

Measurement by Liquid Scintillator of Labelled Compounds (^3H or ^{14}C) Dropped onto Supports

Sonia Apelgot and Maurice Duquesne
Laboratoire Curie de la Fondation Curie - Institut du Radium
11 rue P. et M. Curie. 75231 Paris Cedex 05 (FRANCE)

Abstract

Radioactive compounds dropped onto supports are measured correctly, even if not extracted by liquid scintillator. Their dissolution is not required, it suffices simply to have a contact between the β particles and the liquid scintillator.

The difficulties encountered with paper support exist only in the case of ^3H (and not ^{14}C) and are a consequence of the paper structure itself. These difficulties disappear when the papers are counted wet (and not dried) in a liquid scintillator containing dioxane. Under these conditions, the measuring efficiencies are not very different from those obtained with glass fibres, or with a homogeneous phase; they no longer depend on the size of tritiated compound molecules.

Introduction

An unfavourable prejudice exists against measurement, by liquid scintillator, of labelled compounds dropped onto supports. The principal objection is that, under such conditions, the radioactive compounds dissolved in the liquid scintillator are counted with a greater efficiency than that of the insoluble compounds remaining on this support (1 and 2). But experiments have shown that these assertions are false.

The first supports used were of paper. Under these conditions, experiment shows that measurement efficiencies are, in fact, low when the radioelement is ^3H , and the radioactive compounds are insoluble in the liquid scintillator. From these results, the unfavourable conclusions regarding the general use of supports were perhaps drawn - and these - probably, through analogy to the example of solutions of labelled compounds which are non miscible in the liquid scintillator. However, the studies performed in our laboratory demonstrated that it is the paper structure, and it alone, which is responsible for the results obtained with these supports; the soluble or insoluble characteristic of tritiated compounds does not play any role (3 and 4). By

using glass fibre as a support, the measurement efficiencies are high, even with tritiated compounds insoluble in liquid scintillators.

Materials and Methods

1. Apparatus: Automatic Spectrometer (Intertechnique, France).
2. Liquid scintillators:
 - a) water - non-miscible : NE211 (Nuclear Enterprises, G.B.) or solution prepared at the laboratory, containing toluene (Baker Chemical D.V.), PPO 4 g/l and dimethyl POPOP 0.1 g/l.
 - b) water - miscible (with 10% water) : NE220 (Nuclear Enterprises, G.B.) containing dioxane.

We generally used either 1 or 3 ml of one or the other of these liquid scintillators.

3. Supports:
 - a) paper made with glass fibre (Whatman ref. GF/C or GF/A), called "glass fibre" in this work.
 - b) Whatman paper n° 1 (185 mg/mm² or n° 17 440 mg/mm²); these papers are made from cellulose.
4. Radioactive solutions:
 - a) standard solution of [³H] thymidine (NEN, USA) or of [¹⁴C] thymidine (CEA, France)
 - b) bacteria of a thymineless E. Coli strain, labelled with ³H; by using [³H] thymidine, we obtained bacteria labelled exclusively in their DNA.
 - c) [³H] DNA: prepared from the previously labelled bacteria, according to the technique described by MARMUR (5).

5. Measurement technique:

An aliquot of these radioactive solutions was dropped onto the selected support, which was measured:

- a) wet: as soon as prepared, the support was immersed in liquid scintillator NE220 containing dioxane. Under these conditions, the measured activity increased with time, to attain its equilibrium value in 3 to 6 hours.
- b) dry: the support was dried under an infra-red lamp, cooled and then immersed in a liquid scintillator without dioxane.

6. Amplitude spectra:

The number of photons produced by a β particle of energy E_0 , and consequently the number n of photoelectrons produced on the photocathode of a photomultiplier tube is, in proportion to the energy absorbed: $\bar{n} = KE$. This mean

value, \bar{n} , corresponds to a probability $p(n)$ of having n photoelectrons by absorption of a β particle. The pulse height spectrum at the anode of the photomultiplier tube reflects this distribution. Thus it can be seen that for each β disintegration there corresponds a value of \bar{n} and a mean amplitude $\bar{V}(n)$ of anode pulses. When $n = 1$, one observed the spectrum S1E corresponding to a single photoelectron; when there is a large number of β disintegrations the pulse height spectrum at the anode is the sum of the spectra S1E, S2E, ..., S n E; the mean value of the amplitudes \bar{V}_{exp} , of the experimental spectrum permits deducing of the mean value \bar{n} . The quenching phenomenon, and the poor transmission of photons caused by the paper's thickness, diminish the value of the constant K in the previous formula $\bar{n} = KE$, while the self-absorption phenomenon, in the paper, of the β particles, diminishes the value of E_0 . The net result of these modifications is to diminish the values of \bar{n} and is manifested in a reduction of the anode pulse amplitudes. Consequently, we observe an increase in the intensity of spectrum S1E, to the detriment of that of the intensity of the spectra S2E...S n E. The photomultipliers with porous dynodes (RCA8850) permit the distinguishing of spectra S1E, S2E and S3E, in an amplitude spectrum corresponding to the detection of ^3H (Fig. 1a). In such spectra, quenching and self-absorption manifest themselves by an increase in the ratio $\frac{\text{Intensity S1E}}{\text{Intensity S2E}}$; that is, the ratio $P(1)/P(2)$. The form of spectrum S1E, with a single photoelectron, allows improving of the detection efficiency of a liquid scintillation apparatus (6).

Results

An initial study was made, using solutions of tritiated compounds and paper supports of different nature and thickness. These solutions, dropped onto supports, were dried, then immersed in a liquid scintillator without dioxane. The measurements of the radioactivity show that the efficiencies (Table 1) are:

- high, in the case of bacteria or DNA, though these compounds are insoluble in the liquid scintillator;
- low in the case of thymidine; in this case, the paper thickness does not play any role.

Therefore, such results show that the degree (high or low) of measurement efficiency is independent of solubility or insolubility of the tritiated compounds, in the liquid scintillator. This efficiency depends neither on the nature of the paper used for support, nor on its thickness.

TABLE I.

[³H] Samples dropped onto supports (paper or glass fibre) and counted dry

Samples	Activity (dpm)	liquid scintillator	n°	Whatman paper		Glass fibre		E %	B/A
				counted activity (cpm) with support (B)	support removed	counted activity (cpm) with support (A)	support removed		
[³ H] T.D in H ₂ O	45'000	NE 211	1	2750	12	20'520	445	45,5	0,14
				2915 } 2865 }	15 15	20'320 } 20'555 }	490 690		
[³ H] Bacteria in physiological serum	73'500	NE 211	1	5695(*)	0	38'880(*)	185	44	0,14
				2847 } 2835 }	0 0	19'440 } 20'290 }	130		
[³ H] DNA in H ₂ O	25'890	with Toluene	1	25'445	0	34'620	120	45,5	0,79
				27'420 } 26'430 }	0 0	32'275 } 33'445 }	105		
[³ H] DNA in H ₂ O	25'890	with Toluene	1	7240	0	10'115	65	39	0,74
				7595 } 7415 }	0 0	9'975 } 10'045 }	65		

The [³H] thymidine ([³H] T.D) solution was a standard solution ; the activity of the [³H] bacteria suspension and the [³H] DNA solution was measured by the combustion technique. For each sample, we dropped 0.1 ml of labelled solution or suspension onto a support. They were dried and then immersed in 3 ml of liquid scintillator in the case of [³H] DNA, and in 1 ml in the case of the other radioactive solutions.

E represents the measurement efficiency.

(*) : for this sample, we immersed, in 3 ml of liquid scintillator, 2 papers, each containing 0.1 ml of the radioactive solution.

MEASUREMENT OF LABELLED COMPOUNDS

TABLE II

Counting of [^{14}C] thymidine on dry supports (glass fibre or paper)

Glass fibre		Whatman paper n° 1		
Counted activity (cpm)		Counted activity (cpm)		
with support (B)	support removed	with support (A)	support removed	B/A
40.065	1515	30.260	51	
40.180	1052	30.785	110	
39.795	3057	30.915	41	
average:		average		
40.015	→ E=95%	30.655	→ E=73%	0.77

The [^{14}C] thymidine solution was a standard solution; onto each support we dropped 0.05 ml, which corresponded to 42.120 dpm. Each support was dried and then immersed in 1 ml of a liquid scintillator without dioxane (NE211).

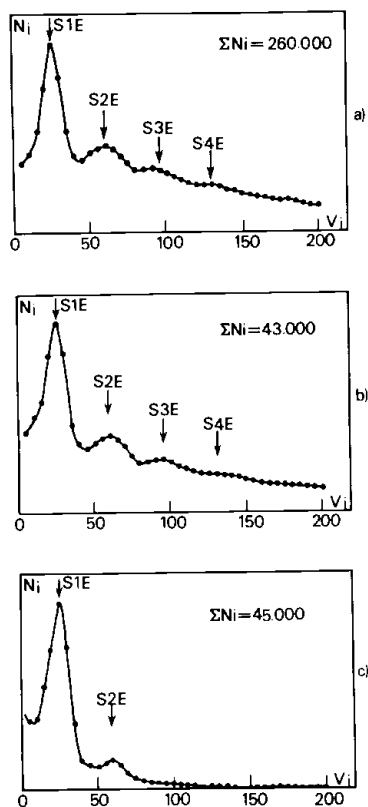


Figure 1: Anode pulse amplitude spectra Ni (V_i) (dry supports)

The same volume of the tritiated standard solution was dropped either onto a paper (spectrum b) or onto a glass fibre (spectra a and c). Each support was dried then immersed in 1 ml of NE211. In the case of the C spectrum, we added 7.5% (v/v) of chloroform, to the sample thus giving the a spectrum. The amplitudes are given in arbitrary units, with the intensities normalised on the S1E spectrum. ΣNi represents the total number of counted pulses.

Conversely, this degree of measurement efficiency depends on the size of tritiated compounds (thymidine or DNA). These phenomena do not exist, either with compounds labelled with ^{14}C (Table II) or with tritiated compounds dropped onto a glass fibre support (Table I). The β self-absorption in the paper, or the paper's action on photon transmission, depend, on the contrary, on the thickness of the paper used and are independent of the size of the tritiated compounds' size and of the nature of the radioactive isotope. None of these phenomena is thus able to explain the results obtained: they are specific to ^3H and paper.

In order to understand the role of the paper, we studied the pulse height spectra of different samples prepared from a same radioactive solution, using, as a support, either paper or glass fibre; the radioactivity of each sample had been previously counted. The pulse height spectra were measured with the special apparatus described in paragraph n° 6 above (Materials and Methods).

For samples of ^3H on paper or glass fibre supports, experiment shows that the pulse height spectra are only slightly modified (Fig. 1a and 1b). The spectral shift of anode pulses to lower amplitudes were previously observed by FURLONG (7). This displacement is expressed by a lowering in the detection efficiency of β particles. To calculate this, we compared, on our spectra, the probability ratios $P(1)/P(2)$, $P(1)/P(3)$, and $P(2)/P(3)$. This calculation provides a detection efficiency of 81%, when the support is glass fibre (Fig. 1a), and 72% when it is paper (Fig. 1b). This decrease of 15% is not at all compatible with that of the counting rate which, for the same samples, differed by a factor of 1/7 (Table III).

In order to interpret these results, we continued the experiments by adding a quantity of chloroform to the sample containing the ^3H dropped onto glass fibre; the volume of chloroform added was such that the drop in the counting rate was similar to the case of paper supports. In the presence of chloroform, the spectral curve (Fig. 1c) is very different from that obtained in the presence of paper (Fig. 1b); the curve shows a definite shift towards the lower amplitude of the SLE spectrum which, this time, accounts perfectly for the observed drop in the counting rate (Table III). It is known that chloroform decreases the detection efficiency by lowering the value of the constant K (p. 3).

Thus, in the presence of paper or of a quantity of chloroform, both of which provoke a similar decrease in the counting rate, the detection efficiencies are different: with chloroform, the counting rate diminishes like the β detection

efficiency while, with paper only the counting rate falls, the β detection efficiency itself being slightly modified (Fig. 1). It is thus a phenomenon of "all or nothing" which can be explained by assuming that a large number of [^3H] molecules are not "registered" by the liquid scintillator. This takes place as if 6 out of 7 β particles were "masked" by the paper, the 7th being correctly detected. For ^3H carried by large molecules, DNA, or ^3H incorporated in bacteria for example, about 75% of the β are detected (Table I and III).

It seemed to us that the difference between the results obtained using glass fibre and those obtained with paper, might be explained by the difference in structure of the supports.

With the aid of documentation generously provided to us by the "Ecole Française de Papeterie" at Grenoble, we were able to formulate the following hypothesis: the paper fibre behaves like a network of capillary tubes, permeable only to aqueous solutions; the glass fibres act like a network of impermeable threads, capable of retaining the diverse molecules only on their surface. In the capillary microstructure of the paper, aqueous solutions carry with them only solutes of small size, radioactive or not, large-sized solutes remaining on the surface. When the radioelement is ^3H , the labelled compounds of small size are not detected for two reasons: firstly, because the liquid scintillator cannot penetrate the capillary microstructure and is never in contact with these molecules; and secondly, because the β particles from the ^3H have a path length which is too short to reach the liquid scintillator. These difficulties do not occur with large-sized molecules remaining on the surface, and thus in contact with the liquid scintillator; nor do they occur with the ^{14}C , whose β particles has more energy (Table II).

This permeability of the capillary microstructure of paper only to aqueous solutions should permit, as experiments have confirmed, better counting efficiencies when this support is measured wet. Under such conditions, a liquid scintillator containing dioxane is necessary, and it was found that ^3H is measured with an acceptable efficiency - about 80% - of that obtained with a glass fibre support (Table IV); moreover, this efficiency is similar to that obtained in the case of bacteria or DNA (Table I). When these supports are dried and immersed in the liquid scintillator, it was observed that measurement efficiencies begin very low and increase with time. Under these conditions, the measured activity slowly approaches its equilibrium

MEASUREMENT OF LABELLED COMPOUNDS

TABLE III

Detection Efficiency of the β of ^3H

Supports	Activity measured (cpm)			R	
	M	I	I/Ir	%	R/R _o
- HCl ₃ glass fibre	254.000	260.000	Ir	81	R _o
+ HCl ₃	38.000	45.000	0.17	19	0.23
paper	36.000	43.000	0.16	72	0.89

0.1 ml of a [^3H] thymidine solution was dropped onto a paper or glass fibre disc. The discs were dried and then immersed in 1 ml of the liquid scintillator NE211. Each sample was first counted in the Intertechnique spectrometer apparatus, then in the apparatus having a photomultiplier with a porous dynode, which permits the registration of the anode pulse amplitude spectra. "M" represents the result of the measurement on the Intertechnique apparatus, and "I" the total number of the counted pulse serving to establish the amplitude spectra; "R" is the efficiency detection of the β calculated from the spectra, as explained in the text.

value (in 7 to 39 days), and in the case of the [^3H] thymidine used, the measured activity is seen in the liquid scintillator. This experiment demonstrates the slow diffusion of liquid scintillators, containing dioxane, in the interstices where water penetrates easily and where liquid scintillators without dioxane (such as NE211) have no access.

Discussion

The study of scintillation spectra demonstrated that paper does not significantly alter the scintillation phenomena (Fig. 1) and that it decreases the measurement efficiencies by only about 15% (Table III). Previous experiments have shown that the difficulties encountered with paper in measurements of ^3H activity were not related to its constituent - cellulose - but to its tubular structure, since these difficulties disappear with the use of electrophoretic membranes, whose only difference from paper lies in its cellulose structure (4). These previous results are confirmed by the present study, since we demonstrated that all these difficulties occur only when the measurements are carried out with dried paper. Paper has a multitude of microholes, and the results obtained can be explained if one assumes that these holes are permeated only by aqueous solutions. This paper specific structure explains that ^3H counting results are of the same kind, regardless of the nature, origin or even thickness of the paper (Table 1 and ref. 8). By measuring these paper supports wet, in a liquid scintillator containing dioxane, the measuring efficiencies have the same magnitude for all tritiated compounds, whatever their size, and they are very much the same as those obtained with glass fibres. Paper thus loses its "peculiarity" - meaning that the [^3H] β particles are detected independently of the tritiated compounds' size; the 20% decrease in the counting rate, which was observed, meshes with the 15% decrease in efficiency detection-deduced from analyses of the scintillation spectra. This paper reduction of the counting efficiency is the same as with ^3H or ^{14}C (Table 1, II and IV).

This study also demonstrated that the tritiated compounds dropped onto supports are, like the [^{14}C] compounds, correctly measured, even when not extracted by the liquid scintillator (Table 1 and II); their dissolution is not necessary and a contact only between the β of ^3H and the liquid scintillator is required. Each time it is possible to establish such a contact, the counting efficiencies are acceptable. Thus it is possible to measure ^3H or ^{14}C contained in bacteria or organs, without destroying them.

MEASUREMENT OF LABELLED COMPOUNDS

TABLE IV
Measurements in a liquid scintillator containing dioxane (NE 220)

Volume dropped (ml)	Measurements with wet supports			Measurements with dry supports								
	Glass fibres with support removed (a ₁)	Paper with support removed (b ₁)	b ₁ / a ₁	Glass fibres with support removed (a ₂)	with support removed t=0 (b ₂)	Paper with support equilibrium (b' ₂)	support removed	b ₂ / a ₂	b' ₂ / a ₂			
0.050	11.690	10.380	9.350	9.050	0.73	12.340	11.885	1.105	8.285 (+)	7.560	0.09	0.67
0.10	23.420	21.960	19.200	18.515	0.83	24.625	23.370	2.360	14.315 (+)	13.015	0.10	0.58
0.15	33.030	31.080	27.505	25.170	0.74	35.480	31.075	3.705	20.800 (*)	18.420	0.10	0.59
0.20	43.030	37.845	36.895	33.980	0.86	45.805	41.850	4.900	32.635 (*)	30.015	0.11	0.71

We utilized an aqueous solution of [³H] thymidine. 0.050 or 0.10 ml were dropped onto support, the other volumes (0.15 and 0.20) onto two supports. The paper utilized here was Whatman n° 17. The measurement of radioactivity made at t = 0 corresponds to that made in the hour following the immersion of the support in NE 220; the equilibrium corresponds to t = 7 days for measurements marked (+); t = 19 days marked (●) and t = 30 days marked (*). Activities are given in cpm.

The cellular membranes are permeated by the solvents (toluene, xylene) common to all liquid scintillators. Since the compounds inside the cells are not very concentrated, they only slightly modify the scintillation processes. Counting of isolated cells is accomplished with a high degree of efficiency, even in the case of ^3H (4). Conversely the compact structure of a biological tissue hinders the scintillation phenomena. However, it suffices to destroy this structure through the use of an enzyme - pronase - to take correct measurements again (8 and 9). As the liquid scintillators pass through the cellular membranes, they extract the cells' soluble compounds and, due to this, become modified. This is the classical "quenching", which is determined by the usual methods. According to the nature of the labelled compounds inside the organs' cells, the samples to be counted, contain radioactive compounds dissolved by the liquid scintillator, and others still "in situ", inside the cells. Experiments show that they are both counted with the same efficiency, that corresponding to the liquid scintillator being modified or not by the compounds, it will have dissolved (8 and 9).

This situation is different from that which exists in another kind of heterogeneous phase measurement, produced by 2 non-miscible liquids. In this case, the solutes are, in fact, shared between the 2 solvents, which form the two phases. The soluble solutes fraction in the liquid scintillator is counted with a greater efficiency than the insoluble fraction in the liquid which, therefore, remains dissolved in its original solvent. This solvent, which is non-miscible in the liquid scintillator, forms a screen between the latter and the radioactive molecules. Thus, a defective contact exists between the β particles and the liquid scintillators.

Conclusions

The compounds labelled with ^3H or ^{14}C and dropped onto supports, are correctly counted each time a contact is possible between the liquid scintillator and the β particles, even if the labelled compounds are insoluble in these scintillators. In the case of ^{14}C , such a contact exists, whatever the nature of the support is; in the case of ^3H , this contact is possible only if the support is of glass fibre, or if the support, when paper, is measured wet.

Acknowledgements

We wish to thank Professor J. Chiaverina (Ecole Française de Papeterie at Grenoble) for the documents and

information he kindly furnished to us, and the Societe Intertechnique, which measured for us some [³H] DNA, and [³H] bacteria samples, by combustion technique.

Bibliography

1. Y. Kobayashi and D.V. Maudsley in "Liquid Scintillation Counting: Recent Developments", p. 189-205 (P.E. Stanley and B.A. Scoggins, Eds.) Academic Press Inc., N.Y. (1974).
2. B.W. Fox, Symposium on "Liquid scintillation counting", Bath (Angleterre) (16 - 19 Sept. 1975) proceedings in press.
3. S. Apelgot and N. Rebeyrotte, Unpublished results.
4. S. Apelgot and M. Duquesne, J. Chim. Phys. 58, 774 (1961).
5. J. Marmur, J. Mol. Biol. 3, 208 (1961).
6. M. Duquesne, C.R. Acad. Sci. Paris, 267, 1347 (1968).
7. M.D. Furlong in "The Current Status of Liquid Scintillation counting", p. 201 (E.D. Bransome, Ed.) Grune and Stratton, New York and London (1970).
8. S. Apelgot, R. Chemama and M. Frilley, Bulletin du Cancer 60, 41 (1973).
9. S. Apelgot, R. Chemama and M. Frilley in "Liquid scintillation counting: Recent developments", p. 249-265, (P.E. Stanley and B.A. Scoggins, Eds.) Academic Press Inc., N.Y. and London (1974).

