Chapter 10

A New Gelifying Agent in Liquid Scintillation Counting

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Several techniques for preparing samples for liquid scintillation counting have been described in the literature. Solid samples of materials (i.e. $Ba^{14}CO_3$, powdered biological material, absorbant of thin-layer chromatography plates, inorganic salts, etc.) that are not soluble in liquid scintillation mixtures may be counted by suspending the material as a powder in the liquid scintillant.

Originally the counting sample was simply shaken and then counted, allowance being made for the settling that occurred. This technique was not valuable because the efficiency and reproductibility was not satisfactory.

More satisfactory results are obtained, however, when a gelifying agent is incorporated in the liquid scintillant to prevent settling. The material is homogeneously suspended in the vial containing the liquid scintillator.

Aluminium stearate has been used at a concentration of 5% in toluene or in xylene,[1] containing the fluors (PPO, POPOP). The aluminium stearate, however, has the disadvantage of making a hard gel, creating a difficult homogeneous distribution of the solid radioactive sample.

Another material, named 'Thixcin' (ricinoleic acid), has been used as a gelifying agent.[2] This material enables the preparation of a certain quantity of gelifying gel in advance, at a concentration of 25 g of 'Thixcin' in powder for 1 litre of scintillating liquid. The samples of the liquid scintillation prepared with this product have a good optical transmission. Nevertheless, its use as a gelifying agent has some disadvantages, in particular because it loses its gelifying properties at room temperature, allowing the suspended product to deposit.

Another product has been described in the literature and used, namely colloidal silica.[3] It has been used under the name of 'Cab-O-Sil'† at a concentration of 4% by

*Pavillon des Isotopes, 20 Bd. d'Yvoy, Geneva, Switzerland.

†Trademark of Cabot Corporation, USA.

weight in the toluene containing fluors. It is widely used for measuring the radioactivity of solid material suspended in gels. For quantities of 100 to 200 mg in 15 ml of scintillating solvent, it does not show any effect on light transmission and can bear quantities up to 1 g of suspended material. Nevertheless, 'Cab-O-Sil' has several disadvantages, like the difficulty in handling when weighing, this material being very bulky and very sensitive to electrostatic charges. Furthermore, at room temperature it is inclined to let the suspended material settle. On the other hand, in certain applications the silica, because of its great reactivity, absorbs polar radioactive material, thus diminishing the efficiency of the measure of the radioactivity by liquid scintillation.

Another gel scintillator has been described.[4] It is formed by adding toluene diisocyanate to a toluene cocktail containing branched aliphatic amines. The toluene diisocyanate used is Hylene TM–65 (Dupont) (mixture approximately 65% toluene 2,4-diisocyanate and 35% toluene 2,6-diisocyanate). Armeen L–11 (Armour Ind. Chem. Co.) was used as the source of branched aliphatic primary amine.

The gel scintillator described in this chapter consists of polyolefine resins which are the result of the polymerisation of ethylene, propylene, butylene and similar products as well as of the co-polymerisation of two or more of these olefines with low molecular weight.

This product is known under the name of 'Poly-Gel-B'.[5] It is in the form of fine opalescent white pellets. Poly-Gel-B pellets are soluble in solvents such as toluene, xylene, etc. when the solvent is heated at a temperature of 60 to 70°C. After cooling at room temperature a homogeneous gel is formed. This product already contains primary and secondary fluors like PPO/POPOP.

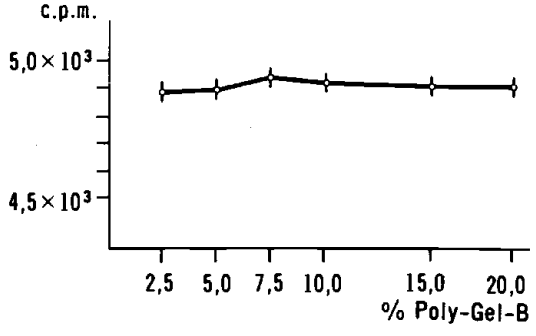

Fig. 1: Concentration effect of Poly-Gel-B in counting efficiency of 50 mg $Ba^{14}CO_3$.

It is currently used at a rate of 10% in the solvent. Figure 1 shows that the concentration has no effect on the counting efficiency of $Ba^{14}CO_3$. The concentration depends on the material to be suspended. Our experiment, however, showed that this 10% quantity is sufficient for $Ba^{14}CO_3$ and for powder of biological material. As shown in this figure, an increase of Poly-Gel-B does not decrease the counting efficiency. If it is necessary to suspend high density material the concentration can be increased.

For one litre of toluene, 100 g of pellets of Poly-Gel-B should be used. The solvent is heated in a water bath to a temperature of 60 to 70°C for a few minutes until the gelifying agent is completely dissolved. The quantity of solvent containing the gelifying agent is

placed hot in the scintillation vial already containing the material to be suspended. One can also add pellets of Poly-Gel-B in amounts of 10% or more directly in the scintillation vial already containing the toluene. The vial, even if capped, is then heated for about five minutes until the gelifying agent completely dissolves. The gel is formed when the solution is brought back to room temperature. In case a quick formation of gel is required, the scintillation vial can be cooled in ice. The gel can be put back into solution by simply heating the vials.

Several radioactive isotopes in suspension can be counted under the form of a gel, i.e. carbon-14 in the form of $Ba^{14}CO_3$ obtained after combustion of biological material, sodium carbonate after precipitation, carbon-14 contained in the powder of biological material, plant material, absorbant of thin-layer chromatography plates like cellulose powder, alumina powder, etc., tritiated compounds as contained in organic material or on thin-layer chromatography absorbant.

Other isotopes can be counted in suspension, i.e. iron-55 or iron-59 in the form of insoluble white ferri-phosphate complex or benzene phosphinate complex, calcium-45, strontium-90 in the form of sulphate, caesium-137 as perchlorate, iodine-131 in the form of silver iodide.

We have compared the counting efficiency of $Ba^{14}CO_3$ using Cab-O-Sil and Poly-Gel-B. Figure 2 shows that Poly-Gel-B is as efficient as the Cab-O-Sil. It even slightly increases the counting efficiency.

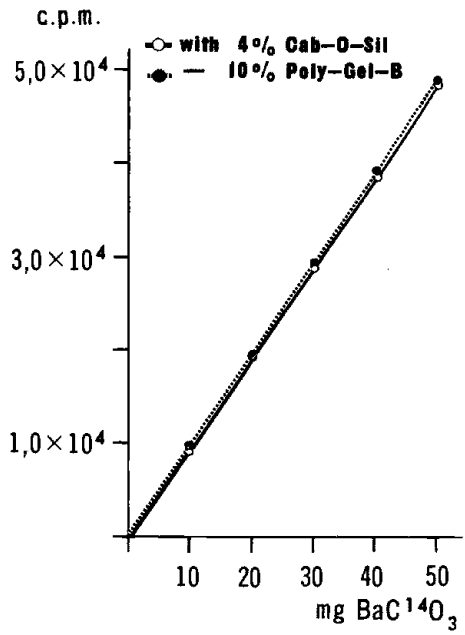

Fig. 2: Comparison of results obtained by suspension scintillation counting of $Ba^{14}CO_3$ with Cab-O-Sil and Poly-Gel-B.

Figure 3 shows a comparison of scintillation counting of rat liver powder in toluene and in suspension in Poly-Gel-B. For quantities over 20 mg, one can see a notable increase of the counting ratio.

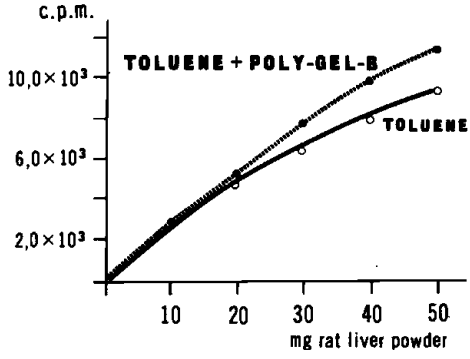

Fig. 3: Comparison of results obtained by scintillation counting of biological material (rat liver powder) in toluene and in suspension with Poly-Gel-B.

The samples thus prepared have shown a perfect suspension without any settling, even after several months at room temperature. This stability at room temperature is important as several liquid scintillation counting spectrometers work at room temperature.

Poly-Gel-B does not show any effect of light transmission and has no quenching effect. The addition of Cab-O-Sil and Poly-Gel-B does not modify the spectra of toluene carbon-14 measured in a Beckman Spectrometer LS–200B in the window-range of 0 to 400 (Fig. 4).

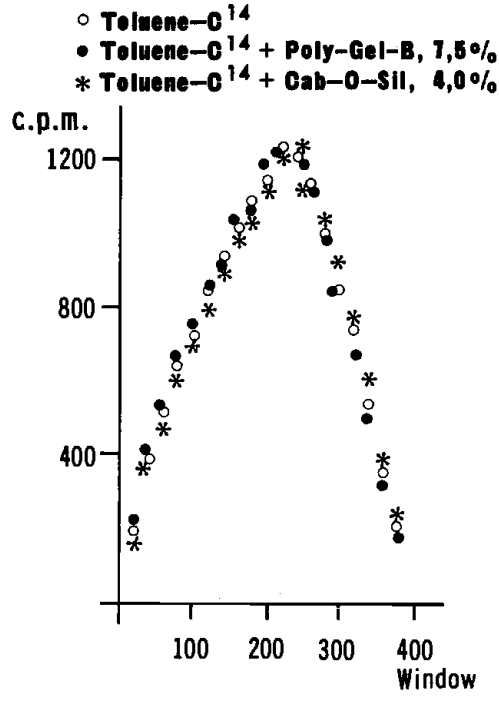

Fig. 4: Effect of Cab-O-Sil and Poly-Gel-B in carbon-14 spectra of homogeneous internal standard of toluene-^{14}C.

Fluors other than PPO/POPOP can be used, i.e. butyl-PBD* associated with the POPOP. As indicated in Fig. 5, there is a slight increase of the counting efficiency of the $Ba^{14}CO_3$ when fluor butyl-PBD/POPOP is used. In contrast, the butyl-PBD alone does not give such good results.

$BaC^{14}O_3$		PPO/POPOP		Butyl-PBD		Butyl-PBD/POPOP	
mg	dpm	cpm	Eff.%	cpm	Eff.%	cpm	Eff.%
10	10 500	9 394	89,4	8 859	84,3	9 591	91,3
20	21 000	18 847	89,7	18 839	89,7	18 924	90,1
30	31 500	28 077	89,1	28 275	89,7	27 680	87,8
40	42 000	37 561	89,4	37 430	89,1	37 022	88,1
50	52 500	47 323	90,1	47 982	91,3	46 835	89,2

Standard material: $BaC^{14}O_3$ ($1{,}05 \times 10^3$ dpm/mg)

Scintillation cocktail:
– toluene, PPO/POPOP (4/0,05 g/l), 10% Poly-Gel-B
– toluene, butyl-PBD/POPOP: (7/005 g/l), 10% Poly-Gel-B

Fig. 5: Counting efficiency of different amounts of $Ba^{14}CO_3$ in a Poly-Gel-B gel scintillator with various fluors.

The *f* value described as the ratio of suspension-counting efficiency to homogeneous internal-standard counting efficiency is determined by using various quantities of $Ba^{14}CO_3$. Figure 6 compares the influence of Cab-O-Sil and Poly-Gel-B on this *f* value. In fact, the *f* value of the $Ba^{14}CO_3$ is better with Poly-Gel-B than with Cab-O-Sil.

	f VALUES		
$BaC^{14}O_3$ mg	CAB-O-SIL PPO/POPOP	POLY-GEL-B PPO/POPOP	POLY-GEL-B Butyl-PBD/POPOP
100	0,95	0,99	0,98
200	0,94	0,97	0,98
300	0,93	0,96	0,94
400	0,91	0,95	0,94
500	0,90	0,95	0,91

***f* : ratio of suspension-counting efficiency to homogeneous internal-standard counting efficiency.**

Standard material: $BaC^{14}O_3$, (105 dpm/mg)
Scintillation cocktail:
— toluene, PPO/POPOP (4/0,05 g/l), 4 % Cab-O-Sil
— toluene, PPO/POPOP (4/0,05 g/l), 7,5 % Poly-Gel-B
— toluene, butyl-PBD/POPOP (7/0,05 g/l), 7,5 % Poly-Gel-B

Fig. 6: Suspension *f* values for different amounts of $Ba^{14}CO_3$ in Cab-O-Sil and Poly-Gel-B.

*2(4'-*t*-butylphenyl)-5-(4''-biphenyl) -1, 3, 4-oxadiazole

Biological material can also be counted in suspension and absolute values can be determined if calibration curves have been prepared in advance. Figure 7 shows that the counting efficiency depends on the volume of scintillation liquid and on the amount of biological powder. When rat liver powder is counted, one can obtain a relative efficiency of about 44% in comparison after determination with oxygen flask combustion. This figure shows also that the quenching calibration factor S is better in the case of measurements of 50 mg in 20 ml of liquid scintillation solution.

Rat liver powder	liquid scintillation solution: 15 ml		liquid scintillation solution: 20 ml	
mg	S	Relat. Eff. %	S	Relat. Eff. %
50	0,542	43,5	0,711	44,5
50	0,599	43,3	0,732	43,9
100	0,388	37,7	0,479	37,1
100	0,336	35,6	0,497	37,3

Scintillation cocktail:
– toluene, PPO/POPOP(4/0,05 g/l),10% Poly-Gel-B
– toluene, butyl-PBD/POPOP(7/0,05 g/l),10% Poly-Gel-B
S: quench calibration factor

Powder of rat liver containing C^{14}:
– Radioactivity of 100 mg: 86900 cpm measured after oxygen flask combustion.

Fig. 7: Counting efficiency of biological material in suspension counting with Poly-Gel-B in various experimental conditions.

The gelifying agent Poly-Gel-B must not be used with cocktails of the Bray type containing dioxane. It can, however, be used with toluene containing 10% ethanol. In the case of measurements of radioactivity of biological material like urine, plasma, etc., another type of gelifying agent can be used, Poly-Gel-B13, which allows the formation of an emulsion.

In conclusion, the use of Poly-Gel-B in liquid scintillation counting as a gelifying agent, presents the following advantages:

(a) The gels obtained are suspended without any settling even at room temperature.
(b) Numerous materials can be gelified provided calibration curves are prepared and absolute measurements can be made.
(c) It allows counting of material of various densities.
(d) These gels prevent some labelled compounds such as lipids from being absorbed on the glass surface of the scintillation vial, thus increasing the counting efficiency.
(e) This gelifying agent does not cause a quenching effect and decline of counting efficiency of ^{14}C ^{3}H labelled substances.
(f) This material is easily handled. It is of low cost and its properties should make the switch from other currently used gel scintillators to Poly-Gel-B easy and advantageous.

ACKNOWLEDGEMENT

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4 J. N. Bollinger, W. A. Mallow, J. W. Register Jr. and D. E. Johnson, *Anal. Chem.* 39, 1508 (1967).
5 A. Benakis, French Patent No. 1,590,762. Other patents, including US, G.B., Japan, Germany and Canada, pending.

DISCUSSION

D. A. Kalbhen: (i) In my own experiments with your Gel I never got counting efficiency above 8% with coloured biological materials, such as red blood cells or liver. How can you get 45% efficiency with liver powder? (ii) What is the advantage of Poly-Gel-B for samples after oxygen combustion?

A. Benakis: (i) I expect that you will not obtain better results if you suspend your red blood cells in Cab-O-Sil! Your results show well that the labelled compound is absorbed by the red blood cells and, under such conditions, a combustion would be preferable. (ii) It still happens in literature references, that people make combustions and after it obtain barium carbonate ^{14}C. In such cases Poly-Gel-B can be really useful in the barium carbonate counting techniques.

A. R. Ware: Regarding some results given by the author using $Ba^{14}CO_3$ standard in which he claims 90% efficiency and extremely good reproducibility, I would like to hear the author's comments on the high efficiency and, more particularly, his views on the effects of self-absorption, etc., caused by variation in the particle size of the suspended material.

A. Benakis: The barium carbonate we have been using was supplied by New England Nuclear Corporation, Boston. It was prepared for instrument calibration. Its specific activity was 1.05×10^3 d.p.m./mg. The high efficiency may be attributed to the physical structure of barium carbonate. In fact, it is known that the barium carbonate counting efficiency in liquid scintillation counting depends on how the product was precipitated. The way barium carbonate is precipitated influences its crystallisation. On the other hand, the barium carbonate difference in counting efficiency was brought to our attention by persons who had been using the suspension technique.
Our results are based on the radioactivity values given by New England Nuclear Corporation. We believe that these values are exact, but we did not have the possibility of controlling them by another technique. I am of the opinion that the barium carbonate technique may be used, but the precipitation conditions should be rigorously established and it is preferable to check the radioactivity of this material with another technique, like by formation of CO_2 and counting in a proportional counter.

B. W. Fox: Comment: I am surprised that the levels of efficiency quoted for this system are so high. I would expect an efficiency of about 40% due to self-absorption factors. Question: Do you have any experience of measuring tissue homogenates in this system?

A. Benakis: I answered Dr. Ware as far as the high efficiency level is concerned. Of course, the efficiency depends on the quantity of barium carbonate suspended. This is

controlled by self-absorption. Self-absorption depends on the quantity and also on the crystalline structure of the barium carbonate.

As to your question on tissue homogenates, we have experience with organic powders, as indicated in the report, but not with tissue homogenates, because Poly-Gel-B is not compatible with aqueous solutions.

Another gelifying agent, Poly-Gel-B13, is used to form emulsions. We are presently perfecting another product: Poly-Gel-H, specially for use with cocktails of the Bray type, containing dioxane. Nevertheless, considering the pigmentation of the tissue homogenates, it is not recommended to measure them without first discolouring them.