

## Chapter 3

# Practical Liquid Scintillation Spectrometry: Organising a Methodology

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## INTRODUCTION

Liquid scintillation counting is one of the most commonly used of the ever increasing variety of analytical techniques available to the modern scientist. The complexity of the liquid scintillation counting (LSC) technique is, however, not generally appreciated. It is therefore not surprising that users encounter disproportionate difficulties which are aggravated by them having little or no knowledge of the physical processes involved and matters are complicated further, we believe, by the tendency of manufacturers to make spectrometers which are designed to be easier and easier to use, i.e. for instruments to be equipped with fewer and fewer controls; the user has only to load the samples and return later to remove the printed output. As the results are well presented and neatly formatted he finds it difficult to believe that they may not be valid. Indeed it is only if his results are quite unexpected that his attention is turned to his use of the LSC technique and the problems inherent therein. With fully automatic instruments, the aware user then discovers the difficulties in ascertaining and correcting for spectrometer drift, chemiluminescence, etc. It was with these problems in mind that we decided to design and produce a computer program package to assist users, expert and novice alike, in the selection of counting conditions and the processing of the resultant data. However, in attempting this, we found increasingly that it was necessary to have a unified numerical description of the whole liquid scintillation counting process. Our extensive program package has since been abandoned, at least for the present, and attention has instead been turned to simulating the LSC process on the computer, thereby bringing together data from quite different sources.

We have confined our attention to simulating soft  $\beta$ -emitters such as  $^3\text{H}$  and  $^{14}\text{C}$ , first since they are the ones most frequently used in the life sciences and second because their physical characteristics encourage a sensitive analysis of the LSC technique.

This chapter deals with the more practical aspects of the project. The preceding chapter dealt in more detail with the simulation itself.

## MATERIALS AND METHODS

### Instruments

Two scintillation spectrometers were employed in this work. One was an early model Packard Model 3375 Tri-Carb system fitted with a detector comprising two flat quartz-faced bialkali EMI 9635 photomultipliers coupled with optical grease to a perspex light pipe and operated at  $20^{\circ}\text{C} \pm 0.5^{\circ}$ . The other was a Searle Analytic Isocap 300 instrument fitted with two bialkali EMI 9805/A photomultipliers which have a stippled (frosted) face of borosilicate glass. Its detector assembly was fitted with reflectors and was operated  $20^{\circ}\text{C} \pm 1^{\circ}$ . Both instruments were set to count in an integral window, i.e. all coincident events from 0–2000 keV. The elevators of both instruments were covered with a disc of black photographic paper to act as a 'perfect' light absorber (see the previous chapter).

Absorption spectra were measured using a Varian Techtron Model 635M spectrophotometer and calibrated within 1 nm using a Holmium standard filter (Philips Part No. 700570). The instrument was set for 0.2 mm slit width and operated in the double beam mode. Spectra of samples were read against toluene in 1.00 cm stoppered cells constructed of Suprasil and plotted on a chart recorder. Fluorescence spectra were recorded on an X-Y plotter from an Aminco Bowman Ratio Spectrofluorometer fitted with a photomultiplier having an S-4 response.

### Chemicals

Commercial supplies of PPO – 2,5-diphenyloxazole – have been reported to have impurities from various sources.<sup>1</sup> It is also thought that its prolonged exposure to sunlight gives rise to quenching impurities. Consequently, PPO used in this study was twice recrystallised which resulted in an increase of  $3^{\circ}\text{C}$  in the melting point to a value of  $71.8^{\circ}\text{C}$ . A further recrystallisation did not change this value. The absorption spectrum in toluene was similar to that reported<sup>2</sup> as was its fluorescence spectrum at the level of  $0.1\ \mu\text{g}/\text{ml}$  in toluene.

Toluene (Pronalys Grade, May and Baker, Australia) was fractionally distilled and the portion boiling between  $110.2^{\circ}\text{C}$  and  $110.6^{\circ}\text{C}$  was collected and used in this work.

The scintillation solution was composed of 8.00 g PPO per litre toluene at  $20^{\circ}\text{C}$ . Its refractive index as measured using a simple Abbe refractometer was 1.5018 thus giving a critical angle of  $41.75^{\circ}$  at a solution-air interface.

Carbon tetrachloride was of spectral grade, obtained from Mallinckrodt, U.S.A., and was used as the chemical quenching agent.

4-Dimethylaminoazobenzene was obtained from Hopkin and Williams, England. A 1.0 mM solution of this compound in the scintillation solution was employed in the colour quenching studies. It was used in preference to methyl orange since both the absorption spectrum and counting efficiency of the treated sample reached a constant value within a few hours; note that with methyl orange, prepared as an alcoholic solution, the samples gave spectra which did not stabilise for at least a day.

### Counting vials

A number of brands of scintillation vials were assessed for their uniformity of diameter, height and weight. Of these, Packard Tru-lite vials were chosen since their measurements exhibited the smallest standard deviation. Ten vials, selected at random, had an average 14.236 g (standard deviation (S.D.) = 0.222) of glass, density  $2.23\ \text{g}/\text{ml}^3$  and refractive index 1.474.<sup>3</sup> The last value gives a critical angle of  $42.7^{\circ}$  for a

glass-air interface. The same vials had an average height to the shoulder of 4.48 cm (S.D. = 0.086), and average diameters (single measurement) at the shoulder, middle and base were 2.716 cm (S.D. = 0.021), 2.718 cm (S.D. = 0.008) and 2.713 cm (S.D. = 0.022) respectively. The average thickness of the curved wall was measured to be 0.1023 cm (S.D. = 0.00435). This thickness was independently estimated by weighing the amount of water necessary to fill the vial to a height of 4.00 cm and assuming the vial had a flat base was found to be 0.096 cm. The average of these two values, 0.099 cm was used in the simulation and the unit of radius was thus taken as 1.260 cm.

All vials were washed with RBS 25 detergent then two rinses of tap water and four rinses of distilled water to ensure maximum cleanliness and to remove any possible quenching agents. Conventional caps were used and these were fitted inside with an aluminium/cork liner covered with a disc of black photographic paper to act as a 'perfect' light absorber (see the previous chapter). The paper was shown to have no quenching agents extractable by the scintillation solution.

### Special vial to assess response of detectors

The relative probability that a photon (of given wavelength) reaches the photocathode of a photomultiplier is a complex function of the geometry of the detector and the point and direction at which the photon leaves the curved wall of the vial. To gain some insight into this response, 20 ml (4.00 cm high) of scintillation solution (spiked with about half a millicurie of  $^3\text{H}$ -toluene) was placed in a vial which was covered entirely with black paper except for a small circular hole (1 mm diameter) 3.95 cm above the inner base of the vial.

It was then placed in the detector of each instrument in turn with the hole directly in front of one photomultiplier. With the coincidence switched off the response of each photomultiplier (above noise) was measured in an integral window. The vial was then unloaded and reloaded so that the small hole pointed at an angle of  $15^\circ$  to the previous one. This was repeated until the vial had been rotated the full  $360^\circ$  (a cardboard protractor was made, taped to the top of the elevator and shielding, and was used to ensure the vial could be accurately placed in all 24 positions from  $0$ - $360^\circ$ ). The vial was then fitted successively with a black paper cover having a 1 mm hole at heights of 3.0, 2.0, 0.5 and 0.05 cm above the base (this choice of heights is explained in Ref. 6), and the response plotted for each photomultiplier in each of the 24 angular positions.

### Standards

Calibrated tritiated and  $^{14}\text{C}$ -labelled hexadecane were purchased from the Radiochemical Centre, Amersham, England, and were certified as having an overall uncertainty of  $\pm 3\%$  and  $\pm 2\%$  respectively. Exactly one litre of the scintillation solution was spiked with a carefully weighed and transferred quantity of the hexadecane standard. The tritiated solution had 155,000 disintegrations  $\text{min}^{-1}$  per 20.0 ml and the  $^{14}\text{C}$ -labelled solution 94,900 disintegrations  $\text{min}^{-1}$  per 20.0 ml. This approach avoided the errors associated with individually spiking 20.0 ml samples with small weights of the standard. 20.0 ml aliquots were then quickly pipetted into tared scintillation vials, capped, and then reweighed to confirm the volume and the height of the liquid column as 4.00 cm. The (air-quenched) samples were then counted to check their efficiency in an integral window using a preset count of 800,000 or 900,000 counts. Samples which did not have a column height between 3.95 and 4.05 were rejected as were those with counting efficiencies which exceeded the total average disintegrations rate (30 vials) by more than 0.75%.

## Quenching studies

Volumes of quenching agent were dispensed into the 20.0 ml aliquots of scintillation solution (after an identical volume of spiked solution had been removed) using a 0.25 ml Gilmont Ultraprecision Micrometer Burette (graduated to the nearest 0.01  $\mu\text{l}$ ). In this way the total sample volume was maintained at 20.0 ml and its new disintegration rate was recalculated. Counting of colour-quenched samples was carried out six hours after the addition of the quencher to ensure that the sample counting rate and its absorption spectrum had reached a stable value. Chemically quenched samples were assessed immediately because they had been found to be quite stable.

After the colour-quenched samples had been counted a portion was removed and its absorption spectrum measured as described previously. The spectrum was dissected into about 20 linear portions between 354 nm and 500 nm. These linearised portions were entered into the computer model for the simulation of colour quenching. A spectrum was also measured for an unquenched (argon-flushed) sample for use in the model for assessing secondary photon emission from PPO.

To obtain an 'unquenched' sample an air-equilibrated standard sample was cooled to about  $-10^{\circ}\text{C}$  and then dry argon was bubbled gently through it until the counting rate did not increase further after it had been brought to  $20^{\circ}\text{C}$ .

## RESULTS AND DISCUSSION

Since counting efficiencies have played an important but not overriding role in validating this model, some considerable effort has therefore been made to produce accurate experimental data. All efficiencies were measured in an integral window, this being simulated in the model by counting all events producing a two-photoelectron coincident event. This was necessary since we cannot satisfactorily simulate discriminator settings due to our lack of information concerning the photomultipliers, amplifiers, pulse perturbations, etc. Counting tritium over an integral window gives, by comparison with the normal case, only slightly higher efficiencies (but with a considerable increase in the background). However, for  $^{14}\text{C}$ , since the normal counting channel is from  $\cong 7.5$  keV to 156 keV, this difference is substantial. For example, when counting at about 96% integral the efficiency in the normal window would be 88–90%, and it is interesting to note that the difference corresponds closely to the amount of the  $\beta$ -spectrum for  $^{14}\text{C}$  lying below 7.5 keV (7.7%). Compare Table 1 of the previous chapter.

A serious deficiency in our early attempts at modelling the LSC process<sup>4</sup> was the lack of information concerning the directional response within the counting chamber; in describing this response it was necessary to have information on the direction taken by the photon after leaving the vial as well as the point at which it left. This problem has been partially overcome by using the special vial described in the previous section.

Figure 1 (a) (b) show this information in diagrammatic form for the Searle Isocap 300 and the Packard Model 3375 Tri-Carb system respectively. Note that the data are normalised to the maximum response for each instrument. It is apparent that light travelling directly towards one photomultiplier has a small, but non-zero, chance of being detected by the other photomultiplier after multiple reflections within the chamber. The response at about  $90^{\circ}$  and  $270^{\circ}$  is less clear-cut but is quite reproducible. Note should be taken of the 'hot-spots' of maximum response on the photocathode. The variation in quantum efficiency across the photocathode is well known,<sup>5</sup> and the current results are consistent with this. Note also the severe fall-off in response for those photons leaving the vial near its base or at the meniscus (note that,

**SEARLE DETECTOR RESPONSE**

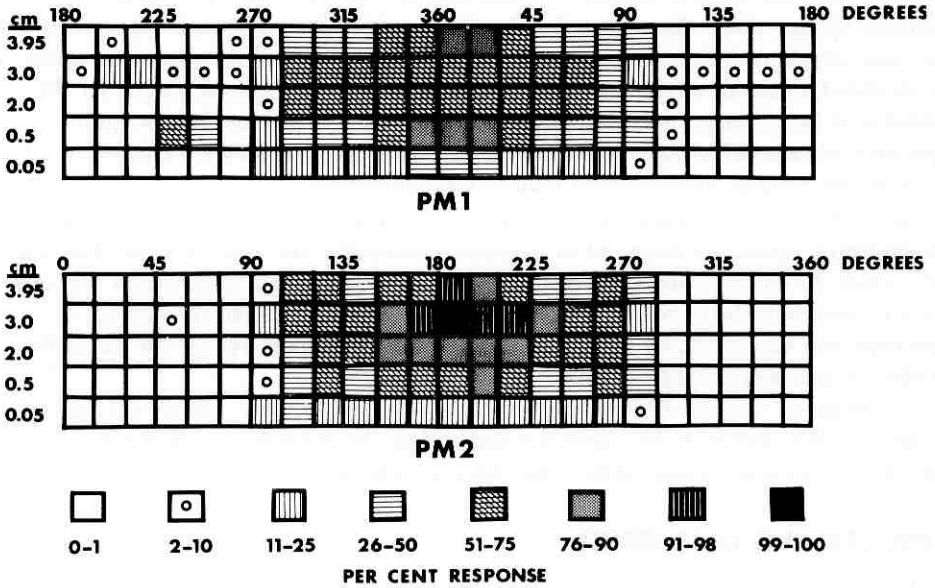


Fig. 1(a). The directional response of the counting chamber of a Searle Isocap 300. See 'Materials and Methods' section for details.

**PACKARD DETECTOR RESPONSE**

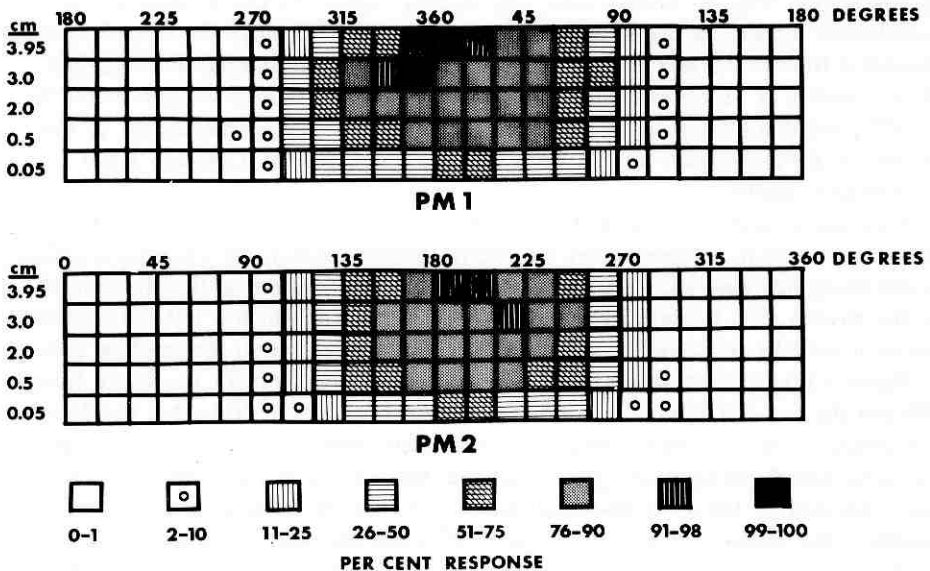


Fig. 1(b). The directional response of the counting chamber of a Packard Model 3375 Tri-Carb system. See 'Materials and Methods' section for details.

Table 1. Comparison percent counting efficiencies obtained by experiment and from the model for conditions of low impurity quenching.

Impurity quenching factor $q$ in model	Searle Isocap 300				Packard Model 3375 Tri-Carb system				
	Experimental		Modelled		Experimental		Modelled		
	Sample 1	Sample 2	$n=2^a$	$n=3$	Sample 1	Sample 2	$n=2^a$	$n=3$	
$^3\text{H}$	1	64.2 <sup>b</sup>	63.9 <sup>b</sup>	67.6	62.4	63.9 <sup>b</sup>	63.9 <sup>b</sup>	70.6	66.2
	0.9			63.9	58.8			68.9	64.4
	0.8			59.4	53.3			64.8	59.3
	0.75	58.5 <sup>c</sup>	59.4 <sup>c</sup>	57.4	52.0	58.2 <sup>c</sup>	58.3 <sup>c</sup>	63.4	58.6
	0.7			55.4	48.7			61.7	56.0
$^{14}\text{C}$	1	96.9 <sup>b</sup>	97.1 <sup>b</sup>	96.9	96.4	97.8 <sup>b</sup>	97.8 <sup>b</sup>	97.8	97.3
	0.75	96.3 <sup>c</sup>	97.0 <sup>c</sup>	95.9	95.4	97.4 <sup>c</sup>	97.7 <sup>c</sup>	96.4	95.9

<sup>a</sup>A coincident pulse must be of at least  $n$  photoelectrons to be counted;  $n$  is therefore the 'pseudo-discriminator' setting.

<sup>b</sup>Argon-purged sample.

<sup>c</sup>Air-quenched sample.

as has been shown elsewhere,<sup>6</sup> the solution can be considered effectively of infinite height in respect of the light leaving the curved walls of the vial).

It is imperative to realise that the approach adopted for this measurement has one severe failing: it does not account for those photons which are reflected back into the vial since they are absorbed by the black photographic paper. In the normal case, however, a photon, once back in the vial, may reach the opposite photomultiplier or be reabsorbed by the solution to begin anew the production of photons.

Table 1 contains two sets of data concerning experimental and modelled efficiencies for both  $^3\text{H}$  and  $^{14}\text{C}$ . Several important features emerge. First, compare the differences in the modelled counting efficiency for  $^3\text{H}$  for 2 and 3 coincident photoelectrons. Such behaviour provides a very sensitive test of the validity of the model, and has frequently been used to gauge its performance during its development. This sensitivity is readily explained by recalling that, in the case of  $^3\text{H}$ , since the number of photons is small, there is a significantly smaller chance of obtaining a three photoelectron event. Compare this with the situation for  $^{14}\text{C}$  where there is very little difference since there are many more photons involved.

Now air quenching causes about a 25% loss in primary photon production,<sup>7</sup> this loss being important for  $^3\text{H}$  counting efficiencies of both two and three photoelectron events, but, on the other hand, having a negligible effect on  $^{14}\text{C}$ .

The reader will no doubt have observed that the tritium data modelled for the Packard instrument are too high when compared with the experimental values, and this is further exemplified in Table 2 (for the meaning of  $q$  see the previous chapter). We suspect that the high values recorded on the Packard unit are due, at least to some extent, to the light transmission and scattering characteristics of the perspex light pipe. Figure 2 details some important spectral characteristics of the detector assembly. Note especially the percentage of light lost in a 0.5 inch thickness of normal perspex as measured in the laboratory. It is not known if this is similar to that installed in the early model instrument used; however, it is believed that a special grade of perspex was used in

Table 2. Percent counting efficiencies obtained for different values of the impurity quenching factor.

Impurity quench factor $q$	$^3\text{H}$		$^{14}\text{C}$	
	Searle Isocap 300	Packard Model 3375 Tri-Carb	Searle Isocap 300	Packard Model 3375 Tri-Carb
1	67.6 <sup>a</sup>	70.6	96.9	97.8
0.9	63.9	68.9	96.5	-
0.8	59.4	64.8	95.7	96.5
0.75	57.4	63.4	95.9	96.4
0.7	55.4	61.7	-	-
0.6	50.7	56.3	94.5	95.5
0.5	45.7	52.2	93.8	94.6
0.4	37.2	42.7	92.8	-
0.3	29.2	34.3	89.5	90.3
0.2	17.3	21.6	84.5	86.3
0.15	-	-	79.5	81.8
0.1	-	-	70.0	72.5
0.075	-	-	61.3	64.9
0.05	-	-	48.2	52.4

<sup>a</sup>Counting efficiencies for all coincident events, i.e. for an integral counting window.

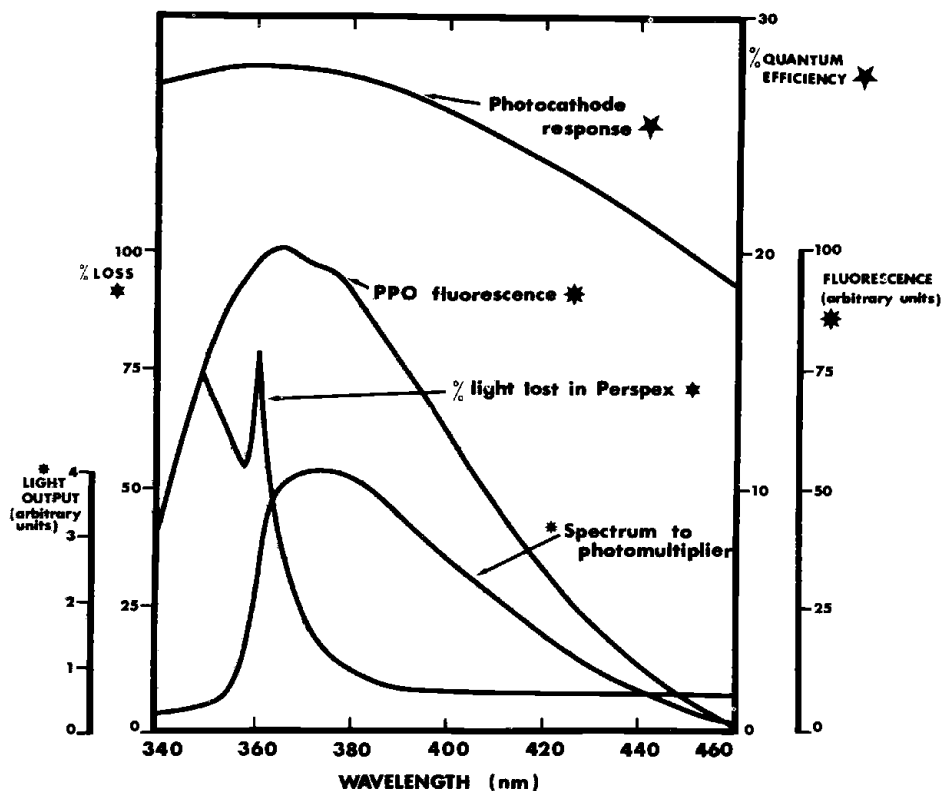


Fig. 2. Some optical characteristics of the scintillation solution as compared to perspex (0.5 in).

spectrometers manufactured after this date — indeed, the later Packard instruments apparently use mirror reflectors. The spectrum to the photomultipliers is the real spectrum leaving the vial and it can be readily seen how the type of perspex measured is poorly matched both to the light emission- and photocathode response-spectra, and would thereby lower the average quantum efficiency observed in practice; this feature, if modelled, would lower the efficiencies predicted by the current model. Because of the complex geometry of the light pipe and the lack of the facilities necessary to characterise it, we decided henceforth to deal only with the Searle Isocap for modelling. The influence on efficiency of the characteristics of individual light pipes in the Packard unit was observed when another light pipe was involved for a short period; this resulted in a decrease of some 3% in the counting efficiency for unquenched  $^3\text{H}$ .

It is also interesting to note in Table 2 that, after removing 95% of the energy available for the production of photons in  $^{14}\text{C}$ , a counting efficiency of about 50% is still recorded whereas, in contrast, an 80% decrease for  $^3\text{H}$  brings the efficiency into a range that today's users would find only marginally acceptable.

As mentioned above, prediction of the tritium efficiency was a useful guide to the model's validity during its period of development. Table 3 indicates the sensitivity of the model to setting the lower pseudo-discriminator in terms of a coincident event of at least  $n$  photons. Counting efficiency decreases drastically as  $n$  increases, and  $q$ , the impurity quenching factor, decreases. Note also the substantial relative increase in average  $\beta$ -energy necessary not only to produce  $n$  coincident photoelectrons, but also

Table 3. Simulation of different 'discriminator' settings for the unquenched and impurity quenched situations (only coincident 'pulses' of at least  $n$  photoelectrons are counted).

	$n = 2$			$n = 3$			$n = 5$			$n = 10$		
	% Effic.	keV <sup>a</sup>	keV	% Effic.	keV	keV	% Effic.	keV	keV	% Effic.	keV	keV
<sup>3</sup> H argon-purged ( $q^b = 0.9$ )	63.9	7.4	7.7	58.8	7.7	8.7	43.3	8.7	14.0	11.1	11.1	
<sup>3</sup> H air-quenched ( $q = 0.8$ )	59.4	7.6	8.0	53.3	8.0	9.1	37.2	9.1	10.9	11.9	11.9	
<sup>3</sup> H impurity-quenched ( $q = 0.5$ )	45.7	8.3	8.9	38.6	8.9	10.3	21.2	10.3	2.0	12.8	12.8	
<sup>14</sup> C argon-purged ( $q = 1$ )	96.9	51.8	52.0	96.4	52.0	52.7	95.0	52.7	90.3	55.0	55.0	
<sup>14</sup> C air-quenched ( $q = 0.75$ )	95.9	51.4	51.7	95.4	51.7	52.8	93.1	52.8	86.5	56.0	56.0	
<sup>14</sup> C impurity-quenched ( $q = 0.4$ )	92.8	53.8	54.5	91.5	54.5	56.6	87.2	56.6	75.6	62.4	62.4	

<sup>a</sup>Average  $\beta$ -energy of events being counted.

<sup>b</sup>Impurity quench factor (see text).

to produce the modelled efficiencies as  $q$  decreases. To a much lesser extent the same is true for  $^{14}\text{C}$ , since here again the substantially larger number of photons involved play an all important part.

In Table 4 we consider some experimental and modelled data for colour quenching. Notice that for moderate amounts of quenching agent (less than 200  $\mu\text{l}$ ) there is reasonable agreement between the experimental and modelled data when  $n = 2$  and  $q = 0.78$  or  $0.75$  for air-quenched samples. However, for  $^{14}\text{C}$ , there is a considerable divergence in these efficiencies with volumes of colour quenching agent in excess of 200  $\mu\text{l}$ ; there are two immediately obvious explanations for this behaviour. First, at these concentrations the 4-dimethylaminoazobenzene may be acting not only as a colour quencher, but also as an impurity or chemical quencher. Second, the model is incorrect, at least in respect of extremely heavy colour quenching (note that the worst case is the sample counted at less than 30% efficiency in a normal  $^{14}\text{C}$  window, and is therefore hardly of practical interest).

The first hypothesis, namely that the model can be used to determine the degree of chemical quenching inherent to various coloured agents, is, in the opinion of the authors, while tantalising, as yet by no means substantiated because of the many simplifying assumptions made in defining the model.<sup>8</sup> It is, however, interesting to

Table 4. Comparison of observed and predicted counting efficiency for colour quenching conditions.

	Coloured agent in 20 ml of solution ( $\mu\text{l}$ )	Observed counting efficiency (%)	Predicted counting efficiency (%) for a pulse of at least $n$ photo-electrons				Difference between expected and observed efficiencies for $n = 2$ $q = 0.78$
			$n = 2$		$n = 3$		
			$q^a = 0.78$	$q = 0.75$	$q = 0.78$	$q = 0.75$	
$^3\text{H}$	7.6	57.5	60.4	58.7	54.3	53.1	2.9
	20.4	55.4	57.7	58.4	51.8	52.7	2.3
	35.2	53.6	57.0	54.9	50.3	48.1	3.4
	52.4	50.6	54.1	53.0	48.0	46.8	3.5
	73.2	48.9	49.9	48.7	43.3	42.5	1.0
	97.0	44.9	48.2	46.4	41.8	39.7	3.3
	115.6	43.0	43.3	43.8	36.5	37.4	0.3
	139.8	40.0	42.5	40.1	35.9	33.2	2.5
	162.4	38.2	40.6	41.7	33.3	35.1	2.4
$^{14}\text{C}$	20.2	95.2	95.6	95.7	94.8	95.1	0.4
	74.6	94.5	94.7	-	93.8	-	0.2
	124.5	93.0	94.1	94.0	93.4	93.2	1.1
	184.3	91.3	93.4	-	92.5	-	2.1
	235.7	88.3	91.6	-	90.4	-	3.3
	297.4	85.0	90.3	89.9	89.3	88.5	5.3
	353.6	82.5	88.3	-	87.1	-	5.8
	406.0	78.9	88.2	-	87.3	-	9.3
	452.9	76.1	86.3	-	85.0	-	10.2
	503.8	74.9	85.5	83.4	84.1	82.2	10.6

<sup>a</sup> $q$  is the impurity quench calibration factor (see text).

note that a spherically symmetric LSC system, described elsewhere,<sup>9,10</sup> can be mathematically modelled with far greater rigour,<sup>6,9</sup> such a system and its associated model might therefore usefully be applied in distinguishing the chemical quenching component of coloured agents.

Consider Figs. 3 and 4. These data deal exclusively with wavelengths of photons and their absorption within the system. Consequently, no distinction can be made on a photon to photon basis between scintillations derived from either  $^3\text{H}$  or  $^{14}\text{C}$ . The data are produced by the model for colour- and chemically quenched samples counting at similar efficiencies, and include information for all photons simulated. In each case the fluorescence spectrum is built-up by the model from a large number of photons and, whilst their shapes are very similar, they are not identical due to the stochastic nature of the model. From about 354 nm to 360 nm the transmittance through 1 cm of the scintillation solution increases from 1% to 50%. This behaviour is not seen in the figures since the absorption spectra are weighted according to the fluorescence spectrum for PPO.

Note that the absorption spectrum for the chemical quenching (Fig. 3) spectrum applies only to PPO and, above 354 nm, is substantially different to that given for an equivalent colour-quenched case (Fig. 4). As a result, the spectra of photons reaching the photomultipliers are substantially different for chemically and colour-quenched samples. Note also that, due to the various photon losses (at the top and base of the vial, the detector, etc.), the sum of the absorption spectrum and the spectrum to the photomultipliers is always less than the fluorescence spectrum. Note finally that the

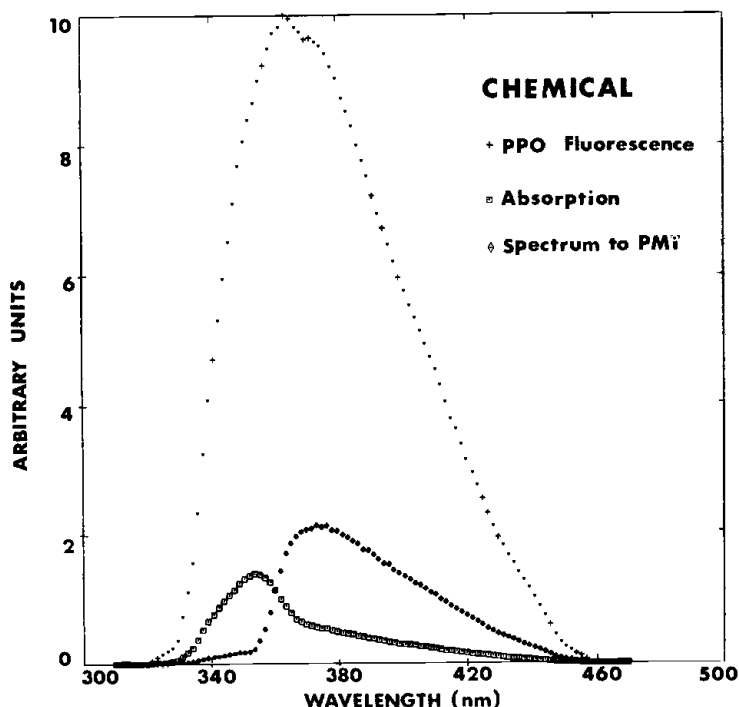


Fig. 3. Spectra generated by the model for the chemically quenched situation. Data derived from 10,000 simulated  $^{14}\text{C}$  events. Integral counting efficiency  $\cong 93\%$ .

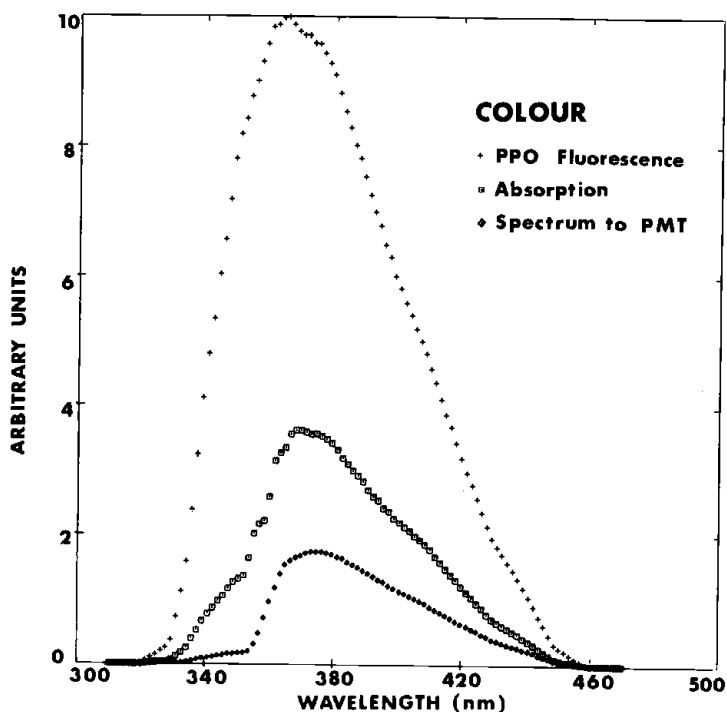


Fig. 4. Spectra generated by the model for the colour-quenched situation. Data derived from 8,000 simulated  $^{14}\text{C}$  events. Integral counting efficiency  $\cong 93\%$ .

comparable counting efficiency in the two systems is a function of the number of primary photons and secondary photons simulated (see Table 1 of the previous chapter).

The advantages of lesser pulse height analysis, at least for  $\beta$ -emitters with energies up to 200 keV, have been demonstrated by Ediss *et al.*<sup>11</sup> and Laney<sup>12</sup> who have emphasised its value when assessing efficiencies of either colour- or chemically quenched samples. Various authors have shown that quenching of  $^{14}\text{C}$  does in fact give distinctly different channels ratio versus efficiency curves when the summed pulse height is employed. However, if the smaller or lesser of the two pulses which enable coincidence is used then the resultant curves are very similar. Neary and Budd<sup>13</sup> have pointed out that, for  $^3\text{H}$ , the difference in pulse height is slight and that this is due to the small number of photons produced. However, for more energetic isotopes such as  $^{14}\text{C}$ , there is a marked difference since more than 10 times as many photons are involved (see Tables 2-4 of the previous chapter), and this permits a more probalistic variation for colour than for chemical quenching.

The model reported herein produces results, similar to those made in the laboratory, which are presented in Figs. 5-8. Data produced by the model (and given in Figs. 5 and 6) predict that there should be, for tritium, little difference between the summed pulse height spectrum for chemical- and colour-quenched samples; the same may be said for the lesser pulse height spectrum. Thus, tritium samples, whether colour- or chemically quenched, should give similar pulse height spectra when counted in an integral window for the same counting efficiency. The channels ratio versus efficiency

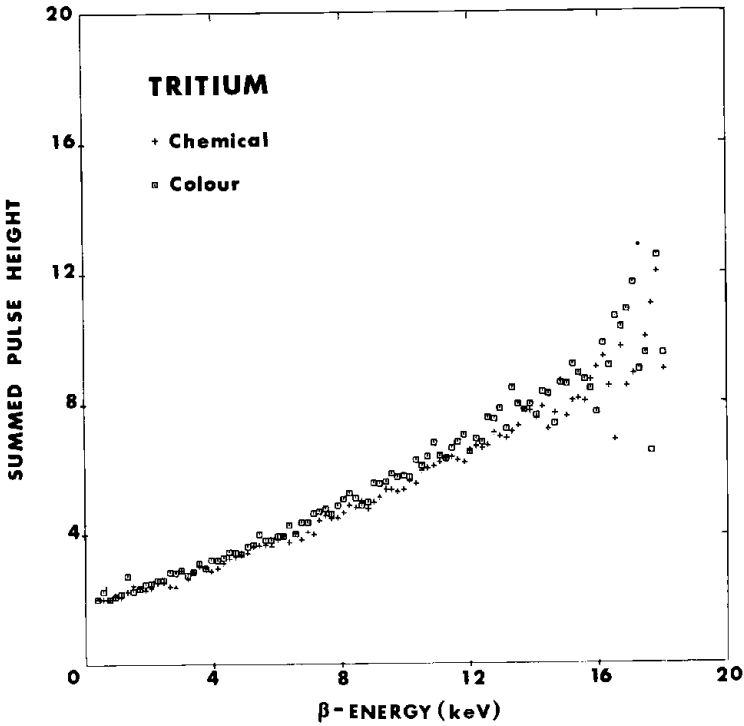


Fig. 5. A plot of average summed pulse height versus  $\beta$ -energy for colour- and chemically quenched tritium events which enable coincidence (note the different scale for the vertical axis when compared with Fig. 6). Integral counting efficiency  $\cong 44\%$ .

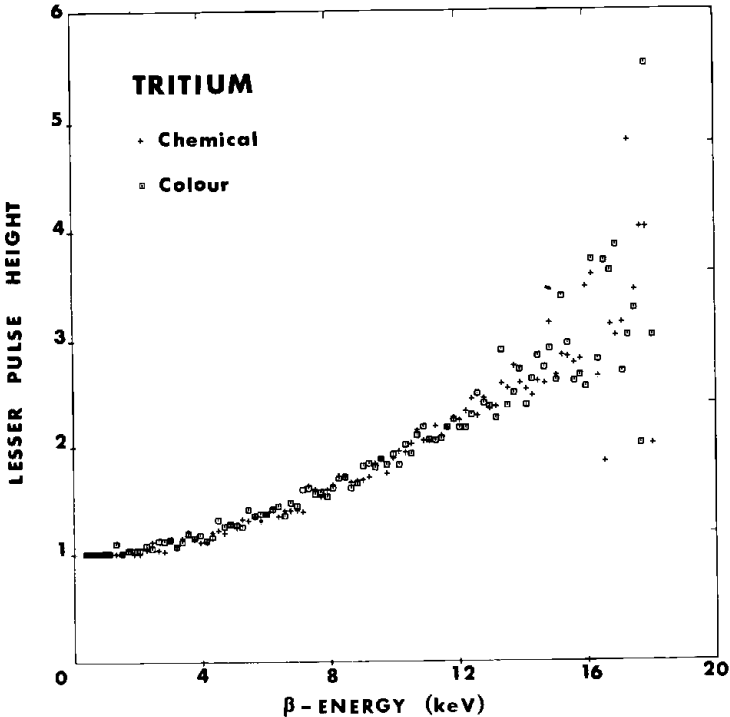


Fig. 6. A plot of average lesser pulse height versus  $\beta$ -energy for colour- and chemically quenched tritium events which enable coincidence (note the different scale for the vertical axis when compared with Fig. 5). Integral counting efficiency  $\cong 44\%$ .

curve should therefore be similar. The fuzziness in the distribution for  $\beta$ -energies above 15 keV is due in part to relatively few (1.3%) events occurring at energies in excess of this.

The situation of  $^{14}\text{C}$  is quite different, as can be seen in Figs. 7 and 8, and, as has been mentioned several times before, this is mainly due to the larger number of photons involved. The position of the scintillation of  $\beta$ -event within the vial, particularly in relation to the curved wall, now becomes of importance and this is discussed in detail elsewhere.<sup>6,9</sup> Figure 7 clearly shows the difference between the summed pulse heights for colour- and chemically quenched  $^{14}\text{C}$  samples counting at 93% (integral), and this confirms that the channels ratio versus efficiency curve would be substantially different. However, in considering the lesser pulse heights for the same samples (Fig. 8), the data for chemical and colour quench lie closely together. This is consistent with the channels ratio versus efficiency curves being similar. Note also that increased fuzziness at high energies, especially for the colour-quenched sample, is due in part to relatively few (8.3%)  $\beta$ -energies occurring above 100 keV.

It is likely for  $\beta$ -emitters which are much more energetic than  $^{14}\text{C}$ ,  $^{32}\text{P}$  for example, that the summed pulse height spectra for colour- and chemically quenched samples will diverge even more, but the situation cannot be treated here since such an emitter cannot be considered to produce a scintillation at a point source.

The number of greater and lesser pulses for photons of different wavelengths are plotted in Figs. 9-12 and included here is the data for both  $^3\text{H}$  and  $^{14}\text{C}$  samples which have been either chemically or colour quenched. (Note that the optical behaviour is independent of the degree of chemical or impurity quenching.) The peak of each

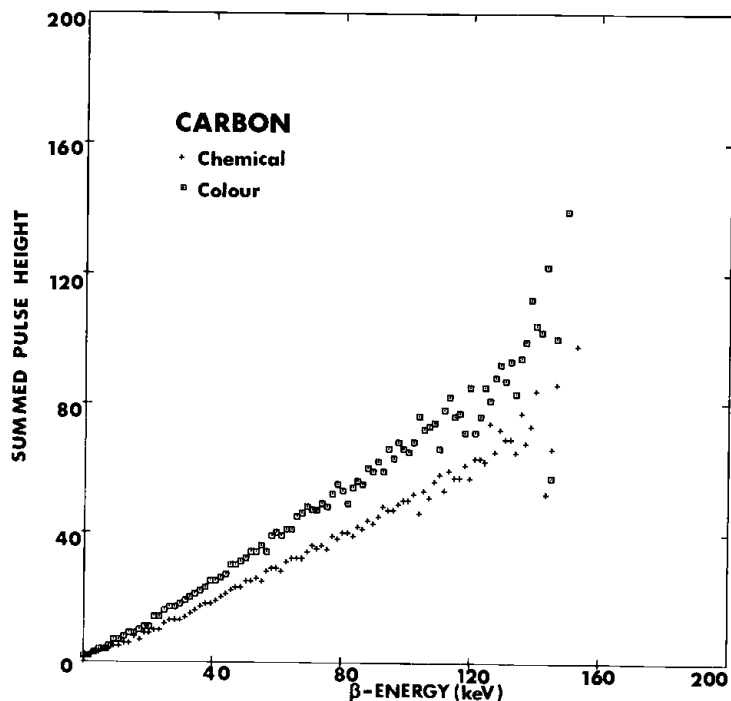


Fig. 7. A plot of average summed pulse height versus  $\beta$ -energy for colour- and chemically quenched  $^{14}\text{C}$  events which enable coincidence. (Note the different scale for the vertical axis when compared with Fig. 8). Integral counting efficiency  $\cong 93\%$ .

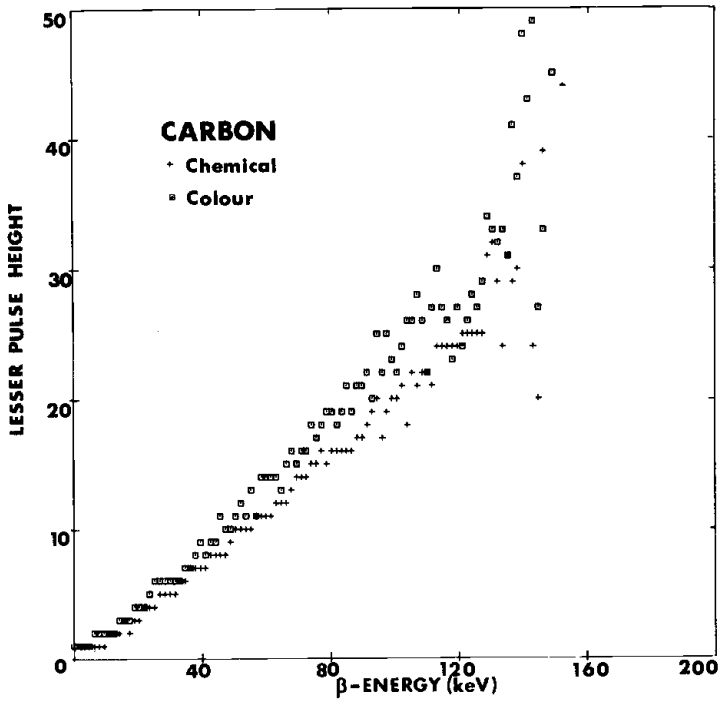


Fig. 8. A plot of average lesser pulse height versus  $\beta$ -energy for colour- and chemically quenched  $^{14}\text{C}$  events (note the different scale for the vertical axis when compared with Fig. 7). Integral counting efficiency  $\cong 93\%$ .

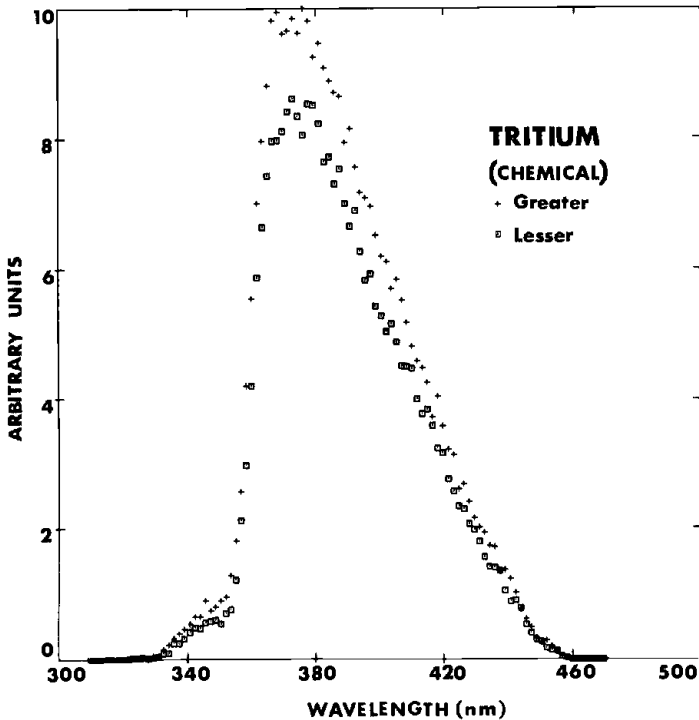


Fig. 9. The relationship between the photon spectra received by the greater and lesser photo-multipliers for chemically quenched tritium events (integral counting efficiency  $\cong 44\%$ ).

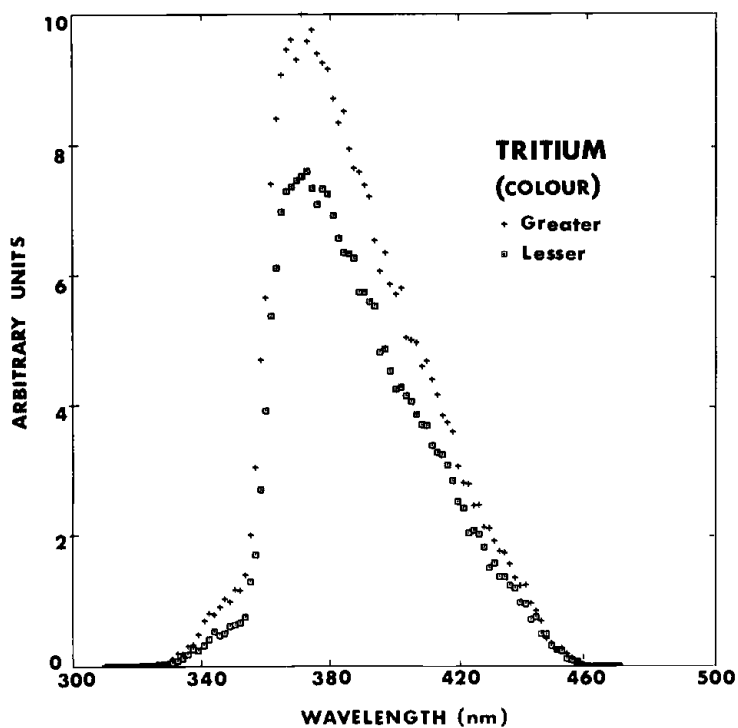


Fig. 10. The relationship between the photon spectra received by the greater and lesser photo-multipliers for colour-quenched tritium events (integral counting efficiency  $\cong 44\%$ ).

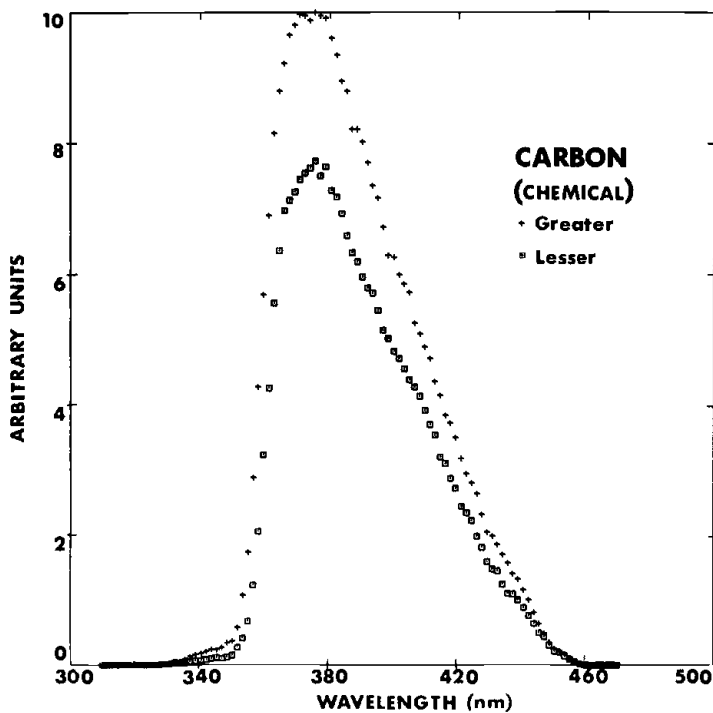


Fig. 11. The relationship between the photon spectra received at the greater and lesser photo-multipliers for chemically quenched  $^{14}\text{C}$  events (integral counting efficiency  $\cong 93\%$ ).

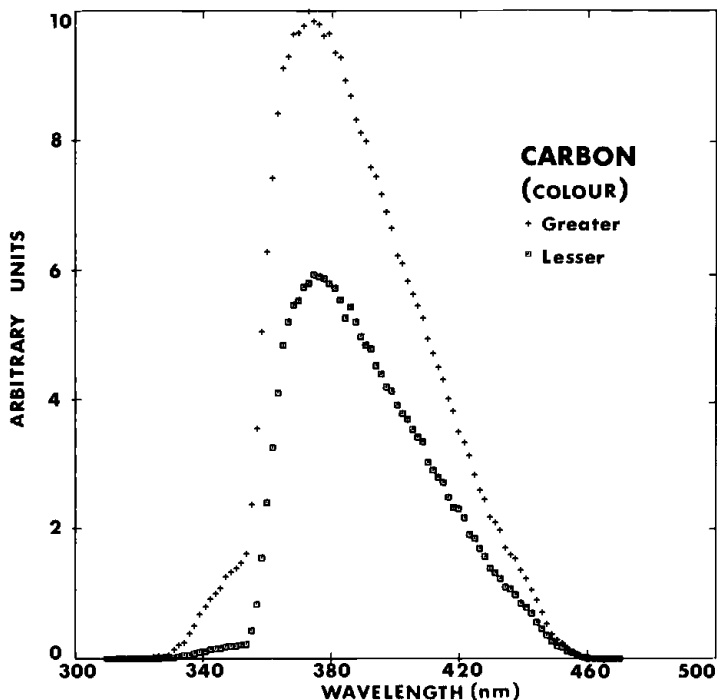


Fig. 12. The relationship between the photon spectra received at the greater and lesser photomultipliers for colour-quenched  $^{14}\text{C}$  events (integral counting efficiency  $\cong 93\%$ ).

curve is reached at about 380 nm. Again these results are for those events enabling coincidence and for the small number of photons leaving the vial in the case of  $^3\text{H}$  there is, as expected, only a small difference between the greater and lesser plots. For  $^{14}\text{C}$ , however, this difference is more marked since there are more photons involved. There is also, for the colour-quenched situation, a noticeable step in the 'greater' distribution below about 355 nm. Relative transmittance is less for wavelengths below this value for chemical since *relatively* more photons absorbed by PPO ( $\lambda < 355$  nm) travel a shorter distance to the photomultipliers. This step, again due to the small number of photons, is just discernable in the case of the colour-quenched  $^3\text{H}$  sample.

## CONCLUSION

In providing numerical data for the mathematical model, it has been necessary to examine carefully, and modify where necessary, the methodology applied in the laboratory even for a system as simple as the PPO-toluene one which has been simulated. The importance of purity of the scintillation chemicals was reaffirmed and time-dependent changes in the optical spectrum for colour-quenched systems have been noted. The effect of small changes in vial geometry has been previously reported<sup>14</sup> and this led us to use vials of the best uniform dimensions to provide optical uniformity from vial to vial. We also report the directional response within the detector chambers of two spectrometers. Perhaps, though, of most importance is the apparent validity of the model

in providing numerical data which is consistent with that found in the laboratory and as has been described in the previous chapter, how it has provided an insight into the complex dynamic interactions (such as total internal reflections) which occur within the system.

In addition, the model itself should be of considerable practical value in allowing workers to make better use of the liquid scintillation spectrometer as well as to assist both beginner and expert alike in their understanding of the LSC process. Furthermore, its use could provide an economical alternative to constructing and testing new designs. Finally, the model should not be overlooked as a tool for fundamental research into the liquid scintillation system; indeed, it has already led the authors to propose a spherical counting vial and detector<sup>9,10</sup> in which a tritium counting efficiency in excess of 80% might well be expected.

## ACKNOWLEDGEMENTS

Acknowledgements given in the previous chapter apply also to the present one. In addition, one of us (Philip E. Stanley) wishes to thank the Department of Agricultural Biochemistry, Waite Agricultural Research Institute, The University of Adelaide, for making available the Packard Model 3375 Tri-Carb system.

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## DISCUSSION

**J. B. Birks:** The fluorescence parameters (spectrum lifetime and quantum yield) of a scintillator solute molecule are independent of the wavelength of excitation. Hence the fluorescence spectrum of a molecule excited by self-absorption is identical with its normal emission spectrum. The fact that the energy of the emitted photon may exceed that of the absorbed photon does not conflict with the conservation of energy. The extra energy is acquired by the excited molecule as thermal energy from the solvent molecules. This process of thermal equilibration occurs in about  $10^{-12}$  s, which is much less than the solute excitation lifetime of about  $10^{-9}$  s.

**F. E. L. ten Haaf:** Concerning the difference between 'summed' and 'lesser' pulse height analysis, have you any quantitative figures on efficiencies of samples produced via the 'lesser' versus the normal method? The shape of the spectrum will influence channels ratio and other methods used to determine efficiencies by comparison to a standard series.

**P. E. Stanley:** This was not considered.

**J. B. Birks:** Concerning the validity of computer modelling of the quenched samples, is there any theoretical justification for lesser pulse height analysis? Its apparent purpose is to make impurity quenching and colour quenching look similar. Impurity quenching obeys a linear Stern-Volmer law, while colour quenching depends exponentially on the scintillator pathlength. The greater of the two coincident pulses experiences the lesser quenching and it would appear to be a more promising candidate than the pulse. The arbitrary discarding of over half the precious experimental data, as in lesser pulse height analysis, requires more justification than it has yet received. Perhaps Dr. Laney would care to comment?

**E. F. Polic:** How were the chemical colour standards chosen to be 'identical' for the spectral comparison?

**P. J. Malcolm:** The instrument was set to count integrally with the lower level discriminator set at the coincidence threshold. Counting efficiencies for both the  $^{14}\text{C}$  chemical-quenched and colour-quenched samples were set at 93% by careful addition of quenching agents.