

LIQUID SCINTILLATION COUNTING TECHNIQUE:
SOME UNUSUAL POSSIBILITIES

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ABSTRACT

Radioactive isotopes emitting either β^+ particles or γ rays of 1 MeV or X rays of about 30 keV can be measured either in pyrex tubes or in filmware bags placed in classical counting vials containing a liquid scintillator medium. For the tube technique, the counting efficiencies were 14% for ^{22}Na , 5% for ^{125}I and 2.5% for ^{64}Cu or ^{65}Zn . For the filmware bag technique, they were 22.5% for ^{22}Na , 12% for ^{125}I and 4% for ^{65}Zn . Both techniques enabled the measurement of 0.1 to 2 ml of aqueous solutions, and these solutions can be recovered, unchanged, for other purposes. On the other hand, mammalian cells can be grown in the glass tubes, and the incorporated radioactivity can be counted directly. In the case of small-sized radioactive molecules, diluted in a large volume of water (case of dialysates), it is possible to measure the radioactivity of 10 ml of such solutions, simply by adding 400 mg of PPO (2,5-diphenyl oxazole). After 1/2 hour shaking, the samples prepared in this manner remained stable for about 24 hours. The counting efficiencies were 25% for ^{22}Na , 22% for ^{64}Cu and 2.2% for ^{65}Zn and ^{125}I . The results obtained for each of these techniques were reproducible. Further, these techniques are inexpensive, rapid and easy to use.

INTRODUCTION

In the liquid scintillation counting technique, it is necessary to mix an aliquot of a radioactive solution with a liquid scintillator either directly or by means of a support. As most of the biological samples are aqueous solutions, and the scintillator solutions are organic, it is necessary to keep the aqueous solution less than 10% of the final mixture; the total volume must be less than 15 ml. This technique is very useful and works correctly most of the time. However, in some of the experiments, two opposite kinds of difficulties were

encountered when the radioactivity was:

1. diluted in large volumes, as is the case for dialysates (1.5 litres and even more, currently);
2. concentrated in very small volumes, in which many tests must be performed.

When the radioactive isotopes were β^+ , X or γ emitters, we were able to develop two new techniques using:

1. either glass tubes or filmware bags placed in counting vials containing 10 to 15 ml of liquid scintillators; filmware bags were already used for ^{32}P ;¹
2. or 400 mg of PPO compound added directly to 10 ml of aqueous solutions.

These techniques were widely used in our experiments with ^{64}Cu , and tested for ^{65}Zn , ^{125}I and ^{22}Na . They are inexpensive, rapid and easy to prepare. The results obtained were reproducible. These qualities are to be taken into account when these techniques are compared with the standard liquid scintillation counting where the counting efficiency is higher.

EXPERIMENTAL

1. Measurement apparatus: automatic spectrometer (Intertechnique -Kontron, France).
2. Liquid scintillator: Biofluor (New England Nuclear, Boston, Mass. USA).
3. Scintillator compound: PPO (Nuclear Enterprises, Edinburg, Scotland).
4. Pyrex tubes and filmware bags: the pyrex tubes were made in our laboratory; the external diameter was 15 mm and the length was 53 mm; their dimensions enabled them to be placed vertically in classical counting vials, and plugged. Filmware bags (Ets Poly Labo Block, France): the bottom was cut to half of its initial size and left for one half hour at 37°C to lose its rigidity. They were filled with the aliquote of radioactive solutions. Most of the air above the liquid surface was expelled, and they were sealed by heat in two different places to improve the security of the sample.

5. Radioactive isotopes: a) ^{64}Cu from Joliot Hospital in Orsay (France); b) ^{65}Zn , ^{22}Na , ^{125}I used in the form of iododeoxyuridine, ^{33}P and ^{55}Fe , from New England Nuclear (Boston, Mass. USA).
6. Rectilinear alternate shaker: maximum speed 280 shakes per minute (Apelex, France).
7. Procedures: the activity of the different solutions made from all the different radioactive isotopes was always determined using the classical liquid scintillation technique. At the same time, aliquots of each solution were measured using the techniques which were being investigated. One tenth to 2 ml of the radioactive solution were placed in either pyrex tubes or in filmware bags. The tubes were stopped with a plastic plug and the filmware bags were sealed as described above. They were placed in glass counting vials with 10 ml of biofluor for the glass tubes and 15 ml for the filmware bags. The surface of the aliquot had to be below that of the liquid scintillator medium. For the PPO technique, 10 ml of an aqueous radioactive solution were mixed with 400 mg of PPO, and shaken for 30 minutes. The 400 mg of PPO was measured with a previously calibrated glass spoon.

For each technique, and each radioactive isotope, the pulse height spectrum was measured to determine the best channel setting.

RESULTS AND DISCUSSION

All the results obtained and decay schemes used are summarized in the table 1. The techniques described were developed in order to overcome difficulties in our experiments with ^{64}Cu due to its short half-life (12.3 hours) and to the small amount available to us. Since the decay scheme of ^{64}Cu is complex we used ^{22}Na , ^{65}Zn , ^{33}P and ^{55}Fe to determine the origin of the different peaks obtained in the ^{64}Cu pulse height spectra.

Pyrex tube technique

Except for ^{22}Na , the efficiencies are low (table 1) and the pulse height spectra are complex (Figure 1). Peak n°3 which exists for ^{65}Zn

TABLE 1 - Characteristics of the different isotopes and the techniques tested

Isotopes	Decay	Particles emitted						Efficiencies of the different techniques			
		β^-	β^+	X	γ	e^- E.C.	Glass tubes	Filmware bags	PPO		
^{22}Na	β^+ and γ		90% 546 keV		8.5% 1.2 MeV				14% (+)	22.5% (+)	25%
^{64}Cu	β^+ , β^- and E.C.	40% 573 keV	19% 659 keV	17% 7 to 8 keV		24% 6.7 to 8 keV			2.7% (+)	11% (*)	22%
^{65}Zn	E.C. and γ		1.7% 330 keV	42% 8.6 to 9.6 keV	49% 1.1 MeV	56% 7.2 to 8.6 keV			2.5% (*) for peak $n^{\circ}2$ & 3	4% (*) for peak $n^{\circ}3$	2.4%
^{125}I	E.C.			46% 27 to 32 keV	6.5% 35 keV	46% 22.5 to 34 keV			5% (+)	12% (+)	2.2%
^{55}Fe	E.C.			27% 6 keV		73% 6 keV			0%	6% (+)	
^{33}P	β^-	100% 250 keV							0.2%	6% (+)	(53%)

(+) - The efficiency is constant from 0.1 to 2 ml but depends on the density of the solution.

(•) - The efficiency is constant from 0.1 to 2 ml but independent of the density of the solution.

(*) - The efficiency depends on the volume and the nature of the solutions.

Each value is the average of 3 independent experiments; many tests were performed for each of them. Values in brackets correspond to slow equilibrium.

and ^{22}Na , corresponds to high energy γ rays (1.1 and 1.2 MeV). Its intensity is correlated to the percentage of γ rays existing in the decay scheme (8.5% for ^{22}Na but 49% for ^{65}Zn). For ^{64}Cu peak n°2 corresponds essentially to the β^+ particles of 659 keV energy.

Experiments with ^{33}P (emitter of weak energy β^- particles (250 keV) have shown that the counting efficiency of such β^- particles is very low (table 1). Experiments with ^{55}Fe have shown that electrons and X rays of 6 keV cannot be measured using this technique. Therefore, peak n°1 cannot be correlated to these kinds of emissions and must correspond to Compton electrons and Cerenkov emission.

For ^{65}Zn , peaks n° 2 and 3 are independent of the volume (from 0.1 to 2 ml) and the density of the solutions measured.

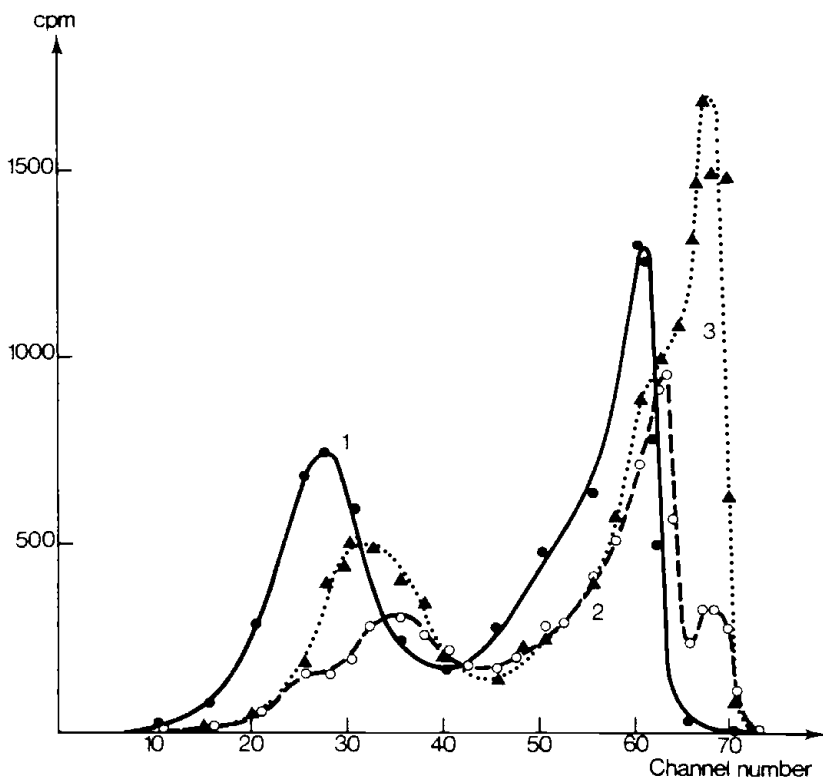


Figure 1. Pulse height spectra for the tube technique.

2 ml per tube of a solution containing 651,400 dpm of ^{64}Cu (—●—); 485,500 dpm of ^{65}Zn (....▲....); 125,000 dpm of ^{22}Na (—○—). In each case, the counted activity was 17,500 dpm.

For ^{125}I , the counting efficiency is 5% and the pulse height spectrum shows only one peak (Figure 2).

Filmware Bag Technique

In this technique, the efficiencies are higher for each radioisotope studied (table 1) and the intensity of each pulse height spectrum peak is also greater (Figure 2) than that observed in the tube technique (Figure 1).

Peak n°1 corresponds to the low energy of the X ray electron capture decay process, (case of ^{55}Fe , ^{65}Zn and ^{64}Cu). Peak n°2 corresponds to the β^- and β^+ particles from 250 keV to 659 keV (case of

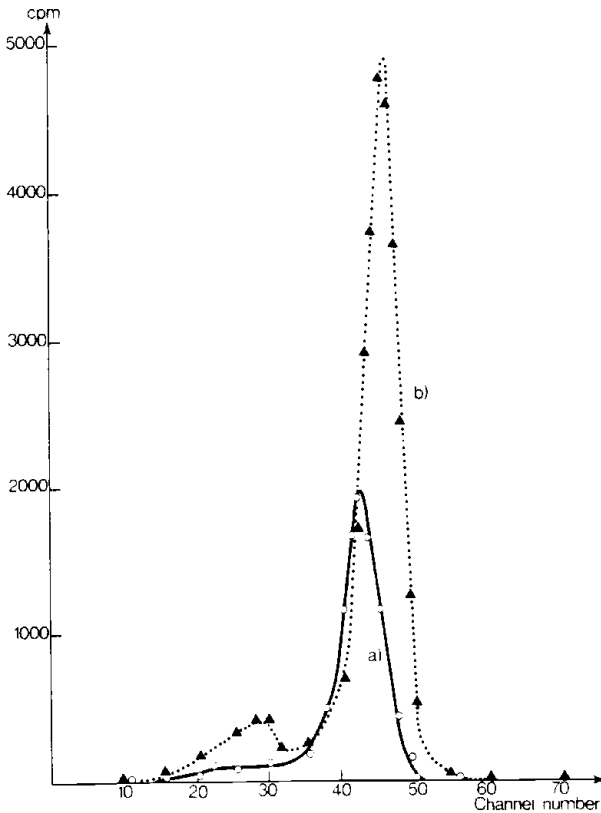


Figure 2. Pulse height spectra for ^{125}I odeoxyuridine.

The solution used had an activity of 97,500 dpm/ml. 2 ml were placed either in a pyrex tube (curve a, —○—) or in a filmware bag (curve b, ...▲...). The counted activity was 9,850 cpm for the tube and 23,300 cpm for the filmware bag.

^{33}P , ^{22}Na and ^{64}Cu). Peak n°3 corresponds to the γ particles of about 1 MeV (case of ^{22}Na and ^{65}Zn). The counting efficiency depends on the volume of the solution being tested as long as either β^- particles (case of ^{33}P and ^{64}Cu) or low energy X rays (case of ^{65}Zn and ^{64}Cu) are concerned, but independent of volume when either β^+ particles (case of ^{22}Na) or γ rays (case of ^{65}Zn , peak n°3) are concerned. Conversely, the density of the solution is important except where high energy γ particles are concerned (peak n°3 of ^{65}Zn).

For ^{125}I , the counting efficiency is 12% (table 1). The main peak of the pulse height spectrum (Figure 3) corresponds to its X rays.

Before using the filmware bags, we tried plastic tubes. We observed that, with such tubes, the counting efficiency is never constant, but increases with time as a proportion of the impregnation of the wall of these tubes with the liquid scintillation medium to which they are permeable. Conversely, the material of the filmware bags is impermeable to this media, so that the counting efficiencies remain stable with time.

PPO Technique

This technique is based on our previous results showing that measurements in non-homogenous phases are possible as long as the energy transfers between the ionizing particles emitted by the radioactive isotopes and the scintillator compounds are possible.² In this PPO technique, two parameters have to be taken into account: efficiency and the equilibrium speed. Among the different scintillator compounds tested, PPO gave the highest counting efficiency. Among all the radioactive isotopes tested, only two could be measured using this technique: ^{22}Na and ^{64}Cu (table 1). For both of them, counting efficiency was high enough and equilibrium was obtained after the shaking time described in Experimental § 7. The pulse height spectra were non specific (Figure 4). To understand the way in which this technique worked, we filtered the samples and measured the activity adsorbed on the PPO and that remaining in the water. We thus observed that this technique allowed only the counting of the molecules adsorbed on the PPO. In the case of small sized compounds labelled with ^{64}Cu and diluted in a large volume of dialysates, this technique works very well. The samples prepared in this manner remained stable for about 24 hours.

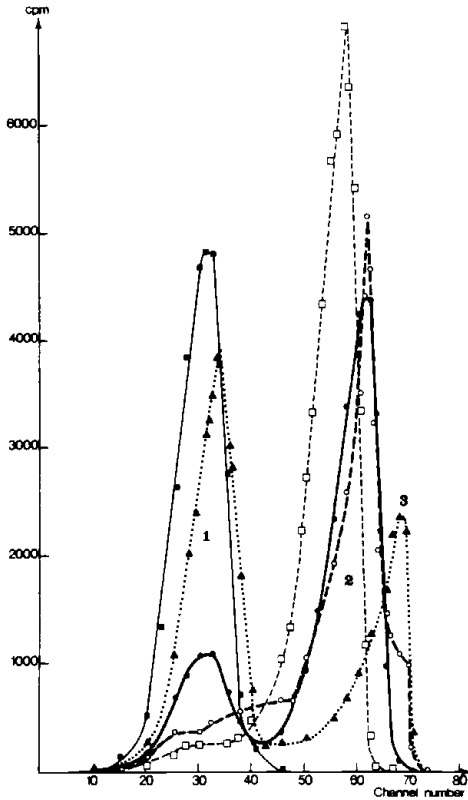


Figure 3. Pulse height spectra for the filmware technique.

1 ml per bag of a solution containing 419,000 dpm of ^{64}Cu (—●—); 485,500 dpm of ^{65}Zn (···▲···); 206,000 dpm of ^{22}Na (—○—); 772,500 dpm of ^{55}Fe (—■—); 713,000 dpm of ^{33}P (—□—). In each case, the counted activity was 46,300 cpm.

CONCLUSION

Beside the inexpensive, rapid and easy characteristics of the techniques described, the tube as well as the filmware bag techniques also have another advantage. They allowed the integral recovery of the counted solutions which can thus be used for many other purposes. Another advantage of the tube technique is that mammalian cells can be grown, washed and counted in the same tube and then used in their totality for different tests.

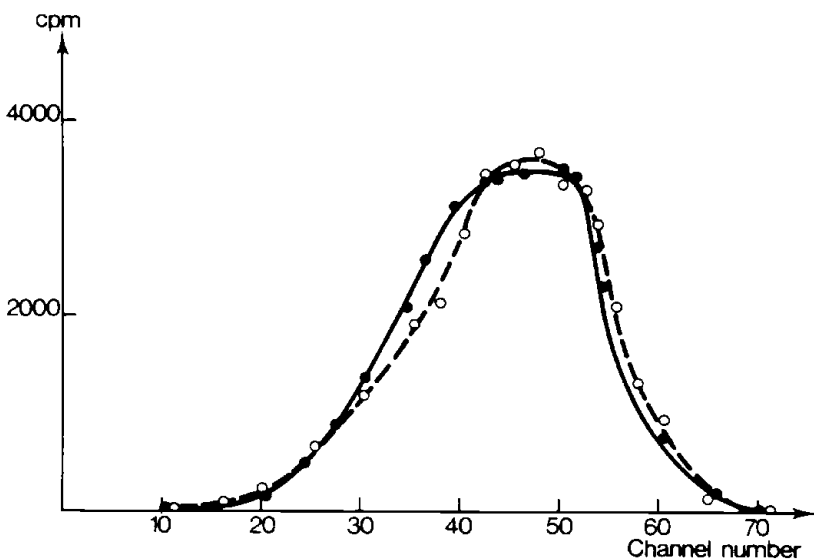


Figure 4. Pulse height spectra for the PPO technique

400 mg of PPO was mixed to 10 ml of an aqueous solution containing either 299,000 dpm of ^{64}Cu (—●—) or 263,000 dpm of ^{22}Na (---○---). The counted activity was 65,700 cpm in each case.

As for the PPO technique, it would probably be useful for liquids containing small-sized molecules, such as urine.

ACKNOWLEDGEMENT

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