

Comparison of the efficacy of biodegradable and non-biodegradable scintillation liquids on the counting of tritium- and [^{14}C]-labeled compounds

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Abstract

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The widespread use of ^3H and ^{14}C in research has generated a large volume of waste mixed with scintillation liquid, requiring an effective control and appropriate storage of liquid radioactive waste. In the present study, we compared the efficacy of three commercially available scintillation liquids, Optiphase HiSafe 3, Ultima-Gold™ AB (biodegradable) and Insta-Gel-XF (non-biodegradable), in terms of [^{14}C]-glucose and [^3H]-thymidine counting efficiency. We also analyzed the effect of the relative amount of water (1.6 to 50%), radioisotope concentration (0.1 to 100 nCi/ml), pH (2 to 10) and color of the solutions (samples containing 0.1 to 1.0 mg/ml of Trypan blue) on the counting efficiency in the presence of these scintillation liquids. There were few significant differences in the efficiency of ^{14}C and ^3H counting obtained with biodegradable or non-biodegradable scintillation liquids. However, there was an 83 and 94% reduction in the efficiency of ^{14}C and ^3H counting, respectively, in samples colored with 1 mg/ml Trypan blue, but not with 0.1 mg/ml, independent of the scintillation liquid used. Considering the low cost of biodegradable scintillation cocktails and their efficacy, these results show that traditional hazardous scintillation fluids may be replaced with the new safe biodegradable fluids without impairment of ^3H and ^{14}C counting efficiency. The use of biodegradable scintillation cocktails minimizes both human and environmental exposure to hazardous solvents. In addition, some biodegradable scintillation liquids can be 40% less expensive than the traditional hazardous cocktails.

Key words

- Biodegradable chemicals
 - Scintillation liquids
 - Liquid waste
 - Color quenching
 - Scintillation counting efficiency
 - Tritium and ^{14}C counting
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Introduction

Radioisotopes commonly used in biomedical research possess low energy and a short range of air or fluid penetration. Therefore, they require direct contact with the scintillation medium and special technology

for efficient indirect detection of radioactivity (1). Among these radioisotopes, tritium (^3H) and radioactive carbon (^{14}C) have many applications in cell biology, pharmacology and clinical research. The widespread use of these isotopes in research requires effective control of the liquid, solid and biological

radioactive waste that results from techniques such as radioimmunoassay or radioligand binding assays. Most of these experiments generate a large volume of ^3H and ^{14}C waste in scintillation liquid.

For an ideal liquid scintillation detector, the amount of light generated must be proportional to the energy transferred and the detection should be perfectly linear. However, an energy transfer loss frequently occurs as a result of absorption of light by solid materials, chromogenic interposition, solution turbidity or pH changes in the scintillation fluid (2,3).

The process of radioactive detection involves scintillation fluids composed of aromatic solvents that increase the efficiency of energy transfer to the organic fluorine compound, improving the detection of light emission. Table 1 lists commercially available scintillation cocktails and their characteristics based on technical charts provided by the manufacturers. The primary and most extensively used scintillation fluid contains 2,5-diphenyloxazole, and is known as PPO. In fact, secondary compounds such as 1,4-bis(5-phenyl-2-oxazolyl)-benzene are also included in the scintillation fluid in order to

absorb the light emitted by the PPO, properly distribute the light and enhance the detection process (4). Toluene, xylene and dioxane are also widely used for this purpose (5). These inflammable and extremely toxic solvents represent a risk to laboratory activities, which is aggravated by the inappropriate storage of large amounts of hazardous liquids in the work place, since these liquids cannot simply be dumped into the public sewage system and their incineration is too costly (5). Finally, the liquid associated with radioisotopes is also considered radioactive waste that must be processed according to radioprotection laws (6).

The Radiological Protection Unit of UNIFESP/Sociedade Paulista para o Desenvolvimento da Medicina/Hospital São Paulo (UNIFESP/SPDM/HSP) recommends standardized procedures that fulfill current legislation and minimize safety problems in research laboratories (available at <http://protecoradiologica.unifesp.br>). Scintillation fluids with special formulations are now commercially available and have been considered safe because of their reduced aromatic content and biodegradability. However, the traditional use of well-established non-bio-

Table 1. Chemical characteristics and properties of scintillation liquids.

	Insta-Gel-XF	Ultima-Gold™ AB	Optiphase HiSafe 3
Biodegradability	No	70% biodegradable after 28 days	80% biodegradable after 28 days
Substance family	1,2,4-Trimethylbenzene mixed, liquids and detergents	Diisopropylnaphthalene mixed, liquids and detergents	Diisopropylnaphthalene mixed, liquids and detergents
Hazards identification	Flammable, harmful by inhalation, irritating to eyes, skin and respiratory system	Flammable, irritating to skin and eyes	Irritating to skin, eyes, and respiratory system
Mutagenic	No	No	No
Carcinogenic	No	No	No
Teratogenic	No	No	No
Recommended discard	Incineration	Discard according to national legislation	Discard according to national legislation
Price for 10 liters	US\$ 380.00	US\$ 383.00	US\$ 226.00

Data obtained from the manufacturer's Material Safety Data Sheet. Prices obtained from March to April, 2003.

degradable scintillation liquids and the resistance to change in laboratory routines hamper the acceptance of biodegradable scintillation liquids as solution for some of the safety problems and for the costly incineration of toxic laboratory wastes.

Therefore, we assessed the efficiency of biodegradable aqueous scintillation liquids and compared them to the liquids conventionally used in our laboratories for which the only disposal option is incineration. The aim of the present study was to determine, on the basis of counting efficiency, appropriate conditions for the use of biodegradable liquids. In addition, we propose to bring to the attention of the scientific community the advantages of biodegradable scintillation liquids over non-biodegradable liquids, because there is guaranteed adequate disposal of biodegradable radioactive waste according to Comissão Nacional de Energia Nuclear (CNEN) rules for radioprotection and environmental conservation.

Material and Method

The influence of the relative amount of aqueous solution, radioisotope concentration, pH and solution color on the detection of radioactivity emitted by [³H]-thymidine or [¹⁴C]-glucose was determined for several commercially available scintillation fluids: Insta-Gel-XF, Ultima-Gold™ AB (Packard Biosciences B.V. Chemical Operations, Ulgersmaweg, Groningen, Netherlands) and Optiphase HiSafe 3 (Perkin Elmer Life Science/Wallac Oy, Mustionkatu, Turku, Finland). All experiments were carried out in quadruplicate. The results are reported as mean ± SEM radioactivity measured as disintegration per minute. Statistical differences among groups were determined by analysis of variance (ANOVA) followed by the Newman-Keuls test, with the level of significance set at $P < 0.05$. When necessary, a linear regression analysis was performed.

Effect of the relative amount of water on counting efficiency

In order to analyze the influence of the relative amount of water solution on counting efficiency, 3700 Bq (100 nCi) [¹⁴C]-glucose or 0.037 MBq (1.0 μCi) [³H]-thymidine was diluted in a final volume of 6 ml containing 0.1 to 3 ml of water and the various scintillation liquids. The aqueous solutions corresponded to 1.6, 8.3, 16.7, 25, 33.3 and 50% of the final volume. The vials were vortexed and the radioactivity was determined with a scintillation counter.

Effect of the concentration of radiolabeled compound on counting efficiency

For the determination of radioactivity as a function of radioisotope concentration, 0.1, 1, 10, and 100 nCi [³H]-thymidine or [¹⁴C]-glucose diluted in 1 ml of water were added to vials containing 5 ml Insta-Gel-XF, Optiphase HiSafe 3 or Ultima-Gold™ AB scintillation fluids. The vials were vortexed and radioactivity was determined with a scintillation counter.

Effect of solution pH on counting efficiency

In this set of experiments, the influence of pH was determined by diluting 100 nCi [³H]-thymidine or [¹⁴C]-glucose in 1 ml of phosphate-buffered saline, pH 7.0, glycine-HCl buffer, pH 2.0, or borate buffer, pH 10.0. Subsequently, 5 ml of the scintillation liquids Insta-Gel-XF, Optiphase HiSafe 3 and Ultima-Gold™ AB were added, vials were vortexed and radioactivity was determined.

Effect of sample color on counting efficiency

The assays were carried out using 100 nCi [³H]-thymidine or [¹⁴C]-glucose diluted in 1 ml of water in the presence or absence of 0.1 or 1 mg/ml Trypan blue. Next, 5 ml of the scintillation liquids Insta-Gel-XF, Optiphase

HiSafe 3 and Ultima-Gold™ AB were added to the vials.

Results

Effect of aqueous solution volume on ¹⁴C and ³H counting efficiency

As shown in Figure 1A, when the proportion of water varied from 0 to 33% of total volume, the efficiency of [¹⁴C]-glucose counting was not significantly changed, regardless of the scintillation fluid used. However, when the samples were diluted in 3.0 ml of water, which corresponds to 50% of the final volume, there was a significant reduction (55%) in counting efficiency with the Optiphase HiSafe 3 scintillation liquid.

The increase in the relative amount of water in the samples induced a linear reduc-

tion in the efficiency of [³H]-thymidine counting, with similar slopes for all scintillation liquids used, as shown in Figure 1B.

Effect of concentration of radioactive samples on ¹⁴C and ³H counting efficiency

Regardless of the scintillation liquid used, the radioactivity detected was proportional to the [¹⁴C]-glucose and [³H]-thymidine concentration used, as shown in Figure 2A and B, respectively.

Effect of sample pH on ¹⁴C and ³H counting efficiency

The changes in solution pH did not influence the efficiency of [¹⁴C]-glucose counting. On the other hand, when Optiphase HiSafe 3 and Ultima-Gold™ AB were used at low pH (2.0), the efficiency of [³H]-thymidine counting was 9 and 20% lower with Ultima-Gold™ AB and Optiphase HiSafe 3 than that obtained using Insta-Gel-XF (Figure 3A and B).

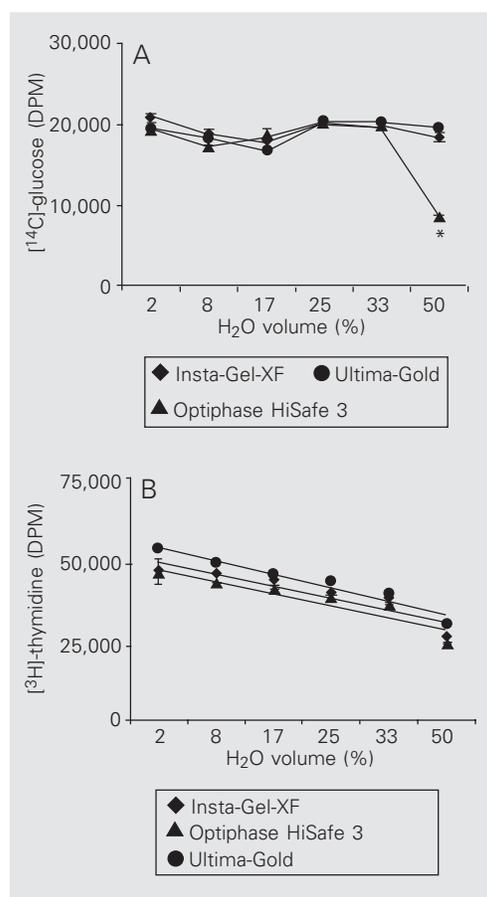
Effect of sample color on ¹⁴C and ³H counting efficiency

In order to analyze the influence of color quenching on the counting efficiency, [¹⁴C]-glucose and [³H]-thymidine were diluted in 1 ml of water containing 0.1 to 1 mg/ml Trypan blue. As shown in Figure 4A, the efficiency of [¹⁴C]-glucose radioactive counting was reduced by 83% in the presence of 1 mg/ml but not of 0.1 mg/ml Trypan blue solution, regardless of the scintillation liquid. Similarly, for [³H]-thymidine samples, the reduction at 1.0 mg/ml Trypan was approximately 94% (Figure 4B).

Discussion

The widespread use of radioisotopes in medical and biological research has generated a large volume of solid, liquid and

Figure 1. Effect of aqueous solution volume on the efficiency of [¹⁴C]-glucose (A) and [³H]-thymidine (B) counting. Each point indicates the mean \pm SEM (N = 4). (The error bars are too small to be visible). *P < 0.05 compared to the Insta-Gel-XF group (ANOVA followed by Newman-Keuls test). The linear reduction in the efficiency of [³H]-thymidine counting (solid lines in panel B) was similar for all scintillation liquids used. DPM = disintegration per minute.



biological wastes that need special treatment for disposal. In order to minimize the production of hazardous radioactive liquid waste we compared the efficacy of non-biodegradable scintillation cocktails containing organic solvents with biodegradable ones. We compared the effectiveness of three commercially available scintillation cocktails on the detection of ^{14}C -glucose and ^3H -thymidine radioactivity and determined the effect of the most common problems associated with the liquid scintillation counting such as water volume variations, sample pH and color quenching.

The present study showed few significant differences in the efficiency of ^{14}C and ^3H counting when using biodegradable scintillation liquids Ultima-Gold™ AB or Optiphase HiSafe 3 compared to a non-biodegradable one (Insta-Gel-XF), indicating that biodegradable cocktails do not impair the detection of radioactivity when used under appropriate conditions.

Except for the lower efficiency of ^3H -thymidine counting using Ultima-Gold™ AB at pH 2.0, replacement of Insta-Gel-XF with biodegradable liquids resulted in a similar counting profile in terms of volume of aqueous solution, concentration of radioisotopes and variations in sample color and pH. The reduced counting efficiency for tritium at extremely low pH (2.0) may have been related to the higher turbidity of the scintillation fluid at acidic pH. This result, however, does not impair the use of Ultima-Gold™ AB, but simply indicates that the sample pH should be corrected before counting.

The most relevant interference with counting efficiency was observed in the colored samples (Figure 4A,B). In general, color quenching results from the attenuation of photons during their passage through the medium (7). The reduction of counting efficiency was observed in samples containing the higher concentration of Trypan blue, 1 mg/ml, regardless of the scintillation liquid used. It is therefore essential to monitor the

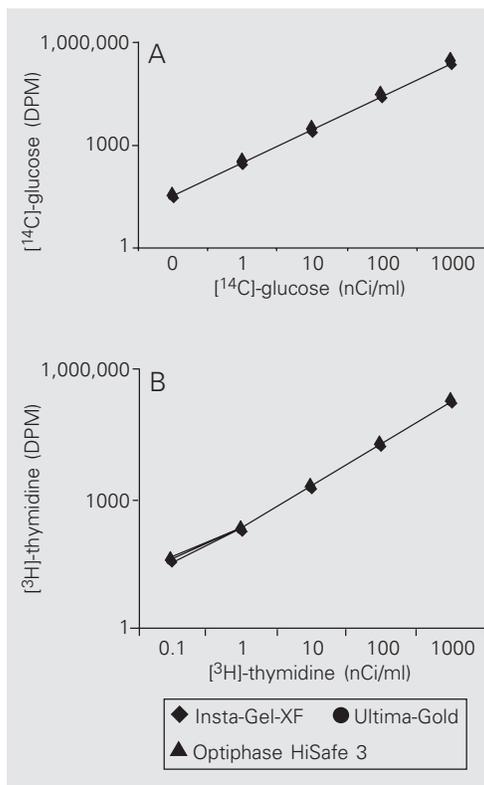


Figure 2. Effect of concentration of radioactive samples on the efficiency of ^{14}C -glucose (A) and ^3H -thymidine (B) counting. Each point indicates the mean \pm SEM (N = 4). (The error bars are too small to be visible). DPM = disintegration per minute.

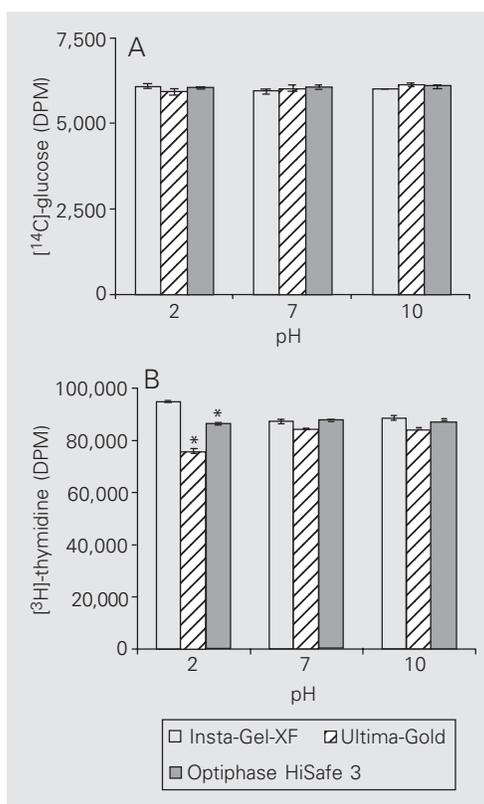
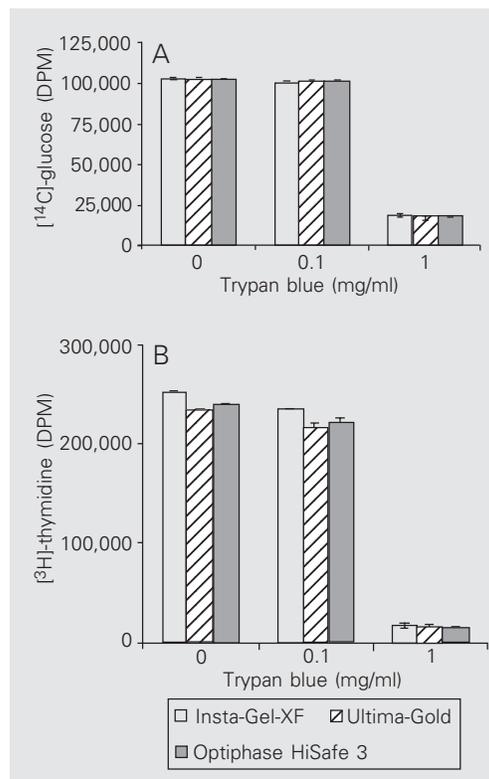


Figure 3. Influence of sample pH on the efficiency of ^{14}C -glucose (A) and ^3H -thymidine (B) counting. Each point represents the mean \pm SEM (N = 4). (The error bars are too small to be visible) *P < 0.05 compared to the Insta-Gel-XF group (ANOVA followed by Newman-Keuls test). DPM = disintegration per minute.

Figure 4. Influence of sample color on the efficiency of [^{14}C]-glucose (A) and [^3H]-thymidine (B) counting. Each bar indicates the mean \pm SEM (N = 4). (The error bars are too small to be visible). DPM = disintegration per minute.



counting efficiency in colored samples, when a precise determination of radioactive molecules is necessary. In this case, standards of known radioactivity should be used to obtain a correction factor and to correct the color quenching.

Our data also showed that the quenching effect on counting efficiency is also dependent on the type of radioisotopes. The higher susceptibility of ^3H compared to ^{14}C might be explained by the lower energy of tritium that reduces the efficiency of energy transfer to the solvent molecules (1).

The fundamental advantage of using biodegradable scintillation cocktails is related to the disposal of radioactive residues. For the non-biodegradable product it is necessary to request the chemical analysis of the waste sample according to the Brazilian Standard Rules and to obtain the industrial waste

registration document required by the environmental agency (Companhia de Tecnologia de Saneamento Ambiental, CETESB) (8,9). It is also relevant to take into account the cost of waste disposal, which includes the storage and treatment of toxic residues. On the other hand, non-hazardous aqueous radioactive wastes that are readily soluble in water may be disposed of via the sanitary sewage system if the concentration and maximum disposal radioactivity limits are observed ($3.7 \times 10^9 \text{ Bq/m}^3$ or $0.1 \mu\text{Ci/ml}$ for ^3H , or $7.4 \times 10^8 \text{ Bq/m}^3$ or $0.02 \mu\text{Ci/ml}$ for ^{14}C) according to the criteria outlined by CNEN. In fact, if the concentration of radioactive waste is below these limits, the waste is no longer characterized as radioactive but is considered to belong to the chemical group of waste defined as Class I Hazardous based on the Brazilian Standard Rules (ABNT NBR-10004 and 10007).

The present results show that traditional hazardous scintillation fluids can be replaced with safe biodegradable ones. The benefits of using environmentally benign fluids include the reduction of both liquid and solid radioactive waste since the containers of hazardous solvents must also be stored for later incineration. In addition to reducing the overall cost of radioactive waste disposal, the use of biodegradable scintillation cocktails minimizes both human and environmental exposure to hazardous solvents. In addition, some biodegradable scintillation liquids can be 40% less expensive than the traditional hazardous cocktails (see Table 1).

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