

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

LOSS OF TRITIATED WATER DURING LIQUID
SCINTILLATION COUNTING WITH SPECIAL
REFERENCE TO POLYETHYLENE-FILM TUBES¹

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Loss of tritiated water (THO) from aqueous samples can occur either as bulk water loss to a desiccating environment without concomitant changes in specific activity or through water vapor exchange between the radiolabeled sample and a humidified environment, the latter decreasing the specific activity of the labeled sample without affecting sample volume. THO is lost from liquid scintillation (LS) vials as a function of vial composition. One half of the label is lost from 20 ml borosilicate glass vials in around 230 days whereas polyethylene vials lose half the THO in 90 days (20 ml vials) or 27 days (3 ml vials) and polyethylene-film tubes (Filmware®) lose the same amount in under 4 days. THO moves directly through the wall of the film tube and appears to diffuse through the polyethylene in contact with the solvent as well as through the air space above the scintillator. The closure seams of the tube do not constitute a significant pathway for THO loss. The composition of the scintillant in the tubes can change the halftime of THO loss from 1.5 day ('Ready-Solv HP',® Beckman) to 4 days ('Liquiscint',® National Diagnostics). The absolute loss of THO activity from sol-gel scintillators in either uncapped glass vials or sealed film tubes decreases as the fraction of unlabeled water in the scintillation solution increases. THO is lost from solutions of tritiated biomolecules in film tubes with two

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rate constants, one reflecting *THO* permeation of the tube and the second indicative of T-H exchange between water and the labeled molecule. Although filmware tubes are highly cost effective for counting most beta emitters they are unsuitable for counting tritiated water or biomolecules with labile tritium.

I. INTRODUCTION

Investigations utilizing tritiated molecules are fraught with numerous methodological problems. Most significant among these are the exchange of tritium between radiolabeled molecules and water and the subsequent loss of tritiated water vapor into the atmosphere (for example see; Jacobs, 1968; Waterfield et al., 1968; Geller and Silberman, 1970; Evans, 1974; Neame and Homewood, 1974; Evans, 1976; Lukas and Redalieu, 1976). This will not only compromise the specific activity of the labeled molecule but, if the storage or reaction vessel is open to the atmosphere, laboratory equipment, reagents and personnel can become contaminated with tritiated water.

The permeability of polyethylene to organic molecules and solvents is well known (Johanson and Lundqvist, 1972; Horrocks, 1974; Horrocks, 1975; Neame, 1975). Decreased activity following tritiated water storage in polyethylene vials has also been reported (Neame, 1975; Muse and Rao, 1976). Although loss of activity might reflect scintillator induced changes in the efficiency of the vial, (Horrocks, 1975; Neame, 1975; Allen, 1976; Horrocks, 1976a; Barson, 1977) there is substantial evidence that tritium diffuses out of the vial in the form of tritiated water. Net weight loss from polyethylene vials containing water has been demonstrated (Leiberman and Moghissi, 1970; Johnson and Lowenthal, 1972) and Horrocks (1975) and Hansen and Yoder (1968) have shown that significant amounts of tritiated water are lost from polyethylene bottles during refrigerated storage. Factors which can influence the rate of tritiated water loss from vials such as scintillator composition, ambient humidity, temperature and vial composition have not been examined in detail.

Attempts to engender maximum cost-effectiveness in liquid scintillation counting are primarily directed toward less expensive counting vials and reduced scintillator volume. Miniature glass and polyethylene vials with sample volumes from 3 to 10 ml are becoming more common and recently a polyethylene-film tube (Filmware [®]), Nalge Corp., Rochester, NY.) has been introduced. Unfortunately little is known of the counting characteristics of these tubes.

The purpose of the present experiments is to examine the loss of tritiated water from various types of counting vials with special reference to the polyethylene-film tubes. The effects of scintillator composition and counting environment on water loss is also considered and the hazard from tritiated water loss as a result of tritium exchange between water and radiolabeled biomolecules is demonstrated.

II. METHODS

1. *Effects of Ambient Humidity on THO and H₂O Exchange*

Small incubation vessels containing 2 ml of water were placed in desiccators containing either 1 liter water or approximately 1 liter silica gel pellets and bulk water loss from the vessels was monitored gravimetrically at timed intervals. Water vapor exchange between the incubation vessel and the surroundings was measured by spiking the incubated samples with THO and counting a 20 λ aliquot withdrawn from the sample at appropriate intervals. Bulk water loss from sealed vials was also determined gravimetrically under desiccated conditions. All experiments were done in triplicate.

2. *Half-time for THO Loss*

Tritiated water (0.18 μC in 50 λ) was added to vials and film tubes containing various scintillation solutions. The vials and tubes were then tightly capped and heat sealed, respectively, and placed in a standard cardboard 20 ml vial tray which in turn was placed in a ventilated fume hood. The samples were removed in groups of not more than 30 and counted to 0.5% error and returned to the hood. The half-time for THO loss was determined from the standard decay equation. The quadruplicate samples were not corrected for tritium decay.

3. *Tritiated Water Exchange in Film Tubes*

Heat sealed film tubes containing 3 ml scintillator (TT-21) and spiked with THO (20 λ) were incubated in an enclosed 300 ml screw-top glass jar with an equal number of unspiked but otherwise identical tubes. At timed intervals all tubes (triplicate samples) were removed, counted and returned to the jar.

4. *Effects of Scintillator Water Load on Tritiated Water Exchange*

The effects of increased water-scintillator ratios on THO exchange in air exposed vessels and film tubes was examined using toluene and a xylene derivative based scintillator. Percent water of the samples was increased from 0.5 percent to 55 percent water which is within the range of sol-gel scintillator capability (Benson 1976). Total volume (scintillator plus water) was maintained constant as was the total tritium activity. All samples were shaken for 30 seconds prior to counting.

5. *Tritium Exchange by Biomolecules*

Exchange of tritium for hydrogen by tritiated biomolecules and subsequent loss of THO into the environment was examined by incubating the labeled biomolecule and scintillator in a sealed film tube at ambient temperature. The samples (in triplicate) were then counted at timed intervals. Three tritiated nucleosides were compared in this manner, adenosine (2,8-³H) from New England Nuclear (Boston, MA.) and guanosine (8-³H) and uridine (5-³H) both from ICN (Irvine, CA.).

6. *Counting Conditions*

The following premixed sol-gel scintillation solutions were used: Ready-Solv HP^R and EP^R, Beckman Instruments Inc., Irvine, CA.; Liquiscint^R, National Diagnostics, Parsippany, N.J. and TT-21^R, Yorktown Research, South Hackensack, N.J. The Ready-Solv HP^R and EP^R and TT-21^R are toluene based where as the solvent for liquiscint is a high flashpoint xylene derivative. All samples were counted at ambient temperature on a Beckman LS 233 liquid scintillation counter to 0.5%, 2 σ error.

III. RESULTS AND DISCUSSION

The well known relationship between bulk water evaporation and unidirectional water vapor efflux is illustrated in figure 1. Under humidified conditions there is no net water loss from the sample but the specific activity of the sample decreases due to the unidirectional efflux of THO. The slight water loss observed is due to the periodic venting of

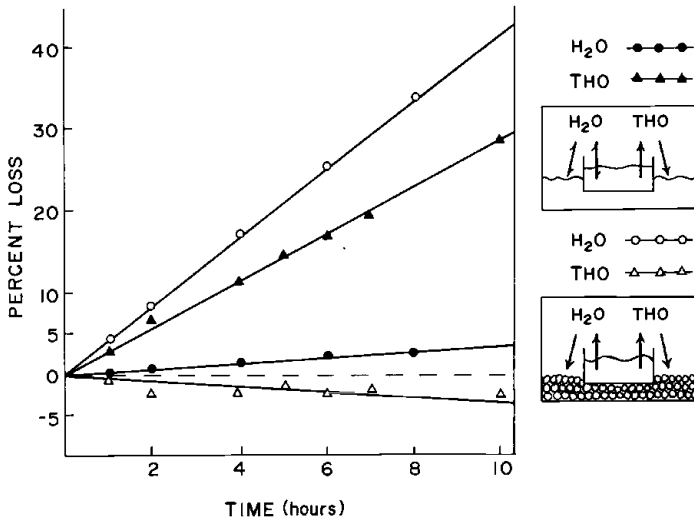


FIGURE 1. Percent loss of bulk water (solid and open circles) and THO (solid and open triangles) into humidified (solid symbols, upper inset) and desiccated (open symbols, lower inset) environments as a function of time.

the desiccator to weigh samples. In a desiccating environment both bulk water evaporation and unidirectional THO efflux are favored and the specific activity remains unchanged. The slight increase in specific activity in the latter environment probably reflects the mass effect of THO vs H₂O vapor. At constant temperature, the efflux of THO is independent of ambient humidity while bulk evaporation is inversely correlated with humidity. Thus, in an aqueous solution tagged with THO, loss of total activity is independent of humidity whereas, in time, the specific activity is least affected in dry environments and progressively decreases as humidity increases. This relationship also exists for water fluxes across water permeable containers.

Loss of THO from various scintillation vials under different conditions is shown in Table 1. Glass vials are relatively impermeable to THO with a half-time of around 8 months. The permeability of the polyethylene vials increases as vial size decreases which presumably reflects the vial thickness. Bulk water loss from 20 ml polyethylene vials in a dehydrated environment was rapid for several days (2 mg/day) then decreased to a steady rate of 0.3 mg/day over the ensuing 15 days which is similar to that reported by Lieberman and Moghissi (1970) and Horrocks (1975). The rate of THO loss

TABLE I. Half-Time for Tritiated Water Loss

| Sample Conditions | | | Half-Time ^a |
|-------------------|--------------------|----------------------|------------------------|
| Vial | Scintillator | Conditions | |
| Glass | TT-21 ^d | Plastic-lined cap | 289.00 \pm 28.50 |
| 20ml | 7ml | | |
| Glass | TT-21 | Foil-lined cap | 230.00 \pm 47.80 |
| 20ml | 7ml | | |
| Poly ^b | TT-21 | Screw cap | 90.30 \pm 1.070 |
| 20ml | 7ml | | |
| Poly ^b | TT-21 | Friction cap | 26.70 \pm 4.800 |
| 3ml | 2ml | | |
| Tube ^c | TT-21 | | 4.97 \pm 0.224 |
| 10ml | 7ml | | |
| Tube | TT-21 | | 3.25 \pm 0.173 |
| 3ml | 3ml | | |
| Tube | TT-21 | | 2.55 \pm 0.087 |
| 10ml | 3ml | | |
| Tube | TT-21 | Small air space | 3.30 \pm 0.104 |
| 10ml | 3ml | | |
| Tube | TT-21 | Large air space | 1.57 \pm 0.044 |
| 10ml | 3ml | | |
| Tube | TT-21 | Seams crimped 2X | 2.83 \pm 0.083 |
| 10ml | 3ml | | |
| Tube | TT-21 | End seams crimped 4X | 3.58 \pm 0.058 |
| 3ml | 3ml | | |
| Tube | HPE ^e | | 1.50 \pm 0.100 |
| 3ml | 3ml | | |
| Tube | EPE ^e | | 2.18 \pm 0.072 |
| 3ml | 3ml | | |
| Tube | LS ^f | | 4.50 \pm 0.083 |
| 3ml | 3ml | | |
| None | TT-21 | Air exposed | 0.29 \pm 0.015 |
| None | HPE ^e | Air exposed | 0.30 \pm 0.092 |
| None | LS ^f | Air exposed | 0.44 \pm 0.023 |

^aValues in days, mean \pm S.E. (N) = 4

^bPolyethylene vial

^cPolyethylene-film tube, Nalge Corp., Rochester, N.Y.

^dYorktown Research, South Hackensack, N.J.

^eBeckman Instruments Inc., Irvine, CA.

^fLiquiscint, National Diagnostics, Parsippany, N.J.

from polyethylene-film tubes is much greater than for any other vial examined and in several instances over 50% of the label was lost in two days. As would be expected, bulk water loss from film tubes was also rapid (3.5 mg/day and 2.7 mg/day for 10 ml and 3 ml tubes respectively). In uncapped vials half the THO is lost in around 8 hours.

Several factors influence the half-time of tritium loss from the film tubes (Table 1). As size of the air space above the scintillator (and therefore the total exchange surface area of the tube) increases, the rate of loss of THO increases. This indicates that THO not only diffuses out of the tube directly from the solvent phase but from the gas phase above the scintillator as well. Solvent systems which increase the water vapor pressure in the enclosed tube by decreasing the solubility coefficient of water, micelle stability or micelle surface area to volume ratio would be expected to decrease the half-time for THO loss. This would explain the difference in half-times between the toluene and xylene derivative based scintillation solutions.

The heat crimped seams of the film tubes are not significant pathways for THO loss. The slight increase in half-time found in tubes sealed 4 times (Table 1) is probably due to the decrease air space above the scintillator.

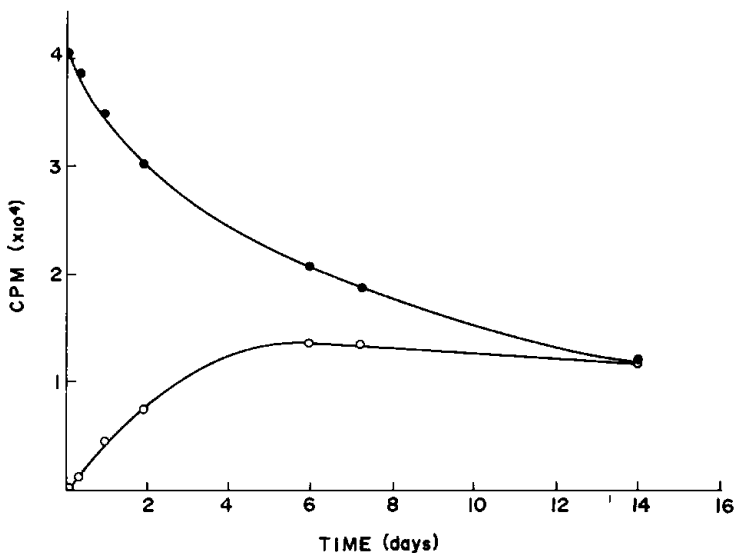


FIGURE 2. Exchange of THO between spiked film tubes (solid circles) and unspiked film tubes (open circles) incubated in an enclosed container.

The decreased activity within the film tubes is due to physical diffusion of tritium out of the tube and not changes in efficiency from scintillator permeation of the tube wall (figure 2). Unspiked tubes progressively increase in tritium activity during incubation with spiked tubes.

As the percent water in the liquid scintillation solution is increased from 0.5 to 30, the half-time for tritium loss from film tubes containing either Liquiscint or TT-21 increases (figure 3). At water concentrations greater than 30%, little additional increase in half-time was observed. Although not evident in figure 3, because of the short half-times, the relationship between half-time of tritium loss and percent water is similar for air exposed scintillator solutions and film tubes (i.e. the half-time increases up to a plateau around 30% water). The increase in half-time (and decrease in tritium efflux rate) from 0 to 30% water presumably is due to the decrease in specific activity in the water micelles and in the water vapor phase. The relative stability in half-time at water concentrations greater than 30% is unexplainable although it may reflect alterations in the gel matrix.

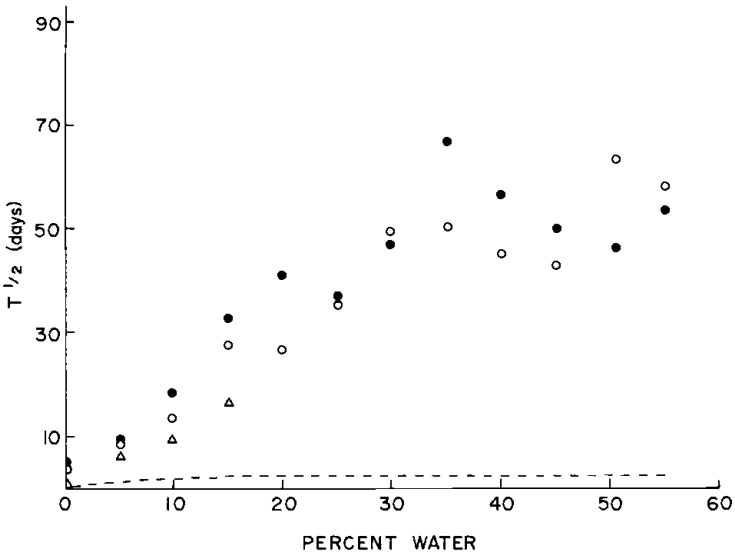


FIGURE 3. Half-time for THO loss from sealed film tubes (symbols) or uncapped vials (dashed line) as a function of the percent water in the scintillator (total volume constant) and scintillator composition. Solid circles, Liquiscint; open circles, TT-21; triangles, Ready-Solv HP.

The absolute water loss (M/l) from either Liquiscint or TT-21 in air increases linearly as percent water increases up to 30% (not shown). Absolute water loss tends toward a plateau between 30 and 55% water. Horrocks (1976b) has shown that in toluene based solutions, as the water content increases from 0 to 15-25% the number of water micelles increases whereas their size remains constant. This will produce a linear increase in total micellar surface area which in turn will linearly increase total water efflux out of the solution as evidenced in the present experiments. If the volume of the micelles increases, the surface area/volume ratio decreases and less surface area relative to total water volume will be available for water vapor exchange. This could account for the plateau above 30% water. However, other factors such as changes in the nature of the phase contact between the solvent and water and/or restriction of water mobility in the more rigid gel matrix may also be significant in limiting water efflux (Benson, personal communication).

Figure 4 shows the changes in activity of film tubes containing either THO or tritiated biomolecules. Activities of both adenosine (2- 8^3H) and uridine (5- 3H) remain constant

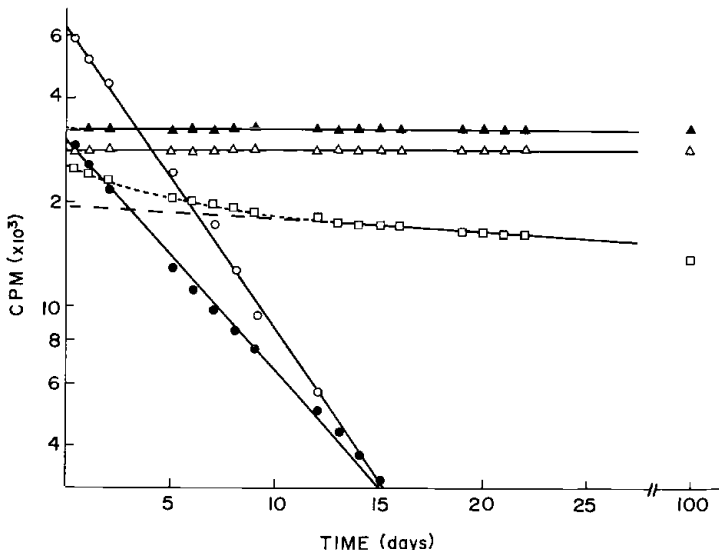


FIGURE 4. Stability of tritiated biomolecules in sealed polyethylene-film tubes. Solid triangles, adenosine (2,8- 3H); open triangles, uridine (5- 3H); open squares, guanosine (8- 3H); solid circles, tritiated water; open circles, extrapolated tritiated water loss from guanosine (displaced upward by a factor of 10).

for the 100 day counting period where as guanosine ($8\text{-}^3\text{H}$) activity decreases with two apparent rate constants. Determination of the fast rate constant by extrapolating the slow constant back to time 0 (dashed line) and subtracting this from the actual counts (dotted line) shows the rate of this component (open circles, displaced by a factor of ten) to be very similar to the efflux rate of THO (solid circles). The slow component is the exchange rate of tritium for hydrogen on the guanosine molecule and has a half-time of 324 days. These results demonstrate the labile nature of tritium in biomolecules and its ultimate release into the environment probably as tritiated water. The rate of environmental contamination is greatly accentuated by the use of the highly permeable film tubes.

The high degree of THO permeability of the polyethylene film-tubes suggests a practical application. It would seem feasible to routinely monitor the tritium exchange of many tritiated molecules by sealing the molecule of interest with a sol-gel scintillator in a film tube and periodically counting the sample. If two decay components exist (as in figure 4) the fast component identifies the degree of tritium exchange prior to use (i.e. indicates the amount of THO in the sample) while the slow component reflects the rate of tritium-hydrogen exchange. The aqueous environment of the micelles should provide a satisfactory solvent for many of the commercially available tritiated biomolecules.

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