</td>
<td>0</td>
<td>17449</td>
<td>0</td>
<td>39728</td>
<td>0</td>
</tr>
<tr>
<td>AMINO ACRIDINE (10)</td>
<td>33045</td>
<td>12111</td>
<td>20636</td>
<td>3187</td>
<td>39006</td>
<td>-725</td>
</tr>
</tbody>
</table>
subtracting the count for the solvent, hexane, 10% DCM in hexane or water, from the solution count.

It can be seen from Table 2.1 that solutions 4, 5 and 6 give a lower count than the count from the solvent alone. This is due to self absorption of the emitted photons.

To investigate this problem further, three aqueous solutions of Rhodamine B of concentration 0.1, 1 and 10 part per million were made up and 15 cm$^3$ of each solution counted using $^{137}$Cs as the excitation source. The results are shown below in Table 2.2.

TABLE 2.2 Fluorescence from Solutions of Rhodamine B

<table>
<thead>
<tr>
<th>Concentration of Rhodamine B (ppm)</th>
<th>Counts (min$^{-1}$)</th>
<th>S-B (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMPTY VIAL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12903</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>23417</td>
<td>-869</td>
</tr>
<tr>
<td>1.0</td>
<td>22548</td>
<td>-1656</td>
</tr>
<tr>
<td>10.0</td>
<td>21761</td>
<td>-11059</td>
</tr>
</tbody>
</table>

Table 2.2 demonstrates the problem of colour quenching in large volume solutions. Clearly, this would be much less of a problem in the small path length of an hplc detector. Furthermore, the results presented above are recorded by coincidence counting. In coincidence counting, two photomultiplier tubes 'view' the polythene vial and a count is recorded when a photon of light enters both tubes at the same time. Thus single photon counting, using one photomultiplier, should be more sensitive to the detection of small quantities of materials provided that the events are single photon events and that the majority of the photons emitted by the solution enter the photomultiplier tube and strike the photocathode.
In Chapter 1, the large range and relatively low ionizing power of a gamma photon was discussed. To investigate the use of bremsstrahlung and gamma photons in short path length flowcells the following experiments were carried out.

2.3 Bremsstrahlung from a Tritium Source

A 1 cm x 1 cm piece of $^3$H neutron activation generator target was attached to the bottom of a 2.5 cm diameter cork by sello-taping aluminium foil on to the base of the cork. Teflon tubing, ID 1 mm, was trapped in between the aluminium foil and the photomultiplier tube. The whole assembly was encased in a lead castle to ensure that the photomultiplier tube was in the dark. The anode of the photomultiplier tube was connected, via a coaxial cable, to the input of a Nuclear-Enterprise SR5 Scaler-Ratemeter.

Hexane, 100 ppm POPCP in hexane, toluene based liquid
scintillant and a strong quinine sulphate solution were passed through the teflon tube, but no increase in counts was observed above the 240 cpm observed when the tube was empty of liquid.

2.4 Bremsstrahlung from a $^{147}$Pm Source

A $1\mu$Ci $^{147}$Pm point source, as shown in Fig. 2.11, was placed in contact with a 2.5 cm x 0.4 cm diameter quartz tube as shown in Fig. 2.9. The ends of the quartz tube were glued to $1/16$ dia, 10 thou ID, stainless steel tubing to allow liquid to enter and leave the quartz tube.

Hexane, 100 ppm POPOP in hexane, toluene liquid scintillator and a strong aqueous solution of quinine sulphate were passed through the flowcell but no increase in background count rate was recorded. When water alone was passed through the flowcell a count rate of 40 000 cps was observed. On placing a thin sheet
of lead between the $^{147}\text{Pr}$ source and the quartz tubing the background count rate fell to 2200 cps; a clear indication that the quartz was itself fluorescing due to excitation by beta-particles emanating from the $^{147}\text{Pr}$.

2.5 Use of $^{57}\text{Co}$ Source

A 5 mCi $^{57}\text{Co}$ source was placed into a lead pot which had a 2 mm diameter hole drilled in its side so that gamma photons could escape from the pot. The hole was positioned so that the gamma photons leaving the pot would pass into the quartz tubing as shown in Fig. 2.10.

Hexane, 100 ppm POPOP in hexane, toluene based scintillant and aqueous quinine sulphate solution were passed through the
quartz tubing but no increase in count rate was observed above the background of 325 cps observed when the flowcell was empty.

2.6 Use of Variable Energy X-ray Source

A variable X-ray source containing 10 mCi of Americium-241 and a number of different target materials (available from the Radiochemical Centre, Amersham) was used instead of the lead pot in Fig. 2.10.

Hexane and toluene based scintillant solution was passed through the quartz tube and the results, using a number of targets, are presented below.

**TABLE 2.3 Fluorescence Using X-rays for Excitation**

<table>
<thead>
<tr>
<th>TARGET</th>
<th>ENERGY (keV)</th>
<th>PHOTON YIELD (photons/s/steradian)</th>
<th>HEXANE (cps)</th>
<th>TOluene SCINTILLANT (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>22.10 24.99</td>
<td>38000</td>
<td>617</td>
<td>1080</td>
</tr>
<tr>
<td>Ba</td>
<td>32.06 36.55</td>
<td>46000</td>
<td>1303</td>
<td>1745</td>
</tr>
<tr>
<td>Tb</td>
<td>44.23 50.65</td>
<td>76000</td>
<td>515</td>
<td>820</td>
</tr>
</tbody>
</table>

**Summary of the Use of External Sources**

One of the major requirements of a liquid chromatographic detector is that it should possess a small dead-volume. Gamma photons clearly produce fluorescence from solvent and solutions and, in relatively large volume solutions, this technique may be developed to enable small quantities of a suitable solute to be determined. However, in small volume (~ 10 μl), small path length (~ 2 mm) flowing systems the ionizing power of a gamma photon is insufficient to cause appreciable excitation within the
solution, with the result that the application of a gamma excitation source to detection in HPLC is of little use.

With this in mind, it was decided to concentrate on the use of a beta-emitter as a fluorescence excitation source.

2.7 The Choice of Beta-Particle Emitting Isotope

A number of such sources are shown in Table 2.4, together with their decay half-lives (125) and the (theoretical) maximum available specific activity.

**TABLE 2.4 Properties of Some Beta Decay Radionuclides**

<table>
<thead>
<tr>
<th>NUCLIDE</th>
<th>$t_{1/2}$ (years)</th>
<th>$E_{\text{max}}$ (keV)</th>
<th>MAXIMUM SPECIFIC ACTIVITY ($Bq g^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3$H</td>
<td>12.5</td>
<td>18</td>
<td>$3.5 \times 10^{14}$</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>5730</td>
<td>156</td>
<td>$1.6 \times 10^{11}$</td>
</tr>
<tr>
<td>$^{32}$P</td>
<td>0.038</td>
<td>1700</td>
<td>$1.1 \times 10^{16}$</td>
</tr>
<tr>
<td>$^{35}$S</td>
<td>0.23</td>
<td>167</td>
<td>$1.6 \times 10^{15}$</td>
</tr>
<tr>
<td>$^{45}$Ca</td>
<td>0.45</td>
<td>255</td>
<td>$6.5 \times 10^{14}$</td>
</tr>
<tr>
<td>$^{63}$Ni</td>
<td>100</td>
<td>63</td>
<td>$2.1 \times 10^{12}$</td>
</tr>
<tr>
<td>$^{90}$Sr</td>
<td>30</td>
<td>500 and 2000</td>
<td>$4.9 \times 10^{12}$</td>
</tr>
<tr>
<td>$^{147}$Pm</td>
<td>2.5</td>
<td>230</td>
<td>$3.6 \times 10^{13}$</td>
</tr>
</tbody>
</table>

To obtain a high sensitivity from a BIF detector, it is clearly desirable that fluorescence detected from eluted materials should be recorded against a dark background, and for this reason those nuclides with $E_{\text{max}}$ (the maximum energy possessed by the beta particle) which lie significantly above the Cerenkov threshold (110) for beta particles cannot be employed as a BIF source.

Of the nuclides shown in Table 2.4, $^3$H, with a half-life of
12.5 years and maximum specific activity of \(3.5 \times 10^{14} \text{ Bq g}^{-1}\) looks a reasonable alternative to \(^{63}\text{Ni}\). However, the low \(E_{\text{max}}\) of \(^3\text{H}\) results in a considerable amount of self-absorption which would probably exclude its use in the HIF detector application.

\(^{14}\text{C}\) is excluded on the grounds of its relatively low specific activity (0.23 'g Ci\(^{-1}\)).

\(^{32}\text{P}, \, ^{45}\text{Ca}\) and \(^{35}\text{S}\) are excluded because of their short half-lives and \(^{90}\text{Sr}\) is excluded because its \(E_{\text{max}}\) lies well above the Cerenkov threshold.

Thus of the pure beta emitters \(^{147}\text{Pm}\) is the most viable alternative to \(^{63}\text{Ni}\). Furthermore, \(^{147}\text{Pm}\) is available as a 1mCi "point source" (code PHC.32, available from the Radiochemical Centre, Amersham), the elements of which are shown schematically in Fig. 2.11.

Fig. 2.11 The 1mCi "Point Source"

Fig. 2.12 represents the production of the source. Promethium (147) carbonate is mixed with an excess of silver and
$Pm_2\left(\text{CO}_3\right)_3 \cdot \text{Ag excess}$

SINTERED

ROLLED

+ SILVER FOIL

ROLLED

CIRCLES PUNCHED OUT

FACE IS 5µm

Fig 2.12
sintered. The resulting block is then rolled into a thin sheet and silver foil added to one surface. The silver foil and the active sheet are rolled so that the former has a final thickness of 5 µm. Circles, of approximate diameter 1.5 mm, are punched out and fixed into stainless steel holders as shown in Fig. 2.11.

Clearly an increase in activity may be achieved by punching out a larger area from the composite sheet. A 20 mCi "strip source" was produced this way and is described later.

A third 147Pm source was available for the BIF investigation and was supplied by New England Nuclear, North Billerica, Massachusetts, U.S.A. The elements of this 5 mCi "point source" are shown schematically in Fig. 2.13.

![Diagram](image)

**Fig. 2.13** The 5 mCi "Point Source"

The source matrix consists of 147Pm as a vitreous ceramic fused to the ceramic source holder, forming a ceramic glass.
Unfortunately, the supplied source was not overplated with a protective coating. Although no $^{147}$Pm activity was found in the mobile phases when this source was used, an extremely high background count rate (350 000 cps from 10% toluene in hexane) was observed indicating that the ceramic was fluorescing under the influence of the beta-particles. In order to decrease the light output, the source was coated with a metal coating using the apparatus shown in Fig. 2.14.

![Apparatus Used for Coating 5 mCi Source](image-url)
The 5 mCi source was placed into the apparatus shown in Fig. 2.14, the base of which was cooled by liquid nitrogen. A coiled length of nichrome wire was attached to the tungsten electrodes and the pressure inside the apparatus reduced to $10^{-7}$ torr by a mercury vapour diffusion pump backed by a rotary pump. A low voltage (DC) of approximately 10V was applied to the wire, which evaporated and coated the source.

After coating the source with metal, the background count rate was reduced to 9000 cps.

2.8 Flowcells Used in BIF

As the nickel-63 flowcell had shown that BIF, as a detection technique in hplc, was viable, and as it had been decided to replace nickel-63 with promethium-147, the primary objective was to design a flowcell which was easily constructed from readily available materials and that the design should be such as to enable the technique to be shown to be as sensitive as possible. With this objective in mind a series of flowcells were designed, around the three sources described previously, and are described below.

Mark I Flowcell

The flowcell was fabricated from stainless steel and is shown in Fig. 2.15. The inlet and outlet 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 x 19 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
Fig 2.15

Mk.1 BIF FLOW CELL
detected) may be calculated from the 2 mm diameter of the hole, containing the $\beta^-$ particle source and the distance between the face of the $\beta^-$ 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 $\mu$l and 10 $\mu$l could be used. Fig. 2.16 shows an enlarged diagram of the active volume of the flowcell.

![Diagram of the active volume of the flowcell]

**Fig. 2.16 Active Volume of the M$\times$1 Flowcell**

To determine the position of the source, with respect to the distance from the spectrosil window, which gave the highest signal to noise ratio, the source was moved into and out of the eluent flow by turning the source positioning screw a $\frac{1}{4}$ of a turn at a time. Background counts were determined, for each position,
when deoxygenated hexane flowed through the cell and signal counts were determined by observing the count rate at peak maximum when a hexane solution containing 100 ppm bis-methyl-styryl-benzene (MSB) was injected into the hexane mobile phase and passed through the flowcell. The counts were recorded on the apparatus shown in Fig. 2.24. To separate the MSB from the injected hexane, a 15 cm 10 μm Lichrosorb column was employed.

**TABLE 2.5 Relationship Between the Position of the Source and Signal Obtained**

<table>
<thead>
<tr>
<th>POSITION OF SOURCE</th>
<th>BACKGROUND(B) (cps)</th>
<th>SIGNAL (cps)</th>
<th>S-B(S) (cps)</th>
<th>$\frac{S}{B^2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20900</td>
<td>57500</td>
<td>36600</td>
<td>253</td>
</tr>
<tr>
<td>$+\frac{1}{4}$</td>
<td>21099</td>
<td>58500</td>
<td>37401</td>
<td>257</td>
</tr>
<tr>
<td>$+\frac{1}{2}$</td>
<td>23060</td>
<td>49000</td>
<td>25940</td>
<td>170</td>
</tr>
<tr>
<td>$-\frac{1}{4}$</td>
<td>20160</td>
<td>54750</td>
<td>34590</td>
<td>244</td>
</tr>
<tr>
<td>$-\frac{1}{2}$</td>
<td>19130</td>
<td>47000</td>
<td>27870</td>
<td>201</td>
</tr>
<tr>
<td>0</td>
<td>20800</td>
<td>57500</td>
<td>36700</td>
<td>254</td>
</tr>
</tbody>
</table>

The 0 position was thought to be where the top surface of the source was flat with the base of the eluent track. However, on closer examination of the flowcell, it was apparent that when the source was moved forward by a $\frac{1}{4}$ turn of the screw, it was in this position that the top surface of the source was flat with the track base.

Two conclusions may be drawn from this experiment. Firstly, there is a small ($\sim 10\%$) increase in the background count rate, when the distance between the source and the cell window is reduced by $\frac{1}{2}$ a turn of the screw. This is due to a larger number of beta particles striking the window thus producing a larger number of
fluorescence photons from the window. Secondly, the largest signal to noise ratio was obtained when the source was flat with the base of the eluent track. This is hardly surprising since movement of the source, either forward or backward, results in a disruption of flow over the active part of the source. Movement forward results in the solution flowing around the sides of the source and therefore less of the solution is excited by the beta-particles. Movement backwards, into the source hole, results in "trapping" hexane immediately above the source and therefore the beta-particles are absorbed by hexane before they are able to enter the solution and induce excitation. This experiment therefore shows the necessity for the solution to enter the active volume of the source. In practice it is necessary for all of the solution to come into direct contact with the surface of the $\beta$-emitting source. With this point in mind, the following flow-cell was designed.

**Mark II Flowcell**

![Flowcell Diagram](image-url)
In the Mark I flowcell the source is perpendicular to the mobile phase flow and, since the mobile phase track depth was 2 mm, it is possible that the $\beta^-$ particles may not be entering the upper volume of solution within the track. The Mark II flowcell (shown in Fig. 2.17) overcomes this problem by positioning the surface of the source directly in front of the eluent inlet tube. The cell body was machined from 4 mm diameter brass rod and a 3 mm diameter hole was drilled in the side to produce the excitation area. 1/16 inch stainless steel tubing, of internal diameter 10 thousandth of an inch, was soldered into the body to act as an eluent inlet. Quartz tubing of 6 mm inside diameter was sealed on to the cell body by an 'O' ring to enable the eluent to be discharged from the cell and to allow fluorescent photons to escape from the cell. As in the Mk I cell, the source position could be adjusted to enable the best signal to noise ratio to be obtained. The active volume of the flowcell may be calculated to be approximately 10 µl.

Several problems are associated with this cell and are detailed below:–

(1) The number of photons that are able to enter the photomultiplier tube is limited to those photons which escape from the cell through the quartz tube window. It is possible to electrolytically silverplate the cell compartment producing a highly reflecting surface, but this was not attempted.

(2) Due to the shape of the cell efficient optical contact with the photomultiplier tube window is not possible.

(3) During the time that the cell was in use it became evident that air bubbles became trapped in the cell compartment. After a period of time these would eventually dissolve in the eluent, but
this was a factor which must be borne in mind when designing future flowcells.

Due, mainly to factor (1), the following flowcell was produced.

Mark III Flowcell

Fig. 2.18

The cell is shown schematically in Fig. 2.18, and was machined from 35 mm diameter teflon rod. One side of the rod was milled flat so that the cell could make efficient optical contact with the photomultiplier window. The area, on the flat face, around
the active volume was reduced to produce a teflon window thickness of approximately 1.5 mm. Eluent inlet and outlet connections were made to the cell by 0.5 mm internal diameter teflon tubing and $\frac{1}{4}$" pipe thread "Cheminert" fittings.

Finally, an aluminium foil reflector was taped around the back of the cell to reflect those photons which are produced in such a direction that they would not enter the photomultiplier tube directly. Chromatograms of 20ng each of anthracene, diphenyl hexatriene and bis-methyl-styryl-benzene are presented in C1 and C2 to demonstrate the use of the aluminium foil reflector.
Clearly it is advantageous to collect as many fluorescent photons as possible, but any increase in collection efficiency of the solute fluorescence is accompanied by an increase in background fluorescence when eluent alone resides in the cell. For example, if the figure of merit for a cell is taken as $S/B^2$, for a given compound, then increasing the collection efficiency by a factor of 2 increases $S/B^2$ by a factor of $\sim 1.41$. Clearly $S/B^2$ may be increased further by optically filtering out the fluorescence from the eluent. This is possible since the wavelength at which eluent fluorescence occurs is, in general, lower than the fluorescent wavelength of the compounds of interest. (see HIF spectra, Chapter 3). Since the body of this cell is made from teflon, the cell itself acts as an optical filter, being unable to transmit wavelengths shorter than $\sim 300$ nm. The absorption spectrum of teflon is shown in Fig. 2.19, and was obtained by spraying teflon on to one face of a 1 cm quartz cell.

Fig 2.19

ABSORPTION SPECTRUM OF TEFOLON
In later experiments it became evident that optical cut-off filters, interposed between the cell and the photomultiplier window, increased $S/B^{1/2}$ still further and therefore the use of teflon as a cell window became less advantageous.

The major disadvantage associated with this cell is its relatively large dead volume ($\sim 38 \mu l$). It is possible to reduce this, but this required workshop facilities which were sometimes not available. Furthermore this cell could not be easily adapted to incorporate the larger activity $^{147}$Pm sources which were now available. Two cells will be described which were capable of taking these sources.

Mark IV Flowcell

Fig. 2.20
The $^{147}$Pm activity is in the form of a 20 mCi "strip" source of overall dimensions 25 mm x 4 mm x 1 mm. The activity is contained in an area 20 mm x 2 mm and is completely covered by silver foil. This source was supplied by the Radiochemical Centre, Amersham, as an experimental source, and is not available for general distribution.

The cell body, track washer and window retaining ring was machined from 33 mm (across flats) hexagonal brass rod. The eluent track washer was milled so that an eluent track 28 mm x 2 mm x 2 mm deep was produced. The eluent inlet and outlet tubes were soldered into the body of the cell so that they were flush with the base of the source retaining rectangle. The cell window was cut from a pyrex glass microscope slide, the optical transmission characteristics of which are shown in Fig. 3.4.

This cell was designed to show that an increase in beta-particle activity resulted in an increase in the fluorescence signal from both the fluorescent solute and from the mobile phase. However, the very large dead volume prevented any serious consideration being given to the cell's use in hplc.

**Mk V Flowcell**

The design of this flowcell is shown in Fig. 2.21. The flowcell body was machined from ptfe rod and screw threaded at each end with $\frac{1}{4}$" pipe thread. One end of the cell was closed with a ptfe plug and the other end was screwed into the reflector adaptor, through which passed a $\frac{1}{16}$" stainless steel (0.008" bore) inlet tube. The inlet tube also passed through a Cheminert T connector, shown in Fig. 2.22, to take the flowcell outlet to waste.
A variation of this flowcell was made by replacing the teflon tube with 6 mm (ID) quartz rod. This cell is shown in Photograph 1.

The flowcell was designed with the following considerations in mind.

1. It must contain the 5 mCi "point source" of $^{147}$Pm as described previously.

2. It must be capable of being adapted to contain the 1 mCi "point source" of $^{147}$Pm used in earlier work. This adaptation is readily achieved by replacing the ptfe cell body by a 50 mm length of ptfe tubing (nominal $\frac{1}{8}$" od) fitted at each end with a "Cheminert" flanged connector for $\frac{1}{4}$" pipe thread fittings. One end of the tubing was reamed out with a 2 mm drill bit so that the 1 mCi $^{147}$Pm source could be fitted into the tubing.

3. It must have an active volume of $<10 \mu l$. This volume may be calculated from the diameter of the ptfe cylinder surrounding the source and the distance between the end of the stainless steel inlet tubing and the face of the source. This latter distance may be varied (by moving the source), and if a distance of 1 mm is chosen, this results in an active volume of $\sim 3.2 \mu l$ for the 1 mCi source and $\sim 9.6 \mu l$ for the 5 mCi source.

4. It must allow efficient detection of the emitted photons at wavelengths down to $\sim 370$ nm (the lowest wavelength cut-off filter used for the suppression of eluent emission). This objective is achieved by the use of a reflector extracted from an Everready torch.
Photograph 1 shows some of the flowcells just described.

PHOTOGRAPH 1

2.9 HPLC Equipment

The HPLC equipment used during this work was similar to that described in Chapter 1 and is shown in Photograph 2. For HIF operation, the mobile phase was deoxygenated by bubbling argon gas, delivered from a cylinder, through the liquid. This is essential in HIF since it is well known that oxygen quenches BIF with high efficiency (see Chapter 1). However, during the operation of the Cerenkov detector it is not necessary to deoxygenate the mobile phase and so this part of the equipment may be dispensed with. The mobile phase was pumped through the column by an Eldex dual piston pump (model AA-94-8) supplied by Owens Polyscience Ltd., Macclesfield, Cheshire. A pressure gauge, 0-4000 psig, was fitted in between the injection valve and the pump, by means of a stainless steel "Cheminert" T piece. The pump fed mobile phase
Photograph 2

Photograph 3
to a Rheodyne valve (model 7125, purchased from ACS, Luton). The valve was fitted with a 20 µl loop which could be completely filled with sample solution or partially filled by using a 0-10 µl calibrated syringe, making possible the injection of different volumes of sample solution. The injection valve was connected to the top of the column by a short length (~ 10 cm) of stainless steel tubing. Various columns were used throughout the project and they are described in the relevant parts of this thesis. The base of the column was connected to the flowcell within the coffin.

Photograph 3 shows the inside of the coffin with the Mk I flowcell in its working position, adjacent to the photomultiplier tube. The flowcell and PM tube window was encased in an aluminium box, placed inside the coffin, to ensure that the environment around the PM tube window was light tight. On the right of the PM tube case can be seen the HV bias, and anode output signal, leads which are connected to the PM tube.

2.10 Photon Detection and Pulse Counting Equipment

Photons emanating from the flow cell entered a quartz window photomultiplier tube (PMT). The PMT was an EMI type 9804 QB, bialkali photo cathode, 13 stage tube which 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. 2.23. The electronic pulses produced at the output were counted using a fast (30 MHz) nuclear counting system which consisted of the following Nuclear Instrument Modules (shown in Photograph 4) supplied by Canberra Ltd. (Farringdon, Great Britain) and connected as shown schematically in Fig. 2.24.
Fig. 2.23

(1) Amplifier, Model 2110 timing filter amplifier

The output pulses from the emitter follower were negative pulses, with a pulse height of between 50-100 mV, a half width of \(\sim 50\) ns and base to base width of \(\sim 100\) ns. These pulses were fed to the input of the timing filter amplifier (TFA), whose main purpose was amplification and pulse shaping, by a coaxial cable with 50\(\Omega\) BNC, UC-1094/U connectors at each end. The front panel of the TFA contained a fine gain control which was a single turn potentiometer which selected a variable gain factor from X0.45 to X1. Coarse gain was achieved by a six-position rotary switch which selected gain factors of X3, X6, X12, X25, X50 and X100. Pulse shaping was achieved by a six-position rotary switch which selected an integrating time constant (10, 20, 50, 100 and 200 ns) to control the rise time of the output pulses. The
FIG. 2.4

BIAS SUPPLY

PMT

FLOWCELL

AMPLIFIER

SHAPER

COUNTER

RECORDER

FILTER

RATEMETER
integrating time constant could be switched to the out position, equivalent to an integrating time of $\approx 2.3$ ns.

A third six-position rotary switch selected differentiation time constants of 10, 20, 50, 100 and 200 ns, to control the decay time of the output pulses.

(2) **Shaper, Model 1433 Discriminator**

The pulses from the TFA were fed, via a short length of coaxial cable, to the shaper which was employed to produce fast logic pulses. The signal input of the shaper accepts negative pulses in the range of $-0.030$ volts to $-6$ volts without saturation and is capable of a count rate $>100$ MHz. On the front panel of the shaper is a threshold control which is continuously variable from 30 mV to 600 mV. In practice, this control was always set to 0.00, corresponding to a threshold of 30 mV, since it was shown that this setting resulted in the largest signal from the BIF flow-cell.

Three BNC connections, on the front panel, provide for two negative and one positive outputs. One negative output was connected to the input of the counter whilst the positive output was connected to the ratemeter. Both connections were again made by coaxial cable and BNC (UG-1094/u) connectors, and the input connection to the ratemeter was terminated with a 50 $\Omega$ terminator connected by a BNC 'T' piece.

(3) **Ratemeter, Model 1481L, Lin/Log.**

The Model 1481L Lin/Log Ratemeter offers a choice of either linear or logarithmic presentation, via a front panel meter, of the average number of pulses per second being received at the front panel input.
In the linear mode, the ratemeter has ten full scale ranges, from 10 to 500,000 counts per second, permitting the choice of a wide input range. In this mode, the input pulse rate may be integrated over any one of four switch-selectable time constants, from 0.5 to 40 seconds.

In the logarithmic mode, the ratemeter has two 3-decade ranges, from $10^1$ to $10^4$ counts per second, and from $10^2$ to $10^5$ counts per second.

On the rear panel of the ratemeter a 0-100 millivolt output was used to drive the pen of a Servoscribe 2S potentiometric recorder. Interposed between the ratemeter and the recorder was an electronic filter/amplifier (as described in Appendix 1), which was used to remove the statistical fluctuations in recorded count rates. The filter also provided amplification and d.c. offset facilities.

(4) Counter, Model 1776 dual Scaler/timer

The counter was used to determine the integrated pulse count under a chromatographic peak. Since the counter had two channels available, one was used for observing the integrated count for a preset time, whilst the other was employed as a timer. The time taken for the whole of peak to pass through the detector was determined from the chart recorder. The timer was then set for greater than this time (e.g. 100 seconds) and as the peak entered the detector, shown by the recorder, the timer was switched on. In all cases, a background integrated count was determined for the preset time, when only mobile phase through the detector. Thus the integrated count, due to the eluted fluorescent material alone was determined. This is shown in Fig. 2.25.
Fig. 2.25

Integrated peak count = observed peak count - background count
In quenched beta-induced fluorescence

Integrated peak count = background count - observed peak count.

The count rate at peak maximum was determined by calibrating pen movement in terms of the observed count rate as shown by the ratemeter.

Photograph 4 shows the module components of the counting system and Photograph 5 shows the whole hplc system.
2.11 BETA INDUCED FLUORESCENCE IN NORMAL PHASE OPERATION

In normal phase hplc, the stationary phase is more polar than the mobile phase. Normal phase is now generally accepted to be adsorption chromatography, the most popular stationary phases being silica and alumina. Components within a sample mixture compete with molecules of the mobile phase for active sites on, and within, the stationary phase and are separated due to the differences between the polarities of the components within the mixture. In general, the most polar solute within the sample mixture is retained by the column for the longest time. The polarity of the mobile phase can be adjusted, either by the use of a different solvent or by the use of binary and tertiary mixtures of solvents, so that suitable retention times (time from injection to elution of peak maximum) of solutes results.

2.12 Response of HIF Detector to Eluted Materials

**Instrument Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>5 μm Lichrosorb, 15 x 0.6 cm.</td>
</tr>
<tr>
<td>Cell type</td>
<td>Mk I</td>
</tr>
<tr>
<td>Eluent</td>
<td>Hexane</td>
</tr>
<tr>
<td>Flowrate</td>
<td>1 cm³ min⁻¹</td>
</tr>
<tr>
<td>TFA</td>
<td>g x 25, 50/50</td>
</tr>
<tr>
<td>Ratemeter</td>
<td>R10⁵ LIN</td>
</tr>
<tr>
<td>Filter</td>
<td>g x 1, cut 0.1</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.2 cm min⁻¹</td>
</tr>
</tbody>
</table>

Compounds shown in Table 3.1, Chapter 3, were dissolved in hexane (Fisons, Loughborough, HPLC grade) and diluted to a final concentration of 100 ppm (wt/vol.). Each resulting solution was injected into the flowing eluent using an Altex model 905 sample injection valve fitted with a 20 μl sample loop. Thus the mass of each compound injected was of the order of 2 μg. Elution was with hexane containing varying amounts of toluene as a polar modifier. In all cases the
eluent was deoxygenated by purging with argon.

The response of the BIF detector is shown in integrated peak counts $\mu g^{-1}$ sample and were obtained by using the method described previously.

Fig. 3.1 shows examples of individual peaks obtained during the experiment described above.

### 2.13 Linearity of Response of the BIF Detector

**Instrument Parameters**

- **Column**: 5 $\mu$m Lichrosorb, 15 x 0.6 cm
- **Cell type**: Mk I
- **Eluent**: Hexane
- **Flowrate**: 1 cm$^3$ min$^{-1}$
- **TFA g x 25, 50/50**
- **Rate meter**: $R_{10}^5$ LIN
- **Filter**: g x 1, Cut 0.1
- **Recorder speed**: 0.2 cm min$^{-1}$

To demonstrate the linearity of response over a range of sample loadings a number of known concentration solutions of p-terphenyl in hexane were prepared. 20 $\mu$l of each solution were injected into deoxygenated hexane and the integrated count response for each peak was determined as described above. The peak count response was determined at least four times for each individual solution and the average peak count response plotted against concentration.

The results are presented graphically in Fig. 3.2.

The limited solubility of p-terphenyl in hexane prevented the extension of the concentration beyond 100 ppm. However, the results for fluorene in hexane are shown in Fig. 3.3.

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. Two techniques have been examined for enhancing this difference in an attempt to improve the sensitivity of the HIF 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 material.

2.14 Effect of Adding Fluorescent Material to Eluting Solvents

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>5 µm Lichrosorb 15 x 0.5 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk I</td>
</tr>
<tr>
<td>Eluent</td>
<td>Hexane, hexane/toluene</td>
</tr>
<tr>
<td>Flowrate</td>
<td>1 cm³ min⁻¹</td>
</tr>
<tr>
<td>TFA</td>
<td>g x 25, 50/50</td>
</tr>
<tr>
<td>Ratemeter</td>
<td>R10⁵ LIN</td>
</tr>
<tr>
<td>Filter</td>
<td>g x 1, Cut 0.1</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.2 cm min⁻¹</td>
</tr>
</tbody>
</table>

20 µl of a 100 ppm solution of bis-methyl-styryl-benzene in hexane were injected into deoxygenated hexane and the integrated peak counts were recorded on the scaler. The background counts, when mobile phase alone flowed through the HIF cell, were determined for the same time that it had taken for the peak to pass through the detector. The actual peak count was calculated by subtracting the background count from the observed peak count.

The above procedure was repeated with deoxygenated 1, 5 and 10% (vol/vol) toluene in hexane and toluene as the eluent.

The results are presented in Table 3.2, Chapter 3. The peak counts/µg are average counts of at least four determinations.

Chromatogram 3 shows the separation obtained by injecting 20 µl of a solution containing 20 ppm each anthracene, diphenyl hexatriene and bis-methyl-styryl-benzene into deoxygenated 0.2% acetonitrile in hexane.
Chromatogram 4 shows the same separation using deoxygenated 10% toluene in hexane as the mobile phase. In both separations the 5 mCi $^{147}Pm$ source was employed in the Mk V flowcell.

The second technique, used to improve the sensitivity of the HIF detector, involves the use of optical cut-off filters. The filters are placed between the flow-cell and the photomultiplier tube in an attempt to filter out the HIF background from the eluent while allowing the fluorescence from eluted materials to be detected normally.

2.15 Effect of Filtering Fluorescence on Response of HIF Detector

**Instrument Parameters**

<table>
<thead>
<tr>
<th>Column</th>
<th>5 µm Lichrosorb 15 x 0.6 cm TFA g x 25, 50/50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk I</td>
</tr>
<tr>
<td>Eluent</td>
<td>10% toluene in hexane</td>
</tr>
<tr>
<td>Flowrate</td>
<td>1 cm³ min⁻¹</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.2 cm min⁻¹</td>
</tr>
</tbody>
</table>

An L39 Nikon camera cut-off filter (λ cut ~ 370 nm, see Fig. 3.4) was placed in between the flow-cell and the photomultiplier tube. A piece of black paper, with a 3 cm diameter hole cut in it, was placed between the filter and the photomultiplier tube to ensure that only light that passed through the filter entered the photomultiplier tube. After allowing the photomultiplier tube to light adapt, deoxygenated hexane was passed through the cell and the background count rate determined. 20 µl of a 100 ppm solution of bis-methyl-stryryl-benzene was injected into the eluent and the observed integrated peak counts determined. The eluent was replaced by deoxygenated 1, 5 and 10% toluene in hexane and toluene and the integrated peak count and background count-rate redetermined for each eluent.
The L39 filter was replaced by a Pyrex microscope slide (λ cut ~ 320 nm, see Fig. 3.4) and the procedure, described above, repeated.

The results are presented in Table 3.3.

2.16 Investigation of Cut-Off Filters

**Instrument Parameters**

<table>
<thead>
<tr>
<th>Column</th>
<th>Waters µParasil 30 x 0.4 cm.</th>
<th>TFA g x 25, 50/50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk V, 1 mCi 147Pm.</td>
<td>Ratemeter R5 x 10^4 LIN</td>
</tr>
<tr>
<td>Eluent</td>
<td>10% toluene in hexane</td>
<td>Filter g x 5, Cut 0.25</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm^3 min^-1</td>
<td>Recorder speed 0.2 cm min^-1</td>
</tr>
</tbody>
</table>

Five camera cut-off filters (LIA, 385, L39, 400, YK2) were available for this investigation. The UV transmission characteristics of each filter are shown in Fig. 3.4.

The LIA filter was placed in between the flowcell and the photomultiplier tube and allowed to light adapt. Deoxygenated 10% toluene in hexane was passed through the flowcell and the background count rate was determined. 20 µl of a solution containing 1 ppm each of anthracene, diphenyl hexatriene and bis-methyl-styryl-benzene in hexane were injected into the eluent and the peak heights (mm) of the separated components determined. The LIA filter was replaced by the 385, L39, 400 and YK2 filters and in each case the background count rate and component peak heights were redetermined. The results are shown in Table 3.4.

The Mk V flowcell uses a reflector to reflect the fluorescence light, from the flowcell, into the PM tube. A filter/reflector may be produced for this flowcell if the reflective coating is made from a material which reflects light of wavelength longer than (say)
370 nm but is transparent to light of shorter wavelength. Fig. 2.26 shows the reflective properties of a selection of materials (126).

Fig. 2.26 shows that titanium dioxide reflects light of wavelength longer than ~400 nm, and therefore may be a suitable material for use as a filter/reflectors in conjunction with the Mk V flowcell.
2.17 Investigation of Reflectors for the Mk V Flowcell

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>5 μm Spherisorb 15 x 0.4 cm.</th>
<th>TFA g x 25, 50/50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk V, 5 mCi $^{147}$Pm.</td>
<td>Rate meter R5 x $10^4$ LIN.</td>
</tr>
<tr>
<td>Eluent</td>
<td>10% toluene in hexane.</td>
<td>Filter g x 10 Cut 0.175.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm$^3$ min$^{-1}$</td>
<td>Recorder speed, 0.5 cm min$^{-1}$.</td>
</tr>
</tbody>
</table>

Three reflectors were available for this investigation:

1. Chrome on glass (as used in a projector);
2. Chrome on plastic, extracted from an Ever Ready torch;
3. As (2) with chrome stripped off and an emulsion of TiO$_2$ painted on.

The L39 cut-off filter was placed in between the flowcell and the PM tube window. Deoxygenated 10% toluene in hexane was passed through the hplc system. The chrome on glass reflector was fitted to the flowcell, and the distance from the end of the reflector to the active area of the source adjusted to be 10 mm. The system was allowed to light adapt and the background count rate determined.

20 μl of a solution containing 0.1 ppm each of diphenyl hexatriene and bis-methyl-styryl-benzene were injected into the mobile phase and the separated peak heights determined.

The background count rate and peak heights were redetermined for distances of 12, 14, 16, 18, 20, 22 and 24 mm from the end of the reflector to the PM tube window.

The experiment was repeated for the chrome on plastic and TiO$_2$ on plastic reflectors and the results are shown in Table 3.5.

Fig. 3.5 shows a graph of S/B$^2$ against distance for the three reflectors.
2.18 Response and Sensitivity of HIF Flowcells

Instrument Parameters

Column

5 μm Spherisorb 15 x 0.4 cm. TFA g x 25, 50/50.

Cell type

All types.

Eluent

10% toluene in hexane.

Flow rate

1 cm³ min⁻¹

Ratemeter R 5 x 10⁴ LIN.

Filter g x 5, Cut 0.175.

Recorder speed. 0.5 cm min⁻¹.

Throughout the investigation of HIF detection in HPLC, the primary objective was the search for a cell system which provided a response and sensitivity which was comparable with currently available fluorescence HPLC detectors. It was therefore decided that, as each cell was developed, the response and sensitivity of the cell could be recorded by separating an injected mixture of anthracene, diphenyl hexatriene and bis-methyl-styryl-benzene into an eluent of 10% toluene in hexane. The eluted peaks of the separated components were recorded on the chart recorder and the response and sensitivity of the flowcell calculated as described on page 5.6.

For the Mk 1 and Mk 2 flowcells, the calculated response was relatively small and therefore 1 ppm (20 ng injected) of each component in the mixture was injected into the eluent. For the other cells, where the relative response is higher, 0.1 ppm (2 ng injected) of each component was separated for purposes of comparison. In all cases the eluent was deoxygenated by purging with argon and the L39 camera filter was interposed between the cell and the photomultiplier tube.

Table 3.6 shows the response and sensitivity of each cell, for the three component mixture. The noise was measured in mV, and taken from the chart trace as the maximum peak to peak variation in the baseline.
Example chromatograms are shown in Chromatograms 5, 6, 7 and 8, Chapter 3.

To enable a comparison to be made with a commercially available detector, a Perkin Elmer 1000 fluorimeter was connected, to the base of the column, in place of the HIF detector. The output of the fluorimeter was fed to the electronic filter, to enable various gains and frequency cut-offs to be selected, thus keeping the time constant comparable with the HIF detector.

The 10% toluene in hexane eluent was replaced by 0.1% acetonitrile in hexane which was deoxygenated by purging with argon. The excitation wavelength could be selected by interposing a narrow band-pass-filter between the excitation source and the flowcell. A 292 nm filter was used initially, to determine the most favourable emission wavelength. Response and sensitivity calculations were made for the three components of the mixture at emission wavelengths of 400, 420, 430 and 450 nm and they are presented in Table 3.7. Since the only other excitation filter available was a 337 nm filter, this was used in place of the 292 nm filter and response and sensitivity calculations made at an emission wavelength of 430 nm. The calculations are shown in Table 3.8.

For the above calculations of $R_c$ and $S_c$ to be valid, it is desirable to demonstrate that the HIF detector is a concentration dependent detector. The following investigation was undertaken to show this was the case.

2.19 Effect of Flow rate on Response of HIF Detector

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>Waters pPorasil 30 x 0.4 cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFA g x 25, 50/50</td>
<td></td>
</tr>
<tr>
<td>Cell type</td>
<td>Mk V, 5 mCi $^{147}$Pm.</td>
</tr>
<tr>
<td>Ratemeter</td>
<td>R5 x $10^5$ LIN.</td>
</tr>
</tbody>
</table>
Eluent 10% toluene in hexane. Filter g x 1, Cut 0.175
Flow rate 1 cm$^3$ min$^{-1}$. Recorder speed, 0.2 cm min$^{-1}$.

10% toluene in hexane was deoxygenated and passed through the column and the BIF detector. The flow rate of the mobile phase was adjusted to 0.750 cm$^3$ min$^{-1}$, by timing the collection of 1 cm$^3$ of mobile phase in a 2 cm$^3$ burette. 20 μl of a 10 ppm solution of diphenyl hexatriene were injected into the mobile phase and the observed integrated peak count recorded as the solution passed through the detector. The background count, when mobile phase alone passed through the cell, was recorded for the same time that the peak count was recorded. The integrated peak count is calculated by subtracting the background count from the observed integrated peak count.

The integrated peak count for diphenyl hexatriene was determined for a number of different flow rates and are shown in Table 3.9. Fig. 3.6 shows a plot of integrated peak counts versus the reciprocal of the flow rate.

2.20 Examples of Chromatograms Using the BIF Detector

(a) Instrument Parameters

Column 5 μm Lichrosorb 15 x 0.6 cm, TFA g x 25, 50/50.
Cell type Mk II, Ratemeter R10$^4$ LIN.
Eluent Hexane, Filter, g x 1, Cut 0.1.
Flow rate 1 cm$^3$ min$^{-1}$. Recorder speed, 0.2 cm min$^{-1}$.

20 μl of a solution of 20 ppm each of anthracene, p-terphenyl, perylene, 1,6-diphenyl-1,3,5-hexatriene and bis-methyl-styryl-benzene were injected into deoxygenated hexane. The resulting chromatogram is shown in Chromatogram 9. It should be noted that when using
hexane (not doped with toluene) the background count rate is sufficiently low as to make the use of a cut-off filter unnecessary.

Chromatogram 10 shows the result of separating a mixture containing 10 ppm of each of the above compounds. The background count rate was 4600 cps.

(b) Instrument Parameters

Column Waters µ Porasil 30 x 0.4 cm. TFA g x 25, 50/50.
Cell type Mk IV.
Ratemeter R10^5 LIN.
Eluent 0.03% acetonitrile in hexane. Filter g x 5 Cut 0.175
Flow rate 1 cm^3 min^{-1} Recorder speed 0.5 cm min^{-1}.

0.03% acetonitrile in hexane was deoxygenated and passed through the hplc system. 20 µl of a solution containing 4 ppm each of anthracene, p-terphenyl, 1,6 diphenyl-1,3,5-hexatriene and bio-methyl-styryl benzene in hexane were injected into the flowing eluent and the resulting chromatogram is shown in Chromatogram 11.

Chromatogram 12 shows the result of separating a solution containing 1 ppm each of the above compounds.

In both cases, no camera cut-off filter was used. The background count rate was 14600 cps.

(c) Instrument Parameters

Column 5 µm Lichrosorb 15 x 0.6 cm. TFA g x 25 50/50.
Cell type Mk I.
Ratemeter R10^5 LIN.
Eluent 1% toluene in hexane. Filter g x 3 Cut 0.1
Flow rate 1 cm^3 min^{-1} Recorder speed 0.2 cm min^{-1}.

A pyrex microscope slide was placed in between the flow cell and the PM tube. 1% toluene in hexane was deoxygenated, by purging with argon, and passed through the hplc system. 20 µl of a solution containing 15 ppm each of fluoranthene, benzo [a] pyrene and 3-methyl
cholanthrene in hexane were injected into the eluent and separated on the column. The resulting chromatogram is shown in Chromatogram 13, and the background count rate was 27500 cps. Chromatogram 14 was recorded when the L39 filter was placed between the flowcell and the PM tube window. In this case the background count rate was 3200 cps.

(d) **Instrument Parameters**

- Column: Waters µPorasil 30 x 0.4 cm, TFA g x 25, 50/50.
- Cell type: Mk IV.
- Eluent: 2% toluene in hexane.
- Flow rate: 1 cm$^3$ min$^{-1}$
- Filter: g x 2 Cut 0.175
- Recorder speed: 0.5 cm min$^{-1}$

1% toluene in hexane was deoxygenated and passed through the hplc system. 20 µl of a solution containing 1 ppm each of fluoranthene, benzo [a] pyrene and 3-methyl cholanthrene in hexane were injected into the eluent. The resulting chromatogram is shown in Chromatogram 15. The chromatogram was obtained using the LIA cut-off filter, the background count rate 18000 cps. Chromatogram 16 shows the same separation recorded at a recorder speed of 0.2 cm min$^{-1}$.

(e) **Instrument Parameters**

- Column: Waters µPorasil 30 x 0.4 cm, TFA g x 25, 50/50.
- Cell type: Mk IV.
- Eluent: 15% toluene in hexane.
- Flow rate: 1 cm$^3$ min$^{-1}$.
- Filter: g x 2 Cut 0.175.
- Recorder speed: 0.5 cm min$^{-1}$

15% toluene in hexane was deoxygenated and passed through the hplc system. 20 µl of a solution containing 1 ppm each of pyrene, diphenyl hexatriene and bis-methyl-styryl-benzene were injected into the eluent and separated on the column. The result is shown in Chromatogram 17.
The chromatogram was recorded using the LIA cut-off filter and the background count rate was 62500 cps.

(f) **Instrument Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Waters µ Porasil 30 x 0.4 cm. TFA g x 25, 50/50.</td>
</tr>
<tr>
<td>Cell type</td>
<td>Mk IV.</td>
</tr>
<tr>
<td>Eluent</td>
<td>15% toluene in hexane. Filter g x 2 Cut 0.175.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm³ min⁻¹. Recorder speed 0.5 cm min⁻¹.</td>
</tr>
</tbody>
</table>

15% toluene in hexane was deoxygenated and passed through the hplc system. 20 µl of a solution containing 1 ppm each of pyrene and benzo[a]pyrene were injected into the eluent and separated on the column. The result is shown in Chromatogram 18. The chromatogram was recorded using cut-off filter and the background count rate was 62500 cps. Chromatogram 19 shows the same separation on gain X1 of the filter/amplifier.

(g) **Instrument Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Waters µ Porasil 30 x 0.4 cm. TFA g x 25, 50/50.</td>
</tr>
<tr>
<td>Cell type</td>
<td>Mk V, 5 mCi ¹⁴⁷Pm.</td>
</tr>
<tr>
<td>Eluent</td>
<td>0.5% Acetonitrile in hexane Filter g x 5 Cut 0.175.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm³ min⁻¹. Recorder speed 0.5 cm min⁻¹.</td>
</tr>
</tbody>
</table>

0.5% acetonitrile in hexane was deoxygenated and passed through the hplc system. 20 µl of a solution containing 880 ppm of p-dimethoxy benzene, 4 ppm NN¹dimethylaniline and 2 ppm PPO-diphenyl oxazole were injected into the flowing eluent and separated on the column. The result is shown in Chromatogram 20. A 390 nm cut-off filter was used to reduce the background count rate to 40000 cps.
2.21 BETA INDUCED FLUORESCENCE IN REVERSED PHASE OPERATION

In reversed phase hplc, the stationary phase is less polar than the mobile phase. Reversed phase is partition chromatography, the liquid stationary phase being bound to an inert solid support.

Components within a sample mixture are partitioned between the mobile phase and the stationary phase. Since a component can only progress down the column when it is in the mobile phase, the component within a mixture which is most soluble (i.e. least polar) in the stationary phase is retained by the column for a longer time. Thus separation, in reversed phase chromatography, is affected due to the different solubilities of the components in a mixture in the stationary phase.

2.22 Response of HIF Detector to Eluted Materials

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>5 μm Spherisorb ODS 25 x 0.4 cm. TFA g x 25 50/50.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk I.</td>
</tr>
<tr>
<td>Eluent</td>
<td>various</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm$^3$ min$^{-1}$</td>
</tr>
<tr>
<td>Ratemeter</td>
<td>R5 x 10$^4$ LIN.</td>
</tr>
<tr>
<td>Filter</td>
<td>g x 1 Cut 0.175.</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.2 cm min$^{-1}$.</td>
</tr>
</tbody>
</table>

Compounds shown in Table 3.10 were dissolved in methanol and 20 μl of each sample was injected into the deoxygenated eluent shown in Table 3.10. In each case, a pyrex microscope slide was placed in between the flowcell and the window of the photomultiplier tube. Table 3.10 shows the background count rate from each mobile phase and the integrated peak count response of each compound normalised to 1 μg of compound.
2.23 Effect of Adding Fluorescent Material

(a) 1-Naphthol

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>5 μm Spherisorb ODS, 15 x 0.4 cm. TFA g x 25, 50/50.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk I.</td>
</tr>
<tr>
<td>Eluent</td>
<td>various</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm$^3$ min$^{-1}$.</td>
</tr>
</tbody>
</table>

Methanol was deoxygenated by purging with argon and passed through the hplc system. 20 μL of a solution containing 100 ppm of 1-Naphthol in methanol were injected into the mobile phase. No peak was detected. The methanol was replaced by 1% toluene in methanol which was deoxygenated with argon. 20 μL of the 1-Naphthol solution were injected and the integrated peak counts recorded. Table 3.11 shows the integrated peak counts from 1-Naphthol and the solvent background count rate when using methanol, 1%, 5% and 10% toluene in methanol and 10% benzyl alcohol in methanol. In all cases, the mobile phase was deoxygenated with argon, and a pyrex microscope slide, used as a cut-off filter, was placed in between the flowcell and the photomultiplier tube window.

(b) Anthranilic Acid

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>5 μm Spherisorb ODS, 1.5 x 0.4 cm. TFA g x 25, 50/50.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk I.</td>
</tr>
<tr>
<td>Eluent</td>
<td>various</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm$^3$ min$^{-1}$.</td>
</tr>
</tbody>
</table>

2% toluene in 15% water/methanol was passed through the hplc system. 150 μL of a solution containing 100 ppm anthranilic acid in methanol were injected into the mobile phase and the integrated peak...
count recorded. The integrated peak count from anthranilic acid was recorded using the following deoxygenated mobile phases: 4%, 6% and 8% toluene in 1% water/methanol. Fig. 3.7 shows a plot of peak counts versus percentage of toluene added to the 1% water/methanol mixture. These results were obtained when using the L39 cut-off filter to reduce the background count.

Table 3.12 shows the integrated peak counts per microgram of injected anthranilic acid resulting from the use of a number of different deoxygenated mobile phases. The use of a pyrex microscope slide or the L39 filter is indicated within the table.

(c) Efficiency of Dopents

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>TFA g x 25, 50/50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Methanol</td>
</tr>
<tr>
<td>Eluent</td>
<td>Ratemeter R5 x 10^4 LIN.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>Filter g x 1 Cut 0.175.</td>
</tr>
<tr>
<td></td>
<td>Recorder Speed. 0.2 cm min^{-1}.</td>
</tr>
</tbody>
</table>

Table 3.13 shows the effect of a number of dopents in methanol on the BIF count from injected anthranilic acid. For these results, the column was removed and methanol continuously pumped through the flowcell. Anthranilic acid, dissolved in methanol, was injected into the mobile phase and the integrated count under the fluorescent peak was recorded (value a). 1% (by weight) solutions of each dopent in methanol were prepared and injected, each in turn. The integrated count under each peak was recorded (value b). Finally, a solution containing 1% (by weight) of each dopent and the same concentration of anthranilic acid in methanol was injected into the mobile phase. The integrated count, under the fluorescent peak for each anthranilic acid in dopent/methanol was recorded (value c).
Thus the total peak count, given by anthranilic acid, in each dopent was calculated from \((c-b) - a\) counts. In this experiment, the mobile phase was not deoxygenated and the L39 cut-off filter was used to reduce the background count rate.

2.24 Examples of Chromatograms

(a) Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>Spherisorb 5 μm ODS, 25x0.4 cm. TFA g x 25, 50/50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk IV</td>
</tr>
<tr>
<td>Eluent</td>
<td>10% toluene in 90% methanol/water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.13 cm(^3)min(^{-1})</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.5 cm min(^{-1}). Filter g x 2 Cut 0.175</td>
</tr>
</tbody>
</table>

The L39 filter was placed in between the Mk IV flowcell and the PM tube window and the system allowed to light adapt. 10% toluene in 90% methanol/10% water was deoxygenated and passed through the hplc system. The background count rate, recorded by the scaler was 4200 cps.

8 μl of a solution containing 400 ppm anthracene, 200 ppm diphenyl hexatriene and 200 ppm of bis-methyl-styryl-benzene in dichloromethane, were injected into the mobile phase and the resulting separation is shown in Chromatogram 21. Chromatogram 22 shows the separation achieved by injecting 5 μl of the same solution mixture.

To demonstrate the sensitivity of BIF in reversed phase chromatography, 5 μl of a solution containing 40 ppm anthracene, 20 ppm diphenyl hexatriene and 20 ppm bis-methyl-styryl-benzene in dichloromethane were injected into the mobile phase and the separation is shown in Chromatogram 23.
The mobile phase was changed to deoxygenated 6.2% toluene in 80% acetonitrile/20% water and 8 µl and 5 µl of the stronger solution mixture injected into the mobile phase. Chromatograms 24 and 25 show the resulting separations.

(b) Instrument Parameters

Column: Spherisorb 5 µm ODS, 25x0.4 cm. TFA g x 25, 50/50.

Cell type: Mk IV. Ratemeter R5 x 10⁴ LIN.

Eluent: 6.2% toluene in 80% acetonitrile/water Filter g x 5 Cut 0.175

Flow rate: 1.13 cm³ min⁻¹ Recorder speed: 0.5 cm min⁻¹.

3 µl of a solution containing 800 ppm anthracene and 250 ppm 2,3-dibenzanthracene in dichloromethane were injected into a deoxygenated mobile phase consisting of 6.2% toluene in 80% acetonitrile/20% water. The background count rate was 4900 cps with the L39 optical filter fitted. The separation is shown in Chromatogram 26.

5 µl of a solution containing 200 ppm pyrene and 70 ppm benz [a] pyrene in dichloromethane were injected into the mobile phase and the separation is shown in Chromatogram 27.
2.25 **QUENCHED BETA-INDUCED FLUORESCENCE (QHIF)**

Throughout the investigation on beta induced fluorescence it was evident that certain compounds that passed through the flowcell reduced the fluorescence photon count rate from the mobile phase. Compounds containing sulphur, chlorine, bromine or iodine atoms, carbonyl or nitro groups are well known to quench the fluorescence observed in liquid scintillation counting (60). The quenching effect was investigated as a technique for the quantitative detection of such species in the hplc mobile phase using the experimental system previously described. Since, to achieve sensitivity in QHIF, the photon count rate from the mobile phase should be as high as possible, no cut-off filter was present between the flowcell and the photomultiplier tube.

2.26 **Response in QHIF Mode of Eluted Materials**

**Instrument Parameters**

<table>
<thead>
<tr>
<th>Column</th>
<th>5 µm Lichrosorb 15 x 0.6 cm.</th>
<th>TFA g x 25, 50/50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>Mk I.</td>
<td>Ratemeter 5 x 10^5 LIN.</td>
</tr>
<tr>
<td>Eluent</td>
<td>various.</td>
<td>Filter g x 3 Cut 0.175.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>2.5 cm³ min⁻¹</td>
<td>Recorder Speed 0.2 cm min⁻¹.</td>
</tr>
</tbody>
</table>

Solutions of a variety of materials, in hexane, were made up and 20 µl of each solution were injected into the hplc system. Table 3.14 shows the integrated peak counts per microgram for each solution in a number of mobile phases. In each case, the mobile phase was deoxygenated by purging with argon. The Table also shows the fluorescent background count rate from the mobile phase.
2.27  **Linearity of Response of QBIF Signal**

**Instrument Parameters**

Column 5 μm Lichrosorb 15 x 0.6 cm.  TFA g x 25, 50/50

Cell type  Mk I.  Ratemeter $R_5 \times 10^5$ LIN.

Eluent  Toluene  Filter $g \times 2$, Cut 0.175.

Flow rate  $2 \, \text{cm}^3 \text{min}^{-1}$  Recorder speed, $0.2 \, \text{cm} \text{min}^{-1}$.

Toluene was deoxygenated with argon and passed through the hplc system. Five solutions containing 200, 400, 600, 800 and 1000 ppm of benzaldehyde in hexane were prepared and 20 μl of each were injected into the mobile phase. In each case the (negative) integrated area under the peak was recorded. Fig. 3.8 shows a plot of peak counts versus mass of benzaldehyde injected for the solutions.

2.28  **Example of a QBIF Chromatogram**

**Instrument Parameters**

Column 5 μm Lichrosorb 15 x 0.6 cm.  TFA g x 25, 50/50.

Cell type  Mk I.  Ratemeter $5 \times 10^5$ LIN.

Eluent 2% acetonitrile in toluene  Filter $g \times 10$, Cut 0.175.

Flow rate  $1 \, \text{cm}^3 \text{min}^{-1}$  Recorder speed

2% acetonitrile in toluene was deoxygenated and passed through the hplc system. 20 μl of a mixture containing 333 ppm each of benzaldehyde, anisaldehyde and propanone in hexane were injected into the mobile phase and separated on the column. The resulting chromatogram is shown in Chromatogram 28.
The hypothesis describing the mechanism involved in the scintillation emission from a solute is derived from studies using ultraviolet photons as the excitation source. The U.V. photons directly excite solute molecules into $S_1$ excitation whereupon they rapidly ($\sim 10^{-9}$ s) return to a vibrational level in $S_0$ by emitting a photon of characteristic energy. As described in Chapter 1, scintillation spectra obtained from a binary mixture, result from transfer of excitation energy from solvent to solute. However, since the emitted spectra energy is the difference between the $S_1$ and $S_0$ energies of the solute concerned the emitted energy should be independent of the source of excitation. This is indeed the case since Langenscheidt (127) has shown that the wavelength of fluorescence spectra is independent of the mode of excitation. In a series of experiments he excited solutions with alpha particles (1 mCi, $^{247}$Am), beta particles (0.5 mCi, $^{147}$Pm), gamma (2 Ci, $^{192}$Ir) and ultraviolet radiation. Furthermore, Birks et al. (128) used beta radiation from $^{32}$P (1.4 mCi) and compared the scintillation spectra from solutions obtained in this way with those obtained under UV excitation from a mercury lamp. They concluded that the spectra were similar to the spectra obtained for direct photo excitation of the solutes.

The objectives of these experiments are as follows:

1. To verify and confirm the independence of the fluorescence wavelength of a solute on the mode of excitation.
2. To verify and confirm that the mechanism of solute excitation is by energy transfer from excited solvent molecules.
3. To show the effect of a cut-off filter on the fluorescence from solutions in toluene,
4. To investigate the phenomenon of quenching in BIF.

To obtain wavelength spectra a Mini-Chrom 1 monochromator, loaned by ACS Ltd., Luton, was used for wavelength selection. The overall dimensions of the monochromator were 10 cm x 4.7 cm x 5.8 cm which resulted in it being easily incorporated into the wooden coffin provided that the aluminium inner box was removed. However, before using the monochromator, several problems had to be overcome:-

1. The monochromator was not equipped with a scanning motor, wavelengths were selected manually by rotating a precision micrometer to alter the grating angle. To automate this, it was necessary to couple the micrometer to a Philips automatic scanner (model PW 4083/01). Within this scanner, a synchronous motor is connected to a helical potentiometer via a system of gears. As the potentiometer is driven by the motor, each revolution of the potentiometer is recorded by a vernier which is visible on the outside of the scanner. Since the total number of possible revolutions is ten, the vernier recorded 0.00 to 10.00 during a full 'scan'. By selecting different gear ratios, total scanning times of 3.5, 14, 24 and 100 minutes could be selected.

The scanner was modified to incorporate a 5V battery so that at each half revolution of the potentiometer a marker pulse (0 or 5V) was recorded on the second pen of the chart recorder. This was necessary so as to graduate the time axis of the chart recorder in a vernier reading scale of nanometers as shown by the micrometer on the monochromator. For example, by adjusting the micrometer on the monochromator to read 150 nm and setting the vernier on the potentiometer to read 0.00, a total wavelength scan of between 150 and 650 nm was obtained when the potentiometer travelled through its
full number of revolutions. In this case a 'step' calibration was obtained every 25 nm corresponding to 0.00 to 0.50 etc. (i.e. every half revolution of the scanner vernier).

2. No details were available of the transmission characteristics of the monochromator. However, these were obtained by positioning the monochromator in the sample beam of a Cecil 5095 high performance scanning spectrophotometer and recording the percentage transmission at 50 nm intervals. As can be seen from Fig. 2.28, this suggested that the maximum transmission of ~10% occurred at a wavelength of ~450 nm. The experiment was repeated and furnished very similar results. Although these results do not give the actual percentage transmission of the monochromator, at a particular wavelength they may be used to determine the ratio of the transmission.

**Fig. 2.28** Transmission of the Monochromator
Fig 2.27

PHOTOGRAPH 6
The monochromator, drive couple and photomultiplier tube were placed into the wooden coffin as shown in Fig. 2.27 and photograph 6. Initial MIF spectra were obtained by standing the 20 mCi ionizing source in a 1 cm x 1 cm quartz cuvette, but this was found to be unsatisfactory since it was not possible to deoxygenate solutions.

In later experiments the cuvette was replaced by the flowcell shown in Fig. 2.29 (the Mk IV flowcell was not used because of its pyrex microscope slide window - see Fig. 3.4).
One advantage of this system was that it was unnecessary to enter the wooden coffin to change the solution that was under examination.

The BIF spectra were recorded by pulse counting, as described previously.

Since the percentage transmission of the monochromator was so low, entrance and exit slits were dispensed with. Although this results in a decrease in resolution, with consequent loss of fine structure, this is acceptable since only peak wavelength and area are of interest in this study.

To record a BIF spectrum, the chart recorder was run on a chart speed of 1 cm min$^{-1}$. The scan time (0.00 → 10.00 = 150 nm → 650 nm on the monochromator) was set at 14 minutes, and the synchronous motor started. As the motor commenced rotating the monochromator micrometer, a marker pulse was recorded on the chart recorder. Similarly, a marker pulse was recorded throughout the scan at 25 nm intervals and at the end of the scan.

Fig. 3.9 shows the BIF spectrum from 1000 ppm fluorene in hexane, recorded without slits in the monochromator. Fig. 3.10 shows the spectrum from the same solution; in this case 600 μm slits were used on the entrance and exit of the monochromator. In both cases the 20 mCi $^{147}$Pm strip source was placed in a quartz 1 cm x 1 cm cuvette which was filled with the test solution.

2.30 Fluorescence Wavelength Independence On Excitation Source

A solution of naphthalene in hexane was placed into a quartz cuvette and a nickel-63 beta source suspended in the solution. The source consisted of 7 mCi of $^{63}$Ni electroplated on to a 2.5 cm
length of 0.05 cm diameter copper wire. The cuvette was placed close to the entrance of the monochromator and the spectrum recorded, as shown in Fig. 3.11.

The $^{63}$Ni source was replaced by the 20 mCi $^{147}$Pm strip source and the solution replaced by a 1% (wt/vol) naphthalene in hexane solution. The spectrum of this solution was recorded and is shown in Fig. 3.12.

Finally, the solution was replaced by a 1% naphthalene in cyclohexane solution. Fig. 3.13 shows the spectrum obtained. Fig. 3.14 shows the fluorescence spectrum obtained under normal UV excitation.

2.3.1 Energy Transfer in the Scintillation Process

The flowcell shown in Fig. 2.29, containing the 20 mCi $^{147}$Pm strip source, was placed next to the entrance slit of the monochromator and the system allowed to light adapt. Hexane was deoxygenated with argon and then pumped through the flowcell. The spectrum of hexane was obtained, over the wavelength range 150 to 650 nm and is shown in Fig. 3.15. Toluene was pumped through the flowcell to clear the system of hexane. A fresh sample of toluene was then deoxygenated and pumped through the flowcell, and the BIF spectrum obtained. The spectrum is shown in Fig. 3.16. Fluorescence spectra were obtained for deoxygenated solutions of 50, 100 and 200 ppm anthracene in toluene and are presented in Figs. 3.17, 3.18 and 3.19.
2.32 Use of a Cut-Off Filter

50 ppm anthracene in toluene was deoxygenated and passed through the flowcell. The fluorescence spectrum of this solution was recorded over the wavelength range 150 to 650 nm and is shown in Fig. 3.20.

The L39 camera cut-off filter, previously used to reduce the background count rate from toluene in hexane mobile phases, was interposed between the flowcell and the entrance of the monochromator and the system allowed to light adapt. The spectrum of the 50 ppm anthracene in toluene was re-recorded and is shown in Fig. 3.21.

2.33 Quenching in BIF Spectra

A solution containing 100 ppm anthracene in toluene was deoxygenated and passed through the flowcell. The spectrum of this solution was recorded and is shown in Fig. 3.22.

0.1% (vol/vol) of carbon tetrachloride was added to the anthracene solution, the solution deoxygenated and the spectrum recorded. The result is shown in Fig. 3.23.

A further 0.1% (vol/vol) of carbon tetrachloride was added to the solution and the solution deoxygenated. Fig. 3.24 shows this spectrum.

The results of these experiments, together with a discussion on BIF, are presented in Chapter 3.
CHAPTER 3. BETA INDUCED FLUORESCENCE — RESULTS AND DISCUSSIONS

3.1 RESPONSE OF BIF DETECTOR IN NORMAL PHASE OPERATION

TABLE 3.1 Response of BIF Detector to Eluted Materials

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Compound</th>
<th>Mobile Phase</th>
<th>Fluorescent Background (cps)</th>
<th>Response (counts/μg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-Terphenyl</td>
<td>Hexane</td>
<td>12 000</td>
<td>813639</td>
</tr>
<tr>
<td>2</td>
<td>Anthracene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>286918</td>
</tr>
<tr>
<td>3</td>
<td>Fluorene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>263070</td>
</tr>
<tr>
<td>4</td>
<td>Diphenylhexatriene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>519972</td>
</tr>
<tr>
<td>5</td>
<td>bis-Methyl-styryl-benzene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>556481</td>
</tr>
<tr>
<td>6</td>
<td>Biphenyl</td>
<td>&quot;</td>
<td>&quot;</td>
<td>50342</td>
</tr>
<tr>
<td>7</td>
<td>Tetraphenylbutadiene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>251625</td>
</tr>
<tr>
<td>8</td>
<td>Naphthalene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>105302</td>
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<tr>
<td>9</td>
<td>Phenanthrene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>44995</td>
</tr>
<tr>
<td>10</td>
<td>Acenaphthene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>153667</td>
</tr>
<tr>
<td>11</td>
<td>Anisole</td>
<td>&quot;</td>
<td>&quot;</td>
<td>38073</td>
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<tr>
<td>12</td>
<td>Indene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>4546</td>
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<td>13</td>
<td>Benzo[a] pyrene</td>
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<td>&quot;</td>
<td>338100</td>
</tr>
<tr>
<td>14</td>
<td>3-Methylcholanthrene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>191800</td>
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<tr>
<td>15</td>
<td>Fluoranthene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>114600</td>
</tr>
<tr>
<td>16</td>
<td>Diphenyloxazole</td>
<td>Toluene</td>
<td>180 000</td>
<td>8726800 (a)</td>
</tr>
<tr>
<td>17</td>
<td>Dibenzofuran</td>
<td>&quot;</td>
<td>&quot;</td>
<td>759600 (a)</td>
</tr>
<tr>
<td>18</td>
<td>bis-Methyl-styryl-benzene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5353500 (a)</td>
</tr>
<tr>
<td>19</td>
<td>bis-Methyl-styryl-benzene</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1903000 (a)</td>
</tr>
</tbody>
</table>

Note: (a) Pyrex microscope slide in front of photomultiplier tube.

Table 3.1 shows the response in counts μg⁻¹ of sample and illustrates the range of materials which may be detected using BIF. Fig. 3.1 presents examples of chromatographic peaks (from 2 μg samples) obtained during the investigation. Note that the small quenching peaks arise due to the injected hexane not being deoxygenated.
Fig 3-1

2 μg SAMPLES

1 2 3 4 5 (SAMPLE NUMBER)

8 10
Once the $\beta^-$ particles have entered the liquid phase they lose
ergy and give rise to the creation of ionised and excited
molecules through both primary and secondary mechanisms. The
processes involved have been extensively studied because of their
relevance to liquid scintillation counting of radiouclides, and have
been described in Chapter 1. However, the details of the rate
processes involved in BIF do differ from those in liquid scintilla-
tion counting as a result of the very low concentrations of
fluorescent material likely to be involved in the chromatographic
system. In particular it should be noted that single photon
emissions occur under normal sample/solvent conditions. The
elementary processes requiring consideration in the BIF system are
collected in Table 3.15 where it will be noted that although solvent
excimers, D, have been considered, excimers and exciplexes involving
the fluorescent sample have not been taken into account. While such
species are of undoubted importance (and considerable theoretical
interest) in some systems, their intervention would not alter the
conclusions to be drawn from the kinetic equations which follow from
the simplified scheme of Table 3.15.

As the $\beta^-$ particles enter the liquid phase they deposit their
energy by causing ionisation and electronic excitation of the
molecules forming the bulk of the liquid (solvent molecules, M).
The direct excitation of the sample molecules, S, will be ignored as
these are likely to be a very small proportion of the total.
Attention will be confined to the (relatively) non-polar solvents
which have been used for normal phase chromatography with BIF
systems, (e.g. hexane, toluene, etc.) then ions initially formed will
capture electrons and the net result of these processes is the
### TABLE 3.15 Basic Processes Involved in HIF (from Chapter 1)

<table>
<thead>
<tr>
<th></th>
<th>Process Description</th>
<th>Rate Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\text{S}^-/\text{S} + \text{M} \rightarrow \text{1}^*_{\text{M}}$</td>
<td>$\frac{d\text{1}^*_{\text{M}}}{dt} = k_0 [\text{M}]$</td>
</tr>
<tr>
<td>16</td>
<td>$\text{1}^<em>_{\text{M}} + \text{M} \leftrightarrow \text{1}^</em>_{\text{D}}$</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>$\text{1}^*_{\text{M}} \rightarrow \text{M}$</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>$\text{1}^<em>_{\text{M}} \rightarrow \text{3}^</em>_{\text{M}}$</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>$\text{3}^*_{\text{M}} \rightarrow \text{M}$</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>$\text{1}^*_{\text{M}} \rightarrow \text{M} + \text{hv}_1$</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>$\text{3}^*_{\text{M}} \rightarrow \text{M} + \text{hv}_2$</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>$\text{1}^<em>_{\text{D}} \rightarrow \text{3}^</em>_{\text{M}} + \text{M}$</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>$\text{1}^*_{\text{D}} \rightarrow 2 \text{M}$</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>$\text{1}^<em>_{\text{D}} \rightarrow 3^</em>_{\text{D}}$</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>$3^<em>_{\text{D}} \leftrightarrow 3^</em>_{\text{M}} + \text{M}$</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>$3^*_{\text{D}} \rightarrow 2 \text{M}$</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>$\text{1}^*_{\text{D}} \rightarrow 2 \text{M} + \text{hv}_3$</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>$3^*_{\text{D}} \rightarrow 2 \text{M} + \text{hv}_4$</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>$\text{1}^<em>_{\text{S}} + \text{S} \rightarrow \text{1}^</em>_{\text{S}} + \text{M}$</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>$\text{1}^<em>_{\text{D}} + \text{S} \rightarrow \text{1}^</em>_{\text{S}} + 2 \text{M}$</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>$\text{1}^*_{\text{S}} \rightarrow \text{S} + \text{hv}_5$</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>$\text{1}^*_{\text{S}} \rightarrow \text{S}$</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>$\text{1}^<em>_{\text{S}} \rightarrow \text{3}^</em>_{\text{S}}$</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>$3^*_{\text{S}} \rightarrow \text{S} + \text{hv}_6$</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>$3^<em>_{\text{S}} \rightarrow \text{3}^</em>_{\text{S}}$</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>$\text{1}^*_{\text{M}} + \text{Q} \rightarrow \text{M} + \text{Q}$</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>$\text{1}^*_{\text{D}} + \text{Q} \rightarrow 2 \text{M} + \text{Q}$</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>$\text{1}^*_{\text{S}} + \text{Q} \rightarrow \text{S} + \text{Q}$</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>$3^*_{\text{S}} + \text{Q} \rightarrow \text{S} + \text{Q}$</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:**

(a) A multi-stage process involving ions and higher excited states. It is assumed that the rate of this process = $k_0 [\text{M}]$, where $k_0 = f (E_0(t))$

(b) Treated as unimolecular for simplicity. In practice processes involved are largely bimolecular, but relatively insensitive to the nature of the collision partner for solvents of interest in this work.
production of highly excited solvent molecules which rapidly (<10^{-11}s) relax to the lowest excited singlet state \((1)\), \(1_M^*\). These molecules may themselves fluoresce \((20)\) transfer their energy to quencher species \((\text{such as oxygen})\) \((39)\) or to the sample species \((29)\), or may undergo an intersystem crossing to yield a triplet species \((18)\).

Alternatively these excited molecules may indulge in the reversible process of excimer formation \((16)\) or undergo a radiationless transition to the ground state \((17)\). The processes of fluorescence \((32)\), quenching \((41)\), relaxation \((33)\) and triplet formation \((34)\) may also occur to excited sample molecules formed by \((29)\) and \((30)\). Any of the triplet species formed may phosphoresce \((21, 28\) and \(37)\) or be quenched \((\text{e.g.} 42)\) or relax to the ground state \((19, 26\) and \(38)\), these latter processes being written as unimolecular for simplicity, although they undoubtedly may also involve bimolecular interactions with non-specific partners.

Under the conditions which apply in the BIF detector phosphorescent emissions may be neglected and the rate of photon emission when mobile phase alone passes through the flow-cell may be written

\[
\dot{p}_m = k_{20} [1_M^*] + k_{27} [1_D^*] \quad (\text{Eq. 3.1})
\]

The rate which applies when a luminescent sample species is present becomes

\[
\dot{p}_m + \dot{p}_s = \dot{p}_m + k_{32} [1_S^*] + k_{37} [3_S^*] \quad (\text{Eq. 3.2})
\]

For most of the materials which have so far been detected using BIF systems the final term in Equation \((3.2)\) may be neglected. For both of the above equations the concentrations of the excited state species may be written by equating their rates of formation with their rates of decay, i.e.
\[ [1]^M = \frac{k_0 [M]}{(k_{50} [M] + 1)(k_{20} + k_{17} + k_{39} [Q] + k_{29} [S])} \]  
(Eq. 3.3)

where it is assumed that \( k_{50} \) is approximately constant over the ranges of \([Q]\) and \([S]\) of interest (i.e. < \(10^{-3}\)M) and

\[ k_{50} = \frac{k_{16}(k_{22} + k_{17} + k_{39} [Q] + k_{29} [S])}{(k_{16} + k_{22} + k_{27} + k_{23} + k_{24} + k_{40} [Q] + k_{30} [S])(k_{20} + k_{17} + k_{16} + k_{39} [Q] + k_{29} [S])} \]

\[ [1]^D = \frac{k_16 [M] [1]^M}{(k_{16} + k_{22} + k_{27} + k_{23} + k_{24} + k_{40} [Q] + k_{30} [S])} \]  
(Eq. 3.4)

\[ [1]^S = \frac{(k_{29} [1]^M + k_{30} [1]^D)}{k_{32} + k_{41} [Q] + k_{33} + k_{34}} \]  
(Eq. 3.5)

Substituting these values for \([1]^M\), \([1]^D\) and \([1]^S\) into Equations (3.1) and (3.2) we obtain

\[ \dot{p}_m (S, Q) = \frac{k_{20} + k_{51} [M]}{(k_{50} [M] + 1)(k_{20} + k_{17} + k_{18} + k_{39} [Q] + k_{29} [S])} k_0 [M] \]  
(Eq. 3.6)

where

\[ k_{51} = \frac{k_{27} k_{16}}{k_{-16}} \]

and

\[ \dot{p}_S = \frac{k_{32}(k_{29} + k_{52} [M])}{(k_{50} [M] + 1)(k_{20} + k_{17} + k_{18} + k_{39} [Q] + k_{29} [S])(k_{32} + k_{41} [Q] + k_{34} + k_{33})} k_0 [M] [S] \]  
(Eq. 3.7)

where

\[ k_{52} = \frac{k_{30} k_{16}}{k_{-16}} \]

Equations (3.6) and (3.7) in conjunction with Equation (3.9) contain the information necessary for an understanding of the properties of a BIF detector, and may be used for deriving expressions for responses and signal-to-noise ratios for the detector operating in normal BIF mode.
3.2 LINEARITY OF RESPONSE

Equation 3.7 shows the dependence of the recorded signal on the sample size. Fig. 3.2 shows the integrated count response for a range of sample loadings for p-terphenyl, where the effect of deoxygenation of the mobile phase may also be seen. Fig. 3.3 demonstrates that the detector response remains linear for sample loadings of up to \( \approx 20 \) \( \mu \)g of fluorene.

Equation 3.3 together with equation 3.7 show that the magnitude of the fluorescence emitted by a suitable solute is dependent upon the solvents ability to form excited molecules and transfer their excitation energy to the solute. Table 3.2 shows the effect of adding toluene to hexane in order to increase these two factors.

**TABLE 3.2** Effect of Adding Fluorescent Material to Eluting Solvent

<table>
<thead>
<tr>
<th>Compound: bis-methyl-styryl-benzene.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eluting Solvent</strong></td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Hexane</td>
</tr>
<tr>
<td>Hexane-toluene (99:1)</td>
</tr>
<tr>
<td>Hexane-toluene (95:5)</td>
</tr>
<tr>
<td>Hexane-toluene (90:10)</td>
</tr>
</tbody>
</table>

*Counting system probably in error through dead time.

3.3 EFFECT OF ADDING FLUORESCENT MATERIAL TO ELUTING SOLVENT

Table 3.2 clearly demonstrates the increase in response obtained when the mobile phase is doped with toluene.

Chromatograms 3 and 4 show the effect, on response, of the addition of toluene to the mobile phase. Both chromatograms were
Fig 3.2
Fig 3.3
Effect of addition of toluene on response.

Chromatogram 3  0.2% acetonitrile in hexane
Chromatogram 4  10% toluene in hexane

20 ng each of

1 Anthracene
2 Diphenyl hexatriene
obtained by injecting 20 µl of a mixture containing 1 ppm each of anthracene, diphenyl hexatriene and bis-methyl-styryl-benzene into the hplc system. Chromatogram 3 was obtained by employing a mobile phase which consisted of 0.2% acetonitrile in hexane and chromatogram 4, 10% toluene in hexane. Both separations were achieved using the same ratemeter (R \(10^5\)) and gain settings (\(g \times 25\)) on the timing filter amplifier, but chromatogram 3 was obtained on gain \(x 10\) on the electronic filter while gain \(x 2\) was used to obtain chromatogram 4. Separation was achieved on a Waters \(\mu\) Porasil 30 cm x 0.5 cm (dia.) column and detected by the Mk V flowcell fitted with the 5 mCi NEN source. An L39 cut-off filter was placed in between the flowcell and the PM tube window when using the toluene/hexane mobile phase; the reasons for this action will be discussed later.

To obtain useful retention times in normal phase hplc, it is necessary to adjust the polarity of the mobile phase. Whilst it is accepted practice to achieve the adjustment by the addition of dichloromethane \((\epsilon^0(\text{Al}_2\text{O}_3) 0.42)\) to (say) hexane, one could equally well add toluene \((\epsilon^0(\text{Al}_2\text{O}_3) 0.29)\) in greater volume to achieve the same result.

3.4 EFFECT OF FILTERING FLUORESCENCE ON RESPONSE

The disadvantage of doping with toluene is that it increases the background countrate, observed when mobile phase alone flows through the cell. The BIF response, \(R_B(S)\), may be written as the difference between the photon detection rate recorded at the peak and that recorded from mobile phase alone, i.e.

\[
R_B(S) = [d_s \hat{P}_s + d_m + P_m]_S - [d_m \hat{P}_m]_{s=0} \quad \text{(Eq. 3.8)}
\]

where \(d_s\) and \(d_m\) are the detection efficiencies for photons emitted
by the sample species and the mobile phase respectively. A decrease in \( \frac{d}{d_m P_m} \) clearly results in an increase in \( R_B(S) \), and may be achieved by placing an optical cut-off filter in between the flow cell and the PM tube window. Table 3.3 shows this effect.

**TABLE 3.3 Effect of Filtering Fluorescence On Response**

<table>
<thead>
<tr>
<th>Compound: bis-methyl-styryl-benzene.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Hexane</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hexane-toluene (99:1)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Hexane-toluene (95:5)</td>
</tr>
<tr>
<td>Hexane-toluene (90:10)</td>
</tr>
<tr>
<td>Toluene</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

* See footnote to Table 3.2.

Since the placing of a cut-off filter in between the flow cell and the PM tube window was shown to result in a dramatic decrease in fluorescence photons from mobile phase alone, it was decided to investigate the effect that five camera cut-off filters had on the background countrate and peak height response, from an injected mixture of anthracene, diphenyl hexatriene and bis-methyl-styryl-benzene.

### 3.5 INVESTIGATION OF CUT-OFF FILTERS

Fig. 3.4 shows the uv transmission characteristics of the filters together with the transmission of the pyrex microscope slide used previously.
Fig 34

Camera Filters
1 Pyrex microscope slide
2 LIA
3 385
4 L39
5 400
6 YK2
Table 3.4 shows the peak heights obtained during the experiment where it should be noted that care was taken to ensure mobile phase flow rate stability, to keep the retention times of the eluting peaks constant.

**TABLE 3.4 Investigation of Cut-off Filters**

<table>
<thead>
<tr>
<th>FILTER</th>
<th>LIA</th>
<th>385</th>
<th>L39</th>
<th>400</th>
<th>YK2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane-toluene (90:10)</td>
<td>Background (cps)</td>
<td>14525</td>
<td>5500</td>
<td>4100</td>
<td>4470</td>
</tr>
<tr>
<td>COMPOUND</td>
<td>peak heights (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>36</td>
<td>43</td>
<td>26</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>DPH</td>
<td>77</td>
<td>107</td>
<td>80</td>
<td>85</td>
<td>11</td>
</tr>
<tr>
<td>MSB</td>
<td>50</td>
<td>76</td>
<td>49</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>Ratio DPH:anthracene</td>
<td>2.14</td>
<td>2.49</td>
<td>3.00</td>
<td>3.86</td>
<td>-</td>
</tr>
<tr>
<td>Ratio MSB:anthracene</td>
<td>1.54</td>
<td>1.41</td>
<td>1.63</td>
<td>1.51</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.4 clearly demonstrates the decrease in toluene fluorescence achieved by using cut-off optical filters. In general, the longer the cut-off wavelength of the filter, the smaller the background count rate. (The small increase in background count rate shown by exchanging the L39 filter for the 400 filter may be attributed to the inability of the 400 filter to cut out all photons of wavelength shorter than ~380 nm).

However, a cut-off wavelength greater than ~400 nm is clearly undesirable since the YK2 filter shows that reduction, or even elimination, of the signal results from a solute which fluoresces in the near uv. Anthracene has a fluorescence peak at ~400 nm (129) and this compound may be used to monitor the reduction in signal resulting from the use of longer wavelength cut-off filters. The ratio of the peak heights from DPH (peak ~450 nm) (129) and MSB (peak ~420 nm)(129) to the peak heights obtained from anthracene, in
general, increase from the LIA filter to the 400 filter showing a reduction in anthracene signal.

3.6 INVESTIGATION OF REFLECTORS USED WITH MK V FLOWCELL

Table 3.5 shows the results of the investigation on the use of reflectors with the Mk·V flowcell. Fig. 3.5 shows a graph of signal-to-noise ratio (calculated as peak height (mm) divided by the square root of the background count rate) for each reflector and each compound at different distances from reflector to source.

<table>
<thead>
<tr>
<th>Table 3.5 Investigation of Reflectors Used with Mk V Flowcell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflectors/Distance from Source (mm)</td>
</tr>
<tr>
<td>--------------------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>24</td>
</tr>
</tbody>
</table>

The largest values of signal-to-noise ratios (0.933 and 0.719) are observed when the chromium on glass reflector is employed at a distance of 18 mm from the source. The chromium on plastic reflector gave maximum signal-to-noise ratios of 0.655 and 0.474 at 20 mm and the TiO₂ on plastic values of 0.676 and 0.482 at 16 mm.

Although the signal-to-noise ratios for the latter two reflectors appear to be similar, it is significant that at these values the peak heights from DPH and MSB decreased by approximately 29% whilst the background decreased by 52% when the TiO₂/plastic reflector replaced
1·00

0·90

Pp Ht

0·80

0·70

0·60

0·50

0·40

0·30

0·20

0·10

0·00

0·00

12
14
16
18
20
22
24

DISTANCE REFLECTOR FROM SOURCE mm

Fig 3·5

1 CHROME ON GLASS
2 CHROME ON PLASTIC
3 TiO₂ ON PLASTIC

--- DPH
--- MSB
the chromium/plastic reflector. Thus it may be concluded that the replacement of the chromium reflective coating by the TiO₂ reflective coating reduces both signal and background, but that the reduction in the latter is greater and is partly effective as a reflector/filter.

3.7 **RESPONSE AND SENSITIVITY OF BIF FLOWCELLS**

Table 3.6 shows the response and sensitivity observed from the flowcells described in Chapter 2.

Recalling, from Chapter 1, that the response \( R_c \) of a concentration dependent detector is defined as

\[
R_c = \frac{A \cdot V}{c \cdot m} \text{ mV/g/cm}^3
\]

where \( A \) is the area of the chromatographic peak (peak height (mV) x peak width (cm) at 0.607 of peak height, \( V \) the flow rate (cm³/min), \( c \) the chart speed (cm min⁻¹) and \( m \) the mass (g) of injected compound. Thus, if the BIF detector is a concentration dependent detector

\[
A \propto \frac{1}{V}
\]

for constant \( R_c \), \( c \) and \( m \).

3.8 **EFFECT OF FLOW RATE ON RESPONSE**

Table 3.9 shows the integrated peak count variation with flow rate, and Fig. 3.6 shows a plot of integrated peak count versus the reciprocal of the flow rate.
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>MK I</th>
<th>MK II</th>
<th>MK III</th>
<th>MK IV</th>
<th>MK V</th>
<th>MK V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>0.039</td>
<td>0.041</td>
<td>0.034</td>
<td>0.171</td>
<td>0.052</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td>12.31</td>
<td>6.83</td>
<td>19.41</td>
<td>2.34</td>
<td>4.61</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.14</td>
<td>0.33</td>
<td>0.20</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>1,6 Diphenylhexatriene</td>
<td>0.210</td>
<td>0.132</td>
<td>0.340</td>
<td>0.584</td>
<td>0.196</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td>2.28</td>
<td>2.12</td>
<td>1.94</td>
<td>0.68</td>
<td>1.22</td>
<td>0.46</td>
</tr>
<tr>
<td>bis-methyl-styryl-benzene</td>
<td>0.101</td>
<td>0.069</td>
<td>0.190</td>
<td>0.380</td>
<td>0.153</td>
<td>0.541</td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td>4.05</td>
<td>3.47</td>
<td>1.05</td>
<td>1.57</td>
<td>0.55</td>
</tr>
<tr>
<td>Background (cps) from Hexane-toluene (90:10)</td>
<td>4000</td>
<td>6250</td>
<td>7400</td>
<td>31100</td>
<td>4100</td>
<td>8950</td>
</tr>
</tbody>
</table>

**Notes:**

(a) The response, $R_c$, is $10^9 \text{ mV/cm}^3$ and is defined in Chapter 1.

(b) The sensitivity, $S_c$, is $10^{-9} \text{ g/cm}^3$ as is defined in Chapter 1.

(c) The noise, $N$, is given in mV and is the peak to peak noise when mobile phase alone flows through the cell.

(d) Examples of chromatograms obtained when using the Mk I, Mk IV and Mk V (5mCi) flowcells are shown in chromatograms 5, 6, 7 and 8.
Chromatogram 5 (Mk. I, 1 mCi) 20 ng each
1 Anthracene
2 Diphenylhexatriene
3 bis-Methyl-styryl-benzene

Chromatogram 6 (Mk IV, 20 mCi) 20 ng each
1 Anthracene
2 Diphenylhexatriene
3 bis-Methyl-styryl-benzene
Chromatogram 7
Mk V (5 mCi)
20 ng each
1 Anthracene
2 Diphenylhexatriene
3 bis-Methyl-styryl-benzene

Chromatogram 8
Mk V (5 mCi)
2 ng each
1 Anthracene
2 Diphenylhexatriene
3 bis-Methyl-styryl-benzene
Waters μ Porasil Column
Fig 3.6
TABLE 3.9 Effect of Flow rate on Response

<table>
<thead>
<tr>
<th>Flow rate (cm$^3$ min$^{-1}$)</th>
<th>Retention time (min)</th>
<th>Peak count (counts x 10$^5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.750</td>
<td>53</td>
<td>61.94</td>
</tr>
<tr>
<td>1.053</td>
<td>40</td>
<td>41.65</td>
</tr>
<tr>
<td>1.579</td>
<td>26.5</td>
<td>27.00</td>
</tr>
<tr>
<td>2.143</td>
<td>20</td>
<td>23.44</td>
</tr>
<tr>
<td>2.727</td>
<td>15</td>
<td>16.75</td>
</tr>
</tbody>
</table>

Before discussing the results shown in Table 3.6, it is appropriate to compare BIF detector responses with those quoted by detector manufacturers for marketed detector, a sample of which is shown below.

<table>
<thead>
<tr>
<th>Detector</th>
<th>Sensitivity ($S_c - g/cm^3$)</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waters R401 RI</td>
<td>5.6 x 10$^{-6}$</td>
<td>benzene</td>
</tr>
<tr>
<td>UV monitor (LDC)</td>
<td>1.6 x 10$^{-8}$</td>
<td>benzene (254 nm)</td>
</tr>
<tr>
<td>Fluorimeter</td>
<td>10$^{-9}$</td>
<td>quinine sulphate</td>
</tr>
</tbody>
</table>

3.9 RESPONSE AND SENSITIVITY OF A "NORMAL" FLUORIMETER

The value of 10$^{-9}$ g/cm$^3$, for the sensitivity of the fluorimeter, appears rather high and it was decided to determine the response and sensitivity of a "normal" fluorimetric detector using the three compounds employed with the BIF detector.

Table 3.7 shows the response and sensitivity for the three compounds when detected by a Perkin Elmer 1000 fluorimeter. The compounds were separated using normal phase hplc employing deoxygenated 0.1% acetonitrile in hexane as the mobile phase and a Lichrosorb
### TABLE 3.7  
Response and Sensitivity of a "Normal" Fluorimeter  
\( \lambda_{\text{ex}} = 292 \text{ nm} \)

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>( \lambda_{\text{f}} )</th>
<th>420 nm</th>
<th>430 nm</th>
<th>450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( R_c \times 10^6 )</td>
<td>( S_c \times 10^{-9} )</td>
<td>( N )</td>
<td>( R_c \times 10^6 )</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.86</td>
<td>2.78</td>
<td>0.0012</td>
<td>0.34</td>
</tr>
<tr>
<td>DPH</td>
<td>7.05</td>
<td>0.34</td>
<td>9.07</td>
<td>0.26</td>
</tr>
<tr>
<td>MSB</td>
<td>9.25</td>
<td>0.26</td>
<td>8.87</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Where the response, \( R_c \), is \( x 10^6 \text{ mV/g/cm}^3 \) and the sensitivity is \( x 10^{-9} \text{ g/cm}^3 \). The noise is again determined as the peak to peak movement (in mV) in the background.
5 μm silica column of dimensions 15 x 0.4 cm (dia). The electronic filter/amplifier was connected in between the fluorimeter and recorder to keep the time constant the same as that employed in the BIF detector.

To achieve maximum sensitivity for each component in the mixture, the excitation wavelength ($\lambda_{\text{ex}}$) and the fluorescence wavelength ($\lambda_{\text{F}}$) must be selected carefully. Only one other excitation filter was available for the fluorimeter and the results of using this excitation wavelength are shown below.

**TABLE 3.8 Response and Sensitivity of a "Normal" Fluorimeter**

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_c$ ($\times 10^6$ mV/g/cm³)</th>
<th>$S_c$ ($\times 10^{-9}$ g/cm³)</th>
<th>$N$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>1.05</td>
<td>0.381</td>
<td>0.0002</td>
</tr>
<tr>
<td>DPH</td>
<td>27.50</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>MSB</td>
<td>22.57</td>
<td>0.018</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.6, together with Table 3.8 show that the Perkin Elmer 1000 fluorimeter is ~7 times more sensitive than the BIF detector for anthracene and is ~30 times more sensitive for both diphenyl hexatriene and bis-methyl-styryl-benzene, for the most sensitive BIF flowcell so far developed.

Returning to Table 3.6, it can be seen that the Mk IV and Mk V flowcells were the most sensitive flowcells. Concerning the design of flowcells, the most important factors involved in attaining sensitivity will now be discussed.
Firstly, the activity and construction of the source utilized to induce BIF from solutions.

In a BIF detector system energy is transferred from a radioactive source to a liquid solution as a result of $\beta^-$ particles escaping from the source and entering the liquid. The fate of the energy deposited in the solution depends almost entirely on the properties of the solution, and this aspect has been considered previously. However, the amount of energy deposited in the liquid, a quantity which is a function of the nature of the radioactive source, the physical properties of the barrier between the decaying radionuclide and the liquid phase, and the detailed design of the flow cell - in particular the path length available to the $\beta^-$ particles.

Unlike the $\alpha$ and $\gamma$ radiations emitted during some radionuclide decays, $\beta^-$ particles are not emitted with discrete energies, but rather with a range of energies varying from zero to a maximum value, $E_{\text{max}}$, which is different for different radioisotopes.

For the mobile phases of importance in HPLC attention is confined to those radionuclides which emit $\beta^-$ particles with energies of no more than $\sim 0.25$ MeV, and even at this energy some Cerenkov radiation will be detected for liquids with a refractive index $>1.4$.

With this upper limit in mind the rate at which energy is deposited in the liquid phase, $E_0$, may be written as

$$E_0(t) = gN(t)f(E,r_1,p_1)h(E,r_2,p_2)E$$

(Eq. 3.9)

where $N(t)$ is the rate of decay of the $N(t)$ atoms of the radionuclide, and depends on the age, $t$, of the source; $g$ is a geometric factor which represents the fraction of $\beta^-$ particles emitted from the source which could enter the liquid; $f(E,r_1,p_1)$ is the barrier factor, which is the fraction of the $\beta^-$ particles' energy not absorbed by the
barrier (thickness $r_1$, density $p_1$) separating the decaying nuclide from the liquid phase, and $h(E, r_2 p_2)$ is the path length factor, which is the fraction of the $\beta^-$ particles energy absorbed by the liquid (density $p_2$) in the available path length (source to wall or window), $r_2$.

The designs of BIF cells have always involved $g \leq 0.5$. Although $g$ could be increased beyond 0.5 the design difficulties which arise in attempting such an improvement are formidable, so that it is assumed $g = 0.5$. The range of $\beta^-$ particles with energies $< 0.25$ MeV in liquids of $p_2 \approx 1$ is (136) $< 0.5$ mm, and in most flow cell designs to date the available path length has been sufficient to allow the assumption that $h(E, r_2 p_2) \approx 1$. The radioactivity law (136) may be introduced to rewrite (3.9) as

$$
\dot{E}_0(t) \approx \frac{1}{2} f(E, r_1, p_1) \times E \dot{\bar{N}}_0 \exp \left( -\frac{\ln 2 \times t}{t_1} \right) \quad \text{(Eq. 3.10)}
$$

where $\dot{\bar{N}}_0$ = initial activity of radioactive source and $t_1$ = half life of radionuclide.

The time dependence of the energy deposition rate is explicitly provided by the exponential factor, and clearly $\dot{E}_0(t)$ declines as the source decays. If a rate of decline in the sensitivity of the detector (which is assumed to be directly proportional to the rate of energy deposition within the flow cell) of 1% per month is acceptable, then a radionuclide with a half life of little over 1 year may be selected. However, if a rate of decline in detector sensitivity of 1% per year is required, then the radionuclide source must have $t_1 > 70$ years. One of the advantages of a BIF detector is that its long term response to a specified sample is related to $\dot{E}_0(t)$, which may always be calculated from $\dot{E}_0(0)$ using

$$
\dot{E}_0(t) = \dot{E}_0(0) \exp \left( -\frac{\ln 2 \times t}{t_1} \right) \quad \text{(Eq. 3.11)}
$$
The final factor of Equation (3.9) requiring examination is the barrier factor, \( f(E, r_1, P_1) \). As stated in Chapter 2, whether or not a physical barrier between the \( \beta^- \)-emitting radionuclide and the solution in the flow cell is required at all is a debatable point, as it may well prove possible to fabricate an uncovered source which does not dissolve to any measurable extent in the eluent which flows over the source during normal use. However, the increasing importance of the safety aspects of laboratory equipment does suggest that there may be considerable difficulty associated with the marketing of a detector which could release significant quantities of radioactive material as a result of a minor operator error—such as the use of an eluent in which the radionuclide was soluble. It therefore seems likely that some kind of barrier will exist between the radionuclide and the liquid phase in any practical BIF excitation source.

If the barrier is considered to be a sheet of material of density \( \rho_1 \), and whose constituent atoms have a mass number and an atomic number of \( A \) and \( Z \) respectively, then from the Bethe equation \( [40] \) we may write the stopping power of the barrier for electrons as

\[
\frac{-dE}{dx} = \frac{2 \pi e^4 \rho_1 N_L Z}{m_0 v^2 A} \left[ \ln \frac{m_0 v^2 E}{21^2(1 - \beta^2)} - \ln 2(\sqrt{1 - \beta^2} - 1 + \beta^2) \\
+ 1 - \beta^2 + (1 - \sqrt{1 - \beta^2})/8 \right] \tag{Eq. 3.12}
\]

where \( N_L = \) Avogadro's constant

- \( E = \) energy of the electron of velocity \( v \)
- \( m_0 = \) rest mass of an electron
- \( e = \) charge of an electron

and \( (1 - \beta^2) = \frac{m_0 c^2}{E + m_0 c^2} \).
The stopping powers of silver foil ($P_1 = 10 \text{ g cm}^{-3}$) for the maximum energy and mean energy $\beta^-$ particles emitted during the decays of $^{63}\text{Ni}$ and $^{147}\text{Pm}$ are, along with the ranges of those $\beta^-$ particles in silver as shown below.

**Stopping powers and mean ranges for $\beta^-$ particles**

<table>
<thead>
<tr>
<th>$\bar{\beta}$ particle energy (keV)</th>
<th>Ag Stopping power (keV/µm)</th>
<th>mean range in Ag (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>230 ($E_{\text{max}}$ for $^{147}\text{Pm}$)</td>
<td>2</td>
<td>53</td>
</tr>
<tr>
<td>75 ($E$ for $^{147}\text{Pm}$)</td>
<td>3.5</td>
<td>11.4</td>
</tr>
<tr>
<td>67 ($E_{\text{max}}$ for $^{63}\text{Ni}$)</td>
<td>4</td>
<td>8.9</td>
</tr>
<tr>
<td>22 ($E$ for $^{63}\text{Ni}$)</td>
<td>13</td>
<td>2.1</td>
</tr>
</tbody>
</table>

The $^{147}\text{Pm}$ source, shown in Fig. 2.11, was protected with a 5 µm silver foil barrier, for which the mean fractional energy loss of $\beta^-$ particles is $\sim 0.2$ so that $f(50, 5, 10) \sim 0.8$. A $^{63}\text{Ni}$ source protected in the same manner would have $f(16, 5, 10) \sim 0.06$, so that from Equation (3.9) it follows that such a $^{63}\text{Ni}$ source is approximately two orders of magnitude less efficient at depositing energy into the flow cell than a $^{147}\text{Pm}$ source of the same activity (for $t=0$ in both cases).

The values of $f(E, r_1, P_1)$ for both isotopes could be increased by the use of thinner barriers or by the use of lower atomic mass
material for the fabrication of the barrier. However, thinner barriers may not be capable of satisfactorily protecting the source unless the radionuclide is in a less soluble form than the carbonate presently used for the $^{147}$Pm source. Furthermore thinner foils are more difficult to make non-transparent and a number of glassy or resin-based $\beta^-$ sources also emit visible light. Lower mass number materials, such as Al or Fe, would be attractive, although there could be difficulties with their long term corrosion resistance in some media.

Secondly, the geometric light collection efficiency (GLCE) is important. In the Mk I flowcell, if the PM tube cathode offers a radius of $a$ cm, perpendicular to the fluorescence light, the GLCE is approximated by

$$\text{GLCE} \approx \frac{1}{2} \left(1 - \frac{d}{(d^2 + a^2)^{\frac{1}{2}}} \right)$$

where $d$ is the distance from the point source to the PM tube photocathode. Substituting $d = 0.4$ cm and $a = 2.54$ cm gives a calculated value of 0.422 for GLCE. However, this value is higher in practice because the silver window of the excitation source acts as a reflector.

The geometrical construction of the Mk II flowcell results in an even lower value of 0.146 for GLCE. However, this flowcell is more sensitive than the Mk I flowcell because all of the solution entering the flowcell enters the "active volume" of the flowcell (see Chapter 2).

The three factors outlined above:-
(a) construction and activity of source,
(b) geometric light collection efficiency and that
(c) the solution must enter the "active volume" of the flowcell, are of fundamental importance in the consideration of flowcell design.
One further consideration must be that of dead volume. Dead volume in BIF flowcells may be defined as the volume of the flowcell before the solution enters the active volume plus the active volume of the flowcell. Large dead volume results in the loss of resolution from chromatographic peaks eluted from the column with retention times that are close together. Small dead volume results in high resolution. Considering the Mk I flowcell, the active volume may be calculated to be approximately 3 µl. Connection of this volume to the column outlet requires (say) 10 cm of 0.02 cm (8 thou) diameter stainless steel tubing, resulting in a total dead volume of approximately 6 µl — a value which compares favourably with current flowcells.

Detector performance in terms of noise and signal will now be discussed, to demonstrate the conditions required to achieve good detector performance in terms of signal-to-noise ratio.

**Noise**

Noise, in the context of a BIF chromatography detector, is the noise-in-(background) signal variety (130), and results almost entirely from the random nature of the radioactivity decay process of the $\beta^-$ source and of the consequent photon detection rate.

Other noise sources may be made negligible by suitable electronic design, and noise-in-(peak) signal will not be considered separately as it will be small for large peaks and equal to the noise-in-(background) signal for very small peaks. The noise in the background signal may be taken as the root variance, $\sigma_m^2$, in the photon detection rate, $\hat{P}_m^2$, and is given by

$$\sigma_m = \left[ \frac{d_m^2 \hat{P}_m(0) + d_B^2 B + I_D}{T} \right]^{\frac{1}{2}}$$

(Eq. 3.13)
where $I_0$ is the PMT dark signal, $P_B$ is the photon emission rate of the cell in the absence of fluorescent eluent, $d_B$ is the detection efficiency of such photons and $T$ is the characteristic time of the photon detection system — given by the sampling time if single photon counting is used or by $\tau/2$ (with $\tau = RC$) if RC current integration is used (130).

While the absolute value of the statistical noise may be reduced by increasing the characteristic time, $T$, of the detection electronics, an upper limit to $T$ (which also determines the response time of the detector) is generally set by chromatographic requirements, and in practice $T \leq 1$ s.

The appropriate expression for $P_m$ and $P_s$ could be taken from equations 3.6 and 3.7 directly, although it is convenient to simplify these equations in the light of practical BIF chromatographic detection. As considerable care is normally exercised in selecting mobile phases which are free of quencher impurities and as such mobile phases are generally deoxygenated before use, it seems reasonable to set $[Q] = 0$. This allows equation 3.8 to be rewritten as:

$$R_B(S) = \frac{k_0[M]}{(k_{50}[M] + 1)(k_{17} + k_{18} + k_{29}[S])}\left\{\frac{d_m(k_{29} + k_{52}[M]k_{32})}{k_{32} + k_{33} + k_{34}} - \frac{d_m k_{29}k_{20} + k_{54}[M]}{k_{17} + k_{18} + k_{20}}\right\}[S] \quad (Eq. 3.14)$$

From equations (3.13) and (3.14) the (peak response) signal-to-noise ratio for normal BIF operation may be written as

$$\frac{R_B(S)}{\sigma_M} = \frac{\hat{P}_m(S)T^\frac{1}{2}[S]}{(I_0 + d_B\hat{P}_B + d_m\hat{P}_m(0))^\frac{1}{2}(k_{20} + k_{51}[M])}\left\{\frac{d_s k_{32}(k_{29} + k_{52}[M])}{k_{32} + k_{33} + k_{34}} - \frac{d_m k_{29}(k_{20} + k_{51}[M])}{k_{17} + k_{18} + k_{20}}\right\} \quad (Eq. 3.15)$$
Consideration of Equation (3.15) allows the determination of conditions required to achieve good detector performance in terms of signal-to-noise. In practice the limit of detection may be taken as that value of \([S]\) which gives rise to a signal-to-noise ratio of 2, and this value will generally be sufficiently small that \(k_{29}[S] \ll (k_{17} + k_{18} + k_{20})\), so that \(\dot{P}_{m}(S) \approx \dot{P}_{m}(0)\). Furthermore, as for conventional fluorescence detector systems, the sample species, \(S\), is generally highly fluorescent compared with the solvent, \(M\), so that the second term in the difference bracket of Equation (3.15) is small compared with the first term. For these reasons a near "sample-independent" limit of detection for highly fluorescent species may be written as

\[
[S]_{\text{min}} = \frac{2}{d \dot{P}_{m}(0)} \left( \frac{k_{20} + k_{51}[M]}{k_{29} + k_{52}[M]} \right) \left( \frac{I_{D} + d \dot{P}_{m}}{T} \right)^{\frac{1}{2}} \quad \text{(Eq. 3.16)}
\]

Even this simplified form shows some sample dependence through \(k_{29}\) which has been found to vary slightly for different sample materials.

Parameters for processes involved in BIF photon emission from

**Toluene and Anthracene** (71, 74, 129)

<table>
<thead>
<tr>
<th>Toluene (M in Table 3.15)</th>
<th>Anthracene (S in Table 3.15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{16} = 5.1 \times 10^{10} \text{ M}^{-1} \text{s}^{-1})</td>
<td>(k_{34} = 5 \times 10^{7} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(k_{-16} = 9.2 \times 10^{11} \text{ s}^{-1})</td>
<td>(k_{32} = 5 \times 10^{7} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(k_{20} = 6.5 \times 10^{5} \text{ s}^{-1})</td>
<td>(k_{37} \sim 10^{6} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(k_{40} = k_{39}, 5.7 \times 10^{10} \text{ M}^{-1} \text{s}^{-1}) (for (Q = \text{oxygen}))</td>
<td>(k_{41} = 10^{10} \text{ M}^{-1} \text{s}^{-1})</td>
</tr>
<tr>
<td>(k_{30} = k_{29}, 5.2 \times 10^{10} \text{ M}^{-1} \text{s}^{-1}) (for (S = \text{anthracene}))</td>
<td>(k_{33} \sim 10^{7} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(k_{18} = 10^{7} \text{ s}^{-1})</td>
<td>(k_{40} = 10^{7} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(k_{21} \sim 10^{-1} \text{ s}^{-1})</td>
<td>(k_{37} \sim 10^{7} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(k_{17} = 3 \times 10^{7} \text{ s}^{-1})</td>
<td>(k_{30} = 10^{6} \text{ s}^{-1})</td>
</tr>
<tr>
<td>(k_{27} \sim 10^{6} \text{ s}^{-1})</td>
<td>(k_{41} = 10^{10} \text{ s}^{-1})</td>
</tr>
</tbody>
</table>
Using the data shown above, the $P_m$ value of $6.5 \times 10^5$ s$^{-1}$, $T = 1$s, $d_s = 0.25$ and $d_m = 6 \times 10^3$, the detection limit may be evaluated as follows:

$$[S]_{\text{min}} = \frac{2}{0.25 \times (6 \times 10^5)} \left( \frac{(6.5 \times 10^5) + (5.5 \times 10^4)}{(5.2 \times 10^{10}) + (5.6 \times 10^5)} \right)^{\frac{1}{2}} \left( \frac{200 + 400 + (6 \times 10^{-3}) (6 \times 10^5)}{1} \right) $$

$$= 1.17 \times 10^{-8} \text{ M} \cdot \text{L}^{-1} $$

For anthracene (Molar mass = 178.2 g)

$$= (1.17 \times 10^{-8})(178.2) \text{ g} \cdot \text{L}^{-1}$$

For a 20 µl sample size,

$$[S]_{\text{min}} = (20 \times 10^{-6}) (1.17 \times 10^{-8})(178.2) \text{ g} \approx 41.7 \text{ pg}$$

A value which agrees well with the detection limit of ~500 pg shown in Table 3.6, if the dilution factor is taken into account (~10, determined independently by a uv monitor).

The advantages offered by choosing mobile phase systems with large values of $P_m$ and $k_{29}$ but small values of $k_{20}$ are clear, and in practice these lead to the selection of aromatic hydrocarbons (131), such as benzene, toluene and p-xylene, as eluents either in pure form or as high molarity (>0.1 M) solutions in (relatively) inert liquids such as hexane.

One of the most promising avenues for the enhancement of HIF sensitivity is the choice of a small value of $d_m$ to minimise the final term in Equation (3.16). In practice this means the optical filtration of the photons emitted from the HIF flow cell to remove those which result from solvent fluorescence. This technique has been applied to the emissions from a toluene based mobile phase, using a number of cut-off filters (see Table 3.4) which reduced $d_m$.
from 0.2 to approximately $6 \times 10^{-3}$ (including photocathode efficiency).

Not surprisingly the photomultiplier dark count or dark current must be kept as small as possible, and it seems likely that small area, bialkali photocathode tubes offer the greatest advantage in this respect. $P_B$, the photon emission rate when the cell contains a non-fluorescent material (such as carbon tetrachloride), may be significant if fluorescent materials (such as ptfe) have been used in the construction of the cell, or if the $\beta^-$ particles are able to reach the cell window and excite fluorescence from the window material. Where $^{147}\text{Pm}$ is used as the excitation source a small amount of Cerenkov radiation may be produced by the highest energy $\beta^-$ particles when eluents of a high refractive index are used (e.g. toluene).

3.10 EXAMPLES OF CHROMATOGRAMS IN NORMAL PHASE OPERATION

In the examples of chromatograms, attention has been focussed on detecting polycyclic aromatic hydrocarbons (PAHs). There are two main reasons for this. Firstly, PAHs occur in crude tar products and they are also formed during the pyrolysis of coal, oil and other forms of organic matter. Contamination of the environment with PAHs may occur through the discharge of industrial wastes, accidental spillages, combustion processes of industry, motor vehicle emissions and in food during preparation and cooking. Lo and Sandi (132) have reviewed the occurrence of PAH residues in food and Harrison et al. (133) have paid particular attention to their distribution in water supplies. The identification and determination of these compounds in various parts of the environment, air, food, water, etc. is of interest as some of them are known to be strongly carcinogenic (134), whilst others are non- or only weakly carcinogenic. Secondly, PAHs
exhibit fluorescence, a property on which the HIF detector relies for its operation.
Chromatogram 9
400 ng each
1 Anthracene
2 p-Terphenyl
3 Perylene
4 Diphenyl hexatriene
5 bis-Methyl-styryl-benzene

Chromatogram 10
200 ng each
1 Anthracene
2 p-Terphenyl
3 Perylene
4 Diphenyl hexatriene
5 bis-Methyl-styryl-benzene
Chromatogram 11
80 ng each
1 Anthracene
2 p-Terphenyl
3 Diphenylhexatriene
4 bis-Methyl-styryl-benzene

Chromatogram 12
20 ng each
1 Anthracene
2 p-Terphenyl
3 Diphenylhexatriene
4 bis-Methyl-styryl-benzene
Chromatogram 13
300 ng each
1 Fluoranthene
2 Benzo [a] pyrene
3 3-Methyl cholanthrene

Chromatogram 14
L39 filter fitted
300 ng each
1 Fluoranthene
2 Benzo [a] pyrene
3 3-Methyl cholanthrene
Chromatogram 15
Chart speed 0.5 cm min^{-1}
20 ng each
1 Fluoranthene
2 Benzo [a] pyrene
3 3-Methyl cholanthrene

Chromatogram 16
Chart speed 0.2 cm min^{-1}
20 ng each
1 Fluoranthene
2 Benzo [a] pyrene
3 3-Methyl cholanthrene
Chromatogram 17
20 ng each
1 Pyrene
2 Diphenyl hexatriene
3 bis-Methyl-styryl-benzene

Chromatogram 18
20 ng each
1 Pyrene
2 Benzo [a] pyrene

Chromatogram 19
20 ng each
1 Pyrene
2 Benzo [a] pyrene
Chromatogram 20

1) 17.6 μg p-Dimethoxybenzene
2) 80 ng NN'Dimethylaniline
3) 40 ng PPO (Diphenylhexazone)
3.11 RESPONSE OF HIF DETECTOR IN REVERSED PHASE OPERATION

### TABLE 3.10 Response of HIF Detector to Eluted Materials

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mobile Phase</th>
<th>Fluorescent Background (cps)</th>
<th>Response (counts/µg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthranilic acid</td>
<td>Methanol</td>
<td>77%</td>
<td>5500</td>
</tr>
<tr>
<td>Carbazole</td>
<td>Water</td>
<td>13%</td>
<td>35000</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>Toluene</td>
<td>8%</td>
<td>25800</td>
</tr>
<tr>
<td>Anthranilic acid</td>
<td>Acetonitrile</td>
<td>78%</td>
<td>14700</td>
</tr>
<tr>
<td>Carbazole</td>
<td>Water</td>
<td>14%</td>
<td>111400</td>
</tr>
<tr>
<td>Indole</td>
<td>p-Xylene</td>
<td>8%</td>
<td>48800</td>
</tr>
<tr>
<td>9-Amino acridine</td>
<td>Methanol</td>
<td></td>
<td>2000</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>Methanol</td>
<td>90%</td>
<td>8000</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>Toluene</td>
<td>10%</td>
<td>63600</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>Methanol</td>
<td>90%</td>
<td>5500</td>
</tr>
<tr>
<td></td>
<td>Bensyl alcohol</td>
<td>10%</td>
<td>25300</td>
</tr>
</tbody>
</table>

To achieve reasonable response in reversed phase HPLC, the mobile phase, methanol or methanol/water mixtures, must be doped with an energy transfer agent such as toluene. Doping does not achieve a similar response to that obtained from the HIF detector when employed in normal phase HPLC using hexane as the mobile phase. For example, carbazole (quantum yield ($\phi$) 0.38 and decay time ($\tau$) 16.2 ns) produces a response of 35000 counts µg⁻¹ in methanol/water/toluene whilst anthracene ($\phi$ 0.36, $\tau$ 4.9 ns) shows eight times this response in hexane. Furthermore, 1-naphthol ($\phi$ 0.21, $\tau$ 10.6 ns) gives a response of 63600 in methanol/toluene whilst naphthalene ($\phi$ 0.23, $\tau$ 96 ns) produces a response ~1.5 times greater in pure hexane.

The presence of water and, to a lesser extent, methanol affects
the mechanism by which the BIF is produced. It has been shown (135) that on the addition of 24% water, the original light intensity drops by more than 70% in a solution of PPO in purified dioxan.

The first step in the radiolysis of water is most probably

\[
H_2O \rightarrow H_2O^+ + e^-
\]

(1)

In neutral solution the electron becomes solvated

\[
\text{e}^- + nH_2O \rightarrow \text{e}^-_{aq}
\]

(2)

The time taken for solvation is 0.2 ps in water, 2 ps in methanol and 10 ps in ethanol (136).

After $10^{-8}$ s the species present in irradiated water are $H_2$, $H_2O_2$, $H^\cdot_{aq}$, $H^+O^-$, $e^-_{aq}$, $OH^-$ and $H_3^0^+$ (136). These species would be present in the flowcell since the residence time of the mobile phase is approximately 0.6 s for a 10 µl flowcell with a mobile phase flow rate of 1 cm$^3$ min$^{-1}$. Species such as $H_2O_2$ would be expected to quench BIF and is a major factor in reducing the photon yield from solution.

The ionization potential of toluene is 8.8 eV (137) and that of water 12.6 eV. Thus one would expect fewer ionized species and secondary electrons to be formed in water than in toluene, for a beta-particle track. The secondary electrons produced in toluene have an important role to play in the overall process of BIF because they cause considerable ionization and excitation of toluene molecules. Secondary electrons produced in water are hydrated electrons and instead of causing further excitation and ionization may interact with other species produced by the radiolysis, e.g.

\[
e^-_{aq} + e^-_{aq} + 2H_2O \rightarrow H_2 + 2OH^-
\]

\[
e^-_{aq} + H_3O^+ \rightarrow H + H_2O
\]
Thus "ionization and excitation amplification", which is present in non-polar solvents such as toluene, is greatly reduced or even absent in polar solvents.

3.12 EFFECT OF ADDING FLUORESCENT MATERIAL TO ELUTING SOLVENT

Table 3.11 and Fig. 3.7 clearly demonstrate that doping the mobile phase with toluene increases the BIF photon yield from a solution. Furthermore, the increase in photon yield is proportional to the quantity of toluene added.

Table 3.12 also shows the effect of adding toluene or p-xylene to a number of different reversed phase mobile phases and also the use of the L39 cut-off filter and the pyrex microscope slide.

A major problem with the use of toluene, as an energy transfer agent, is its low solubility in methanol/water mixtures. Saturation of 80% methanol/20% water occurs on the addition of approximately 8% (vol/vol) of toluene. More polar solvents such as benzyl alcohol
EFFECT OF ADDED TOLUENE ON ANTHRANILIC ACID RESPONSE

PEAK COUNTS $\times 10^4$

% ADDED TOLUENE

Fig 3.7
TABLE 3.12 Response from Anthranilic Acid in Reversed Phase Mobile Phases

1 µg sample. Solvents deoxygenated with Argon

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>PEAK COUNTS/µg</th>
<th>SOLVENT</th>
<th>SOLVENT c.p.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthranilic Acid</td>
<td>13081</td>
<td>75% MeOH, 22% H₂O, 3% Tol</td>
<td>3350¹</td>
</tr>
<tr>
<td>Anthranilic Acid</td>
<td>33383</td>
<td>77% MeOH, 15% H₂O, 8% Tol</td>
<td>5500¹</td>
</tr>
<tr>
<td>Anthranilic Acid</td>
<td>68967</td>
<td>78% CH₃CN, 14% H₂O, 8% p-Xyl</td>
<td>14750¹</td>
</tr>
<tr>
<td>Anthranilic Acid</td>
<td>69764</td>
<td>87% CH₃CN, 5% H₂O, 8% p-Xyl</td>
<td>16300¹</td>
</tr>
<tr>
<td>Anthranilic Acid</td>
<td>70663</td>
<td>87% CH₃CN, 5% H₂O, 8% p-Xyl</td>
<td>2250²</td>
</tr>
</tbody>
</table>

1. Microscope Slide
2. L39 filter

are expected to be more soluble in such mixtures and Table 3.13 shows the relative efficiencies of a number of dopents.

TABLE 3.13 Relative Efficiency of Dopents

<table>
<thead>
<tr>
<th>DOPENT</th>
<th>PEAK COUNTS (µg⁻¹)</th>
<th>RELATIVE EFFICIENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorobenzene</td>
<td>684</td>
<td>0.52</td>
</tr>
<tr>
<td>Benzene</td>
<td>718</td>
<td>0.55</td>
</tr>
<tr>
<td>Ethyl benzene</td>
<td>720</td>
<td>0.55</td>
</tr>
<tr>
<td>Cumene</td>
<td>763</td>
<td>0.58</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>1267</td>
<td>0.98</td>
</tr>
<tr>
<td>Toluene</td>
<td>1299</td>
<td>1.00</td>
</tr>
<tr>
<td>Mesitylene</td>
<td>1570</td>
<td>1.21</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>1811</td>
<td>1.39</td>
</tr>
<tr>
<td>Diphenyl methane</td>
<td>1841</td>
<td>1.42</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>1845</td>
<td>2.19</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>7440</td>
<td>5.72</td>
</tr>
</tbody>
</table>
3.13 EXAMPLES OF CHROMATOGRAMS IN REVERSED PHASE OPERATION

Chromatograms 21 to 27 show the performance of the BIF detector in reversed phase chromatography. Several important points may be noted from these chromatograms. Firstly, calculations of $R_c$ and $S_c$, taken from a chromatographic separation similar to chromatogram 23, showed that the sensitivity of BIF in the reversed phase system (10% toluene in 90% methanol, 10% water) was 47 times less sensitive for anthracene, 108 times less sensitive for diphenyl hexatriene and 29 times less sensitive for bis-methyl-styryl-benzene than in normal phase (10% toluene in hexane) chromatography.

Secondly, in reversed phase chromatography, the most polar compound elutes from the column first which is the reverse of the elution order found in normal phase chromatography. Throughout the investigation on the use of the BIF detector in reversed phase chromatography, the order of elution of the components was found to be the same as that observed in normal phase chromatography suggesting that under these conditions the separation was by adsorption and not partition.
Chromatogram 21

1  3.2 µg Anthracene
2  1.6 µg Diphenylhexatriene
3  1.6 µg bis-Methyl-styryl-benzene

Mobile phase 10% toluene in 90% methanol/10% water.

Chromatogram 22

1  2 µg Anthracene
2  1 µg Diphenylhexatriene
3  1 µg bis-Methyl-styryl-benzene
Chromatogram 23

1. 0.2 µg Anthracene
2. 0.1 µg Diphenylhexatriene
3. 0.1 µg bis-Methyl-styryl-benzene

Mobile phase 10% toluene in 90% methanol/10% water.
Chromatogram 24

1  3.2 µg Anthracene  
2  1.6 µg Diphenylhexatriene  
3  1.6 µg bis-Methyl-styryl-benzene

Chromatogram 25

1  2 µg Anthracene  
2  1 µg Diphenylhexatriene  
3  1 µg bis-Methyl-styryl-benzene

Mobile phase 6.25% toluene in 80% acetonitrile/20% water.
Chromatogram 26
1 2.4 µg Anthracene
2 0.75 µg Benzanthracene
Mobile phase 6.25% toluene in 80% acetonitrile/20% water.

Chromatogram 27
1 1 µg Pyrene
2 0.35 µg Benzo [a] pyrene
Mobile phase 6.25% toluene in 80% acetonitrile/20% water.
3.14 QUENCHED BETA-INDUCED FLUORESCENCE (QHIF)

The Detection of Fluorescence Quenchers

The principle of quenching outlined in Chapter 1 may be used for
the detection of quencher species eluting from an hplc column. If
a deoxygenated toluene/hexane mixture is used as the mobile phase,
then the peak magnitude of the quenched beta induced fluorescence
(QHIF) signal recorded by the photon detection apparatus is given by

\[ R_q(Q) = \frac{d_m}{d_m} \left( \hat{p}_m ([Q]) - \hat{p}_m ([Q] = 0) \right) \]  

(Eq. 3.17)

where \([Q]\) is the peak concentration of quenching material in the
detector flowcell, and \(d_m\) is the efficiency with which the mobile
phase emissions are detected.

Equations (3.6) and (3.17) may be combined to yield

\[ R_q(Q) = \frac{d_m}{d_m} \left( k_{20} + k_{51}[M] \right) k_0[M] \cdot k_{39} [Q] \]  

(Eq. 3.18)

i.e. a negative going signal with a peak magnitude which is a function
of the (flow cell) concentration of the quenching species, \(Q\).

3.15 RESPONSE IN QHIF MODE OF ELUTED MATERIALS

The QHIF signal recorded following the separation of benzaldehyde,
anisaldehyde and propanone (6 \(\mu\)g of each in a 20 \(\mu\)l sample volume of
hexane solvent) on a 15 x 0.6 cm column of 5 \(\mu\)m Lichrosorb eluted with
a deoxygenated 98% toluene/2% acetonitrile mobile phase at a flow rate
of 1 ml min\(^{-1}\) is shown in Chromatogram 28. (The first peak is of the
air saturated sample solvent.) The integrated responses (peak areas
expressed as -photons) obtained from a variety of samples eluted in
toluene based mobile phases are collected in Table 3.14.

Clearly QHIF can be used to detect the elution of species which
would be expected to quench the fluorescence of toluene, e.g.
Chromatogram 28 QHIF

1 Hexane

~ 6 µg each of
2 Benzaldehyde
3 Anisaldehyde
4 Propanone

Mobile phase 2% acetonitrile in toluene.
molecules with highly polar functional groupings.

**TABLE 3.14 Response in QBIF Mode of Eluted Materials**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mobile Phase</th>
<th>Fluorescent Background (cps)</th>
<th>Response (counts/μg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-dibromoethane</td>
<td>hexane</td>
<td>12,000</td>
<td>6,740</td>
</tr>
<tr>
<td>iodoethane</td>
<td></td>
<td></td>
<td>2,660</td>
</tr>
<tr>
<td>1-bromopropane</td>
<td></td>
<td></td>
<td>2,130</td>
</tr>
<tr>
<td>tetrachloroethene</td>
<td></td>
<td></td>
<td>6,800</td>
</tr>
<tr>
<td>1,2-dichloroethane</td>
<td></td>
<td></td>
<td>1,660</td>
</tr>
<tr>
<td>nitrobenzene</td>
<td></td>
<td></td>
<td>11,670</td>
</tr>
<tr>
<td>chlorobenzene</td>
<td></td>
<td></td>
<td>7,460</td>
</tr>
<tr>
<td>p-dichlorobenzene</td>
<td></td>
<td></td>
<td>7,700</td>
</tr>
<tr>
<td>hexachlorobenzene</td>
<td></td>
<td></td>
<td>7,650</td>
</tr>
<tr>
<td>3-chlorotoluene</td>
<td></td>
<td></td>
<td>8,060</td>
</tr>
<tr>
<td>4-chlorotoluene</td>
<td></td>
<td></td>
<td>6,600</td>
</tr>
<tr>
<td>thiophen</td>
<td></td>
<td></td>
<td>5,050</td>
</tr>
<tr>
<td>propanone</td>
<td>5% acetonitrile</td>
<td>354,000</td>
<td>84,800</td>
</tr>
<tr>
<td>butan-2-one</td>
<td>9% toluene</td>
<td></td>
<td>67,500</td>
</tr>
<tr>
<td>ethanol</td>
<td></td>
<td></td>
<td>119,400</td>
</tr>
<tr>
<td>anisaldehyde</td>
<td></td>
<td></td>
<td>182,600</td>
</tr>
<tr>
<td>cinnamaldehyde</td>
<td></td>
<td></td>
<td>268,400</td>
</tr>
<tr>
<td>benzaldehyde</td>
<td>toluene</td>
<td>428,000</td>
<td>268,700</td>
</tr>
<tr>
<td>nitropropane</td>
<td></td>
<td></td>
<td>167,200</td>
</tr>
<tr>
<td>nitrobenzene</td>
<td>90% hexane</td>
<td>350,000</td>
<td>235,400</td>
</tr>
<tr>
<td>10% toluene</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
It thus becomes relevant to consider the sensitivity of the BIF technique for the detection of "good" quenchers, which may be considered to be those species which quench the excited mobile phase molecules efficiently and for which \( k_{39} > 10^{10} \text{ M}^{-1} \text{ s}^{-1} \). As before the noise in the recorded signal may be taken as the root variance of the photon detection rate, \( \sigma_m^2 \), where

\[
\sigma_m = \left[ \frac{I_d + \hat{P}_{dB} + \hat{P}_m}{T} \right]^{1/2}
\]

(Eq. 3.19)

where \( I_d \) is the dark signal, \( \hat{P}_{dB} \) the signal detected from sources other than solvent fluorescence, and \( T \) is characteristic time of the detector electronics (see below).

For QBIF no effort has been made to reduce \( d_m \) to zero (as was the case in normal BIF) so that, for a toluene based mobile phase

\[
\dot{P}_m \cdot d_m \gg (I_d + \hat{P}_{dB}) \quad \text{and we may assume}
\]

\[
\sigma_m = \left[ \frac{\hat{P}_m}{T} \right]^{1/2}
\]

(Eq. 3.20)

The signal-to-(mobile phase) noise ratio for QBIF may be written from Equations (3.18) and (3.20):

\[
\frac{R_Q(Q)}{\sigma_m} = \left[ \frac{\frac{d_m}{(k_{20} + k_{51}[M])} k_0 [M] T \tau_m}{(k_{50}[M] + 1) (k_{17} + k_{18} + k_{20} + k_{39}[Q])} \right]^{1/2}
\]

(Eq. 3.21)

where \( \tau_m = (k_{17} + k_{18} + k_{20})^{-1} \), the lifetime of the fluorescing species.

Equation (3.21) illustrates that the signal-to-noise ratio, and so the sensitivity, of the QBIF detector may be increased by increasing the characteristic time, \( T \), of the detection system. If the technique of single photon counting is used then \( T \) is the sampling time \((130)\), whereas if the measurement of anode current is used to quantify the photon detection rate then \( T = \tau/2 \), where \( \tau \) is the RC time constant of the integrating circuitry \((130)\).

In normal mode BIF systems single photon counting provides the
best method of determining the photon detection rate (at least as far as statistical noise is concerned). However, this technique has severe limitations for the QHIF mode, as the signal-to-noise ratio is governed by Equation (3.21) which, when written as

$$\frac{R(Q)}{\sigma_m} = (d_m T_m)^{\frac{1}{2}} \frac{k_{39}[Q]}{k_{17} + k_{18} + k_{20} + k_{39}[Q]}$$  

(Eq. 3.22)

illustrates the desirability of operating with $d_m T_m$ (i.e. the photon detection rate) as large as possible. Single photon counting becomes susceptible to errors resulting from pulse pile-up at counting rates in excess of $10^6 \text{ s}^{-1}$, and the lower value of $T$ inherent in using RC integration for anode current measurement is more than offset by the improvement which becomes possible when photon detection rates in excess of $10^8 \text{ s}^{-1}$ may be used. However, limitations on the value of $T$ are set by the chromatographic requirement that the time constant for detector response be small compared with the peak rise-time (138). This time constant (governed by $\tau = RC$) must generally be kept below 2 seconds, so that $T \leq 1 \text{ s}$.

The limit of detection of the QHIF detector, which may be taken as the concentration of a "good" quencher which may be detected with a signal-to-noise ratio of 2, may also be evaluated from Equation (3.22). If toluene based mobile phases are considered, i.e.

$$(k_{17} + k_{18} + k_{20}) \approx 4.4 \times 10^7 \text{ s}^{-1} \text{ and } k_{39} \approx 5 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$$

and a detection system for which $d_m T = 0.2$, then the limits of detection for various eluent emission rates are given in Table 3.15 where the detected concentrations refer to $[Q]$ in the detector flowcell. In a typical modern chromatographic system operated with a microparticulate column, dilution factors of between 10 and 20 are common for retained peaks (i.e. the ratio between the $[Q]$ of the sample
injected and the maximum \([Q]\) in the detector during peak elution).
Assuming an average dilution factor of 15, and a BIF detector flow cell volume of 10 \(\mu l\), the detection limit in terms of number of moles of \(Q\) may also be calculated, and the corresponding values are recorded in Table 3.15.

**TABLE 3.15 Theoretical limits of detection of quenchers in QBIF detector operated with toluene based eluents**

<table>
<thead>
<tr>
<th>Toluene emission ((a))</th>
<th>Limits of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>minimum detected concentration /M typical minimum sample size /mole</td>
</tr>
<tr>
<td>10(^5)</td>
<td>1.24 (\times) 10(^{-5})</td>
</tr>
<tr>
<td>3 (\times) 10(^5)</td>
<td>7.19 (\times) 10(^{-6})</td>
</tr>
<tr>
<td>10(^6)</td>
<td>3.94 (\times) 10(^{-6})</td>
</tr>
<tr>
<td>3 (\times) 10(^6)</td>
<td>2.27 (\times) 10(^{-6})</td>
</tr>
<tr>
<td>10(^7)</td>
<td>1.24 (\times) 10(^{-6})</td>
</tr>
<tr>
<td>10(^8)</td>
<td>3.94 (\times) 10(^{-7})</td>
</tr>
</tbody>
</table>

Notes

(a) photon detection rate is generally \(\sim 20-30\%\) of this value; in these calculations \(d_m = 0.2\).

(b) close to the rates obtained with the BIF system operated with a 1 mCi \(^{147}\)Pm source. The predicted limit of detection is equivalent to \(\sim 150\) ng of nitrotoluene, which agrees well with the experimental value of \(\sim 200\) ng.

3.16 LINEARITY OF RESPONSE WITH CONCENTRATION

Equation (3.18) shows how the magnitude of the QBIF response, \(R_q(Q)\), varies with the concentration of the quenching species, \([Q]\), in the detector flow cell. Even for efficient quenchers, for which \(k_{39} > 10^{10} \text{ M}^{-1} \text{ s}^{-1}\), \((k_{17} + k_{18} + k_{20}) \gg k_{39}[Q]\) for \([Q] < 10^{-4}\) M, so that at the detector concentrations of interest in most hplc analyses Equation (3.18) approximates to
\[
R_Q(Q) \approx \frac{-d_m(k_{17} + k_{51}[M])k_0[M]k_{39}}{(k_5[M] + 1)(k_{17} + k_{18} + k_{20})^2}[Q]
\] (Eq. 3.23)

which may also be written

\[
R_Q(Q) \approx -d_m \dot{p}_m \tau_m k_{39}[Q]
\] (Eq. 3.24)

Equation (3.24) predicts a linear dependence of the QBIF response with quencher concentration, although a departure from linearity is expected at high values of \([Q]\) as the approximations which led to Equation (3.23) breakdown. Furthermore from Equation (3.18) it can be seen that the departure from linearity at high quencher concentrations will appear as smaller responses than predicted by Equation (3.23).

That these predictions are borne out in practice may be seen in Figure 3.8 where the QBIF response recorded by the detector system for samples of benzaldehyde is shown as a function of benzaldehyde loading of the column. Clearly the detector response is a linear function of column loading up to \(\sim 20 \mu g (\sim 7 \times 10^{-3} M)\), and, with a column dilution factor of \(\sim 20\) in this case, this corresponds to a detector cell peak concentration of \(\sim 3.5 \times 10^{-4} M\), in good agreement with the limitations previously placed on the linear region.
Peak Counts
$\times 10^6$

Mass of Benzaldehyde (µg)

Fig 3.8
3.17 BETA-INDUCED FLUORESCENCE SPECTRA

Figs. 3.11 to 3.13 and 3.15 to 3.24 show the uncorrected BIF spectra obtained during this investigation. Fig. 3.14 is the uv excited spectrum of naphthalene in hexane (129).

3.18 FLUORESCENCE WAVELENGTH INDEPENDENCE OF EXCITATION SOURCE

Fig. 3.11 shows the BIF spectrum obtained from naphthalene in hexane excited by beta-particles from $^{63}$Ni. Fig. 3.12 and Fig. 3.13 show the naphthalene spectra obtained by excitation with $^{147}$Pm and Fig. 3.14 by uv radiation. The relevant wavelengths are tabulated in Table 3.16.

TABLE 3.16

<table>
<thead>
<tr>
<th>Figure</th>
<th>Excitation Source</th>
<th>Start Peak (nm)</th>
<th>Peak (nm)</th>
<th>End Peak (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.11</td>
<td>$^{63}$Ni</td>
<td>303</td>
<td>347</td>
<td>431</td>
</tr>
<tr>
<td>3.12</td>
<td>$^{147}$Pm</td>
<td>303</td>
<td>335</td>
<td>428</td>
</tr>
<tr>
<td>3.13</td>
<td>$^{147}$Pm</td>
<td>306</td>
<td>335</td>
<td>435</td>
</tr>
<tr>
<td>3.14</td>
<td>uv</td>
<td>301</td>
<td>333</td>
<td>430</td>
</tr>
</tbody>
</table>

Note:– wavelengths quoted are $\pm$ 5 nm.

Table 3.16 clearly demonstrates that the wavelength of the fluorescence spectrum of a solute remains constant when excited by beta-particles or uv radiation.

3.19 ENERGY TRANSFER IN THE SCINTILLATION PROCESS

Table 1.3 and Fig. 1.5 (Chapter 1), together with Figs. 3.16 to 3.19, demonstrate energy transfer in BIF. Fig. 3.15 is a scan of hexane, which represents background. These spectra have been discussed previously in Chapter 1.
Fig. 3.9
Fluorene in hexane
No monochromator slits.

Fig. 3.10
Fluorene in hexane
600 um monochromator slits.
Fig. 3.11
Naphthalene in hexane, 7 mCi Nickel-63 source.

Fig. 3.12
Naphthalene in hexane, 20 mCi Promethium-147 source.
Fig. 3.13
Naphthalene in cyclo hexane.
20 mCi Promethium-147 source.

Fig. 3.14
Naphthalene in hexane
UV excitation
ex 290 nm
Ex slit 20 nm
Em slit 20 nm
Perkin-Elmer
MPF 44 B
Fig. 3.15
Hexane

Fig. 3.16
Toluene

Fig. 3.17
-50 ppm
Anthracene in
Toluene

Hexane
Fig. 3.18
100 ppm Anthracene in Toluene

Fig. 3.19
200 ppm Anthracene in Toluene
3.20 USE OF A CUT-OFF FILTER

Figure 3.20 shows the spectrum obtained from 50 ppm anthracene in toluene and Fig. 3.21 shows the same spectrum obtained when the L39 cut-off filter was interposed between the flowcell and the entrance of the monochromator.

As described previously, a reduction in background noise is obtained by reducing the BIF count rate obtained from mobile phase alone. These figures clearly demonstrate that the toluene fluorescence peak is not observed when the L39 filter is employed.

3.21 QUENCHING IN BIF SPECTRA

Figures 3.22 to 3.24 demonstrate quenching in BIF. Addition of carbon tetrachloride to a solution of anthracene in toluene reduces the fluorescence yield from both anthracene and toluene. Upon increasing the carbon tetrachloride concentration to 0.2% (vol/vol) the toluene peak almost disappears whilst the anthracene peak is further reduced in intensity.
Fig. 3.20
50 ppm Anthracene in Toluene

Fig. 3.21
50 ppm Anthracene in Toluene with L39 cut-off filter in between flow cell and PM tube window.
Fig. 3.23
100 ppm Anthracene in Toluene -
0.1% CCl₄.

Fig. 3.22
100 ppm Anthracene in Toluene.

Fig. 3.24
100 ppm Anthracene in Toluene + 0.2% CCl₄.
3.22 Errors in Method and Technique

Errors in the method are similar to those stated in 5.6 (Chapter 5), and may arise from:

1. Preparation of sample,
2. Injection of sample,
3. Variation in flow rate,
4. Measurement of integrated peak count, and
5. Determination of $R_c$ and $S_c$.

1. Sample solutions were generally prepared by weighing 0.01 g of material, dissolving in solvent, and diluting to a final volume of 100 cm$^3$. Weighing errors should be negligible and dilution to final volume introduces an error of (say) $\pm 0.25$ cm$^3$, equivalent to $\pm 0.25\%$.

Constant volumes (20 µl) were injected into the hplc system, during the BIF work, making necessary the dilution of the stock solution. The dilution may introduce an error of $\pm 2\%$.

2. A 20 µl loop, on the injection valve, was completely filled during each injection and therefore it is unlikely that the injection of sample introduced a random error.

3. Care was taken to ensure that mobile phase flow rate remained constant throughout a particular investigation and therefore negligible error was introduced from this source.

4. The response of the detector to eluted materials is shown in the Tables as integrated counts per microgram of compound. The results shown in the Tables are average values of at least four determinations. Although normal statistical methods may be applied to calculate the variance and standard deviation from
the four determinations, the error in each determination in the
Tables may be calculated from

\[
\text{Response (R)} = \text{Signal (S)} - \text{Background (B)}
\]

Each peak and background count rate was counted for 100
seconds and thus

\[
\text{error in } R = \left[ (R + 100B) + 100B \right]^{1/2} = (200B)^{1/2}
\]

if it is assumed that the error in R is small compared to the
error in B. As the background count rate is shown in each
Table, the error in R may be calculated.

5. If B is the background count rate, the total count rate at the
limit of detection (the response required to produce a signal that
is 2 x the level of noise in the background), is

\[
= 2\sqrt{B} + B
\]

and the response, \( R_c \), at the limit of detection is

\[
R_c = K\left[ B + 2\sqrt{B} \right] - KB
\]

and the error range = \( K\left[ (B+2\sqrt{B}) + B \right]^{1/2} = K\left[ 2B + 2\sqrt{B} \right]^{1/2} \)

The sensitivity, \( S_c \), is

\[
= \frac{2Nd}{R_c} \pm \frac{X}{R_c} \left[ K(2B+2\sqrt{B})^{1/2} \right] \left( \frac{(K\sqrt{2B})^2}{2K} \right)
\]

If \( 2\sqrt{B} \) is neglected in \( (2B + 2\sqrt{B}) \), then

\[
S_c = S_c \pm \frac{X}{K} \frac{\sqrt{2B}}{4B}
\]

\[
= S_c \pm \frac{X}{2K} \frac{\sqrt{2B}}{2\sqrt{2B}}
\]
\[ \bar{S}_c = \frac{2 \text{Nd.c.m}}{V.t \, 2 \, \sqrt{2B}} [\text{Vvt}^2] \]
\[ \bar{S}_c = \frac{\text{Nd.c.m}}{V.t \, 2 \, \sqrt{2B}} \]
\[ \text{Nd.c.m} \left( \frac{1}{\sqrt{B}} \right) \pm \left( \frac{1}{\sqrt{2B}} \right) \]

Taking the value of 31100 cps as the background for the MKIV flowcell (see Table 3.6), the \( \bar{S}_c \) and the error in \( \bar{S}_c \) may be represented by

\[ \frac{1}{\sqrt{31100}} \pm \frac{1}{\sqrt{2 \times 31100}} \]
\[ = (5.67 \pm 4.00) \times 10^{-3} \]
\[ \text{i.e. the error in } \bar{S}_c \]
\[ = \pm 70\% \]

and thus \( \bar{S}_c \) for the MKIV flowcell is

- Anthracene \( (2.34 \pm 1.64) \times 10^{-9} \text{ g/cm}^3 \)
- 1,6-Diphenylhexatriene \( (0.68 \pm 0.47) \times 10^{-9} \text{ g/cm}^3 \)
- bis-Methyl-styryl-benzene \( (1.05 \pm 0.73) \times 10^{-9} \text{ g/cm}^3 \)
CHAPTER 4.  CERENKOV PHOTON ABSORPTION AS A DETECTION TECHNIQUE

4.1 Preliminary Investigation

The most popular form of detection system in HPLC is the U.V. absorption monitor, generally based on a mercury or deuterium lamp source. While not the most sensitive detection system for some compounds, the U.V. monitor combines the advantages of relative simplicity and good sensitivity for a large number of compounds. However, the conventional U.V. 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 the present detector system photons are produced by the Cerenkov effect (see Chapter 1) from energetic electrons resulting from the decay of a radioisotope. A beta decay nuclide which emits electrons with an energy above the Cerenkov threshold \( \sim 0.26 \text{ MeV} \) 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 wavelengths. 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 the present work, strontium-90 was chosen as the source of energetic electrons (see Table 2.4). \(^{90}\text{Sr}\) 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, U.K. The source chosen was a 1 mCi "point source" (Code number SIF 32), mounted on a 10 mm x 2 mm diameter cylindrical stainless-steel holder and covered by a 50 \(\mu\)m stainless-steel window, which effectively protected the radioisotope from the mobile phases which passed over the window during some of the experiments. In later experiments a 10 mCi "point source" (Code number SIF 1174), of the same dimensions as the 1 mCi source, was used as the source.

In the majority of the experiments that will be described, pulse counting, using the nuclear pulse counting system described previously for the BIF detector, was used to determine the emitted photon count rate. This technique 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, it is a method of obtaining precisely quantified data with apparatus
already available in the laboratory. An alternative technique to pulse counting will be described.

4.2 Angle of Maximum Photon Emission

Fig. 4.1

A 6 mm (OD) piece of quartz tubing was sealed at one end to produce a quartz rod of \( \sim 6 \) mm diameter, of length 20 mm. A 1 mCi strontium 90 "point source" was placed inside a brass source holder and then into the quartz tube such that the active end of the source touched the quartz rod centrally. Black photographic paper, with a small hole in it, was glued on to a 20 mm length of brass tubing of internal diameter 4 mm, and then placed on to the window of the photomultiplier tube. The quartz, containing the source, was clamped into a retort clamp and the height of position X (as
shown in Fig. 4.1) was adjusted so that it was horizontal with the centre of the brass tube. The quartz tube was then set at an angle (\(\theta\)) 0° to the horizontal, ensuring that the vertical distance (X) had not changed. The quartz and retort stand was then placed inside the wooden coffin and allowed to light adapt. The pulse count was recorded for five separate counts of 100 seconds. The recorded counts were averaged to counts per second.

The above procedure was repeated for angles of \(\theta\) between 10 and 90°, each time ensuring that distance X remained constant. The results of this investigation are presented graphically in Fig. 5.1, Chapter 5.

4.3 Photon Emission as Function of Beta-Particle Path Length

The apparatus used in Fig. 4.1 was set up with distance X, 40 mm from the base of the wooden coffin. The quartz rod was set at an angle of 30° to the horizontal, and in this position the bottom of the quartz rod was 31 mm from the coffin base. The centre of the brass tube was 37 mm from the base. A black paper screen, 32 mm high, was placed in between the quartz and the end of the brass tube and the system then allowed to light adapt. The pulse count was recorded for five separate 100 second counts and averaged to counts per second.

The black paper screen was raised by placing metal washers underneath the screen and the system allowed to light adapt.

The count was recorded for each raised position of the screen. The distance the screen was raised was between 32 and 41 mm from the base of the coffin.

The results are shown graphically in Fig. 5.2.
4.4 Cerenkov Wavelength Spectrum

The apparatus shown in Fig. 2.27 was used to obtain Cerenkov wavelength spectra. However, instead of pulse counting, it was decided to employ current measurement for the detection of photons, to avoid distortion expected from multiphoton events. The photon counting preamplifier circuit was replaced by that shown in Fig. 4.2 and the output voltage from this circuit fed directly to the electronic filter/amplifier. The analogue voltage output from the amplifier drove the pen on the chart recorder. The advantages of this method of photon detection are described later.

The 1 mCi $^{90}\text{Sr}$ point source was attached to a quartz 1 cm x 1 cm cuvette which was positioned on the entrance slit of the monochromator. The spectrum of this system was recorded. The cuvette was filled with distilled water and the Cerenkov spectrum recorded. The result is shown in Fig. 5.3.

![Cerenkov Wavelength Spectrum Diagram](image)

**Fig. 4.2**
Absorption region

window

window retaining ring & bolts

flow

source

Fig 4.3
Fig 4.4 Transmission of Flowcell Window

Top trace - air/air

Bottom trace - spectrosil A window/air
4.5 Design of the Flowcell

A schematic diagram of the flowcell is shown in Fig. 4.3. The flowcell was machined from brass, having a 10 mm x 1.5 mm diameter flow hole closed at one end by the face of the strontium source. The other end of the flowcell was closed by a 20 mm x 2 mm Spectrosil A window. The transmission characteristics of the window are shown in Fig. 4.4, and were obtained on a Cecil CE 5095 high performance scanning spectrophotometer with air as reference.

Inlet and outlet tubes of 0.01 inch bore stainless-steel were soldered into the brass body of the cell.

A second flowcell was also fabricated. In this cell the dimensions of the flowhole were 10 mm x 2.5 mm diameter.

4.6 Pulse Counting and Chart Calibration

Fig. 4.5
When the mobile phase passes through the flowcell the production of Cerenkov photons gives rise to pulses at the photomultiplier tube base, the rate of which is shown by the ratemeter, and recorded on the chart recorder as $I_0$, shown in Fig. 4.5. When photon absorbing material passes through the flowcell the recorded rate is lowered and the chart recorder shows a chromatogram of conventional appearance. The maximum of the eluted peak is at count rate $I$.

Quantitative detector responses reported below are given in $I_p$, the difference between the number of pulses per second detected when only mobile phase is present in the flowcell ($I_0$) and the minimum number of pulses per second ($I$) 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, namely that this allows direct comparisons to be made between different radioactive sources and different flowcells without the risk of confusion arising through the variation of other instrumental parameters. The percentage absorption, due to an absorbing material within the flowcell is given by

$$\% A = \frac{I_p}{I_0} \times 100$$

and the conventional absorption defined as

$$A = \log \frac{I_0}{I}$$

This method of presenting results requires that the peak height be measured in millimetres (or millivolts) and then converted to counts per second. To facilitate this, it is necessary to calibrate the distance the pen moves, on the chart recorder, in terms of the rate of arrival of pulses at the ratemeter. This is readily carried out by decreasing the bias voltage to the photomultiplier tube and
measuring the distance the pen moves, and the pulse count per second shown by the scaler. An example of chart calibration is shown in Fig. 4.6, and the results plotted graphically in Fig. 4.7, for different filter/amplifier gains.

Alternatively, and providing that the decrease in count rate is linear with pen movement, the distance $I_0$ may be taken as 0 mm (count rate $X$ cps) and the photomultiplier tube voltage decreased to a suitable distance $Y$ mm (from $I_0$) and count rate $Z$ (cps) recorded.
Ip (cps) is then given by

\[ \text{Ip (cps)} = \left( \frac{x-y}{y} \right) \times \text{pk ht (mm)} \]

When calculating the percentage absorption, due to a peak, the result remains constant and is independent of filter/amplifier gain setting. For example:

**TABLE 4.1 Percentage Absorption at Various Gain Settings**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peak height (mm)</th>
<th>Ip (cps)</th>
<th>I (cps)</th>
<th>Io (cps)</th>
<th>Gain</th>
<th>% Absorption (100 Ip/Io)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>139</td>
<td>11300</td>
<td>65350</td>
<td>76950</td>
<td>x5</td>
<td>14.7</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>28</td>
<td>10950</td>
<td>66000</td>
<td>76950</td>
<td>x1</td>
<td>14.2</td>
</tr>
<tr>
<td>Cl-benzene</td>
<td>162</td>
<td>63000</td>
<td>13150</td>
<td>76950</td>
<td>x5</td>
<td>17.1</td>
</tr>
<tr>
<td>Cl-benzene</td>
<td>74</td>
<td>63800</td>
<td>13950</td>
<td>76950</td>
<td>x2</td>
<td>18.1</td>
</tr>
</tbody>
</table>

4.7 The Use of Narrow-band Pass Interference Filters

**Instrument Parameters**

- **Column**: None
- **Cell type**: 2.5 x 10 mm, 1 mCi $^{90}$Sr
- **Eluent**: Methanol
- **Flow rate**: 1 cm$^3$ min$^{-1}$

The flow cell was placed close to the PM tube window and the system allowed to light adapt. Methanol was passed through the hplc system and the background count rate recorded. The pen movement was calibrated in count rate as described previously. 1 to 10 µl samples of a solution containing 100 ppm of carbazole in methanol were injected into the mobile phase and the separate peaks recorded on the chart recorder.
A 254 nm interference filter (extracted from an Altex UV monitor) was placed over the flowcell window and the system allowed to light adapt. The background count rate was recorded and because this was considerably lower than that observed without a filter, the ratemeter was set to $R_5 \times 10^4$ and filter to gain $\times 20$. The pen movement was calibrated in count rate. 1 to 10 µl of the above solution were separately injected into the mobile phase and the peaks recorded. The results of this experiment are shown in Table 5.1.

### 4.8 Comparison of the 1.5 mm and 2.5 mm Diameter Flowcells

**Instrument Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1.5 mm and 2.5 mm Diameter Flowcells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Spherisorb 5 µm ODS, 15 x 0.5 cm. TFA Tennelec g x 32.</td>
</tr>
<tr>
<td>Cell type</td>
<td>1.5 mm and 2.5 mm x 10 mm</td>
</tr>
<tr>
<td>1 mCi $^{90}$Sr</td>
<td>Ratemeter $R_{10^4}$ cps, LIN.</td>
</tr>
<tr>
<td>Optical filter</td>
<td>254 nm.</td>
</tr>
<tr>
<td>Filter</td>
<td>g x 5 and g x 2, 0.175.</td>
</tr>
<tr>
<td>Eluent</td>
<td>Methanol</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>120 mm hr$^{-1}$.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm$^3$ min$^{-1}$.</td>
</tr>
</tbody>
</table>

The 1 mCi $^{90}$Sr point source was placed into the 1.5 mm (dia) x 10 mm flowcell and the 254 nm interference filter placed over the flowcell window. The flowcell was placed into the wooden coffin, close to the PM tube window, and the system allowed to light adapt.

Methanol was passed through the hplc system and the background count rate recorded. The pen movement of the chart recorder was calibrated in count rate.

A solution containing 100 ppm of carbazole in methanol was prepared and 1 to 10 µl of this solution were separately injected into the mobile phase, and the resulting peaks recorded.
The experiment was repeated using the 2.5 mm (dia) x 10 mm flowcell. In this case the gain setting on the filter/amplifier was at gain x 2. Care was taken to ensure that the retention times for the carbazole peaks remained constant throughout the investigation.

The results of this experiment are presented in Table 5.2.

4.9 Comparison of Pulse Counting with Current Measurement

The Photomultiplier Tube

A typical photomultiplier tube (PM tube) consists of two major elements, a photo-sensitive layer called the photocathode and an electron multiplier structure to which the photocathode is coupled.

Incident light photons striking the photocathode are absorbed and transfer energy to electrons within the photoemissive material of the photocathode. These electrons migrate to the surface of the photocathode where those with sufficient energy escape from the surface.

The multiplier portion of the PM tube is based on the phenomenon of secondary electron emission. Electrons emitted from the photocathode are accelerated and caused to strike the surface of an electrode, called a dynode. The energy deposited by an incident electron can result in the re-emission of more than one electron from the dynode surface. The dynode is held at a positive potential with respect to the photocathode and the number of electrons emitted from the dynode is a function of incident electron energy and number of electrons striking the dynode. The multiplication factor, $g$, of a single dynode, is defined as the ratio of the number of
electrons emitted to a primary incident electron and in order to achieve very large electron gains, all PM tubes employ multiple dynodes.

Electrons leaving the photocathode are attracted to the first dynode which produces $\delta$ electrons for each photoelectron. The secondary electrons that are produced at the surface of the first dynode are accelerated, again by an electrostatic field, to a second similar dynode, which again re-emits a greater number of electrons than that absorbed by the dynode. This process can be repeated many times resulting in an overall electron gain of typically $10^6$ for a ten stage PM tube. The overall gain is defined as

$$\text{overall gain} = \propto \cdot \delta^N$$

where $\propto$ is the fraction of photoelectrons collected by the multiplier structure, $\delta$ is as defined previously and $N$ the number of stages provided in the multiplier section.

After amplification through the multiplier structure, the $10^6$ electrons serve as the charge signal for the original scintillation event. This charge is conventionally collected at the anode or output stage of the multiplier structure.

**Photon Counting**

![Diagram of a photomultiplier tube](image)
Fig. 4.8 shows the simplified wiring diagram for the base of the PM tube, which utilizes positive high voltage and a grounded photocathode. The anode is at a dc potential, equal to the supply voltage, and signal pulses must therefore be capacitively coupled from the anode to allow the pulse component to be passed on at ground potential to succeeding electronic devices. The load resistor (R) is chosen so that the resulting anode circuit time constant (RC) is of proper magnitude.

Photons striking the photocathode cause a current pulse to be generated from the PM tube anode. Each pulse of electrons is integrated by the anode circuit to produce a voltage equal to \( Q/C \), the ratio of the collected electron charge to the anode circuit capacitance. The shape of the voltage pulse produced at the output of the circuit is determined by the magnitude of the anode time constant. If the time constant is large compared to the decay time of the scintillator emitting the photons, the resulting voltage pulse is of relatively long duration and is acceptable when current pulse rates are not excessively high. When the time constant is made small compared with the scintillation decay time, the result is a much faster pulse, an advantage when high pulse count rates are encountered. In this case the voltage amplitude is

\[
v = \frac{\lambda}{\rho} \cdot \frac{Q}{C}
\]

where \( \lambda \) is the scintillator decay constant and \( \rho \) the reciprocal of the anode time constant.

In BIF, the fluorescence from the mobile phase is optically filtered which results in a relatively low pulse rate from the PM tube anode. The time constant for the anode circuit, as shown in Fig. 2.23, is of the order of 300 ns which results in the pulse shape
shown in Fig. 4.9 and no pulse overlap is observed at the count rates observed in BIF.

![Diagram showing pulse height and time characteristics](image)

**Fig. 4.9**

The pulse amplitude depends upon the number of photons within a scintillation pulse striking the photocathode, and the gain of the photomultiplier tube. The rate at which the voltage pulses are emitted from the anode circuit depends upon the rate at which the scintillation pulses strike the photocathode.

The voltage pulses are amplified and fed to a voltage discriminator. An input voltage pulse, in excess of 30 mV, produces a logic pulse of 0.5 V which is recorded by the ratemeter.

In BIF it is assumed that single photon events occur (i.e. along each beta-particle track a single photon only is produced within a 100 ns time period). With relatively constant rate of decay of the radioisotope, voltage pulses are produced at a rate which is proportional to the rate at which the single photons are emitted from the
flowcell. When a fluorescent material passes through the flowcell, the rate of production of single photons increases with consequent increase in the rate of production of voltage pulses. However, it should be noted that the amplitude of the voltage pulses remains the same.

It is likely that the production of Cerenkov photons, within a flowcell, occurs by multiphoton events and not by single photon events. This results in a large number of photons (a packet of photons) striking the photocathode at the same time. Absorption of all photons within a packet reduces the pulse count rate as shown by the ratemeter. However, since the ratemeter records logic pulses emanating from the discriminator, no reduction in count rate is observed if an absorber reduces the number of photons within a photon packet. Furthermore, with the high pulse count rate observed in Cerenkov photon production care must be taken to ensure that pulse overlap does not occur.

Thus the efficiency of recording the absorption of Cerenkov photons must be in question and an alternative measurement technique should be found. An alternative to pulse counting is current measurement.

Current Measurement

In Fig. 4.10, the photocathode is held at a negative potential with respect to earth, and must be isolated from ground. The anode of the PM tube is at ground potential and current pulses, emanating from the anode, are fed to the integrating operational amplifier. The output of the operational amplifier is a voltage which follows the time average current and not the area of the individual current
Fig. 4.10

pulses, as these are too fast for the op amp to follow. The output, for constant intensity light falling on the photocathode, is shown in Fig. 4.11.

Fig. 4.11

Since the area of a current pulse is proportional to the number of photons within a packet, any reduction in this number reduces the voltage at the output of the circuit. The output voltage is fed directly to the filter/amplifier (see Appendix 1) where it may be filtered, amplified or offset.
With the above considerations in mind, it was decided to compare logarithmic and linear pulse counting and current integration as absorption detection techniques.

**Logarithmic Pulse Counting**

**Instrument Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>5 μm ODS, 25 x 0.4 cm.</td>
</tr>
<tr>
<td>TFA</td>
<td>None.</td>
</tr>
<tr>
<td>Cell type</td>
<td>2.5 x 10 mm, 1 mCi Sr</td>
</tr>
<tr>
<td>Ratemeter</td>
<td>$R_10^5$ LOG.</td>
</tr>
<tr>
<td>Eluent</td>
<td>Methanol</td>
</tr>
<tr>
<td>Filter g x 3 Cut</td>
<td>2.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm$^3$ min$^{-1}$</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.5 cm min$^{-1}$.</td>
</tr>
</tbody>
</table>

The 1 mCi strontium-90 point source was placed into the 2.5 x 10 mm flowcell and the 254 nm filter placed in between the flowcell and the PM tube.

The anode of the PM tube was connected to the pulse counting pre-amplifier, the output of which was fed to the pulse counting detection system as described in Chapter 2. The logarithmic range of the ratemeter was selected.

When using current integration as a detection technique, no count rate is observed since the scaler is inoperative. Since the object of these experiments was to compare the signal-to-noise ratios of the three methods of detection, an alternative to using the square root of the background count rate, for calculating the noise, was required. In all three detection methods, the background may be determined by switching off the HV supply to the PM tube and setting the recorder pen to 0 mm on the chart paper. On switching on the HV supply, the background is the distance moved by the pen if the gain of the filter/amplifier is taken into account.
Methanol was passed through the HPLC system and the background recorded. 20 µL of a solution of 10 ppm carbazole in methanol were injected into the mobile phase and the height of the resultant peak recorded. The procedure was repeated with 20, 40, 60, 80 and 100 ppm solutions of carbazole in methanol.

**Linear Pulse Counting**

The experiment, just described, was repeated with the ratemeter output in the linear mode, and on scale R 5 x 10⁴.

**Current Integration**

**Instrument Parameters**

<table>
<thead>
<tr>
<th>Column</th>
<th>5 µm ODS, 25 x 0.4 cm</th>
<th>TFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>2.5 x 10 cm, 1 mCi ⁹⁰Sr</td>
<td>Ratemeter</td>
</tr>
<tr>
<td>Eluent</td>
<td>Methanol</td>
<td>Filter g x 0.3 Cut 2.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm³ min⁻¹</td>
<td>Recorder speed. 0.5 cm min⁻¹.</td>
</tr>
</tbody>
</table>

The experiment described above was repeated, but in place of the pulse counting preamplifier, the current integration preamplifier was used, the output of which was fed to the filter/amplifier.

Finally, the 1 mCi ⁹⁰Sr point source was replaced by a 10 mCi ⁹⁰Sr point source and the experiments repeated. The results of this series of experiments are shown in Tables 5.3 to 5.6.

It should be noted that in some cases the Canberra timing/filter amplifier (TFA) was not available. In such cases a Tennelec amplifier (model TC 216) was substituted. When this was not available, the output of the pulse counting preamp was fed directly to the discriminator.
Fig 4.12. Transmission of Acetone
4.10 Investigation of Signal and Background

Voltage pulses observed by the ratemeter result from Cerenkov photons entering the PM tube, fluorescence photons emitted from the flowcell window, filter and PM tube window by absorption of radiation (beta-particles or bremsstrahlung), and thermionic emission (dark current) from electrons within the PM tube, and ionizing radiation entering the PM tube. Thus the observed total background count rate is given by

\[
\text{Count}_{\text{observed total}} = \sum \text{Count}_{\text{C,Photons}} + \text{Count}_{\text{fluorescence}} + \text{Count}_{\text{thermionic emission}} + \text{Count}_{\text{Radiation}}
\]

\[
C_{\text{OT}} = \sum C_{\text{CP}} + C_F + C_{\text{TE}} + C_R
\]

To determine the number of voltage pulses \(C_{\text{CP}}\) resulting from Cerenkov photons it is necessary to pass acetone through the flowcell. The absorption spectrum of acetone is shown in Fig. 4.12.

If a 254 nm interference filter is placed in between the flowcell and the PM tube window, Cerenkov photons are absorbed by the acetone and the resulting count rate is

\[
\text{Acetone} \quad C_{T,A} = \sum C_F + C_{\text{TE}} + C_R
\]

and

\[
C_{\text{CP}} = C_{\text{OT}} - C_{T,A}
\]

The contribution of \(C_{\text{TE}} + C_R\) to the total count rate may be determined by placing black paper in between the filter and the PM tube window. Thus

\[
\text{Black Paper} \quad C_{T,BP} = C_{\text{TE}} + C_R
\]
By calculation, the fluorescence contribution from filter and flowcell window is given by

\[ C_F = (C_F + \frac{1}{2} (C_{TE} + C_R)) - (C_{TE} + C_R) \]

\[ = C_{T,A} - C_{T,BP} \]

which is the difference between the count rate when acetone is passing through the flowcell and black paper is in between the filter and PM tube window.

Finally, the dark current count rate, \( C_{TE} \), may be determined by removing the flowcell from the coffin. Thus

Cell Removed \[ C_{CR} = C_{TE} \]

and \[ C_R = C_{T,BP} - C_{TE} \]

\[ = C_{T,BP} - C_{CR} \]

An increase in percentage absorption, with consequent increase in sensitivity, may be achieved by reducing the value of the observed background count rate to that resulting from Cerenkov photons alone.

The dark current count, which results from thermionic emission of electrons from within the PM tube, may be reduced by cooling the tube, but since this is a minor contribution to the total count rate, this action was not considered worth while during the present work. A reduction in flowcell window and filter fluorescence may be achieved by reducing the energy of the ionizing radiation emitted from the source but this is undesirable as it would result in a reduction in the number of Cerenkov photons produced within the flowcell. However, since the fluorescence is emitted isotropically, moving the flowcell away from the PM tube should reduce the number of fluorescent photons striking the photocathode. Furthermore,
this should also reduce the amount of ionizing radiation entering the PM tube. It was decided to investigate the effect that moving the cell away from the PM tube had on signal and background count rate.

To reduce the amount of ionizing radiation emanating from the flowcell, the flowcell was placed in a lead pot as shown in Fig. 4.13. The lead pot was placed on to a wooden platform, shown in Fig. 4.14, which was calibrated in centimetres so that the distance between the flowcell and the PM tube window could be reasonably accurately determined.

The wooden platform and the lead pot, containing the flowcell, were placed in the inner container of the coffin with the filter touching the PM tube window, and the system allowed to light adapt.

**Instrument Parameters**

| Column  | 5 µm ODS, 15 cm x 0.4 cm dia. | TFA | None |
| Cell type | 2.5 x 10 mm, 10 mCi²³⁰Sr. | Ratemeter | 10⁵ cps LIN. |
| Eluent | 80/20 MeOH/H₂O and Acetone. | Filter | g x 5, 0.175. |
| Flow rate | 1 cm³ min⁻¹. | Recorder speed, 0.5 cm min⁻¹. |

A mobile phase consisting of 80% methanol and 20% water (vol/vol) was passed through the hplc system and the background count rate recorded. 20 µl of a solution containing 100 ppm each of naphthalene, anthracene and biphenyl in methanol were injected into the mobile phase and the separated peaks recorded on the chart recorder. The pen movement was calibrated in count rate as previously described.

The lead pot was moved 1 cm from the PM tube window and the peaks from the mixture recorded. The peaks were also recorded for
distances of 2, 3, 4, 5 and 8 cm between the lead pot and PM tube window.

Black paper was placed in between the lead pot and the PM tube window and the count rate recorded when the pot was 0, 1, 2, 3, 4, 5 and 8 cm from the window.

Finally, the black paper was removed and the count rate recorded when acetone passed through the flowcell which was 0, 1, 2, 3, 4, 5 and 8 cm away from the window.

The results of this experiment are shown in Tables 5.7 and 5.8.

4.11 Investigation of Cerenkov Photon Numbers Using Optical Filters

Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>None</th>
<th>TFA</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>2.5 x 10 mm, 10 mCi $^{90}$Sr</td>
<td>Ratemeter</td>
<td>-</td>
</tr>
<tr>
<td>Eluent</td>
<td>Acetone and Water</td>
<td>Filter</td>
<td>-</td>
</tr>
<tr>
<td>Flow rate</td>
<td>$1 \text{ cm}^3 \text{ min}^{-1}$</td>
<td>Recorder speed</td>
<td>-</td>
</tr>
</tbody>
</table>

The 254 nm filter was placed in between the flowcell and the PM tube window and the system allowed to light adapt. Acetone was passed through the flowcell and the count rate was recorded by the scaler. The cell and filter were moved 1 cm away from the PM tube window and the count rate re-recorded. The count rate was then recorded for distances of 2, 3, 4, 5 and 8 cm from the flowcell to the PM tube window.

The acetone was replaced by distilled water and the procedure repeated.

The 254 nm filter was replaced by the 200 nm filter and the whole procedure repeated.
Finally, it was required that the relative transmission areas of the two filters be determined. This was achieved by cutting out and weighing the transmission areas recorded on a Cecil CE 5095 high performance scanning spectrophotometer, and are shown below in Table 4.2.

**TABLE 4.2 Transmission Areas of Optical Filters**

<table>
<thead>
<tr>
<th>Mass of transmission area (g)</th>
<th>Ratio of areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 nm</td>
<td>254 nm</td>
</tr>
<tr>
<td>0.0381</td>
<td>0.0714</td>
</tr>
<tr>
<td>254/200</td>
<td>1.874</td>
</tr>
</tbody>
</table>

The results of this experiment are shown in Tables 5.9 and 5.10.

### 4.12 Photon Numbers and Mobile Phase Flowrate

**Instrument Parameters**

- **Column**: 5 µ ODS, 15 x 0.4 cm.  
- **TFA**: None
- **Cell type**: 2.5 x 10 mm, 10 mCi$^{90}$Sr  
- **Ratemeter**: None
- **Eluent**: Distilled water,  
- **Filter**: None
- **Flow rate**: various,  
- **Recorder speed**: None

A 200 nm was placed over the window of the flowcell. The flowcell was placed 8 cm away from the PM tube window and the system allowed to light adapt. Distilled water was passed through the hplc system at a flow rate of 0.87 cm$^3$min$^{-1}$ and the photon count recorded for 100 seconds by the scaler. The 100 second count was recorded on five separate occasions.

The procedure was repeated for mobile phase flow rates of 2.0, 3.0, 3.53, 4.0 and 5.0 cm$^3$min$^{-1}$ and the results are presented in Table 5.11, where it should be noted that the photon count rate, for a particular flow rate, is an average of all readings.
4.13 CERENKOV ABSORPTION IN NORMAL PHASE OPERATION

4.14 Response of Detector to Eluted Materials

Instrument Parameters

Column 5 μm Lichrosorb, 15 x 0.6 cm. Tennelec, g x 32.
Cell type 2.5 x 10 mm, 1mCi\(^{90}\)Sr, 10mCi\(^{90}\)Sr. Ratemeter 5 x 10\(^4\), LIN.
Eluent Hexane
Flow rate 1 cm\(^3\)min\(^{-1}\)

The 1mCi strontium-90 point source was placed into the flowcell. A 254 nm filter was placed over the window of the flowcell, which was then placed next to the PM tube window. The system was allowed to light adapt. Hexane (Fisons, hplc grade) was passed through the hplc system. Compounds shown in Table 5.12 were dissolved in hexane and 20 μl of each sample injected into the mobile phase. The pen movement of the chart recorder was calibrated in count rate.

The 1mCi\(^{90}\)Sr was exchanged for the 10mCi\(^{90}\)Sr source and the procedure, described above, was repeated on R10\(^5\) and filter gain x 1. Table 5.12 shows the results of this experiment and Fig. 5.8 shows examples of peaks obtained.

4.15 Linearity of Response for Biphenyl

Instrument Parameters

Column 5 μm Lichrosorb, 15 x 0.6 cm. TFA Tennelec, g x 32.
Cell type 2.5 x 10 mm, 1mCi\(^{90}\)Sr. Ratemeter 5 x 10\(^4\), LIN.
Eluent Hexane
Flow rate 1 cm\(^3\)min\(^{-1}\)

A 254 nm filter was placed between the flowcell and the PM tube window and hexane passed through the hplc system. A 100 ppm solution
of biphenyl in hexane was prepared and the peaks resulting from the injection of 2, 4, 6, 8, 10 and 20 µl were recorded. The pen movement of the chart recorder was calibrated in count rate.

The results of this experiment are shown in Table 5.13, and Fig. 5.9 shows a plot of absorption against mass of biphenyl injected into the mobile phase.

4.16 Example Chromatogram

Instrument Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>5 µm Lichrosorb, 15 x 0.6 cm</td>
</tr>
<tr>
<td>Cell type</td>
<td>2.5 x 10 mm, 1mCi 90Sr</td>
</tr>
<tr>
<td>Optical filter</td>
<td>254 mm</td>
</tr>
<tr>
<td>Eluent</td>
<td>Hexane</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm³ min⁻¹</td>
</tr>
<tr>
<td>TFA</td>
<td>g x 25, 50/50</td>
</tr>
<tr>
<td>Ratemeter</td>
<td>5 x 10⁴ LIN</td>
</tr>
<tr>
<td>Filter</td>
<td>g x 5 Cut 0.1</td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.2 cm min⁻¹</td>
</tr>
</tbody>
</table>

10 µl of a solution containing 500 ppm each of nitrobenzene and p-nitrotoluene were injected into the mobile phase and the separated peaks recorded on the pen recorder.

Chromatogram 29 shows the result of the separation.
4.17 CERENKOV ABSORPTION IN REVERSED PHASE OPERATION

4.18 Response of Detector to Eluted Material

Instrument Parameters

Column 5 μm ODS, 15 x 0.4 cm. TFA Tennelec g x 32.
Cell type 2.5 x 10 mm, 1mCi 90Sr. Ratemeter R x 10^4 LIN.
Optical Filter 254 nm. Filter g x 1, cut 0.175.
Eluent Methanol. Recorder speed. 0.2 cm min⁻¹.
Flow rate 1 cm³ min⁻¹.

20 μl of a methanol solution of each compound shown in Table 5.14 were injected into the mobile phase and each peak recorded on the chart recorder. The background count rate was recorded and the pen movement of the recorder calibrated in count rate. Fig. 5.10 shows examples of peaks obtained.

A limited number of samples which do not absorb strongly at 254 nm were passed through the detector fitted with a 200 nm filter. In this experiment, the mobile phase was distilled water and the column was dispensed with.

The results are shown in Table 5.15, where it should be noted that the background count rate is low (5600 cps) because no timing/filter amplifier was present.

4.19 Examples of Chromatograms

(a) Instrument Parameters

Column 10 μm Spherisorb ODS, 25 x 0.4 cm. TFA Tennelec g x 32.
Cell type 2.5 x 10 mm, 1mCi 90Sr. Ratemeter R x 10^4 LIN.
Optical Filter 254 nm. Filter g x 1 and g x 2, cut 0.175.
Eluent 60% acetonitrile 40% water. Recorder speed. 0.5 cm min⁻¹.
Flow rate 2 cm³ min⁻¹.
A solution containing 4 g l\(^{-1}\) each of dimethyl, diethyl, diallyl and diisopropyl phthalates in methanol was prepared. 5 \(\mu\)l of the solution were injected into the mobile phase and the separated peaks recorded on the pen recorder.

Chromatogram 30 shows the recording and Chromatogram 31 presents a recording of the separation obtained by using an Altex model 150 UV monitor (range 0.5 AFS), connected in series with, and after, the Čerenkov detector.

(b) Instrument Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>10 (\mu)m Spherisorb ODS, 25 x 0.4 cm</td>
</tr>
<tr>
<td>Cell type</td>
<td>2.5 x 10 mm, 1mCi(^{90})Sr.</td>
</tr>
<tr>
<td>Optical filter</td>
<td>254 nm.</td>
</tr>
<tr>
<td>Eluent</td>
<td>35% acetonitrile 65% water.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>2 cm(^3)min(^{-1}).</td>
</tr>
<tr>
<td>TFA Tennelec</td>
<td>g x 32</td>
</tr>
<tr>
<td>Ratemeter R10(^4) LIN.</td>
<td></td>
</tr>
<tr>
<td>Filter g x 1 Cut 0.175.</td>
<td></td>
</tr>
<tr>
<td>Recorder speed</td>
<td>0.5 cm min(^{-1}).</td>
</tr>
</tbody>
</table>

Bendiocarb (2,2 dimethyl-1,3-benzodioxol-4-ol methyl carbamate) and carbaryl (1-naphthyl N-methyl carbamate) are widely used as contact insecticides. The separation of a mixture containing the two insecticides was effected as follows.

5 \(\mu\)l of a solution containing 2.7 mg cm\(^{-3}\) of bendiocarb and 1.6 mg cm\(^{-3}\) of carbaryl, in acetonitrile, were injected into the mobile phase. Chromatogram 32 shows the separation together with that obtained from the UV monitor operated on range 0.5 A.F.S.D.

(c) Instrument Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>5 (\mu)m Spherisorb 15 x 0.5 cm</td>
</tr>
<tr>
<td>Cell type</td>
<td>2.5 x 10 mm, 10 mCi(^{90})Sr</td>
</tr>
<tr>
<td>Optical filter</td>
<td>254 nm.</td>
</tr>
<tr>
<td>TFA g x 25, 50/50.</td>
<td></td>
</tr>
<tr>
<td>Ratemeter R10(^5) LIN.</td>
<td></td>
</tr>
<tr>
<td>Filter g x 2 Cut 0.175.</td>
<td></td>
</tr>
</tbody>
</table>
Eluent 80% methanol 20% water. Recorder speed 0.5 cm min\(^{-1}\).
Flow rate 1 cm\(^3\) min\(^{-1}\).

A solution containing 100 ppm each of naphthalene, biphenyl and anthracene in methanol was prepared. 20 µl of this solution were injected into the mobile phase and resulting separation is shown in Chromatogram 33.

(d) Instrument Parameters

Column 10 µm Spherisorb ODS 25 x 0.4 cm. TFA Tennelec g x 32.
Cell type 2.5 x 10 mm, 1mCi\(^{90}\)Sr. Ratemeter R10\(^4\) LIN.
Optical filter 254 nm. Filter g x 1.
Eluent Methanol. Recorder speed 0.5 cm min\(^{-1}\).
Flow rate 1 cm\(^3\) min\(^{-1}\).

8 µl of a solution known to contain vitamin A and vitamin D\(_2\) (palmitates) in methanol were injected into the hplc system and the resulting chromatogram is shown in Chromatogram 34. Unfortunately, no knowledge of the approximate concentration of this solution was known since it was supplied by A.C.S. Ltd. and used by them when undertaking an analysis of these vitamins, in pharmaceutical creams.

(e) Instrument Parameters

Column 5 µm Spherisorb-amino, 25 x 0.5 cm. TFA Tennelec g x 32.
Cell type 2.5 x 10 mm, 1mCi\(^{90}\)Sr. Ratemeter R10\(^4\) LIN.
Optical filter 254 nm. Filter g x 1, Cut 0.175
Eluent 30% methanol 70% of 2% acetic acid in water. Recorder speed, 0.5 cm min\(^{-1}\).
Flow rate 2.0 cm\(^3\) min\(^{-1}\).

Furosemide (5-(aminosulphonyl)-4-chloro-2-[(2-furanyl methyl) amino] benzoic acid) is a diuretic which when absorbed into the
body encourages the production of urine by the kidneys and rids the body of accumulated fluid.

Furosemide, and an isomer of furosemide, are produced from 2-sulphanyl-3-chloro-5-amino benzoic acid. Separation of these three components was achieved by reversed phase chromatography employing a Spherisorb-amino column.

5 µl of a solution containing 1700 ppm of starting material, 1500 ppm of furosemide and 700 ppm of furosemide isomer were injected into the mobile phase and the resulting separation is shown in Chromatogram 35.

(f) **Instrument Parameters**

- Column: 5 µm Spherisorb, 25 x 0.5 cm. TFA g x 25, 50/50.
- Cell type: 2.5 x 10 mm, 1 mCi 90Sr.
- Optical filter: 254 nm.
- Eluent: 60% methanol 40% water.
- Flow rate: 0.5 cm³ min⁻¹.

Chromatogram 36 shows the result of injecting 20 µl of a methanol mixture containing 2000 ppm of toluene, 6000 ppm of p-xylene and 1,3,5 trimethyl benzene and 8000 ppm of 1,2,3,4 tetramethyl benzene into the hplc system.

(g) **Instrument Parameters**

- Column: 5 µm Spherisorb ODS, 25 x 0.5 cm. TFA g x 25, 50/50.
- Cell type: 2.5 x 10 mm, 1 mCi 90Sr.
- Optical filter: 254 nm.
- Eluent: 50% 0.01% ammonium carbonate 50% methanol.
- Flow rate: 1 cm³ min⁻¹.
Standard solutions containing 4000 ppm of aspirin, 2000 ppm of caffeine and 1000 ppm of phenacetin, in methanol, were prepared and 1 to 10 µl of each solution were injected into the mobile phase. The resulting peak heights, for each individual injection, are shown in Tables 5.16, 5.17 and 5.18. The pen movement of the chart recorder was calibrated in count rate and Figs. 5.11 and 5.12 show plots of absorbence against mass of compound injected.

Figs. 5.13 and 5.14 show plots of peak height against mass of compound injected obtained from a U.V. monitor which was connected in between the Cerenkov detector and mobile phase waste reservoir.

An A.P.C. tablet (Boots Co.) was crushed, dissolved in warm distilled water, filtered and made up to a final volume of 100 cm³. 5 and 10 µl aliquotes of this solution were injected into the mobile phase and the resulting Cerenkov chromatograms are shown in Chromatogram 37. Table 5.19 presents quantitative data resulting from the separation. Table 5.20 shows the quantitative data obtained from the UV monitor.

(h) Instrument Parameters

<table>
<thead>
<tr>
<th>Column</th>
<th>Spherisorb 5 µm ODS, 25 x 0.5 cm. TFA None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>2.5 x 10 mm, 10 mCi ^90^Sr. Ratemeter R10^5^ LIN.</td>
</tr>
<tr>
<td>Optical filter</td>
<td>200 nm. Filter g x 2, Cut 0.5.</td>
</tr>
<tr>
<td>Eluent</td>
<td>Water. Recorder speed. 0.2 cm min^-1.</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 cm² min^-1.</td>
</tr>
</tbody>
</table>

20 µl of a solution containing 100 ppm each of sodium saccharin and sodium benzoate in water were injected into the mobile phase and the resulting separation is shown in Chromatogram 38.
The results of these experiments, together with a discussion on Cerenkov absorption, are presented in Chapter 5.
CHAPTER 5. CERENKOV PHOTON ABSORPTION - RESULTS AND DISCUSSION

5.1 Preliminary Investigation

In Chapter 1, the mechanism by which Cerenkov photons are produced was briefly described and the Cerenkov relationship stated (Eq. 1.10).

The experiment described in 4.2 (Chapter 4) was designed to determine the angle of maximum photon emission from a quartz rod to determine whether it is possible to use a Cerenkov light source external to the flowcell.

5.2 Angle of Maximum Photon Emission

![Diagram](image)

**Fig. 5.0**

where \[\text{---} \rightarrow \text{----} \] is the beta-particle path

\[\text{---} \rightarrow \] is the Cerenkov photon path

In the quartz rod, shown in Fig. 5.0, the maximum number of photons should be generated at angle \(\theta\) to the longest beta-particle
path length, i.e. parallel to the rod wall. For $\frac{1}{2} E_{\text{max}}$ for $^{90}\text{Y}$, $\beta = 0.9151$. Taking an average value of 1.5025 for the refractive index of quartz over the wavelength range 200 to 600 nm, then

$$\cos \theta = \frac{1}{0.9151 \times 1.5025}$$

and therefore $\theta = 43^\circ 27'$

If the most probable angle of $\theta$ is $43^\circ 27'$ in quartz, the angle of incidence ($i$) to the quartz/air interface is $45^\circ 33'$.

For total internal reflection to occur at the interface,

$$\frac{\sin i}{\sin 90} = \frac{1}{a_{\text{ng}}} = 0.6656$$

$$\therefore \quad i = 41^\circ 44'$$

Thus, light striking the interface at angles of incidence greater than this value is totally internally reflected. For light to escape from the quartz wall, $\theta$ must be greater than $58^\circ 16'$. As it is not possible to attain this value of $\theta$, by even the most energetic $^{90}\text{Y}$ beta-particles ($E_{\text{max}} = 2270$ keV), and considering that $\theta$ decreases to $0^\circ$ along the track of the beta-particle, no Cerenkov light, generated by beta-particles which pass straight down the rod, escapes from the rod. Thus the geometric arrangement (shown in Fig. 5.0) is unsatisfactory if the objective is to generate the maximum number of Cerenkov photons. This argument is continued in Chapter 6.

Fig. 5.1 does show an angular dependence for the number of photons emitted from the quartz rod (Fig. 5.0). The maximum number of photons are emitted at an angle ($\theta'$) of $30^\circ$ to the horizontal, and must be generated from beta-particles which track in directions other than parallel to the quartz rod walls.
Fig 5.1

COUNTS PER SECOND
$\times 10^4$

ANGLE FROM HORIZONTAL $\theta$ IN FIG 4.1

Fig 5.2

COUNTS PER SECOND
$\times 10^4$

NUMBER OF WASHERS
5.3 Photon Emission as Function of Beta-Particle Path Length

Fig. 5.2 shows that when the quartz rod is totally uncovered the count rate is 80000 cps. Elimination of Cerenkov radiation from the base of the quartz rod decreases the count rate to 55000 cps and thus it may be concluded that a high percentage of the total light emanating from the rod is emitted from a considerable distance along the track of the beta-particle.

Fig. 5.02 shows the experimental arrangement of the investigation. This conclusion is rather surprising since the path length of a maximum energy beta-particle from $^{90}Y$ (2270 keV) should be approximately 5 mm ($139$) in quartz ($p = 2.2 \text{ g cm}^{-3}$). It may be concluded therefore that one cannot assume that the path length, through an absorbing material, is fixed when the excitation source is used internally in a flowcell system.
5.4 Cerenkov Wavelength Spectrum

Fig. 5.3 shows the uncorrected spectra obtained by placing a 1 mCi $^{90}$Sr source next to a 1 cm x 1 cm quartz cuvette. Spectrum 1 was obtained when the cuvette was empty; 2 when filled with water; and 3 when the $^{90}$Sr source was removed from the cuvette.

With reference to the transmission characteristics of the monochromator (Fig. 2.28) and with the knowledge that the photocathode efficiency is relatively low at low wavelengths, it is concluded that the intensity of the Cerenkov radiation is greater at shorter wavelengths (200 to 250 nm) and that the radiation is a continuum from $< 200$ nm to $> 700$ nm.

5.5 The Use of Narrowband Pass Interference Filters

Quantitative methods based on the absorption of electromagnetic radiation involve the measurement of the reduction in intensity of the radiation on passing through an absorbing medium. The degree of absorption is determined by comparing the intensity of the transmitted beam when no absorbing species is present, i.e. mobile phase alone, with that transmitted by the sample. For monochromatic, collimated radiation, the reduction in intensity is related to the concentration of the absorbing species and to the thickness of the absorbing medium, both relations being embodied in the Beer-Lambert law:

$$\log_{10} \left( \frac{I_0}{I} \right) = A = \varepsilon c l$$

where $\log_{10}(\frac{I_0}{I})$ is defined as the absorbance $A$, and $\varepsilon$ is a constant known as the molar absorptivity, the value of which depends upon the nature of the absorbing species and the wavelength of the incident radiation. $c$ is the concentration of the absorbing species and $l$ the thickness of the absorbing species.
Fig. 5.3 Cerenkov Spectra $^{90}$Sr source.
1 Empty cuvette
2 Cuvette filled with water
3 Cuvette (no source)
Since \( \mathcal{E} \) is constant and \( l \) remains fixed in the flowcell system,

\[
\log \left( \frac{I_0}{I} \right) = A \propto c
\]

For the above relationship to hold true, monochromatic radiation must be employed and is achieved by the use of narrow bandpass interference filters, which allows selection of a narrow wavelength range.

Fig. 5.4 shows a graph of peak height versus mass of carbazole injected from the data presented in Table 5.1. Clearly there is not a linear relationship between these two factors. However, if the absorbance is plotted against carbazole mass (see Fig. 5.5, Plot 1), a linear relationship is observed when employing the 254 nm filter.

It should be noted that the graph does not pass through the origin. This is due to a small contribution to \( I_0 \) cps (the transmitted light when no absorbing material is present), caused by stray light or stray radiation entering the PM tube (see investigation of signal and background). The pen movement of the chart recorder was calibrated, in this instance, by recording the background count rate and then reducing this count rate and noting the distance the pen moved. Thus the reduction in count rate \( (I_p) \), due to an absorbing material in the flowcell, may be calculated from

\[
I_p = \frac{(I_0 + S) - (I + S)}{X_{mm}} = \frac{I_0 - I}{X_{mm}}
\]

where \( X_{mm} \) is the peak height of the absorbing material, and \( I \) is the count rate when absorbing material is present.

And

\[
I + S = (I_0 + S) - I_p
\]

\[
I = I_0 - I_p
\]

Now, \( S \) may be calculated from Plot 1 (Fig. 5.5) by observing that the graph passes through the y axis at \( A = 0.009 \).

Thus

\[
A = \log \left( \frac{I_0}{I} \right)
\]
<table>
<thead>
<tr>
<th>Carbazole Mass (µg)</th>
<th>Peak ht (nm)</th>
<th>$I_p$ (cps)</th>
<th>$I$ (cps)</th>
<th>$\frac{100 \times I_p}{I_o}$</th>
<th>$\log \left( \frac{I_o}{I} \right)$</th>
</tr>
</thead>
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<tr>
<td><strong>NO FILTER</strong></td>
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<td></td>
<td></td>
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<tr>
<td>0.0</td>
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<td>57123</td>
<td>246381</td>
<td>18.82</td>
<td>0.091</td>
</tr>
<tr>
<td><strong>254 nm FILTER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0</td>
<td>5826(10)</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>0.1</td>
<td>30.0</td>
<td>358</td>
<td>5468</td>
<td>6.15</td>
<td>0.027</td>
</tr>
<tr>
<td>0.2</td>
<td>53.5</td>
<td>639</td>
<td>5187</td>
<td>10.97</td>
<td>0.050</td>
</tr>
<tr>
<td>0.3</td>
<td>70.0</td>
<td>836</td>
<td>4990</td>
<td>14.35</td>
<td>0.067</td>
</tr>
<tr>
<td>0.4</td>
<td>88.0</td>
<td>1032</td>
<td>4774</td>
<td>18.06</td>
<td>0.087</td>
</tr>
<tr>
<td>0.5</td>
<td>108.0</td>
<td>1291</td>
<td>4535</td>
<td>22.16</td>
<td>0.109</td>
</tr>
<tr>
<td>0.6</td>
<td>122.0</td>
<td>1458</td>
<td>4368</td>
<td>25.04</td>
<td>0.125</td>
</tr>
<tr>
<td>0.7</td>
<td>138.0</td>
<td>1649</td>
<td>4177</td>
<td>28.30</td>
<td>0.144</td>
</tr>
<tr>
<td>0.8</td>
<td>152.0</td>
<td>1816</td>
<td>4010</td>
<td>31.17</td>
<td>0.162</td>
</tr>
<tr>
<td>0.9</td>
<td>162.0</td>
<td>1936</td>
<td>3890</td>
<td>33.23</td>
<td>0.175</td>
</tr>
<tr>
<td>1.0</td>
<td>177.5</td>
<td>2121</td>
<td>3705</td>
<td>36.40</td>
<td>0.200</td>
</tr>
</tbody>
</table>
Fig 5.4

PEAK HEIGHT (mm)

MASS OF CARBAZOLE (µg)

FILTER

NO FILTER
**Fig 5.5**

- **FILTER PLOT 1**
- **PLOT 2**
- **NO FILTER**

**Axes:**
- **Absorbance**
- **Mass of Carbazole (μg)**

The graph illustrates the relationship between absorbance and mass of carbazole for different filter conditions.
\[
I = \frac{5826}{S}
\]

\[
I = 5706 = \text{true } I_0
\]

and \( S = 5826 - 5706 = 120 \) cps.

A plot of \( A = \log \left( \frac{I_0 - S}{I} \right) \) is given in Plot 2, Fig. 5.5 and it is observed that this graph passes through the origin. It is as a result of this complication that an investigation of background and signal is described later.

A linear relationship is also observed, between masses of 0.0 and 0.6 \( \mu \)g, when the filter is absent. Further increase in carbazole mass results in departure from linearity.

The use of a short wavelength interference filter eliminates the possibility of beta-induced fluorescence from the compound under investigation.

Finally, it should be noted that when mobile phase alone flows through the cell, Cerenkov photons are generated in the wavelength range 200–600 nm. Compounds do not absorb photons over the whole of this wavelength range and an increase in absorption (see \( \% A \), Table 5.1), is made possible by selecting a narrow wavelength range over which the compound of interest strongly absorbs.

5.6 Errors in the Method and Technique

Errors may arise from the

1. preparation of sample,
2. injection of sample,
3. variation in flow rate of the mobile phase,
4. measurement of peak height,
5. measurement of background count rate \( (I_o) \),
6. measurement of transmitted light \( (I) \) from the calibration graph, and
7. calculation of the absorbed light \( (I_p) \), by an absorbing material.

Taking Table 5.1 as an example,

1. Samples solutions are generally prepared by weighing 0.01 g of material, dissolving in solvent and diluting to a final volume of 100 cm\(^3\). Since electronic balances weigh to four decimal places, errors in weighing should be negligible. Dilution to final volume introduces an error of (say) \( \pm 0.25 \text{ cm}^3 \), equivalent to \( \pm 0.25\% \).

2. Varying volumes of sample solution (prepared in 1) are injected into the mobile phase, thus varying the mass of sample detected. Taking the worst possible case of injecting 1 \( \mu l \) from a 10 \( \mu l \) graduated syringe, which can be read to \( \pm 0.05 \mu l \) introduces an error of \( \pm 0.5\% \).

3. The results and calculations presented in this Chapter rely on constant retention times, since if this value varies in a particular investigation, peak height varies and the measurement of \( I_p \) and \( I \) become invalid. Therefore care was taken to ensure that the mobile phase flow rate remained constant by careful measurement of this parameter.

4. Measurement of peak height can be made to \( \pm 0.25 \text{ mm} \) and therefore, in the smallest peak in Table 5.1, the error is \( \pm 1.5\% \).

5. The error in the background count rate measurement is the square root of the count rate when a 1s time constant is selected,
the percentage error decreasing as the background count rate increases. Thus the error in 5826 cps is ± 76, which is ± 1.3%.

6. & 7. As stated in Chapter 4, I and $I_p$ may be determined in two separate ways,

(a) $I_o$ is observed from the scaler. The HV supply, to the PM tube is reduced by increments, and I and the distance the pen moves on the chart recorder are measured for each increment. A graph is then plotted of I versus pen movement.

The peak height of an absorbing material is measured and I determined from the graph. $I_p$ is then calculated from $(I_o - I)$.

For the worst cases in Table 5.1,

The error in $I = (3705)^{\frac{1}{2}} = \pm 60.9 = \pm 1.6\%$.

The error in peak height = ± 1.3%

The error in calculating $I_p = \pm (5826 + 3705)^{\frac{1}{2}}$

= ± 97.6

= ± 4.6% + ± 1.3%

= ± 6.1%.

(b) $I_o$ is observed from the scaler. The HV supply to the PM tube is reduced by a single increment and I (Xcps) and the distance the pen moves (Ymm) on the chart recorder measured.

The absorbed count rate ($I_p$) is then calculated from the peak height of the absorbing material, i.e.

$I_p = (\frac{X - Z}{Y}) \times \text{peak height}$.

and $I = I_o - I_p$.

The worst possible errors are
\[ \text{in } I_p = \frac{(5826 + 3705)^{\frac{1}{2}}}{\pm 0.14\%} \times \pm 1.61\% \]
\[ = \frac{\pm 4.6\%}{\pm 0.14\%} \times \pm 1.61\% \]
\[ = \pm 6.35\% \]

\[ \text{in } I = (5826 + 358)^{\frac{1}{2}} \]
\[ = \pm 78 \text{ cps} \]
\[ = \pm 1.27\% \]

Thus the maximum error in calculating \( \% A \) is
\[ = \pm 6.3\% \]
\[ = \pm 1.3\% \]
\[ = \pm 7.6\% \]

and in \( A \)
\[ = \pm 1.3\% \]
\[ = \pm 1.6\% \]
\[ = \pm 2.9\% \]

Thus the total error in \( \% A \) is the sum of the worst possible percentage errors
\[ = \pm 14.4\% \]

and in \( A = \pm 9.6\% \)

For brevity, no values of errors are given in most of the tables in this Chapter, but may be easily calculated from the statements contained above. Where possible, error bars have been included in graphs.
5.7 Comparison of the 1.5 mm and 2.5 mm Diameter Flowcells

Table 5.2 presents the response of the Cerenkov absorption detector to various masses of carbazole when employing a 1.5 mm (diameter) x 10 mm flowcell and a 2.5 mm (diameter) x 10 mm flowcell. Fig. 5.6 shows a graph of absorbance versus mass of carbazole for the two flowcells, where it may be noted that both sets of results have been plotted.

Fig. 5.7 presents a selection of the chromatographic peaks obtained from the investigation of the 2.5 mm x 10 mm flowcell.

Table 5.2 and Fig. 5.6 clearly demonstrate that the absorbance and % absorption remain constant, for the same mass of injected carbazole, and is independent of the original number of Cerenkov photons present (I₀).

If the signal to noise ratio is taken as the figure of merit for comparative purposes, and the noise as the square root of the background count rate (Iₒ), then

<table>
<thead>
<tr>
<th>Cell</th>
<th>background noise in A</th>
<th>A (from 1 pg sample)</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 mm</td>
<td>4000</td>
<td>± 0.0069</td>
<td>12.2</td>
</tr>
<tr>
<td>2.5 mm</td>
<td>8450</td>
<td>± 0.0047</td>
<td>18.0</td>
</tr>
</tbody>
</table>

it can be clearly demonstrated that an increase in signal-to-noise ratio is readily achieved by increasing the background count rate. An increase in the latter is achieved by the use of a more active ⁹⁰Sr source and this was investigated during the following comparative study of detection methods.
**TABLE 5.2** Comparison of 1.5 mm (dia) and 2.5 mm (dia) Flowcells

<table>
<thead>
<tr>
<th>Carbazole pg</th>
<th>Peak height (mm)</th>
<th>( I_p ) (cps)</th>
<th>( I ) (cps)</th>
<th>( % A )</th>
<th>( A )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100 ( \frac{I_p}{I_0} )</td>
<td>( \log(\frac{I_0}{I}) )</td>
</tr>
<tr>
<td><strong>1.5 mm (dia) x 10 mm Flowcell</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>4000 (( I_0 ))</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>0.1</td>
<td>22.5</td>
<td>182</td>
<td>3818</td>
<td>4.6</td>
<td>0.02</td>
</tr>
<tr>
<td>0.2</td>
<td>41.0</td>
<td>332</td>
<td>3668</td>
<td>8.3</td>
<td>0.03</td>
</tr>
<tr>
<td>0.3</td>
<td>58.5</td>
<td>474</td>
<td>3526</td>
<td>11.8</td>
<td>0.05</td>
</tr>
<tr>
<td>0.4</td>
<td>75.0</td>
<td>608</td>
<td>3392</td>
<td>15.2</td>
<td>0.07</td>
</tr>
<tr>
<td>0.5</td>
<td>91.0</td>
<td>738</td>
<td>3262</td>
<td>18.4</td>
<td>0.08</td>
</tr>
<tr>
<td>0.6</td>
<td>107.0</td>
<td>868</td>
<td>3132</td>
<td>21.7</td>
<td>0.10</td>
</tr>
<tr>
<td>0.7</td>
<td>125.5</td>
<td>1018</td>
<td>2982</td>
<td>25.4</td>
<td>0.13</td>
</tr>
<tr>
<td>0.8</td>
<td>143.0</td>
<td>1160</td>
<td>2840</td>
<td>29.0</td>
<td>0.15</td>
</tr>
<tr>
<td>0.9</td>
<td>149.5</td>
<td>1212</td>
<td>2788</td>
<td>30.3</td>
<td>0.16</td>
</tr>
<tr>
<td>1.0</td>
<td>153.0</td>
<td>1241</td>
<td>2759</td>
<td>31.0</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>2.5 mm (dia) x 10 mm Flowcell</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>8450 (( I_0 ))</td>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>0.1</td>
<td>21.0</td>
<td>372</td>
<td>8078</td>
<td>4.4</td>
<td>0.02</td>
</tr>
<tr>
<td>0.2</td>
<td>39.25</td>
<td>697</td>
<td>7753</td>
<td>8.2</td>
<td>0.03</td>
</tr>
<tr>
<td>0.3</td>
<td>55.0</td>
<td>976</td>
<td>7474</td>
<td>11.6</td>
<td>0.05</td>
</tr>
<tr>
<td>0.4</td>
<td>72.0</td>
<td>1278</td>
<td>7172</td>
<td>15.1</td>
<td>0.07</td>
</tr>
<tr>
<td>0.5</td>
<td>89.5</td>
<td>1589</td>
<td>6861</td>
<td>18.8</td>
<td>0.09</td>
</tr>
<tr>
<td>0.6</td>
<td>107.25</td>
<td>1904</td>
<td>6546</td>
<td>22.5</td>
<td>0.11</td>
</tr>
<tr>
<td>0.7</td>
<td>121.5</td>
<td>2157</td>
<td>6293</td>
<td>25.5</td>
<td>0.13</td>
</tr>
<tr>
<td>0.8</td>
<td>132.0</td>
<td>2343</td>
<td>6107</td>
<td>27.7</td>
<td>0.14</td>
</tr>
<tr>
<td>0.9</td>
<td>143.5</td>
<td>2547</td>
<td>5903</td>
<td>30.1</td>
<td>0.16</td>
</tr>
<tr>
<td>1.0</td>
<td>154.0</td>
<td>2733</td>
<td>5717</td>
<td>32.3</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Fig 5-6

ABSORBANCE

MASS OF CARBAZOLE µg
CARBAZOLE SAMPLES

2.5 x 10 mm CELL

Fig 5.7
5.8 Comparison of Pulse Counting with Current Measurement

**TABLE 5.3** 1 mCi $^{90}$Sr, 0-2.0 $\mu$g Carbazole

Noise

<table>
<thead>
<tr>
<th>Mass ($\mu$g)</th>
<th>LOG</th>
<th>LIN</th>
<th>INT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pkht (mm)</td>
<td>Normalised (mm)</td>
<td>S/N</td>
</tr>
<tr>
<td>0.20</td>
<td>16</td>
<td>16</td>
<td>23.0</td>
</tr>
<tr>
<td>0.40</td>
<td>32</td>
<td>32</td>
<td>46.0</td>
</tr>
<tr>
<td>0.80</td>
<td>59</td>
<td>59</td>
<td>84.3</td>
</tr>
<tr>
<td>1.20</td>
<td>84</td>
<td>84</td>
<td>120.7</td>
</tr>
<tr>
<td>1.60</td>
<td>102</td>
<td>102</td>
<td>146.5</td>
</tr>
<tr>
<td>2.00</td>
<td>119</td>
<td>119</td>
<td>171.0</td>
</tr>
<tr>
<td>Noise</td>
<td>0.696</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 5.4** 1 mCi $^{90}$Sr, 0-0.4 $\mu$g Carbazole

Noise

<table>
<thead>
<tr>
<th>Mass ($\mu$g)</th>
<th>LOG</th>
<th>LIN</th>
<th>INT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pkht (mm)</td>
<td>Normalised (mm)</td>
<td>S/N</td>
</tr>
<tr>
<td>0.08</td>
<td>24</td>
<td>24</td>
<td>10.3</td>
</tr>
<tr>
<td>0.16</td>
<td>44</td>
<td>44</td>
<td>19.0</td>
</tr>
<tr>
<td>0.20</td>
<td>54</td>
<td>54</td>
<td>23.3</td>
</tr>
<tr>
<td>0.24</td>
<td>65</td>
<td>65</td>
<td>28.0</td>
</tr>
<tr>
<td>0.32</td>
<td>85</td>
<td>85</td>
<td>36.6</td>
</tr>
<tr>
<td>0.40</td>
<td>106</td>
<td>106</td>
<td>45.7</td>
</tr>
<tr>
<td>Noise</td>
<td>2.320</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 5.5  
10 m Ci $^{90}$Sr, 0-2.0 µg Carbazole

#### Noise

Lin $\text{R} \, 5 \times 10^4 \text{ cps, } gx1(100\text{mV}), 146 \text{ mV} = 292 \text{ mm (gx1)} = 292 \pm 1.081 \text{ mm}$

Log $\text{R} \, 10^5 \text{ cps, } gx3(100\text{mV}), 118 \text{ mV} = 236 \text{ mm (gx1)} = 708 \pm 0.147 \text{ mm}$

Int $\text{gx1}(0.5 \text{ V}), 740 \text{ mV} = 1480 \text{ mm (gx1)} = 296 \pm 1.514 \text{ mm (0.5 V)}$

| Mass (µg) | LOG | | | | LIN | | | | INT | |
|---|---|---||---|---||---|---||---|---|
| Pkht (mm) | Normalised (mm) | S/N | Pkht (mm) | Normalised (mm) | S/N | Pkht (mm) | Normalised (mm) | S/N |
| 0.2 | 14 | 14 | 47.6 | 37 | 5.0 | 17.0 | 37 | 3.6 | 12.2 |
| 0.4 | 28 | 28 | 95.2 | 61 | 8.3 | 28.2 | 62.5 | 6.1 | 20.7 |
| 0.8 | 58 | 58 | 197.3 | 116 | 15.8 | 53.7 | 115 | 11.2 | 38.1 |
| 1.2 | 86 | 86 | 292.5 | 146 | 19.8 | 67.3 | 144.5 | 14.0 | 47.6 |
| 1.6 | 113.5 | 113.5 | 386.0 | 161 | 21.9 | 74.5 | 162 | 15.7 | 53.4 |
| 2.0 | 121 | 121 | 411.6 | 182 | 24.7 | 84.0 | 177 | 17.2 | 58.5 |
| Noise | 0.294 | 0.294 | 0.294 | 0.294 | 0.294 |

### TABLE 5.6  
10 m Ci $^{90}$Sr, 0-0.4 µg Carbazole

#### Noise

Lin $\text{R} \, 5 \times 10^4 \text{ cps, } gx1(100\text{mV}), 146 \text{ mV} = 292 \text{ mm (gx1)} = 292 \pm 1.081 \text{ mm}$

Log $\text{R} \, 10^5 \text{ cps, } g \times 10(100\text{mV}), 118 \text{ mV} = 236 \text{ mm (gx1)} = 2360 \pm 0.491 \text{ mm}$

Int $g \times 1(0.5 \text{ V}), 740 \text{ mV} = 1480 \text{ mm (gx1)} = 296 \pm 1.514 \text{ mm (0.5 V)}$

| Mass (µg) | LOG | | | | LIN | | | | INT | |
|---|---|---||---|---||---|---||---|---|
| Pkht (mm) | Normalised (mm) | S/N | Pkht (mm) | Normalised (mm) | S/N | Pkht (mm) | Normalised (mm) | S/N |
| 0.08 | 19 | 19 | 19.3 | 14 | 6.4 | 6.5 | 14.5 | 4.7 | 4.8 |
| 0.16 | 38 | 38 | 38.7 | 29 | 13.2 | 13.4 | 28 | 9.1 | 9.3 |
| 0.20 | 44.5 | 44.5 | 45.3 | 34.5 | 15.7 | 16.0 | 36 | 11.7 | 11.9 |
| 0.24 | 52.5 | 52.5 | 53.5 | 39.5 | 17.9 | 18.2 | 42 | 13.6 | 13.8 |
| 0.32 | 72 | 72 | 73.3 | 50 | 22.7 | 23.1 | 53 | 17.2 | 17.5 |
| 0.40 | 89 | 89 | 90.6 | 61 | 27.7 | 28.2 | 63 | 20.4 | 20.8 |
| Noise | 0.982 | 0.982 | 0.982 | 0.982 | 0.982 |
Tables 5.3 to 5.6 present the results obtained during the investigation and comparison of Log arithmetic (LOG) and Linear (LIN) pulse counting and current integration (INT) measurement. These Tables require the following explanation before interpretation.

1. Calculation of Noise on Ratemeter Linear Range

Let the background \( (I_0) \) be \( X \) mm on range \( R \), gain on the filter \( X_1 \), and integration time 1s

Then

\[
\text{Countrate} = \frac{X \times R}{200}
\]

and the standard deviation, \( \sigma \), is given by

\[
\sigma = \left( \frac{XR}{200} \right)^{1/2}
\]

Therefore, the background and the noise in the background is given by

\[
x \pm \frac{200XR}{R}^{1/2}
\]

\[
= x \pm \left( \frac{200X}{R} \right)^{1/2}
\]

For example, in the investigation of 0-2.0 \( \mu \)g carbazole using the 1 mCi \( ^{90} \)Sr source, the background was determined as 37 mV = 74 mm.

Thus

background and noise = \[ 74 \pm \frac{200 \times 74}{5 \times 10^4} \] \[ = 74 \pm 0.544 \text{ mm} \]

Therefore, on gain \( x \) 3, the background and the noise in the background is

\[ = 222 \pm 1.632 \text{ mm} \]

The peak to peak noise is therefore 3.264 mm, which agrees with estimates taken from the chart record.
2. Calculation of Noise on Ratemeter Log Range

Let the background \((I_o)\) be \(X\) mm on the \(10^5\) cps range, (note that the scale is from \(10^2\) cps to \(10^5\) cps),
then background \(= X\) mm \(= K \log \frac{I}{10^2}\)

The standard deviation is
\[ \pm K \tan h^{-1}\left(\frac{1}{2}\right) \]

where
\[ I = 10^{\frac{X+2k}{k}} \]
\[ = 10^{\frac{X}{2k}} \]
\[ = \pm k \tan h^{-1}\left(\frac{-2k - X}{2k}\right) \log_e(10) \]

If \(I = 10^5\) and \(X = 200\) mm then
\[ k = \frac{200}{\log \frac{10^5}{10^2}} \]
\[ = 66.7 \text{ mm} \]

For example, in the investigation of 0–2.0 \(\mu\)g carbazole using the 1 \(\text{mCi}\) \(^{90}\text{Sr}\) source, the background was determined as 93 mV = 186 mm.
Thus
\[ 186 \text{ mm} \pm 66.7 \times \tan h^{-1}\left(\frac{133.3 - 186}{133.3}\right) \log_e(10) \]
\[ = 186 \pm 0.116 \text{ mm} \]

Therefore on gain \(\times 3\), the background and the noise in the background is
\[ = 558 \pm 0.348 \text{ mm} \]

The peak to peak noise is therefore 0.696 mm, again in good agreement with values estimated from the chart record.
3. **Calculation of Noise in Current Measurement**

On the Linear range, the rate of arrival of pulses (R) at the discriminator is

\[ R = \left( \frac{5 \times 10^4}{100} \right) \times 37 \text{ mV} \]

and the background signal (X) is given by

\[ X = k' R \]

the noise in the current measurement therefore is given by

\[ N = k' \sqrt{2 R} \]

and

\[ X = k' (R \pm \sqrt{2 R}) \]

The background, on g x 1 is 342 mm, therefore

\[ 342 = k' \left( \frac{5 \times 10^4}{100} \right) \times 37 \]

and \[ k' = 0.0185 \text{ mm} \]

Thus the background and noise is

\[ = 342 \pm 3.56 \text{ mm on g x 1} \]

and \[ = 103 \pm 1.068 \text{ mm on g x 0.3} \]

The peak to peak noise is therefore 2.136 mm.

4. The observed peak heights have been normalised to a constant noise value. For example, in Table 5.3, all peak heights have been normalised to a peak to peak noise value of 0.696 mm.

5. The signal-to-noise ratio was calculated as the normalised peak height divided by the peak to peak noise value.

Tables 5.3 to 5.6 show that the highest values of signal-to-noise ratios are obtained when employing pulse counting with the ratemeter in the Logarithmic mode and that pulse counting with the ratemeter in the Linear mode gives marginally higher values than does current measurement.
The ratios of signal-to-noise ratio for the 10 m Ci source divided by the signal-to-noise ratio for the 1 m Ci source is, for the Linear mode, $1.73 \pm 0.11$, a value which is confirmed in Section 5.13.

5.9 Investigation of Signal and Background

Table 5.7 shows the results of the investigation on the background count rate for the 2.5 x 10 mm flowcell containing the 10 m Ci $^{90}$Sr and fitted with the 254 nm filter.

As described in Chapter 4, an increase in sensitivity is observed if the background counted pulses are those which result only from Cerenkov photons and not from other sources, such as fluorescence from the cell window or optical filter, or radiation entering the PM tube window.

From Table 5.7 it is observed that on moving the flowcell 8 cm away from the PM tube window, the count rate resulting from fluorescence ($C_F$) decreases by 80% and the count rate due to radiation entering the PM tube ($C_R$) decreases by 64%. Movement of the flowcell through the same distance reduces the observed total count rate ($C_{OT}$) by 34% and the count rate from Cerenkov photons alone ($C_{CP} = C_{OT} - C_{T,A}$) by 26%. This experiment therefore clearly demonstrates that an increase in absorption is possible simply by moving the flowcell away from the PM tube window.

Table 5.8 shows the results of the investigation on the signal, calculated as True % Absorption and Observed % Absorption. The former represents the percentage reduction in pulse count rate from Cerenkov photons alone, and varies by a maximum of 4% throughout the
8 cm movement of the flowcell. However, the latter, which represents the reduction in pulse count rate obtained from all sources increases by between 10 and 16% on moving the flowcell 8 cm away from the PM tube window.

**TABLE 5.7 Investigation of Background**

<table>
<thead>
<tr>
<th>Distance Pot away from PM tube (cm)</th>
<th>( C_{OT} ) (cps)</th>
<th>( C_{T,A} ) (cps)</th>
<th>( C_{T,BP} ) (cps)</th>
<th>( C_F ) (cps)</th>
<th>( C_R ) (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32811</td>
<td>5780</td>
<td>2463</td>
<td>3317</td>
<td>2263</td>
</tr>
<tr>
<td>1</td>
<td>31035</td>
<td>4468</td>
<td>1973</td>
<td>2495</td>
<td>1773</td>
</tr>
<tr>
<td>2</td>
<td>29685</td>
<td>3448</td>
<td>1653</td>
<td>1795</td>
<td>1453</td>
</tr>
<tr>
<td>3</td>
<td>28496</td>
<td>2840</td>
<td>1413</td>
<td>1427</td>
<td>1213</td>
</tr>
<tr>
<td>4</td>
<td>27198</td>
<td>2458</td>
<td>1245</td>
<td>1213</td>
<td>1045</td>
</tr>
<tr>
<td>5</td>
<td>26211</td>
<td>2155</td>
<td>1145</td>
<td>1010</td>
<td>945</td>
</tr>
<tr>
<td>8</td>
<td>21645</td>
<td>1682</td>
<td>1016</td>
<td>666</td>
<td>816</td>
</tr>
</tbody>
</table>

**Notes**

- \( C_{OT} = \) the observed total count
- \( C_{T,A} = \) total count when acetone flows through the cell
- \( C_{T,BP} = \) total count when black paper is in front of PM tube
- \( C_F = \) count contribution from fluorescence
- \( C_R = \) count contribution from radiation entering PM tube

The dark current count rate \( (C_{CR}) \) was determined to be 200 cps.
**TABLE 5.8 Investigation of Signal**

<table>
<thead>
<tr>
<th>Distance Pot away from PM tube (cm)</th>
<th>Compound</th>
<th>Peak height (mm)</th>
<th>Peak height I (cps)</th>
<th>True photon background ( C_{CP} = C_{OT} - C_{T,A} )</th>
<th>True % Absorption ( \frac{C_{CP}}{100 (C_{OT} - I)} )</th>
<th>Observed % Absorption ( \frac{C_{OT}}{100 (C_{OT} - I)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>A</td>
<td>103</td>
<td>21410</td>
<td>27031</td>
<td>42.2</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>158</td>
<td>15285</td>
<td></td>
<td>64.8</td>
<td>53.4</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>111</td>
<td>20519</td>
<td></td>
<td>45.5</td>
<td>37.3</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>101</td>
<td>19787</td>
<td>26567</td>
<td>42.3</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>155</td>
<td>13773</td>
<td></td>
<td>64.9</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>110</td>
<td>18784</td>
<td></td>
<td>46.1</td>
<td>39.5</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>100</td>
<td>18548</td>
<td>26237</td>
<td>42.4</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>156</td>
<td>12311</td>
<td></td>
<td>66.2</td>
<td>58.5</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>110</td>
<td>17434</td>
<td></td>
<td>46.6</td>
<td>41.3</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>99</td>
<td>17470</td>
<td>25656</td>
<td>43.0</td>
<td>38.7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>154</td>
<td>11345</td>
<td></td>
<td>66.8</td>
<td>60.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>108</td>
<td>16458</td>
<td></td>
<td>46.9</td>
<td>42.2</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>94</td>
<td>16729</td>
<td>24740</td>
<td>42.3</td>
<td>38.5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>148</td>
<td>10715</td>
<td></td>
<td>66.6</td>
<td>60.6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>103</td>
<td>15727</td>
<td></td>
<td>46.3</td>
<td>42.2</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>93</td>
<td>15854</td>
<td>24056</td>
<td>43.0</td>
<td>39.5</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>145</td>
<td>10062</td>
<td></td>
<td>67.1</td>
<td>61.6</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>101</td>
<td>14963</td>
<td></td>
<td>46.7</td>
<td>42.9</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>78</td>
<td>12958</td>
<td>19963</td>
<td>43.5</td>
<td>40.1</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>121</td>
<td>8169</td>
<td></td>
<td>67.5</td>
<td>62.2</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>80</td>
<td>12736</td>
<td></td>
<td>44.6</td>
<td>41.1</td>
</tr>
</tbody>
</table>
5.10 **Investigation of Cerenkov Photon Numbers Using Optical Filters**

Table 5.9 shows the count rate observed when water and acetone pass through the flowcell fitted with, firstly, the 254 nm filter and then the 200 nm filter. The true Cerenkov count rate is the difference between the two observed count rates at each wavelength.

Table 5.10 shows the true photon count rate adjusted for the transmission areas of the two optical filters, and the ratio of these count rates for the two filters, at different distances from flowcell to PM tube window. The decrease in this ratio (from 2.64 to 2.50) due to moving the flowcell 8 cm away from the PM tube window may be attributed to the larger decrease in true photon count rate (24.7%) observed from the use of the 200 nm filter (254 nm decrease is 20.6%).

Calculation of the photon numbers from Eq. 1.14, yields a value of 3.21 (per decay) for the number of Cerenkov photons within a wavelength range of 208 ± 5 nm divided by the number of photons within a wavelength range of 262 ± 5 nm after these numbers have been adjusted for the areas of the filters. This value differs from the observed value of 2.64 ± 0.04 and is most probably due to the method of calculation.

5.11 **Photon Numbers and Mobile Phase Flow Rate**

It is clearly desirable that the background count rate remains constant, if the mobile phase flow rate varies, so that adjustment of the chromatographic baseline is unnecessary. Table 5.11 shows that the photon count rate remains constant, with statistical noise, for a number of different mobile phase flow rates.
TABLE 5.9  Photon Numbers at 254 nm and 200 nm

<table>
<thead>
<tr>
<th>Distance of Cell from PM Tube (cm)</th>
<th>254 nm Filter</th>
<th>200 nm Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOBILE PHASE</td>
<td>TRUE PHOTON</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>Acetone</td>
</tr>
<tr>
<td>0</td>
<td>28516</td>
<td>5780</td>
</tr>
<tr>
<td>1</td>
<td>27283</td>
<td>4468</td>
</tr>
<tr>
<td>2</td>
<td>26070</td>
<td>3448</td>
</tr>
<tr>
<td>3</td>
<td>25215</td>
<td>2840</td>
</tr>
<tr>
<td>4</td>
<td>24495</td>
<td>2458</td>
</tr>
<tr>
<td>5</td>
<td>23660</td>
<td>2155</td>
</tr>
<tr>
<td>8</td>
<td>19723</td>
<td>1682</td>
</tr>
</tbody>
</table>

TABLE 5.10  Ratio of True Photon Numbers

<table>
<thead>
<tr>
<th>Distance of Cell from PM Tube (cm)</th>
<th>TRUE PHOTON COUNT RATE ADJUSTED FOR AREA OF FILTER</th>
<th>RATIO OF COUNT RATE 200/254</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 nm</td>
<td>254 nm</td>
</tr>
<tr>
<td>0</td>
<td>60018</td>
<td>22736</td>
</tr>
<tr>
<td>1</td>
<td>58742</td>
<td>22815</td>
</tr>
<tr>
<td>2</td>
<td>58324</td>
<td>22622</td>
</tr>
<tr>
<td>3</td>
<td>57110</td>
<td>22375</td>
</tr>
<tr>
<td>4</td>
<td>55435</td>
<td>22037</td>
</tr>
<tr>
<td>5</td>
<td>52862</td>
<td>21505</td>
</tr>
<tr>
<td>8</td>
<td>45206</td>
<td>18041</td>
</tr>
<tr>
<td>Mobile Phase Flow Rate $(\text{cm}^3\text{min}^{-1})$</td>
<td>Photon Count Rate (cps)</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------</td>
<td></td>
</tr>
<tr>
<td>0.87</td>
<td>26850</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>27030</td>
<td></td>
</tr>
<tr>
<td>3.00</td>
<td>27012</td>
<td></td>
</tr>
<tr>
<td>3.53</td>
<td>26980</td>
<td></td>
</tr>
<tr>
<td>4.00</td>
<td>26870</td>
<td></td>
</tr>
<tr>
<td>5.00</td>
<td>27005</td>
<td></td>
</tr>
</tbody>
</table>

Note: The errors in the photon count rate are $\pm\sqrt{\text{cps}} = 164\text{ cps}$. 
5.12 CERENKOV ABSORPTION IN NORMAL PHASE OPERATION

5.13 Response of Detector to Eluted Materials

Table 5.12 shows the response of the Cerenkov absorption detector to a range of eluted materials, and Fig. 5.8 presents examples of peaks obtained during the investigation.

From the table, it may be deduced that those compounds which possess a high value of molar absorptivity at 254 nm show high values of percentage absorption of Cerenkov radiation. For example, the molar absorptivity of biphenyl is approximately 15000 (129) at 254 nm and a 2 µg sample shows a percentage absorption of 62.9%. These values may be compared with acenaphthene (molar absorptivity approximately 950 (129), percentage absorption 21.3%).

The table also shows that, with the exception of samples 13, 14 and 17, the percentage absorption remains similar, if the 10 m Ci source is exchanged for the 1 m Ci source. However, the signal-to-noise ratio (calculated as peak height (mm) divided by peak to peak noise (mm)) for the 10 m Ci source is $1.60 \pm 0.26$ times larger than that for the 1 m Ci source.
TABLE 5.12 Response to Eluted Material

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>COMPOUND</th>
<th>MASS (µg)</th>
<th>(1) m Ci (^{90})Sr (I_0 = 10300) cps</th>
<th></th>
<th>(10) m Ci (^{90})Sr (I_0 = 76950) cps</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(P_{kht}) (mm)</td>
<td>(I_P) (cps)</td>
<td>(% A_{100} I_P)</td>
<td>(s/N)</td>
</tr>
<tr>
<td>1</td>
<td>Biphenyl</td>
<td>2</td>
<td>173</td>
<td>6475</td>
<td>62.9</td>
<td>84.8</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>1</td>
<td>108</td>
<td>3875</td>
<td>37.6</td>
<td>52.9</td>
</tr>
<tr>
<td>3</td>
<td>Acenaphthene</td>
<td>2</td>
<td>66</td>
<td>2200</td>
<td>21.3</td>
<td>32.3</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>1</td>
<td>31.5</td>
<td>850</td>
<td>8.2</td>
<td>15.4</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.5</td>
<td>14.4</td>
<td>550</td>
<td>5.3</td>
<td>7.0</td>
</tr>
<tr>
<td>6</td>
<td>Anthracene</td>
<td>2</td>
<td>133</td>
<td>4875</td>
<td>47.3</td>
<td>65.2</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>1</td>
<td>107</td>
<td>3850</td>
<td>37.4</td>
<td>52.5</td>
</tr>
<tr>
<td>8</td>
<td>Fluorene</td>
<td>2</td>
<td>FSD</td>
<td>7100</td>
<td>68.9</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>1</td>
<td>120</td>
<td>4300</td>
<td>41.7</td>
<td>58.8</td>
</tr>
<tr>
<td>10</td>
<td>Naphthalene</td>
<td>2</td>
<td>111</td>
<td>4000</td>
<td>38.8</td>
<td>54.4</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>1</td>
<td>55</td>
<td>1775</td>
<td>17.2</td>
<td>26.7</td>
</tr>
<tr>
<td>12</td>
<td>Chlorobenzene</td>
<td>20</td>
<td>67.5</td>
<td>2225</td>
<td>21.6</td>
<td>32.8</td>
</tr>
<tr>
<td>13</td>
<td>p-Dichlorobenzene</td>
<td>20</td>
<td>76.5</td>
<td>2625</td>
<td>25.5</td>
<td>37.5</td>
</tr>
<tr>
<td>14</td>
<td>Toluene</td>
<td>20</td>
<td>90</td>
<td>3150</td>
<td>30.6</td>
<td>44.1</td>
</tr>
<tr>
<td>15</td>
<td>4-Chlorotoluene</td>
<td>20</td>
<td>92</td>
<td>3250</td>
<td>31.6</td>
<td>45.1</td>
</tr>
<tr>
<td>16</td>
<td>Fluorobenzene</td>
<td>2</td>
<td>26</td>
<td>600</td>
<td>5.8</td>
<td>12.7</td>
</tr>
<tr>
<td>17</td>
<td>Nitrobenzene</td>
<td>10</td>
<td>156</td>
<td>5800</td>
<td>56.3</td>
<td>76.5</td>
</tr>
<tr>
<td>18</td>
<td>p-Nitrotoluene</td>
<td>10</td>
<td>161</td>
<td>5975</td>
<td>58.0</td>
<td>78.9</td>
</tr>
<tr>
<td>19</td>
<td>Nitronaphthalene</td>
<td>10</td>
<td>134</td>
<td>4900</td>
<td>47.6</td>
<td>65.7</td>
</tr>
<tr>
<td>20</td>
<td>Hexachlorobenzene</td>
<td>20</td>
<td>185</td>
<td>7150</td>
<td>69.4</td>
<td>90.7</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>5</td>
<td>93</td>
<td>3275</td>
<td>31.6</td>
<td>45.6</td>
</tr>
</tbody>
</table>

**Notes:**
1. Calculation of the noise (N) in the background gave values of 2.040 mm for the \(1\) m Ci source and 0.794 mm for the \(10\) m Ci source (for details of calculation see footnote to Tables 5.3 to 5.6), taking values of \(10300\) cps = 259.6 mm, for the \(1\) m Ci source and 76950 cps = 198.5 mm for the \(10\) m Ci source.
2. Errors may be calculated as previously described.
5.14 Linearity of Response for Biphenyl

Table 5.13 and Fig. 5.9 demonstrate that the response of the Cerenkov detector is a linear function of mass of biphenyl injected into the hplc system, over the mass range 0.0 to 2.0 μg.

**TABLE 5.13 Response for Biphenyl**

<table>
<thead>
<tr>
<th>Mass of Biphenyl (μg)</th>
<th>Peak height (nm)</th>
<th>I (cps)</th>
<th>I_p (cps)</th>
<th>% A ( \frac{100 I_p}{I_o} )</th>
<th>A ( \log\left( \frac{I_0}{I} \right) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>10300 ((I_o))</td>
<td>0</td>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td>0.2</td>
<td>28.0</td>
<td>9600</td>
<td>700</td>
<td>6.8</td>
<td>0.030</td>
</tr>
<tr>
<td>0.4</td>
<td>52.0</td>
<td>8650</td>
<td>1650</td>
<td>16.0</td>
<td>0.076</td>
</tr>
<tr>
<td>0.6</td>
<td>73.0</td>
<td>7850</td>
<td>2450</td>
<td>23.8</td>
<td>0.118</td>
</tr>
<tr>
<td>0.8</td>
<td>91.0</td>
<td>7150</td>
<td>3150</td>
<td>30.6</td>
<td>0.159</td>
</tr>
<tr>
<td>1.0</td>
<td>105.0</td>
<td>6550</td>
<td>3750</td>
<td>36.4</td>
<td>0.196</td>
</tr>
<tr>
<td>2.0</td>
<td>168.0</td>
<td>4100</td>
<td>6200</td>
<td>60.2</td>
<td>0.400</td>
</tr>
</tbody>
</table>

Notes: 1. Mobile phase hexane.
2. 254 nm optical filter.

5.15 Example Chromatogram

Chromatogram 29 shows the result of separating 10 μg of nitrobenzene (peak 1) and 10 μg of p-nitrotoluene (peak 2) on a 5 μ Lichrosorb column using hexane as mobile phase.
Chromatogram 29

10 µg each

1 Nitrobenzene
2 p-Nitrotoluene
5.16 Cerenkov Absorption in Reversed Phase Operation

5.17 Response of Detector to Eluted Material

Table 5.14 shows the response of the Cerenkov absorption detector to a number of eluted materials. Fig. 5.10 presents examples of chromatographic peaks obtained during the investigation.

**TABLE 5.14 Response to Eluted Materials - 254 nm**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compound</th>
<th>Mass (µg)</th>
<th>Peak height (mm)</th>
<th>( I_p ) (cps)</th>
<th>( % ) Abs [\frac{I_p}{I_o}]</th>
<th>( s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbazole</td>
<td>1</td>
<td>107</td>
<td>4350</td>
<td>53.7</td>
<td>29.7</td>
</tr>
<tr>
<td>2</td>
<td>Ascorbic acid</td>
<td>2</td>
<td>29</td>
<td>1300</td>
<td>16.1</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>Salicylic acid</td>
<td>2</td>
<td>12</td>
<td>650</td>
<td>8.0</td>
<td>3.3</td>
</tr>
<tr>
<td>4</td>
<td>Indole</td>
<td>2</td>
<td>116</td>
<td>4675</td>
<td>57.7</td>
<td>32.2</td>
</tr>
<tr>
<td>5</td>
<td>Aniline</td>
<td>2</td>
<td>37.5</td>
<td>1532</td>
<td>18.9</td>
<td>10.4</td>
</tr>
<tr>
<td>6</td>
<td>9-Aminoacridine</td>
<td>2</td>
<td>82</td>
<td>3350</td>
<td>41.4</td>
<td>22.8</td>
</tr>
<tr>
<td>7</td>
<td>Chlorobenzene</td>
<td>20</td>
<td>49</td>
<td>2100</td>
<td>25.9</td>
<td>13.6</td>
</tr>
<tr>
<td>8</td>
<td>p-Dichlorobenzene</td>
<td>20</td>
<td>55</td>
<td>2300</td>
<td>28.4</td>
<td>15.3</td>
</tr>
<tr>
<td>9</td>
<td>4-Chlorotoluene</td>
<td>20</td>
<td>68</td>
<td>2800</td>
<td>34.6</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Notes:

1. 1 mCi \(^{90}\)Sr source.
2. 254 nm filter.
3. Mobile phase – methanol, \( I_o = 8100 \) cps.
4. Peak to peak noise (N) = 3.6 mm.

Table 5.15 shows the response of the detector at 200 nm to a limited number of samples, which do not absorb strongly at 254 nm.
Fig. 5.10

SAMPLE

9

8

7

4

1
TABLE 5.15  Response to Eluted Material - 200 mm.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mass (µg)</th>
<th>Peak height (mm)</th>
<th>I_p (cps)</th>
<th>% Abs 100 I_p/I_o</th>
<th>s/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Nitrate</td>
<td>2</td>
<td>83</td>
<td>4220</td>
<td>75.4</td>
<td>27.7</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>39</td>
<td>2600</td>
<td>46.4</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>59 (x3)</td>
<td>300</td>
<td>5.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Sodium Nitrite</td>
<td>2</td>
<td>85</td>
<td>4430</td>
<td>79.1</td>
<td>28.3</td>
</tr>
<tr>
<td>Sodium Bromide</td>
<td>2</td>
<td>68</td>
<td>3350</td>
<td>59.8</td>
<td>22.7</td>
</tr>
</tbody>
</table>

Notes: 1. 1 mCi $^{90}$Sr source.
2. 200 mm filter.
3. Mobile phase - water, $I_o$ = 5600 cps.
4. The peak-to-peak noise value taken as 3.0 mm, and calculated as previously described.

5.18 Examples of Chromatograms

Chromatogram 30 and Chromatograms 32 to 36 demonstrate separations that can be achieved in reversed phase chromatography and detected by the Čerenkov absorption detector. Chromatogram 31 is the detection of the separation shown in Chromatogram 30 detected by a u.v. monitor. The details of the chromatography and instrumental settings to achieve these separations are described in Chapter 4.

Tables 5.16, 5.17 and 5.18 present the results of the calibration of detector response for aspirin, phenacetin and caffeine. Figs. 5.11 and 5.12 are graphs of absorption versus mass for the three components.

Figs. 5.13 and 5.14 are graphs of peak height (mm) versus mass for the three components detected by a u.v. monitor. Fig. 5.11 shows that
departure of Cerenkov response from linearity occurs for aspirin at masses greater than approximately 20 μg. This departure is confirmed (in Fig. 5.13) by the u.v. monitor. Fig. 5.12 shows that departure of Cerenkov response from linearity occurs for phenacetin at masses greater than approximately 6 μg. Again a result that is confirmed (Fig. 5.14) by the u.v. monitor. The Cerenkov response for caffeine, shown in Fig. 5.11, shows no linearity whereas the u.v. response shows linearity over the whole mass range of 0 to 20 μg. Since the u.v. monitor shows linearity it can only be assumed that during this particular investigation the calibration of pen movement, in terms of detected pulse rate, changed from that at the beginning of the investigation.

Chromatogram 37 shows the separation of 5 and 10 μl of a solution of an A.P.C. tablet and Table 5.19 presents the quantitative data obtained from the separation. Table 5.20 presents the quantitative data obtained by detecting the separation with a u.v. monitor.

Finally, Chromatogram 38 shows the separation of sodium saccharin and sodium benzoate detected by monitoring the Cerenkov absorption at 200 μm.
Chromatogram 30

20 µg each of
1 dimethyl-
2 diethyl-
3 diallyl-
4 diisopropyl-
phthalates

Mobile phase
60% acetonitrile 40% water.
Chromatogram 31 - UV monitor
20 μg each of
1 dimethyl-
2 diethyl-
3 diallyl-
4 diisopropyl-
phthalates.

Chromatogram 32
1 13.5 μg bendiocarb
2 8.0 μg carboryl
Chromatogram 33
2 μg each of
1 Naphthalene
2 Biphenyl
3 Anthracene

Chromatogram 34
1 Vitamin D₂ palmitate
2 Vitamin A palmitate
Chromatogram 35
1 8.5 µg starting material
2 7.5 µg furosemide
3 3.5 µg furosemide isomer

Chromatogram 36
1 0.04 mg toluene
2 0.12 mg p-xylene
3 0.12 mg 1,3,5 trimethylbenzene
4 0.16 mg 1,2,3,4 tetramethylbenzene
Chromatogram 37
Separation of
1 aspirin
2 caffeine
3 phenacetin
### TABLE 5.16  Aspirin Calibration.

<table>
<thead>
<tr>
<th>Aspirin (µg)</th>
<th>Peak height (mm)</th>
<th>(I_p) (cps)</th>
<th>(I) (cps)</th>
<th>(% A = \frac{I_p}{I_o})</th>
<th>(A = \log \left(\frac{I_o}{I}\right))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5970 ((I_o))</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>720</td>
<td>5250</td>
<td>12.1</td>
<td>0.056</td>
</tr>
<tr>
<td>8</td>
<td>33</td>
<td>1320</td>
<td>4650</td>
<td>22.1</td>
<td>0.108</td>
</tr>
<tr>
<td>12</td>
<td>48</td>
<td>1920</td>
<td>4050</td>
<td>32.2</td>
<td>0.168</td>
</tr>
<tr>
<td>16</td>
<td>56</td>
<td>2240</td>
<td>3730</td>
<td>37.5</td>
<td>0.204</td>
</tr>
<tr>
<td>20</td>
<td>68</td>
<td>2720</td>
<td>3250</td>
<td>45.6</td>
<td>0.264</td>
</tr>
<tr>
<td>24</td>
<td>73</td>
<td>2920</td>
<td>3050</td>
<td>48.9</td>
<td>0.292</td>
</tr>
<tr>
<td>28</td>
<td>82</td>
<td>3280</td>
<td>2690</td>
<td>54.9</td>
<td>0.346</td>
</tr>
<tr>
<td>32</td>
<td>87</td>
<td>3480</td>
<td>2490</td>
<td>58.3</td>
<td>0.380</td>
</tr>
<tr>
<td>36</td>
<td>91</td>
<td>3640</td>
<td>2330</td>
<td>61.0</td>
<td>0.409</td>
</tr>
<tr>
<td>40</td>
<td>96</td>
<td>3840</td>
<td>2130</td>
<td>64.3</td>
<td>0.447</td>
</tr>
</tbody>
</table>

### TABLE 5.17  Phenacetin Calibration.

<table>
<thead>
<tr>
<th>Phenacetin (µg)</th>
<th>Peak height (mm)</th>
<th>(I_p) (cps)</th>
<th>(I) (cps)</th>
<th>(% A = \frac{I_p}{I_o})</th>
<th>(A = \log \left(\frac{I_o}{I}\right))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5970 ((I_o))</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>14.5</td>
<td>580</td>
<td>5390</td>
<td>9.7</td>
<td>0.044</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>1080</td>
<td>4890</td>
<td>18.1</td>
<td>0.086</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>1800</td>
<td>4170</td>
<td>30.1</td>
<td>0.155</td>
</tr>
<tr>
<td>4</td>
<td>55.5</td>
<td>2220</td>
<td>3750</td>
<td>37.2</td>
<td>0.202</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>2680</td>
<td>3290</td>
<td>44.9</td>
<td>0.259</td>
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<tr>
<td>6</td>
<td>76.5</td>
<td>3060</td>
<td>2910</td>
<td>51.2</td>
<td>0.312</td>
</tr>
<tr>
<td>7</td>
<td>82</td>
<td>3280</td>
<td>2690</td>
<td>54.9</td>
<td>0.346</td>
</tr>
<tr>
<td>8</td>
<td>85.5</td>
<td>3420</td>
<td>2550</td>
<td>57.3</td>
<td>0.369</td>
</tr>
<tr>
<td>9</td>
<td>91</td>
<td>3640</td>
<td>2330</td>
<td>60.9</td>
<td>0.409</td>
</tr>
<tr>
<td>10</td>
<td>96</td>
<td>3840</td>
<td>2130</td>
<td>64.3</td>
<td>0.447</td>
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</tbody>
</table>
### Table 5.18  Caffeine Calibration

<table>
<thead>
<tr>
<th>Caffeine (µg)</th>
<th>Peak height (mm)</th>
<th>( I_p ) (cps)</th>
<th>( I ) (cps)</th>
<th>( \frac{A \times 100}{I_p/I_o} )</th>
<th>( \log \left( \frac{I_o}{I} \right) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5970 ( I_o )</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>760</td>
<td>5210</td>
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<td>0.059</td>
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<tr>
<td>2</td>
<td>29.5</td>
<td>1180</td>
<td>4790</td>
<td>19.8</td>
<td>0.096</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
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<td>4330</td>
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<td>0.139</td>
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<td>4</td>
<td>49</td>
<td>1960</td>
<td>4010</td>
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<td>0.173</td>
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<td>62</td>
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<td>41.5</td>
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<td>3270</td>
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<td>0.261</td>
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<tr>
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<td>72</td>
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<td>3090</td>
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<td>0.286</td>
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<tr>
<td>9</td>
<td>75.5</td>
<td>3020</td>
<td>2950</td>
<td>50.6</td>
<td>0.306</td>
</tr>
<tr>
<td>10</td>
<td>79.5</td>
<td>3180</td>
<td>2790</td>
<td>53.3</td>
<td>0.330</td>
</tr>
<tr>
<td>12</td>
<td>84</td>
<td>3360</td>
<td>2610</td>
<td>56.3</td>
<td>0.359</td>
</tr>
<tr>
<td>14</td>
<td>88</td>
<td>3520</td>
<td>2450</td>
<td>59.0</td>
<td>0.387</td>
</tr>
<tr>
<td>16</td>
<td>92</td>
<td>3680</td>
<td>2290</td>
<td>61.6</td>
<td>0.416</td>
</tr>
<tr>
<td>18</td>
<td>96</td>
<td>3840</td>
<td>2130</td>
<td>64.3</td>
<td>0.447</td>
</tr>
<tr>
<td>20</td>
<td>99.5</td>
<td>3980</td>
<td>1990</td>
<td>66.7</td>
<td>0.477</td>
</tr>
</tbody>
</table>
Fig 5.11

Fig 5.12
Fig 5.13

Fig 5.14
### TABLE 5.19 APC Tablet - Cerenkov Absorption Detector

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peak height (mm)</th>
<th>( I_P ) (cps)</th>
<th>( I ) (cps)</th>
<th>( % A )</th>
<th>A (100 ( I_P ) / ( I ))</th>
<th>( \mu g )</th>
<th>Tablet (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>32.5</td>
<td>1300</td>
<td>4670</td>
<td>21.8</td>
<td>0.106</td>
<td>8.00</td>
<td>160 ± 22</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>64.5</td>
<td>2580</td>
<td>3390</td>
<td>43.2</td>
<td>0.246</td>
<td>4.70</td>
<td>94 ± 13</td>
</tr>
<tr>
<td>Caffeine</td>
<td>14.5</td>
<td>580</td>
<td>5390</td>
<td>9.7</td>
<td>0.044</td>
<td>0.75</td>
<td>15 ± 2</td>
</tr>
<tr>
<td><strong>10 ( \mu l ) Injected</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>53.0</td>
<td>2120</td>
<td>3850</td>
<td>35.5</td>
<td>0.190</td>
<td>15.00</td>
<td>145 ± 20</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>89.5</td>
<td>3580</td>
<td>2390</td>
<td>60.0</td>
<td>0.397</td>
<td>8.50</td>
<td>85 ± 12</td>
</tr>
<tr>
<td>Caffeine</td>
<td>21.0</td>
<td>840</td>
<td>5130</td>
<td>14.1</td>
<td>0.066</td>
<td>1.25</td>
<td>12.5 ± 2</td>
</tr>
</tbody>
</table>

Note: Errors calculated for mass in the tablet as described previously.

### TABLE 5.20 APC Tablet - UV Absorption Monitor

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peak height on R 0.5 A.F.S.D. (mm)</th>
<th>( \mu g )</th>
<th>Tablet (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>23</td>
<td>7.75</td>
<td>155</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>90</td>
<td>4.60</td>
<td>92</td>
</tr>
<tr>
<td>Caffeine</td>
<td>7</td>
<td>0.75</td>
<td>15</td>
</tr>
<tr>
<td><strong>10 ( \mu l ) Injected</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>46</td>
<td>15.80</td>
<td>158</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>161</td>
<td>8.30</td>
<td>83</td>
</tr>
<tr>
<td>Caffeine</td>
<td>10</td>
<td>1.30</td>
<td>13</td>
</tr>
</tbody>
</table>
Chromatogram 38

1 2 μg sodium saccharin
2 2 μg sodium benzoate
6.1 Beta-Induced Fluorescence

Chapters 2 and 3 have demonstrated that beta-induced fluorescence is a viable detection technique for use in high pressure liquid chromatography. The detector, developed so far, possesses many of the characteristics of an "ideal" detector, as quoted in Chapter 1. The greatest advantage of the BIF detector is its possible sensitivity. However, it is unlikely that any large increase in this parameter will be achieved by a further change in the design of the flowcell. The sensitivity of the technique is increased by an increase in the signal observed from a fluorescent solute or, by a decrease in noise which is achieved by decreasing the background count rate. Considering the latter problem first. BIF solution processes appear to be optimised by doping the mobile phase with an energy transfer agent such as toluene. However, gas liquid chromatographic analysis of toluene, using flame-ionisation detection, indicated a considerable quantity of p-xylene present in the toluene. The presence of such impurities leads to an increase in background count rate and therefore the removal of such impurities requires investigation. Reduction in background is also achieved by optically filtering out the fluorescence from the mobile phase. The use of the titanium dioxide filter/reflector warrants further investigation. Increase in signal is achieved by the use of a more active source. The present study employed a 1 m Ci promethium-147 source and in later studies a 5 m Ci source. The latter source had to be covered with a metallic coating before use to reduce the fluorescence from the ceramic source. However, this was only partly successful because it was evident that this coating was slowly removed by the mobile phase which resulted in a
rise in the background count rate. Very recently an experimental 100 mCi point source was introduced into the Mk V flowcell. Calculation of \( R \) and \( S_c \) for the detection of the three component mixture yields the following values:

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R ) (mV/g/cm(^3))</th>
<th>( S_c ) (g/cm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene</td>
<td>( 1 \times 10^9 )</td>
<td>( 0.4 \times 10^{-9} )</td>
</tr>
<tr>
<td>DPH</td>
<td>( 3.4 \times 10^9 )</td>
<td>( 1.2 \times 10^{-11} )</td>
</tr>
<tr>
<td>MSB</td>
<td>( 2.5 \times 10^9 )</td>
<td>( 1.6 \times 10^{-10} )</td>
</tr>
</tbody>
</table>

taking the peak to peak noise value from the chart as 0.2 mV. This is most promising if the background count rate, from 1% toluene in hexane, can be reduced considerably from the present 100000 cps.

Compounds detected in the present study were mainly polyaromatic hydrocarbons. Further investigation of other inherently fluorescent compounds is desirable so as to extend the range of compounds detected by BIF. As stated in Chapter 1, fluorimetric detection is extended by the technique of derivatization of the non-fluorescent compound, producing a fluorescent compound. Since this is such an important extension to normal fluorimetric analysis, it must be shown that BIF is capable of detecting such derivatives.

Quenched beta-induced fluorescence has been shown to be a successful technique for extending the basic BIF detection method. However, the lack of sensitivity may prove to be a major problem. Toluene has formed an essential part of the mobile phase systems used in most of the work. However, the quantum yield of toluene is poor (\( \sim 0.17(129) \)), in comparison to highly fluorescent species, and the lifetime of toluene (\( \sim 34 \) ns (129)) — which is essentially
the time available for quenching collisions to occur - is much shorter than is desirable. For these reasons, the potential of doping hexane/toluene mobile phases with a number of fluorescent dopants which show higher quantum yields and longer lifetimes than toluene should be examined, for enhancing the sensitivity with which quenchers may be detected.

The study of beta-induced fluorescence spectra yielded relevant information on the basic processes of the technique. Information and quantitative data on processes such as energy transfer are fundamental in the understanding of the technique, and this line of research should be further investigated.

Finally, it should be noted that when using the 1 mCi promethium-147 point source, no radioactivity was found in the mobile phase. After a considerable amount (30 l) of hexane and toluene had passed through the flowcell, the radioactive source fell out of its stainless steel holder (see Fig. 2.11), due to the mobile phase slowly dissolving the fixative used to retain the source in its holder. The 5 mCi source, provided by NEN, was found to be equally leak proof when mobile phase flowed over it. However, as the source was fabricated from ceramic material which was brittle, considerable care had to be used when handling the source with tweezers.

Some aspects of the investigation of beta-induced fluorescence and quenched beta-induced fluorescence have been published as follows:

1. Beta-Induced Fluorescence as a detection technique for liquid chromatography. I Prelim. Exps., D.J. Malcolme-Lawes,


6.2 Cerenkov Photon Absorption

The absorption of Cerenkov photons has been shown to be a useful detection technique for use in high pressure liquid chromatography. Provided that stray light/radiation is reduced to a minimum, it has been demonstrated that the detector possesses a linear response with increasing mass (up to 20 µg in the case of aspirin) of absorbing material. In this study it was necessary to calculate the absorbance, for each mass of material detected, to produce linearity. This problem may be overcome by suitable electronic design to enable an output of $\log_{10}(\frac{I_0}{I})$ to be delivered to a chart recorder. Although no rigorous comparison of Cerenkov detector performance with u.v. monitor performance was undertaken in this study, comparison of the chromatographic traces obtained by using the two detectors demonstrates that the response and sensitivity of the Cerenkov detector falls short of that achieved by the u.v. monitor. Section 5.7 (Chapter 5) has shown that an increase in sensitivity is achieved by generating a larger number of Cerenkov photons. As it is undesirable to increase the $^{90}$Sr activity beyond the 10 mCi used in parts of this study and, for chromatographic reasons, it is undesirable to increase the diameter of the flowcell beyond the 2.5 mm (flowcell volume approximately 50 µl) there appears little reward in pursuing with the use of a radioactive source inside the flow cell. It may be concluded that the use of a Cerenkov light source, external to the flowcell, should be investigated. The experiments using the quartz rod demonstrate that care must be taken in designing such a light source. A possible answer is shown in Fig. 6.1.
Fig. 6.1

Fig. 6.1 shows a solid quartz marble, drilled to its centre so that the radioactive source may be placed inside the marble. The surface of the marble should be coated with an aluminium reflective coating, a small section of which has been scratched away to enable Cerenkov photons to escape.

The advantage of this system is that, except for those beta-particles which travel into the stainless steel source holder, all the beta-particles emanating from the source enter the quartz and the maximum number of Cerenkov photons are generated. The photons, thus produced, are reflected by the aluminium coating until they escape through the non-aluminised section on the surface of the marble. The photons may travel a considerable distance inside the marble before eventually escaping and therefore it is essential that the marble be fabricated from high grade quartz which does not absorb light of wavelength greater than (say) 180 nm.

The disadvantage of this system is in the loss of simplicity when the radioactive source is used internally in the flowcell. The use of the light source shown in Fig. 6.1 requires focussing optics if it is to be used to full advantage.
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AN ELECTRONIC FILTER FOR RADIOACTIVITY DETECTORS IN CHROMATOGRAPHY SYSTEMS

D. J. MALCOLME-LAWES, S. MASSEY, P. WARWICK

Nuclear Chemistry Laboratory, Loughborough University of Technology, Leicestershire (U.K.)

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A simple low-pass filter system is described for use with chromatographic detectors based on the counting of radioactivity. Typical results are presented which demonstrate that the filter can be used to remove the statistical fluctuations in recorded count rates without the excessive peak broadening which results from using large time constants on conventional ratemeters.

Introduction

In most forms of radiochromatography, the radiochromatogram can be obtained as either a chart record of count rate as a function of time or as a histogram built up by counting fractions of material after the separation. For example in gas or liquid chromatography either a radioactivity detector may be placed so that the gas or liquid flow from the column passes through the detector for activity monitoring, or fractions of eluted material may be collected for subsequent activity estimation. After the development of a separation by thin layer chromatography the t.l.c. plate may be scanned with a radioactivity detector, or alternatively the thin layer material may be removed by scraping short lengths of material into individual sample bottles for counting.

While fraction collecting and subsequent counting offer the chromatographer the advantage of an unlimited choice of counting times, and consequently a high degree of control over the statistical uncertainty in the final result, the technique does have a number of drawbacks. Apart from the obvious effort and time required to collect and to count each fraction, there are the disadvantages of using many sample bottles and, in the case of samples assayed by liquid scintillation counting, a considerable quantity of scintillant. Furthermore samples mixed with scintillant are generally rendered non-recoverable.

On the other hand flow counting or scanning to quantify radiochromatograms, while providing immediate results, does require a higher minimum activity level and
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suffers from the problem of statistical fluctuations in the count rate being recorded as a form of noise. If a radiochromatogram is recorded digitally then the procedures for minimising the effects of such statistical noise are relatively straightforward. However much radiochromatography is actually carried out with the results appearing only as an analogue signal (i.e. a current or voltage proportional to the instantaneous count rate) which is recorded on a chart recorder.

In this paper we describe a simple and inexpensive way of reducing statistical noise fluctuations in count rate records and demonstrate the technique with examples of h.p.l.c. radiochromatograms and t.l.c. plate scans.

The approach

In principle a count-rate meter produces an analogue signal proportional to the instantaneous count-rate. In practice however ratemeters obtain their analogue signal by averaging the count-rate over a preset time, and most have facilities for selecting this time constant. While statistical fluctuations in both the background count-rate (i.e. the baseline) and signals from active material may be smoothed out by selecting a large value for the time constant, unfortunately such a procedure leads to a dramatic distortion of the peak size and shape for radioactive components. This may be seen in Fig. 1 where two radiochromatograms are shown. Both were obtained by scanning a single t.l.c. plate under identical conditions, except that for (a) the ratemeter time constant was set to 10 seconds, while for (b) it was set to 0.1 seconds.

In practice the peak shape and size are most faithfully recorded by using the smallest value of the ratemeter time constant, although the statistical variation in the baseline count rate will, of course, be at its most rapid under these conditions. Our approach has been to minimise this statistical fluctuation, without distorting the peak shape, by filtering the analogue signal to remove the rapid statistical fluctuations while passing the more slowly varying component of the ratemeter signal to the chart recorder. To this end we have constructed a low-pass filter, with amplification and d.c. offset facilities, which can be inserted between a ratemeter and a chart recorder. The filter system and typical results obtained from it are described below.

The filter system

To be of value in the present application the filter unit had to be designed to pass d.c. levels between 0 and 5 volts, and to pass varying signal levels with characteristic frequencies from zero up to a user-chosen cut-off frequency, f_c. Higher
Fig. 1. Thin layer chromatogram scan of a five component $^{14}$C-labelled mixture with rate meter time constant set to (a) 10 s and (b) 0.1 s.

frequencies should be sharply attenuated. There are several ways of approaching this goal. We chose to use a low-gain controlled source active filter showing a Butterworth response. The low-gain controlled source active filter design was chosen because it could be easily assembled from readily available components of common values, because the cut-off frequency could be selected by switching a small number of components, and because the low-gain configuration is inherently stable and free of the risk of noise pick-up. The Butterworth frequency response has a relatively poor attenuation rate around the cut-off frequency, but does have a maximally flat response at lower frequencies. As a flat low frequency response is of obvious importance for this application, the filter was designed for a Butterworth response and the higher frequency attenuation improved by utilising two filter networks in series.

A schematic diagram of the system is shown in Fig. 2, where power supply circuits have been omitted. (A regulated ±15 V supply was used, based on the RC4195 NB regulator.) The circuit consists of two consecutive second order Butterworth voltage-controlled voltage sources, each built around one half of a dual operational amplifier (a 747). Each operational amplifier is operated with a d.c. gain of approximately 1.5, and the output of the second stage is taken through a preset voltage divided (VR1) which is set up so that the voltage taken from the slider is
equal to the voltage input to the first stage (i.e. so that the overall voltage gain of the two filter stages is unity).

The two filter stages are identical and have cut-off frequencies (the frequency at which the power gain is 3 dB) selected by a single four-pole six-way switch S1. With the C/f values of 2.2, 4.7, 10, 22, 47, 100 μF, the cut-off frequencies available were nominally \( f_c = 2, 1, 0.5, 0.25, 0.1 \) and 0.05 Hz respectively. As each stage has a 12 dB/octave roll-off above \( f_c \) the overall filter action is a nominal 24 dB/octave or 80 dB/decade.

The filtered signal taken from VR1 is passed to a summing amplifier operated at unity gain in the inverting mode. Also taken to this amplifier is a signal from the potentiometer VR2, which may be used to back-off the d.c. level of the rate-meter analogue signal level before this signal is amplified by the next stage.

Because our unit is operated with systems giving outputs of both 0–100 mV and 0–5 volts, S2 allows the response of the summing amplifier to VR2 to be selected between ±1 and ±10 volts. VR2 is a ten turn helipot. The final stage is an inverting amplifier with a gain selected by S3 to be 0.1, 0.33, 1, 3.3, 10, 33 or 100.

The unit was constructed on a single circuit board and boxed, together with the ±15 V, 50 mA power supply, in a pressed steel case showing four external controls; cut-off frequency selector switch S1; d.c. offset control (VR2); d.c. offset range selector switch S2; and d.c. gain selector switch S3. Two 747 dual op. amps were used in the prototype, and although it would probably be better to use instrumentation amplifiers, we have had no difficulties using these inexpensive de-
Results and discussion

The filter system described above was initially designed for use with a h.p.l.c. chromatograph operating with a beta-induced fluorescence (b.i.f.) detector for the detection of non-radioactive materials. One characteristic of this type of detection system is a relatively high signal level from the h.p.l.c. solvent. The analogue output from our b.i.f. detector is via a ratemeter (Nuclear Enterprises Ltd.; Model 4678) which has its operating time constant selected by choosing the percentage standard deviation desired for the analogue signal. Fig. 3 shows the detector response to

\[ \text{J. Radioanal. Chem. 57 (1980)} \]
20 ng samples of p-terphenyl with selected standard deviations of (a) 3% and (b) 1% (the smallest value available). In both cases the count rate recorded from solvent (hexane) alone, which is \( \sim 9 \cdot 10^3 \) cps, leaves the recorded baseline well up the chart. Fig. 3 (c) shows the effect of inserting the filter unit between the ratemeter and recorder and repeating run (b) with a cut-off frequency of 0.1 Hz selected and a d.c. gain of unity. Clearly the quality of the recorded peak is dramatically improved, both in terms of its outline — which allows for improved quantitation, — and the signal to noise ratio — which allows for tighter error limits on results.

Fig. 3 (d) shows the further improvement in the recorded signal obtained when run (c) is repeated using a d.c. gain of X3 and with the baseline level offset to the lower edge of the chart paper. This much greater signal is clearly easier to measure, although of course offering no signal to noise ratio improvement.

For more conventional radiochromatography we have a filter system connected to our \( \gamma \)-scintillation detecting h.p.l.c. unit, which uses an SRS (Nuclear Enterprises Ltd.) scaler-ratemeter. We have also connected a filter system to a t.l.c. plate scanner operated with a \( ^3 \)H/\( ^14 \)C GM scanning head and a ratemeter unit (all Panax Nucleonics). The results obtained from both systems are sufficiently similar that we show here only traces obtained by scanning a t.l.c. plate on which there are three fully resolvable \( ^14 \)C-labelled components.

Fig. 4 shows the ratemeter record obtained using a time constant of (a) 10 seconds and (b) 1 second, both without the aid of the filter system. In (b) the three
peaks are fully resolved but are difficult to quantify owing to statistical fluctuations. In (a) the larger time constant has smoothed out the statistical fluctuations on the count rate record, but at the cost of introducing long tails on the peaks so that the recorder resolution has deteriorated — again making the peak areas difficult to quantify. Fig. 4 (c) shows the effect of including the filter system, operating at a d.c. gain of unity and a cut-off frequency of 0.1 Hz, on the radiochromatogram recorded in (b). Again the statistical fluctuations have been smoothed out, but this time the peaks have not been broadened so much that the recorded resolution is impaired.

In each of the examples given above, and in many others recorded in our laboratory, the filter system has offered an improvement in signal-to-(statistical) noise ratio at a smaller cost in terms of peak distortion than can be obtained using the simple time constant selectors on the ratemeters at our disposal. The basis of this improvement is clearly that the frequency response of the filter system declines at 80 dB/decade above the selected cut-off frequency, whereas that of most conventional ratemeters declines more slowly — for the simplest RC smoothing circuit the rate of decline is 20 dB/decade.

The authors acknowledge the support of the Science Research Council and the National Research Development Corporation.

References
## APPENDIX II.

**Sources and Grades of Chemicals Used**

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Fisons - Fisons Scientific Apparatus, Bishop Meadow Road, Loughborough, Leicestershire.

Aldrich - Aldrich Chemical Co. Ltd., The Old Brickyard, New Road, Gillingham, Dorset.

Sigma - Sigma London Chemical Co. Ltd., Fancy Road, Poole, Dorset.

A.C.S. Ltd. - Applied Chromatography Systems Ltd., Concorde House, Concorde Street, Luton. (Compounds donated as gifts).

L.G.C. - Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ (Compounds donated as gifts).