

MEASUREMENT OF ^{125}I , ^{131}I and OTHER γ -EMITTING
NUCLIDES BY LIQUID SCINTILLATION COUNTING

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Other papers in this volume attest to the increasing interest in the biomedical sector in radioimmunoassays and radio-ligand assays of an ever-increasing list of polypeptides, glycopolypeptides, steroids, and pharmaceuticals. In a few procedures the substance of interest is labelled with ^3H but in most, iodination with high specific activity ^{125}I and occasionally ^{131}I , is carried out. It is still customary to count such samples in "gamma" counters where the scintillations (photofluorescence) emanate from a NaI crystal surrounding a shielded well.

We have recently summarized the evidence dating from 1956 that ^{125}I and ^{131}I can be counted at high efficiency and with little trouble by liquid scintillation counting. Calculation of quenchcorrection curves, of spill-over factors, and selection of optimum channels on any modern liquid scintillation counter are essentially the same as in the usual LS counting of $\beta\text{s}(1)$. This is not surprising since, as Birks has pointed out, the principal effect of γ rays is a yield of Compton electrons in a broad spectrum of 0 to 75 percent of the γ ray energy (2). If energetic enough, X-rays may also excite dissolved scintillators. The k-X-ray (E_{max} 27 keV) resulting from the decay of ^{125}I by electron capture thus contributes a second peak (see the Figures below) discernible from the low energy Compton peak of ^{125}I . We have made an effort to examine a number of γ emitters for their detection efficiency in counting solutions of toluene - 10% solubilizer - Biosolv BBS-3 with or without a standard primary scintillator PPO 7g/L (3).

Isotope	Principal Emission	E _{max}	Absolute Counting Efficiency	
	On Decay		With PPO	Without PPO
¹²⁵ I	γ	0.035	73	6.4
	k-X-ray	0.027		
⁵⁷ Co	γ	0.12	66	12.1
	γ	0.14		
⁸⁵ Sr	γ	0.51	68	14
¹³¹ I	γ	0.36		
	β	0.61	100	97

Table 1: Maximum efficiencies γ -emitting isotopes in a wide channel (0-1000) of a Beckman LS-150 liquid scintillation system.

Air equilibrated, in toluene - 10% BBS-3 with and without PPO 7g/L. Calibrated standards were generously provided by the Amersham-Searle Corporation.

Effects of Sample Geometry on Counting Efficiency

The results shown in Table 1 do not allow the systematic analysis of γ s in LS counting we had hoped for. Unfortunately these are the only γ -emitters for which we have been able to obtain accurately calibrated standards.

The results do suggest that with more energetic γ s, much of the radiation escapes the counting vial and therefore escapes detection. Addition of high electron-density materials to the scintillation solvent, eg. tetrabutyltin or tetrabutyl lead as suggested by Ashcroft (4) for counting ¹²⁵I (oddly enough, a procedure offering little advantage to ¹²⁵I counting efficiency) does not seem to be suitable for higher energy γ s, however (5). It is obvious that an analysis of γ counting by liquid scintillation, sufficiently systematic to indicate optimum conditions for counting any particular nuclide is still needed.

We have previously emphasized (6) the converse problem that the absorption of low energy β s emitted by samples counted on solid supports may result in erroneously low

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estimates of sample radioactivity. We have carried out several experiments designed to assess the magnitude of either problem in ^{125}I and ^{131}I counting. For the sake of comparison we made up samples (without the primary scintillator PPO) and counted them in a well (NaI crystal) counter in solution and on two solid supports.

Sample	Absolute Efficiency (%)	
	^{125}I	^{131}I
Aqueous solution	80	20
Toluene	70	22
Cellulose nitrate	78	19
Filter Paper (Whatman #1)	64	19

Table 2. γ counting of ^{125}I and ^{131}I : effect of sample geometry.

Duplicate samples of ^{125}I -insulin (11,100 DPM) and ^{131}I -NaI (45,600 DPM) were dissolved in toluene-solubilizer or 15 mm spots were air dried on 20 mm discs. Each sample was placed in a perspex tube 18 x 100 mm and counted in the well of a Nuclear Chicago Model 1085 scintillation detector with a 2 in. NaI (Li) crystal for 10 min. Absolute activity was determined by reference to a standard quench correction curve after counting the same samples on a wide (0-1000) Channel of a Beckman LS-150 liquid scintillation system.

Table 2 shows that in the type of standard "gamma-counter" the first problem: inadequate attenuation of γ s, is very significant: ^{131}I efficiency was only a fifth of that obtained in LS counting. This loss of γ s through penetration of the crystal detector is well known (7). The variation in efficiencies of ^{125}I detection could be attributed to the other problems: Compton electrons from ^{125}I were absorbed by the toluene-BBS-3 solution and by the solid supports, particularly the filter paper.

^{125}I L.S. Counter, Settings			
A. ^{125}I	LS-150 0-1000	ISOCAP $^{131}\text{I}/^{125}\text{I}^*$	LS-150 $(^3\text{H})/(^{14}\text{C})^*$
Toluene - 10% BBS-3	73	77	69
Cellulose Nitrate	62	65	57
Filter Paper (Whatman #1)	40	42	37
B. ^{131}I			
Toluene - 10% BBS-3 Solubilizer	100	81	63
Cellulose Nitrate	83	67	46
Filter Paper (Whatman #1)	85	68	48

Table 3. Efficiency of ^{125}I and ^{131}I in liquid scintillation counting.

Duplicate samples were dissolved in counting solution or dried onto discs as in Table 2. The discs were placed on the bottom of vials filled with toluene-PP0,7g/L. In the Beckman LS-150 system, ^{14}C and ^3H isosets were used to count ^{131}I and ^{125}I respectively. The efficiencies determined were quite similar to those with ^{14}C and ^3H programs on the Nuclear Chicago Mark II Liquid Scintillation System used to generate the spectra shown in figures 1 and 2. The efficiency figures for the Nuclear Chicago Isocap system were obtained from the appropriate fixed channel in the preset $^{131}\text{I}/^{125}\text{I}$ program. The higher efficiency of ^{125}I (than the LS-150) is attributable to greater efficiency of the phototubes in the Isocap; the lower efficiency of ^{131}I to a narrower channel.

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In liquid scintillation counting the two problems still exist, and are if anything more significant than in "γ" (NaI) scintillation counting, as Table 3 shows. The solid supports cause very significant decreases in counting efficiency which as Figure 1 and Table 4 show is due to absorption with ^{125}I and to both problems with ^{131}I :

	Channel		Ratio
	" ^{131}I "	" ^{125}I "	
Toluene - PPO 7g/L - 10% BBS Solubilizer	376,000	134,000	0.36
Cellulose Nitrate	314,000	113,000	0.36
Filter paper (Whatman #1)	313,000	132,000	0.42

Table 4. Effect of Sample Geometry on ^{131}I channels ratio. Samples of ^{131}I in solution or on discs (see Table 3 - legend) were counted in the fixed $^{131}\text{I}/^{125}\text{I}$ channels of a Nuclear Chicago ISOCAP system.

The loss of ^{131}I efficiency with samples dried onto cellulose nitrate filters was at least partly due to loss of energetic βs or γs from the vial before they could interact with the scintillator: PPO. The lack of spectral distortion in Figure 1 and the unchanged $^{131}\text{I}/^{125}\text{I}$ channel ratio (Table 4) is strong evidence. Suspending the disc in the vial rather than letting it lie flat on the bottom might well improve the situation. The greater absorptive properties of the filter paper (6) through attenuating the β and Compton electron spectra of ^{131}I may have actually increased the counting efficiency. The resultant pulse bright shift, although hard to discern in Fig.1 because of the broad spectrum of ^{131}I is evident in an increased $^{131}\text{I}/^{125}\text{I}$ channels ratio (Table 4).

With the lower energy Compton Spectra of ^{125}I , absorption of β energy by the solid supports is the predominant cause of decreased efficiency. In Figure 2, the resulting spectral distortions are evident as the peak from the 27 kev (E_{max}) k-X-rays becomes less defined as efficiency drops. We have not yet had the opportunity to determine whether the changes in channels ratios resulting from this pulse height shift fall into the same domain on a channels ratio-quench correction curve as does impurity quenching. Our prejudice, based on experience with absorption of ^{14}C β s by solid supports (6) is that the two relationships will be dissimilar. Monitoring and correcting for ^{125}I absorption in samples on solid supports promises then, to be fairly complex.

CONCLUSIONS

In " γ " counting (by scintillation of NaI crystals in response to γ rays), the measurement of γ emission by liquid scintillation is subject to decreased efficiency from inadequate attenuation of the radiation by the detector or from absorption of radiation by the sample. An inadequate yield of Compton electrons and of X-rays (from isotopes decaying by electron capture) is difficult to detect and will be significantly effected by the geometry of the sample in the counting vial. Samples on solid supports therefore may be measured with considerably less efficiency than identical samples in solution. It seems at least at the present time, when the problem has not yet been adequately explored, that the effects of absorption of Compton electron energy by solid supports is a complex phenomenon which will be difficult to estimate in any specific group of samples.

These findings obviously result in a dictum to those who employ liquid scintillation counting in measuring iodine isotopes or other γ emitting nuclides: it is of paramount importance that standard curves be determined with samples prepared in exactly the same manner as unknown samples are. Otherwise there may be sometimes unpredictable systematic errors in measurement of the substance of interest.

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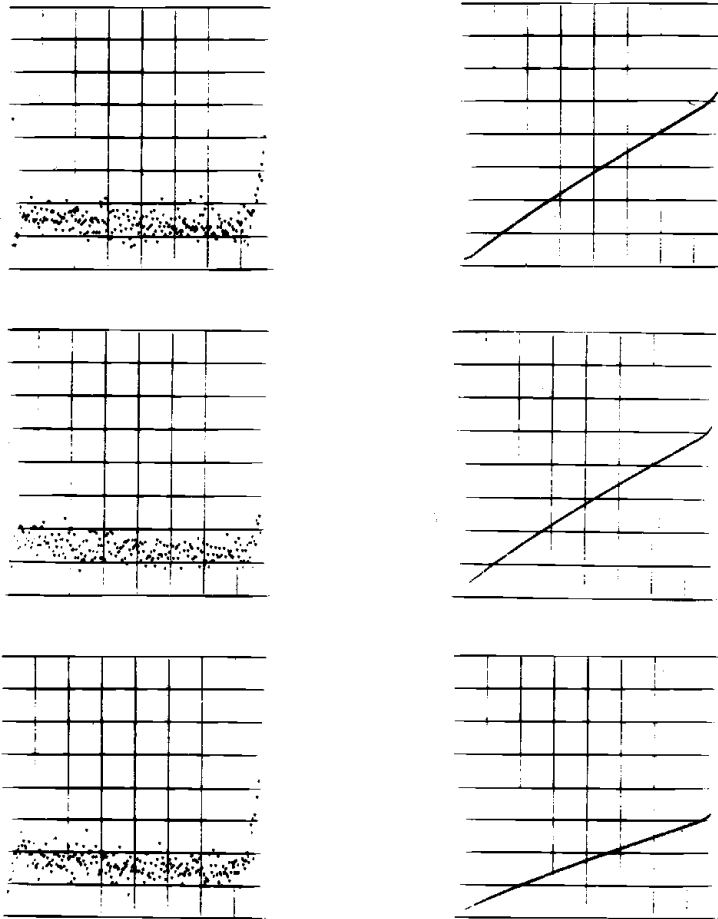


Figure 1. Spectra of ^{131}I : Effect of solid supports obtained with a Nuclear Chicago Mark II liquid scintillation counter interfaced to a Nuclear Chicago 4096 multichannel analyzer (See Ref. 3).

Raw data are displayed on the left; integrals of the raw data on the right. In descending order: 376,000 DPM ^{131}I -new/rare NaI in//toluene-PP0,7g/L-N5 solubilizer 10%: 512 counts/channel/1.5 min. full scale - dried onto a 20 mm cellulose nitrate filter disc placed on the bottom of a vial filled with toluene - PP0, 7g/L 512 counts/channel/2 min. full scale - dried onto a 20 mm. filter paper disc (Whatman #1) 512 counts/channel/1.5 min. full scale.

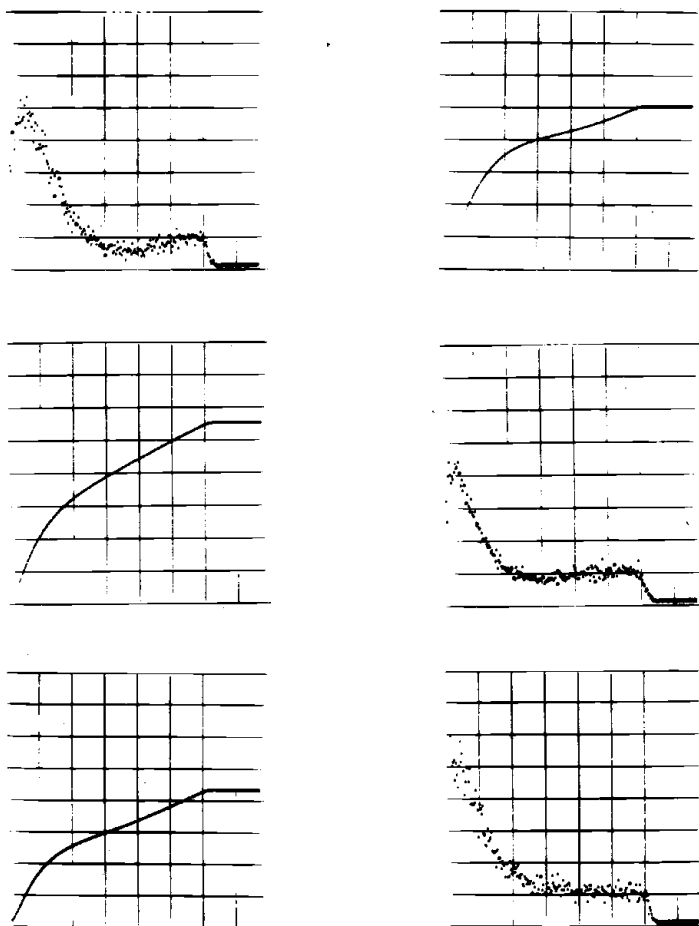


Figure 2. Spectra of ^{125}I : Effect of solid supports.

Raw data and integrals of the raw data. Samples of ^{125}I -insulin (362,000 DPM) were as described in the legend of Fig. 1: in descending order:

- toluene - PPO, 7g/L - 10% Biosolv-BBS-3
512 counts/channel/2 min. full scale
- on a 20 mm Cellulose nitrate disc in toluene
- PPO, 7g./L 512 counts/channel/2 min. full scale
- on a 20 mm filter paper (Whatman #1) filter paper
disc in toluene - PPO, 7g./L. 256 counts/channel/2min.
full scale.

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