Scintillation Counting Techniques In The Isotope Dilution Analysis For Sucrose

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Introduction

The Spreckels research laboratory currently uses an isotope dilution (ID) analytical technique to determine the true sucrose content of various materials. The ID method, as originally reported (3)[2], utilized a gas flow proportional counting system to detect radiation. This system was adequate for the analysis of a small number of samples where the counting time was unimportant. However, the limited sample capacity of the proportional counter did not permit the analysis of a large number of samples.

The ID method has been modified to use a liquid scintillation counting technique. The modification has expanded the general usefulness of the method with no loss in analytical accuracy.

This paper details the scintillation counting technique used in the ID method. Included are preparation of the scintillation solvent, the activity of standards, preparation of vials for counting and counting procedures. Factors that affect the accuracy of the method are also discussed.

Method and Materials

Extraction and purification of the sucrose from samples were reported earlier (3) and are essentially unchanged i.e., barium precipitation and carbonation, purification by ion exchange treatment and crystallization from methanol.

Standards: The radioactive standard sucrose is prepared by diluting 0.5 microcuries of sucrose-^{14}C with 1000 grams of nonradioactive sucrose. The sucrose-^{14}C and sucrose-^{12}C are dissolved in the minimum amount of distilled water, concentrated on a steam bath, and recrystallized from distilled methanol. A portion of this standard is added to the extraction sample so that the resultant mixture has about a 1:1 ratio of radioactive to nonradioactive sucrose.

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[2] Numbers in parentheses refer to literature cited.

A counting standard is prepared in the same manner as the sample. In this case however, the sample is an accurately weighed quantity of pure nonradioactive sucrose. The ratio of radioactive to nonradioactive sucrose is accurately determined and held at a 1:1 ratio.

Comparison of the activities of the counting standard and the pure sucrose recovered from the sample is the basis of the isotope dilution method. The ratio of activities is a direct measure of the sucrose originally present in the material being analyzed.

Preparation of Scintillation Mixture: A sample prepared for liquid scintillation counting includes at least three components: the material being counted, a solvent and a scintillator. Often a fourth component is added to facilitate sample preparation. Usually the physical or chemical nature of the sample, rather than the isotope in question, determines the solvents and additives used in sample preparation. We have found the system(1) described below to be well suited for sucrose analysis.

Components of the scintillation mixture include: Reagent grade p-dioxane; Naphthalene-recrystallized from alcohol; 2,5 diphenyloxazole (PPO). Normal mixture composition by weight is 100 p-dioxane, 4.5 naphthalene, 1.0 PPO.

The mixture is treated with 10% by weight activated carbon(2) and filtered through a 0.45 micron Millipore filter. It is advisable to prepare a fresh scintillation solution for each new group of samples to be counted. Scintillation solutions that have yellowed or set for a prolonged period of time are not reliable.

It should be noted that the scintillation solution described does not contain a secondary scintillator e.g., wavelength shifter. We have found, that for our particular scintillation counter, inclusion of a secondary scintillator does not significantly increase our counting efficiency. This situation will not be true for all counters. Maximum counting efficiency for any counter will result when the wavelength of the light emitted by the scintillator most nearly matches the counter's photomultiplier response. Use of a secondary scintillator with our scintillation mixture will, therefore, depend on the characteristics of the counter to be used.

Preparation of Samples for Counting: The choice of the amount of sucrose that can be used in scintillation counting is restricted by the limited solubility of sucrose in the organic scintillation solvent. To increase its solubility, sucrose is dissolved in water. Water is a good solubilizer, but it is also a strong chemical quencher i.e., interferes with the transfer of energy between the site of an event and a molecule of scintillator. Inclusion of

water in the scintillation mixture will naturally lower the counting efficiency. However, if a constant amount of water is used for all samples, and the counting rate remains sufficiently high to insure good statistics, the loss in counting efficiency is of little consequence.

We have found that 0.2000 grams of sucrose dissolved in 5.0 milliliters of deionized water will form a homogeneous mixture with the scintillation solvent and will remain in solution at reduced counting temperatures e.g., 50°F.

The sucrose is accurately weighed into glass vials for counting. Five milliliters of deionized water are pipetted into each vial. The vials are capped and warmed in a water bath until the sucrose is completely dissolved. After the vials have cooled, 15 milliliters of scintillation solution are pipetted into each vial. Each vial is capped, agitated for homogeneous mixing, and wiped with lens paper. The vials are then ready to count.

Extreme care should be taken to see that insoluble material does not enter the vial with the water. To prevent this the deionized water is filtered through a 0.45 micron Millipore filter before use.

The use of a constant amount of solution in all vials requires precise delivery of both water and scintillation solvent to the vials. Nonuniform delivery will result in greater than normal variation in average count rate between consecutive vials of the same sample. Automatic pipettes with good delivery precision are essential for dispensing scintillation solvent and water.

The choice of the number of standards and samples to be counted and the counting time required for any particular accuracy of sucrose determination is based, in theory, on the activity of the radioactive component. However, certain variables can limit the precision of the test. We have found that the physical and chemical nonuniformity of all commercially available glass vials can be one such limiting error. The magnitude of this error can be reduced by using vials that have been selected for their uniformity of counting. A simple, yet novel vial selection procedure is given below. It should be remembered that vial selection is not a necessary part of the liquid scintillation technique. However, selection of vials will reduce the number of replications required and, therefore, the time necessary to achieve the desired analytical precision.

Vials can be selected for their uniformity of counting if each vial is counted with the same source of radioactivity. Variations in count rates between vials due only to the random nature of radioactive decay follow a known function and can be approximated. Therefore, it is possible to make a comparison of vials

where there is a reasonable probability that any detectable non-uniformity of counting is attributable to the physical or chemical nature of the vials.

If a weighed amount of a homogeneous radioactive scintillation solution is added to each vial, then all vials can be counted with the same source of radioactivity.

The random nature of radioactive decay is described by a Poisson distribution function and the expected variation due to this randomness is approximated by:

$$\text{Eq. [1]} \quad \sigma_p = \frac{\sqrt{\text{Total Counts}}}{\text{Total Time}}$$

The expected variation due to the random nature of radioactive decay can, therefore, be reduced by increasing the total counting time per vial.

The resultant counts of the vials will be distributed in a normal fashion. An example of such a distribution is given in Figure 1. The count interval in the distribution is twice the expected standard deviation of counts due to the randomness of radioactive decay calculated in terms of counts per minute per gram of scintillation solution. The 2σ value for the count interval allows a 95% assurance that the vials in any interval are uniform to 0.05% in counting. Vials in any one interval are considered sufficiently uniform for use.

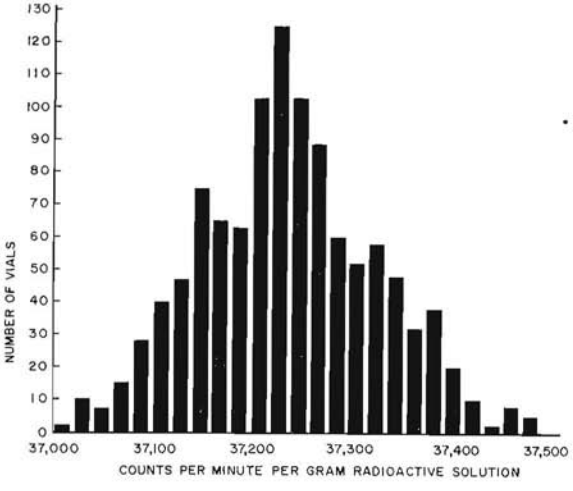

Figure 1.—Distribution of vials by count rate.

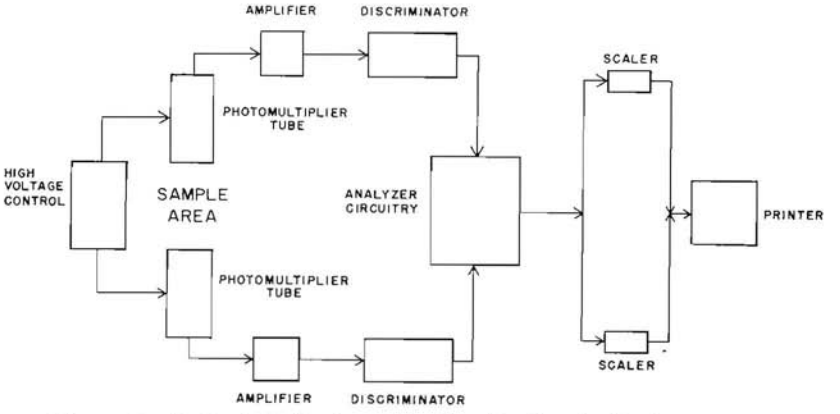

Figure 2.—A simplified schematic of a liquid scintillation counter.

Counting Procedure: Samples and standards are counted in a liquid scintillation counter. A simplified schematic of a modern scintillation counter is given in Figure 2.

It is generally accepted that a more efficient count is obtained if the sample temperature is lowered. This axiom was developed from early work in the field of liquid scintillation counting when counters were less sophisticated. Modern instruments have photomultiplier tubes that are less sensitive to thermal excitation, and ambient counting temperatures are now common. The choice of counting temperature should, therefore, be based on the particular scintillation counter.

The modern scintillation counters allow a choice of counting procedures. Two counting procedures, the channels ratio and balance point methods, are equally applicable to the ID method.

The channels ratio procedure does not give maximum counting efficiency, but it does provide a constant check on sample uniformity. This procedure is normally used for counting samples that vary in their degree of quench. If it is desired to prepare samples that are uniformly quenched, as in the ID procedure, then this counting method is a good check on sample preparation. Once sample preparation is standardized it is better to count the samples with the highest counting efficiency.

The balance point counting procedure insures maximum counting efficiency for samples of uniform composition. The energy discriminator settings used in this procedure will depend on the liquid scintillation counter to be used.

Calculation for percentage sucrose in the sample is the same as for proportional counting and has been described earlier (3). An example of typical counting data and calculations is given in Table 1.

Table 1.—Liquid scintillation counting data and percent sucrose calculation.

Sample	Molasses (beet)			Counting standard		
Vial No.	Wt Sample in vial gm	Total counts 10 min	Avg CPM 0.2000 gm	Wt std in vial gm	Total counts 10 min	Avg CPM 0.2000 gm
1	0.2003	1,682,300	167,978	0.2002	1,676,510	167,484
2	0.1998	1,685,720	168,741	0.2001	1,676,370	167,553
3	0.2000	1,683,970	168,397	0.2003	1,678,090	167,558
4	0.1999	1,686,300	168,714	0.2001	1,670,350	166,952
5	0.1998	1,686,610	168,829	0.2004	1,675,020	167,168
6	0.2002	1,687,130	168,544	0.2000	1,672,060	167,206
7	0.1999	1,682,530	168,337	0.2001	1,673,370	167,253
8	0.2000	1,686,120	168,612	0.1996	1,670,230	167,358
9	0.1998	1,685,500	168,719	0.2000	1,672,630	167,266
10	0.2000	1,681,930	168,193	0.2004	1,671,990	166,865

$W^{14C} = 6.0016$ $R_1 = 167{,}266$ $\sigma_{R_1} = \pm 74$ $a_1 = 0.00044$

$W_s = 12.0017$ $R_2 = 168{,}506$ $\sigma_{R_2} = \pm 87$ $a_2 = 0.00052$

$$\% \text{ Sucrose} = \frac{100\ W^{14C}}{W_s}\left(\gamma\ \frac{R_1}{R_2} - 1\right) \text{ and}$$

$$\sigma_{\% \text{ Sucrose}} = \frac{100\ W^{14C}}{W_s} \cdot \gamma\ \frac{R_1}{R_2} \cdot \sqrt{a_1^2 + a_2^2}$$

$$\% \text{ Sucrose} = \left(\frac{6.0016}{12.0017}\right)\ (100)\ (0.98528) = 49.27$$

$$\sigma_{\% \text{ Sucrose}} = \left(\frac{6.00}{12.00}\right)\ (100)\ (1.985)\ (0.00068) = \pm\ 0.07$$

where:

W^{14C} = weight of standard sucrose added to the extraction sample
W = weight of the extraction sample
R_1 = average counting rate(CPM) of the counting standard
R_2 = average counting rate(CPM) of the sample
a_1 = relative error of the counting standard
a_2 = relative error of the sample
γ = weight ratio $W^{14C} : W^{12C} \therefore W^{14C} = 2.0000$

A comparison of results by proportional and liquid scintillation counting techniques is given in Table 2. The data show no significant difference in percentage sucrose or analytical error between the two methods.

Table 2.—A comparison of results by proportional and liquid scintillation counting techniques in the ID determination of sucrose.

Beet sample	% Sucrose $\pm\ \sigma_{\bar{X}}$	
	Proportional	Liquid scintillation
1	12.44 ± 0.02	12.44 ± 0.03
2	12.52 ± 0.03	12.54 ± 0.02
3	12.75 ± 0.06	12.75 ± 0.02
4	12.66 ± 0.02	12.69 ± 0.02

Summary

The use of a liquid scintillation counting technique in the isotope dilution analysis for sucrose is described. Included are

preparation of the scintillation mixture, activity of standards, preparation of vials for counting and counting procedures. Examples of typical counting data and analytical results are also given.

Literature Cited

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