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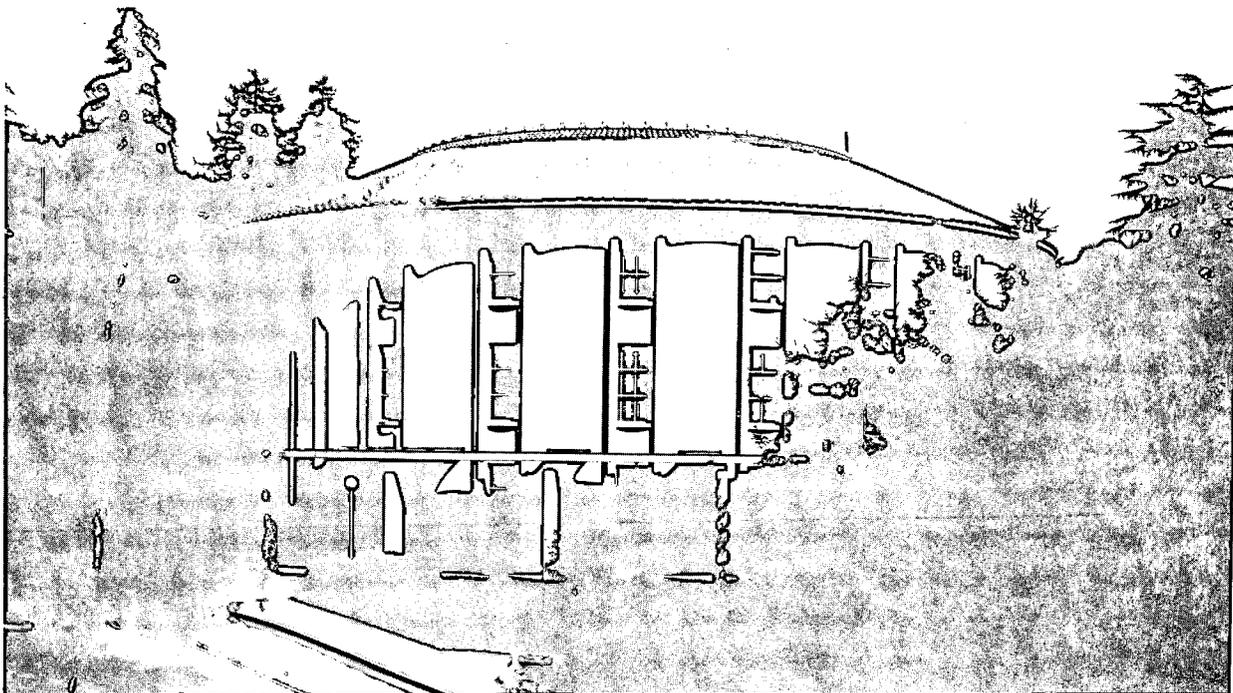
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H. Morimoto and P.G. Williams

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Design and Operations at the National Tritium Labelling Facility

Hiromi Morimoto and Philip G. Williams

National Tritium Labelling Facility, Chemical Biodynamics Division, Lawrence Berkeley
Laboratory, University of California, Berkeley, CA 94720, U.S.A.

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DESIGN AND OPERATIONS AT THE NATIONAL TRITIUM LABELLING FACILITY

Hiromi Morimoto and Philip G. Williams
National Tritium Labelling Facility, Chemical Biodynamics Division
Lawrence Berkeley Laboratory, University of California
Berkeley, California 94720.
Phone (510) 486-4373, Facsimile (510) 486-4877

ABSTRACT

The National Tritium Labelling Facility (NTLF) is a multipurpose facility engaged in tritium labelling research.¹ It offers to the biomedical research community a fully equipped laboratory for the synthesis and analysis of tritium labelled compounds. The design of the tritiation system, its operations and some labelling techniques are presented.

INTRODUCTION

The National Tritium Labelling Facility was established at Lawrence Berkeley Laboratory in 1982 and is funded by the National Institutes of Health. Its aims are to investigate and promote tritium labelling and analysis, to provide a high level labelling facility for long term collaborative projects, to provide a tritium labelling service to the biomedical community, and to educate and train investigators in the safe use of tritium.

TRITIUM LABORATORY AND OPERATIONS

The tritiation laboratory is a 5x8 m room equipped with a central tritium source box, flanked by two tritiation reaction manifold boxes, each accessible via a pass-through window to its own workup box. Exhaust air from the workboxes is passed through a 10x24 cm column of 40 mesh silica gel to adsorb tritiated water. At a flow rate of 0.28 m³/min, this column retains tritiated water for approximately one hour before break-through.² After passing through the silica gel, the total effluent from the workboxes is exhausted to a common high flow stack (283 m³/min, 10,000 cfm). A diagram outlining airflow is given in Figure 1. Both room and total stack effluent concentrations are sampled and measured by dedicated ion chambers, and recorded on a continuous strip recorder.

Inputs to four other ion chambers can be directed to the process and are used for real time process monitoring. With these six ion chambers the experimenter may independently monitor tritium concentration in the room, in total effluent, and in two workboxes before and after removal of tritiated water by the columns of silica gel.

Tritium gas is purchased from EG&G Mound Applied Technologies, Miamisburg, Ohio, in 10,000 Curie lots (ca. 1 g), bound as UT₃ on a uranium bed. It is liberated, measured, and transferred onto a smaller uranium bed (capacity 25,000 Ci, see Figure 2) for storage. Before use, the radiolysis product of tritium, ³He, is evacuated from the container while it is at room temperature. Heating of the bed to 350°C causes desorption of the tritium gas; cooling of the bed to room temperature causes rapid readsorption.

The tritium gas liberated by heating the UT₃ bed is passed into the tritiation system (Figure 3). This system is an all stainless steel manifold, comprised of welded 6.35 mm (0.25 inch) 316 stainless steel tubing (1.65 mm or 0.065 inch wall), and 6.35 mm stainless steel Nupro bellows valves. It has connections to inert gases, to a tritium-hydrogen mixture, and to two reaction ports for tritium chemistry. The standard Nupro valve tips have been replaced with stainless steel tips, and the ring gaskets replaced by flat copper gaskets. The tritiation system is modular in design to expedite replacement of worn or non-functional parts, and the modules are connected by Cajon VCR fittings. Vacuum is maintained by two turbo-molecular pumps, and by three oil free pumps, each of the latter having diaphragm and molecular drag stages. The turbo-molecular pumps are calibrated with Pirani gauges.

For tritiation reactions, tritium is used in the form of tritium gas, as highly tritiated water (T_2O), or as other useful labelling reagents. Accordingly, the tritium trapping system must take account of tritium in all these physical and chemical forms.

Tritium that remains unused during chemical reactions is trapped and recovered during a multistage process (Figure 4). First the reaction flask is frozen in liquid nitrogen to remove tritiated water and other solvents from the gas phase, and the free tritium gas is adsorbed onto a charcoal bed held at $-196^\circ C$. The capacity of cold, 14 mesh, activated coconut charcoal (Fisher Scientific) for adsorption of hydrogen gas is very large: in this case 3 g of

charcoal at liquid nitrogen temperature adsorbs 270 cm^3 of H_2 , the equivalent of ca. 700 Ci of tritium gas. Note that in preliminary studies with H_2 , evacuation of the cooled charcoal container caused removal of approximately one third of the adsorbed hydrogen. In the second stage of T_2 recovery the charcoal bed is isolated from the tritiation vessel and warmed to room temperature. This results in complete desorption of the tritium gas, which is then adsorbed onto the secondary uranium bed. Our initial studies show that more than 90% of tritium from reactions may be recovered in this manner, and captured on the secondary uranium bed (U bed B). This tritium is sent to EG&G Mound Applied Technologies for purification and reuse as 100% T_2 gas.

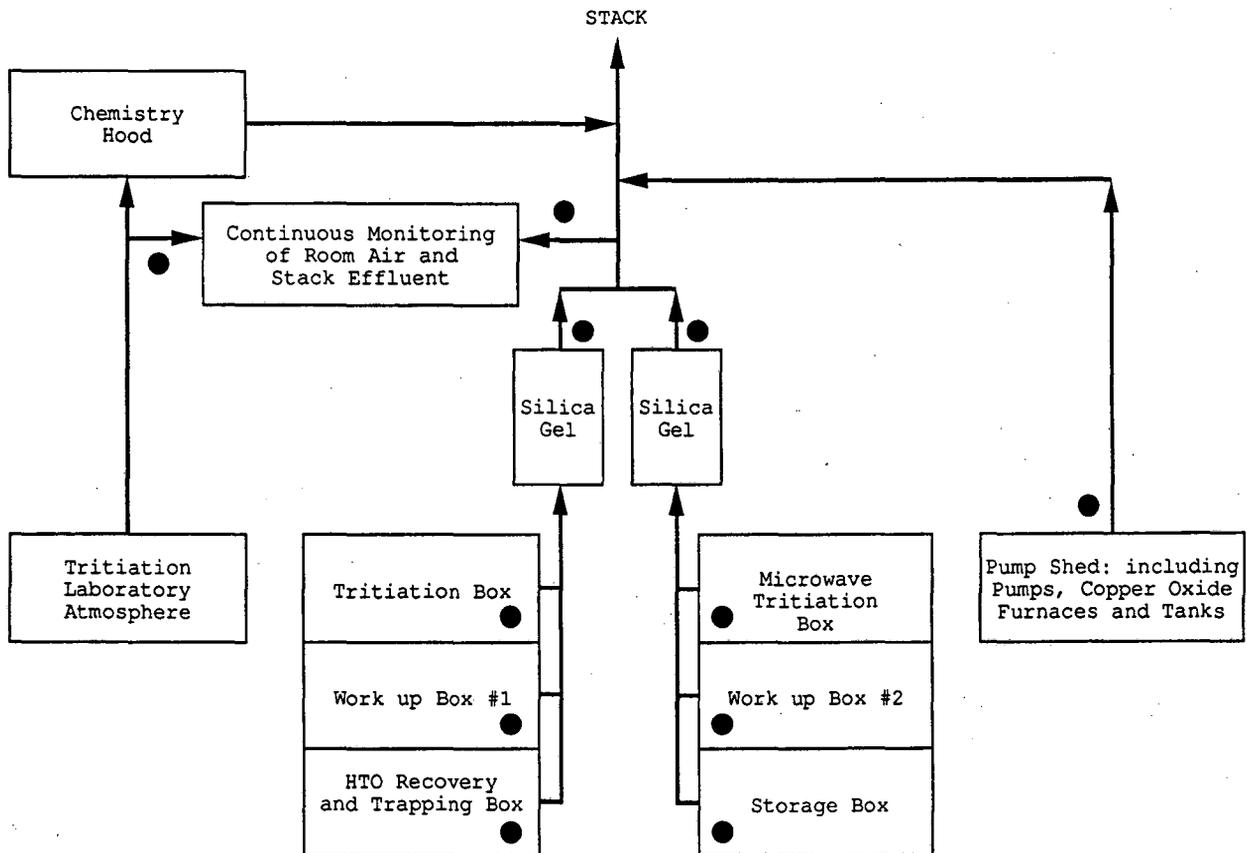


Figure 1: Schematic of the airflow and monitoring points in the labelling laboratory at the NTLF. Monitoring points are indicated by filled circles. The tritium level in the laboratory air and in the stack effluent are continuously recorded on a strip chart.

WASTE HANDLING

The waste tritiated water, solvents, and other reactants are condensed in a liquid nitrogen cooled trap, then the gas stream is passed to the tritium trapping system. This is a duplicative system that allows regeneration of one loop while the other is being used. After the gas stream is collected in a 0.05 m³ cylinder, overnight recirculation of the cylinder contents through a copper oxide tube heated to 600°C converts any gas to tritiated water, and this HTO is condensed by means of an in-line Dewar cooled to - 30°C.

The requirements for waste disposal are thus limited to low level waste in the form of tritiated water, solvent and side products. The tritiated water from the recirculation system is of too low a level and quantity for reuse.

The change over from oil vane pumps to turbo-molecular pumps has eliminated the large reservoir of volatile tritium in oil as a potential source of tritium release, and the necessity for disposal of highly tritiated pump oil as mixed waste.

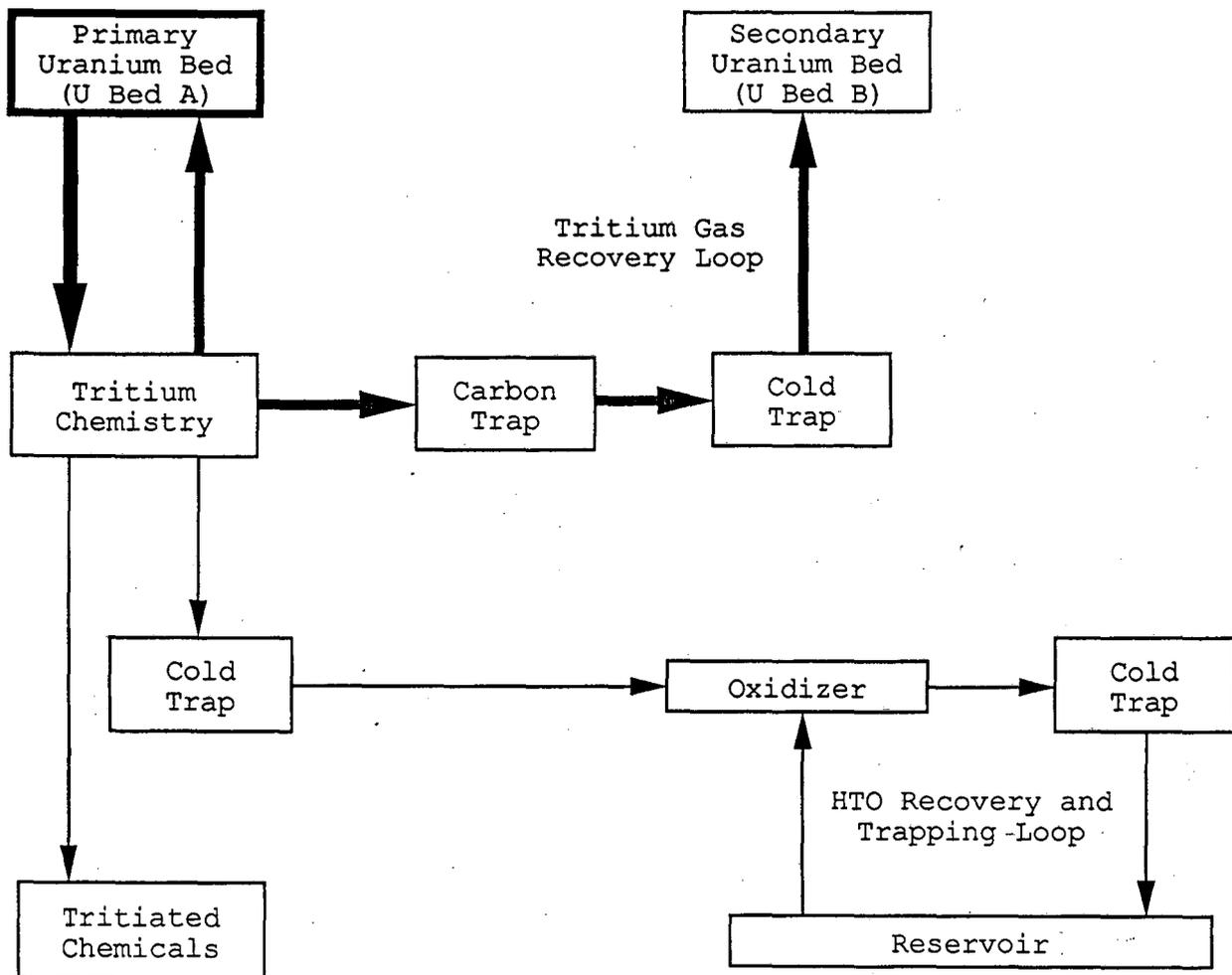


Figure 4: Schematic of the use and recovery of tritium gas and tritiated solvents.

LABELLING CHEMISTRY AND ANALYSIS

Tritium labelling to high specific activity (Table 1) has been limited primarily to addition of tritium to double bonds, and to tritiodahalogenation. Recently the NTLF has developed new tritide reagents at full specific activity,³ i.e. lithium triethylborotritide (Supertritide), lithium tri-sec-butyl borotritide (Selectride), and lithium aluminium tritide (LiAlT₄). These have been shown to be selective yet highly reactive reagents, transferring their full specific activity to the products.

In addition to the development of labelling reagents and methodologies,³⁻⁶ the NTLF offers the biomedical community a fully equipped and safe laboratory for the synthesis of tritium labelled chemicals. Since its inception in 1982 the Facility has hosted 122 visits by 104 scientists who have labelled 249 compounds for biomedical research. Also, the NTLF staff conduct long term collaborative projects such as the direct measurement of acid or base catalyzed

exchange rates in a mixture of compounds,^{7,8} the measurement of the Swain-Schaad exponent for the base catalyzed enolization of acetone,⁹ and the interaction of ligands with proteins such as maltose binding protein¹⁰ and α -chymotrypsin.¹¹

CONCLUSION

The NTLF is a unique facility that promotes the use and analysis of tritium. It offers its expertise and technology for tritium labelling of chemicals in a safe and well equipped laboratory. Its multipurpose program provides a User service facility, engages in long term collaborative projects, and has developed a number of new labelling methods.

ACKNOWLEDGEMENTS

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TABLE 1: Reagents, Precursors, Products and Specific Activities for Common Tritium Labelling Techniques.

REAGENT	PRECURSOR	PRODUCT	SPECIFIC ACTIVITY (Ci/mmmole)
Tritium gas	unsaturated	saturated	57
	aryl halides	dehalogenated	28
	sugars	C-1 labelled	3-15
	benzylic compounds	benzylic- ³ H	3-15
Tritide reagents ³	esters	alcohols	28-57
	epoxides		
	aldehydes		
	amides aryl & alkyl halides	amines dehalogenated	
Methylene diiodide ⁴	unsaturated	cyclopropyl	low
Methyl iodide ⁵	nor-precursor	methylated	60-80
Tritiated water ^{6,5}	exchangeable	exchanged	up to 25
	lithiated		

⁵ Tritiated water may be formed by the reaction of tritium gas and PtO₂. NMR analysis of a reaction using 22 μ moles of PtO₂, showed that the three water species were formed in the following ratio; T₂O / HTO / H₂O = 67 / 23 / 10. The calculated specific activity of the tritiated water was 42.1 Ci/mmmol (T₂O = 57.5 Ci/mmmol), or 73% of the maximum theoretical level.

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