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DESIGN AND EVALUATION OF A FREEZE-DRY APPARATUS FOR
REMOVING FREE WATER FOR TRITIUM ANALYSIS

by

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Proposed for Publication in
Environmental International

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DESIGN AND EVALUATION OF A SIMPLE AND RAPID FREEZE-DRY APPARATUS
FOR REMOVING FREE WATER FOR TRITIUM ANALYSIS*

by

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ABSTRACT

A freeze-dry apparatus was constructed to remove free water from environmental samples for tritium analysis. The apparatus is self-contained, can process eight samples simultaneously, and, to avoid replenishment of the cold bath, uses a refrigeration system. Large samples (200 to 300 g) can be dried in about three days. To avoid fractionation of tritium during the distillation process, it was necessary to take the sample to dryness.

Introduction

To determine the tritium concentration of free water in biota or sediment, the water from a sample must be extracted. To analyze biological samples for "combined tritium"**) requires the removal of free water prior to combustion of the sample. Usually the best method to extract free water is the lyophilization or freeze-dry

* The information contained in this article was developed during the course of work under Contract No. DE-AC09-76SR00001 with the U. S. Department of Energy.

**) See footnote on the next page.

technique^{1,2,3}. Freeze-drying is the drying of frozen material through the sublimation of ice. The general procedure^{3,4} is to: 1) increase the surface area of the sample prior to drying by shredding, cutting into small pieces, or shell freezing (liquid samples); 2) freeze the sample before vacuum is applied; 3) supply heat to the sample in order to support continued distillation; and 4) collect the water from the sample in a condensing trap. The purpose of this paper is to describe a freeze-dry apparatus that can easily dry eight samples simultaneously, be operated unattended, and avoid sample transfer problems. Also, some of the problems such as freeze-dry design, isotopic fractionation, and sample temperature during distillation and/or sublimation will be discussed.

Apparatus

The freeze-dry apparatus consists of a number of components (Figure 1). The circular insulator tank is constructed of 0.16-cm-thick aluminum and insulated with polyurethane foam between the outer and inner tanks. The inner tank contains ~40 L of Freon[®] TMC (E. I. du Pont de Nemours & Co.), a nonflammable fluorocarbon

** Combined tritium - Includes bound tritiated water, labile and nonlabile tritium. Bound tritiated water is loosely defined as that water that does not freeze and is considered to comprise between 5 to 10% of the total water in animal tissue^{4,5}. Labile tritium is usually attached to a biological molecule via oxygen or nitrogen groups and undergoes rapid exchange with water. Nonlabile tritium is attached to a carbon atom and is not readily exchangeable with tritium in water.

with a freezing point of -88°C and low toxicity (threshold limit value of 330 ppm). The chill coil is supported in the middle of the tank, which in turn is placed on top of the castered refrigeration unit. Eight sample support brackets are evenly spaced around the circumference of the tank, and support brackets for the vacuum manifold are attached to the outside of the tank.

The detail of the distillation system is shown in Figure 2. The sample container is a commercial apparatus (Multitainer, FTS Systems, Inc.) with the lid modified to fit the 2.54-cm-OD glass connecting tube. A 2.54-cm-OD "Cajon" tube fitted with a 1.27-cm male threads was used to make this modification. The sample container can accommodate storage bottles or loose samples. The collection flask has a volume of ~ 500 mL and has a side tube for connection to the vacuum manifold. The center of the connecting tube has an inlet for release of vacuum. The air supplied to release the vacuum in the distillation system is dried with silica gel to prevent tritium contamination from the atmosphere. All fittings are "O" ringed except for the vacuum-tube connection. The "O"-ring fittings require less maintenance and have fewer operational problems than the more commonly used ground-glass grease fittings.

The circular vacuum manifold has a diameter of 40.64 cm and is constructed from 2.54-cm-OD stainless-steel tubing. It has eight evenly spaced "bellows-sealed" valves attached to the periphery, and has connections for the vacuum pump and thermocouple

vacuum gauge. The vacuum manifold is supported directly above the chill tank by support brackets attached to the sides of the insulated tank.

Discussion and Results

Several parameters were considered in the construction of the freeze-dry apparatus and in the drying process. To avoid contamination of the sample from exchange of tritiated atmospheric moisture with the sample, the freeze-dry-sample containers were made large enough to hold loose samples or wide mouth storage bottles up to 1 L. By this design, the bottle containing the sample is placed directly into the sample container thereby minimizing the contamination potential. To avoid contamination of the outside of the bottle, it is doubly bagged with polyethylene bags at the time the sample is collected. This bottle is weighed, before and after freeze-drying, to determine the water loss and is used to store the dried sample.

In the design of the distillation system, tubing lengths, diameters, and temperature of the condensing traps had to be considered. When the mean free path of the H₂O molecule is greater than the dimensions of the chamber or tubing, resistance to flow will come from tubing or chamber walls. At normal freeze-dry pressures, 0.5 to 1 mm Hg, the mean free path of the water molecules is about 1 mm, whereas in air it is about 10⁻⁴ mm.⁵

Therefore, the function of a vacuum in a freeze-dry system is to increase the mean free path of the water molecules by lowering the collision frequency with air molecules. If the mean free path of the water molecule at 0.5 to 1 mm Hg is about 1 mm, then the pumping speed (L/min) is primarily a function of tubing dimensions of greater than 1 mm. For instance, a tube 2.54 cm in diameter will have a pumping speed 10 times greater than one of 1.27 cm. The 2.54-cm tube is a readily available tube diameter and would have an estimated pumping of about 20 to 30 L/min for the system as designed. A total tubing length of 40.6 cm was required to connect the freeze-dry-sample container to the condensing trap.

The pressure driving force moving the water from the sample to the condensing trap is the temperature differential. The maximum possible pressure differential between a sample of temperature -10°C (vapor pressure of ice 1.95 mm Hg) and a condensing trap of 0°K (-273°C) is ~ 1.95 mm Hg. If the condensing trap were at -60°C (0.008 mm Hg) and the sample at -10°C , the pressure difference would be ~ 1.94 mm Hg, and for a condensing trap at -30°C (0.286 mm Hg) it would be ~ 1.66 mm Hg. A temperature difference of 20°C (a -10°C sample and a -30°C condensing trap) would result in $\sim 85\%$ of the maximum theoretical pressure driving force. A condensing-trap bath temperature of -40°C would result in nearly 90% of the maximum pressuring driving force and was easily within the capability of the refrigeration system selected.

The temperature during freeze-drying of environmental samples for determination of tritium in the free water is uncontrolled, resulting in a drying process that is a mix of freeze-drying and

vacuum distillation. This process probably does not affect the measured concentration of tritium in the free water collected if the sample is taken to dryness.

The samples goes through three phases during the drying process: 1) the initial freezing, 2) the temperature change during drying and 3) final drying at room temperature. For most freeze-drying techniques, the sample is frozen prior to placing it in the freeze-dry system. Frequently, a room-temperature sample is placed in the freeze-dry apparatus and the freezing of the sample is accomplished by evaporation of the first 10 to 15% of the free water in a sample which is 80% water. This is done out of convenience sometimes, but more often to reduce the condensation of atmospheric moisture on the cold sample that ends up in the freeze-dryer.

The sample changes temperature during the drying process. As a sample dries from the outside toward its center, the boundary that separates the dry layer from the frozen layer moves during the drying process. In order to support the movement of this boundary, heat must be supplied to the frozen layer to maintain sublimation. This heat must travel through the dry layer, hence creating a temperature gradient. As the sample dries, more of the sample is at room temperature. A typical temperature-time curve for the drying of fish flesh at room temperature with the condensing trap at -40°C (0.1 mm Hg) is shown in Figure 3. The shape of the curve and temperature is dependent on the location of the

thermocouple in the sample.⁶ This thermocouple was located 2 to 4 mm deep in a piece of fish flesh (160 g) ~14 mm thick. The sample froze at this thermocouple location within 3 hours to a temperature of -9°C and had a short thermal arrest period of ~1 hour. Then the sample slowly warmed to room temperature over a period of ~60 hours. A temperature of -20 to -25°C has been measured in other fish showing that the temperature is a function of thermocouple location. A temperature differential of 10 to 20°C is expected between the condensing trap and sample.⁵

The temperature that a sample has throughout its drying process is a function of its geometry, mass, thickness, rate of evaporation, and its relation to the heat supplied from the sample container walls.

Isotope Effect

In general, no isotopic fractionation of tritium is expected during freeze-drying if the sample remains frozen during the drying process.³ As discussed earlier, a large portion of the sample can be at room temperature during the drying process and fractionation can occur with the residual bound water or during evaporation if the sample is frozen by water evaporation. This fractionation effect can be reduced by taking the sample to dryness.

The importance of taking a sample to dryness can be approximated by using a formula that expresses the change in tritium concentration as water is being distilled without freezing. The derivation is as follows:

Assuming there are only two species of water present, namely H₂O and HTO, we know by the gas law that the molar ratio of each species in the gaseous form is equal to the ratio of their individual pressures or

$$\frac{\frac{n_T^V}{V}}{\frac{n_H^V}{V}} = \frac{P_T}{P_H} = \chi \quad (1)$$

where:

n_T^V - number of moles of HTO in the gas phase

n_H^V - number of moles of H₂O in the gas phase

P_T & P_H - respective pressures of HTO and H₂O

χ - gas phase fractionation ratio

From Raoult's Law, the pressures are related to the number of moles in the liquid by

$$P_T = P_T^* n_T^L \text{ and } P_H = P_H^* n_H^L \quad (2)$$

where P_T^* & P_H^* are the respective vapor pressures and n_T and n_H are the liquid fractions.

The total number of moles of each is:

$$n_T = n_T^L + n_T^V \quad (3)$$

$$n_H = n_H^L + n_H^V$$

Substituting 2) and 3) into 1) we obtain:

$$\chi = \frac{\frac{n_T^V}{V}}{\frac{n_H^V}{V}} = \frac{P_T^* (n_T - n_T^V)}{P_H^* (n_H - n_H^V)} \quad (4)$$

From 4) we obtain

$$\chi = \frac{P_T^* \left(\frac{n_T}{n_H} - \frac{n_T^V}{n_H^V} \right)}{P_H^* \left(1 - \frac{n_H^V}{n_H} \right)} \quad (5)$$

$\frac{n_H^V}{n_H}$ - represents the fraction evaporated F

$\frac{n_T}{n_H}$ - initial liquid ratio χ_0

Rearranging and substituting equation 5 we obtain a relationship describing the change in the isotopic composition of the gas phase with fraction of water distilled.

$$\frac{\chi}{\chi_0} = \frac{\frac{P_T^*}{P_H^*}}{1 - F \left(1 - \frac{P_T^*}{P_H^*} \right)} \quad (6)$$

The partial pressure ratio, $\frac{P_T^*}{P_H^*}$, for water as a liquid is

is obtained from $n \frac{P_H^*}{P_T^*} = \frac{37813.2}{T^2} + \frac{-136.751}{T} + 0.12409$ where T is

the absolute temperature.⁷ Figure 4 was constructed to show the change in concentration with the fraction of water evaporated at four different temperatures. If only the first 10% of the water is collected for a tritium measurement the concentration would be about 10% less than the actual value. By taking the sample to 90% dryness a reduction of only 2 to 3% would be expected and is

easily within the measurement error for most tritium measurements. Hence, it is important that the sample be taken as close to dryness as possible.

Tritium concentrations were measured in various water fractions collected from fish during freeze-drying to determine if fractionation was occurring. The data were plotted to show the tritium concentration in the series of fractions of free water distilled and not the tritium concentration of individual fractions. Figure 5 shows that fractionation did occur and that by taking the fish all the way to dryness, the final tritium concentration is nearly the same as the water from which the fish were taken (31.2 ± 1.4 pCi/mL fish, 31.0 ± 1.4 pCi/mL water). The line in Figure 5 is similar to the theoretical ones shown in Figure 4. As expected, the tritium concentrations in the first fractions were lower than the final distilled fractions: 29.8 ± 1.0 pCi/mL and 33.0 ± 1.0 pCi/mL for averaged first and final fraction concentrations.

More evidence was obtained on isotopic fractionation by measuring the deuterium concentrations on the same water fractions that the tritium was measured. If tritium was being fractionated, a linear relationship would exist between the deuterium and tritium concentration. Deuterium concentrations in the samples for different fractions follow the same pattern as tritium (Figure 6). The calculated deuterium-tritium fractionation concentrations at 25°C was calculated using a slight modification of equation 6,

the appropriate HTO-HDO vapor pressures, and deuterium and tritium concentrations in the pond water from which the fish were removed.

The freeze-dry apparatus was tested to determine its effectiveness in drying fish and sediment samples. The water-recovery efficiencies for fish flesh and sediment were good. Water recoveries were $99.5 \pm 0.6\%$ and $99.2 \pm 0.5\%$ for 100- and 200-g samples of wet fish flesh and sediments, respectively. These samples were dried to constant weight in about 48 to 72 hours.

In this system, all samples are freeze-dried by a vacuum connection to the common vacuum manifold. This design poses the possibility of sample cross-contamination. Measurements of cross-contamination were made by freeze drying a tritium-spiked water sample (100 mL, 250 pCi/mL) adjacent to blank water samples (10 mL) in the apparatus for 24 hours. A smaller blank volume was used to increase sensitivity. For the 24-hour distillation, a tritium contamination of 0.2% was measured. This level of cross-contamination is small, and since most samples are usually within a concentration factor of 10, cross-contamination with this design is negligible.

Acknowledgements

I would like to thank Dr. R. M. Wallace of Savannah River Laboratory for his assistance.

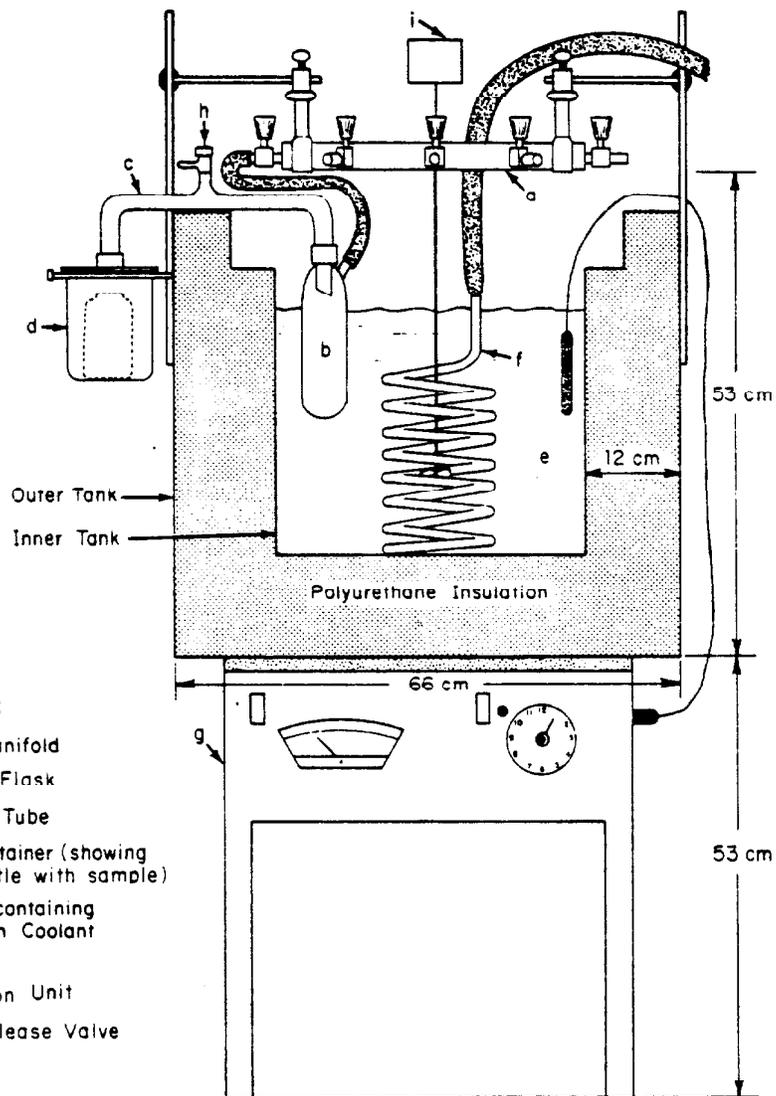


FIGURE 1. Freeze-Dry Apparatus

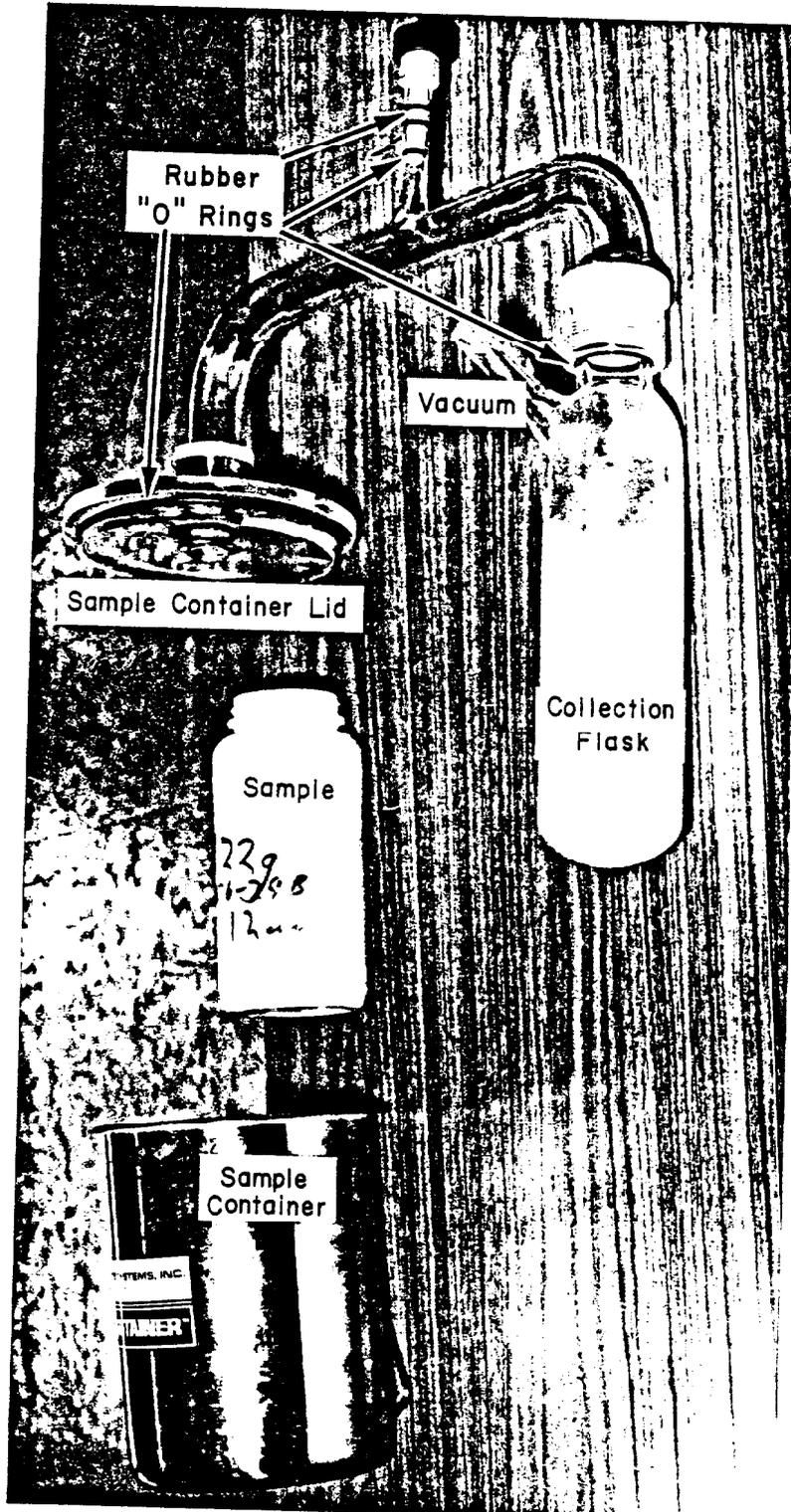


FIGURE 2. Detail of Sample System

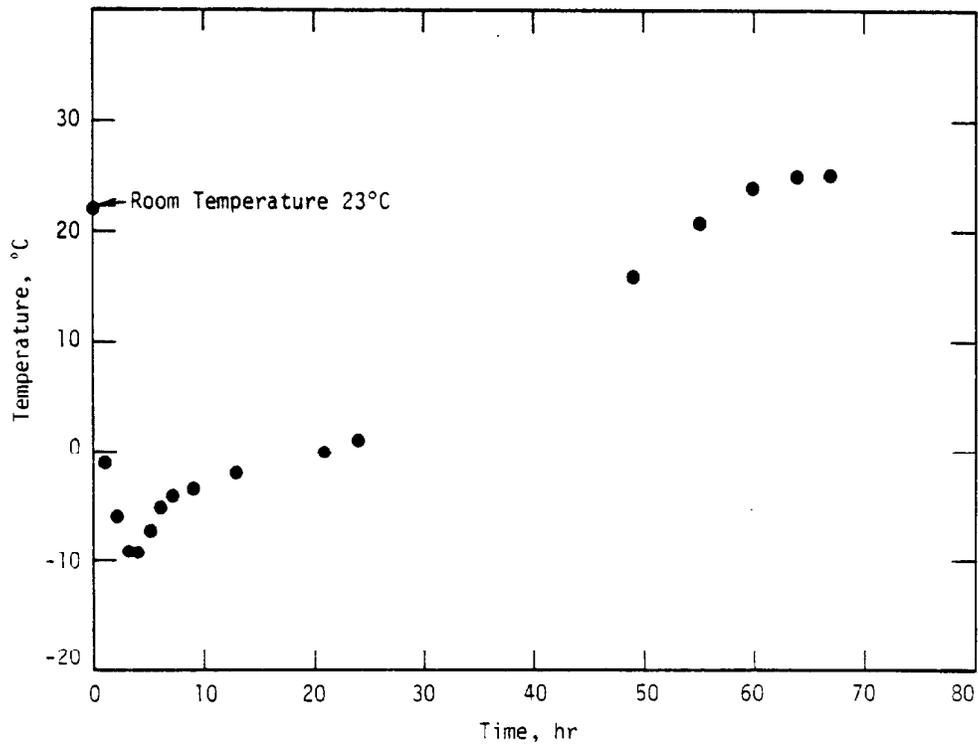


FIGURE 3. Temperature in a Sample of Fish Undergoing Evaporative Freezing, then Freeze-Drying. Room Temperature Varied during Freeze-Drying and Distillation.

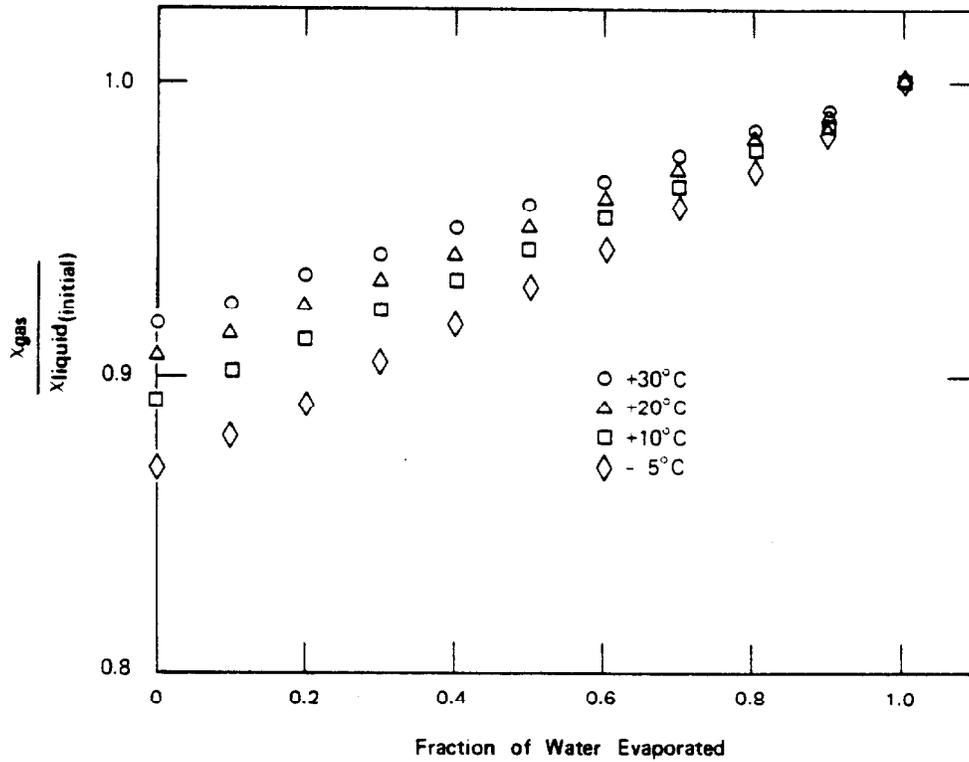


FIGURE 4. Change in Tritium Concentration in Evaporated Water with Fraction of Water Evaporated

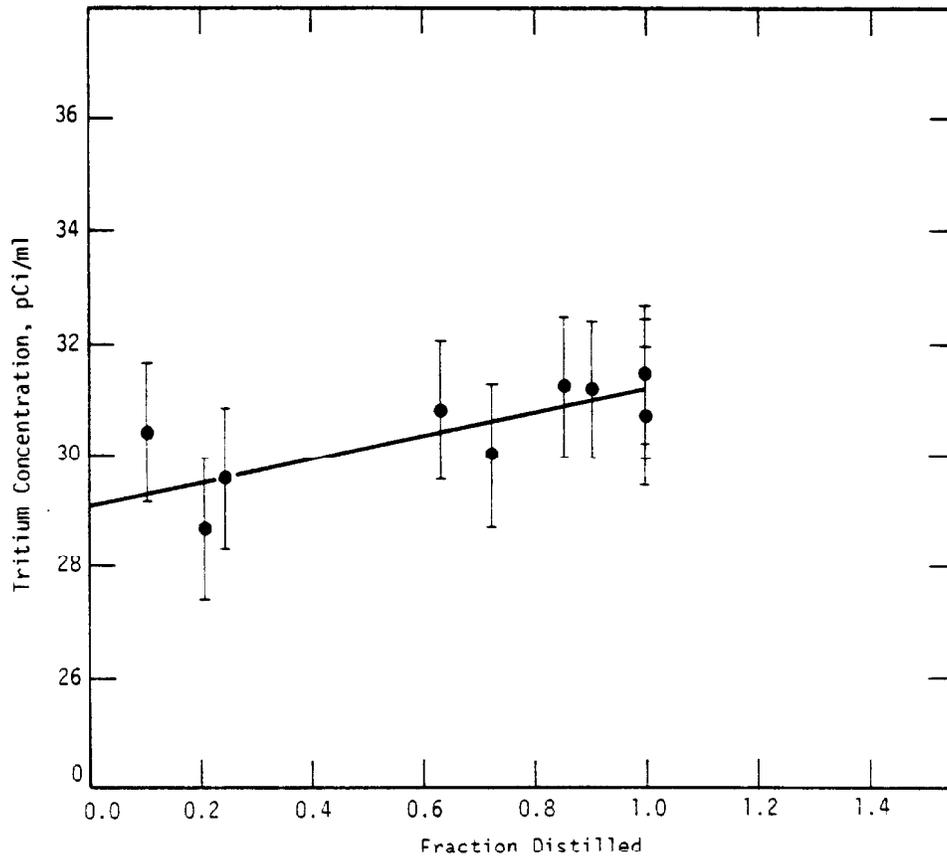


FIGURE 5. Concentration of Tritium in Various Collected Water Fractions. Confidence Level is 2σ .

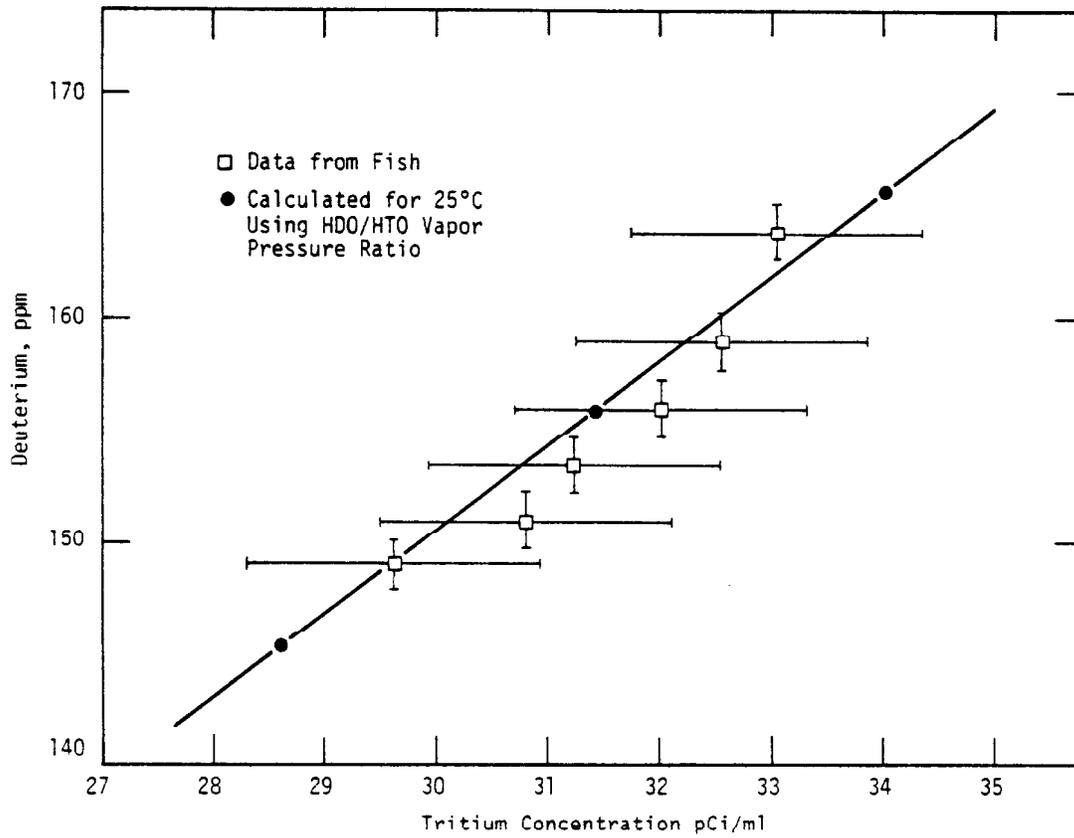


FIGURE 6. Fractionation of Tritium and Deuterium during Freeze-Drying of a Fish Sample. Confidence Level is 2σ .

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