

# A Routine, Automated Synthesis of Oxygen-15-Labeled Butanol for Positron Tomography

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The use of labeled butanol for autoradiographic and positron tomographic measurement of cerebral blood flow has been well established using radiocarbon labels. The advantages of the short half-life of oxygen-15 ( $^{15}\text{O}$ ) in doing sequential flow studies are also recognized. An automated procedure has been developed for the routine rapid and sequential synthesis of  $^{15}\text{O}$ -labeled butanol in amounts and with purity suitable for use in positron tomography. Butanol can now replace  $^{15}\text{O}$ -labeled water, which is commonly used for routine applications. The  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction is used, with 8 MeV deuterons on a nitrogen target containing 0.2% oxygen. Labeled oxygen is reacted with tri-n-butylborane by passing the gas over an alumina support which holds the reagent. Washing with water through small C-18-bonded phase silica cartridges eliminates labeled water and the majority of boron-containing impurities. Injectible labeled butanol is collected at 2.5 min after the end of bombardment. The yield is 6 mCi per microampere of saturated bombardment, measured at the end of synthesis. Injectible product up to 250 mCi can be obtained at 10-min intervals.

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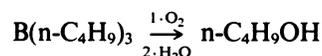
Labeled butanol has been shown to be a more accurate regional cerebral blood flow tracer than labeled water (1-8). However, a very short-lived tracer is required for routine clinical positron tomographic (PET) studies. The 2-min half-life of  $^{15}\text{O}$  allows studies at 10-min intervals and reduces radiation dose. Therefore, sequential studies with improved accuracy would be achievable with  $^{15}\text{O}$ -butanol.

We previously described (9) a labeling procedure based on the work of Kabalka (10,11). A 50/50 product mix of  $^{15}\text{O}$ -butanol and water was obtained in 40% chemical yield. Subsequently, Ido reported (12) the use of C-18 cartridges for both reagent support and purification with similar results.

While reinstalling a butanol synthesis, we had diffi-

culty reproducing product yield and purity using both methods. This was undoubtedly due to particulars of the synthetic apparatus, but indicated a fundamental difficulty with the technique. We describe here a new and automated method and examine the effects of several parameters on the synthesis.

The synthesis uses the published reaction (9-11):



The reaction is exothermic and very rapid. This new method incorporates the suggestion of Ido (12) to use a solid support to allow higher flow rates. Rapid washing with water completes the hydrolysis and washes the butanol onto a pair of C-18 cartridges for  $^{15}\text{O}$  water removal. Butanol (radiochemically 99+% pure) is then eluted with 10% ethanol in saline. Up to 250 mCi of butanol can be produced.

## MATERIALS AND METHODS

Tri-n-butylborane (Aldrich or Alfa) was stored under dry argon. Silica, alumina, and C-18 Sep-Paks (Waters) were prepared by flushing with dry argon. Silica and alumina were obtained from Universal Scientific and Aldrich. A Hewlett-Packard 5890 gas chromatograph with an Alltech RSL-150 capillary column was used with flame ionization detection. Conditions: 70°C, He flow 5 ml/min. Retention times: water and EtOH, 0.69 min; sec-butanol, 0.97 min; n-butanol, 1.3 min. A Spectra-physics 4270/8700 HPLC was used with an Alltech Econosil C-18 column, 15%  $\text{CH}_3\text{CN}/0.01\text{ M}$   $\text{NH}_4\text{OAc}$ , 2 ml/min, with Knauer refractive index and Beckman 170 radioactivity detectors. Retention times of radioactive products were: water, 1.45 min; sec-butanol, 4.4 min; and n-butanol, 5.7 min. Sterility and pyrogen tests were performed according to U.S.P. procedures.

## Preparation and Synthesis

The alumina cartridge was injected with 75  $\mu\text{l}$  (0.3 mmol) tri-n-butylborane under dry argon. It was sealed with sleeve-septom stoppers and stored under argon.

Previously used cartridges can be reused up to ten times. They are recycled by washing with ethanol and acetonitrile and drying in vacuum. Old cartridges are eventually discarded due to mechanical weakness.

Oxygen-15 was produced by the  $^{14}\text{N}(\text{d},\text{n})^{15}\text{O}$  reaction with 8 MeV deuterons on nitrogen gas containing 0.2% oxygen.

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The target (0.25 l) was pressurized to 6 atm and holds ~0.15 mmol of oxygen.

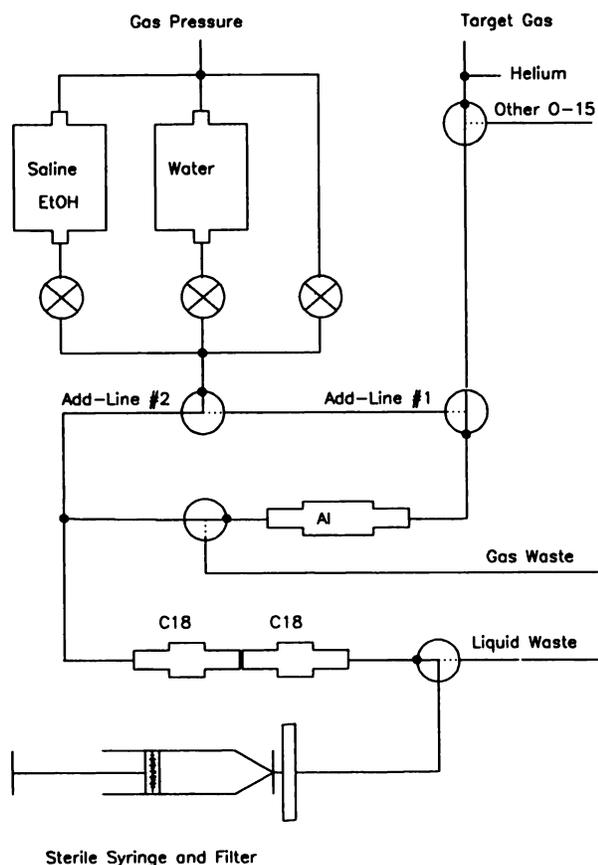
The automated synthetic system is shown in Figure 1. Two reverse-phase cartridges were joined with a short glass tube and placed in the helium-purged system. The reagent cartridge was installed at the end of bombardment, minimizing exposure to air.

Target gas was emptied through the reagent in 30 sec at 3 l/min, using an additional pulse of gas to flush the system. Water (1 ml) was then injected (Line 1) onto the reagent cartridge. After 3 sec, it was followed by 3 ml of water and 5 ml of air, moving butanol from the reagent to the C-18 cartridges. The C-18 material was then washed (Line 2) with 3 × (0.5 ml water + 10 ml air). Oxygen-15-butanol was then eluted through a sterilizing filter with 5 ml of 10% ETOH/saline. The product was obtained within 2.5 min after the end of bombardment.

All variables in the procedure were investigated in over 400 experiments. We report here those experiments which represent alterations of the above optimum procedure.

## RESULTS

Table 1 summarizes the results obtained using the optimum procedure in a routine clinical setting. A 10-min bombardment of 15 microamps typically produced ~400 mCi of oxygen at end of bombardment (EOB). From this amount, over 100 mCi of injectable butanol



**FIGURE 1**  
Butanol synthesis apparatus.

**TABLE 1**  
Results Obtained Using the Optimum Procedure\*

Results	Range	Best
Trapped O <sub>2</sub> activity	250-300	330
Chemical yield (%)	80-90	94
Radiochemical yield (%)	16-25	28
% Activity retained on alumina	15-18	7
% Activity to waste (H <sub>2</sub> O + BuOH)	10-15	6
% Activity retained on C-18	10-12	8
% Recovered injectable BuOH	55-65	84
mCi Recovered injectable BuOH	50-70	84

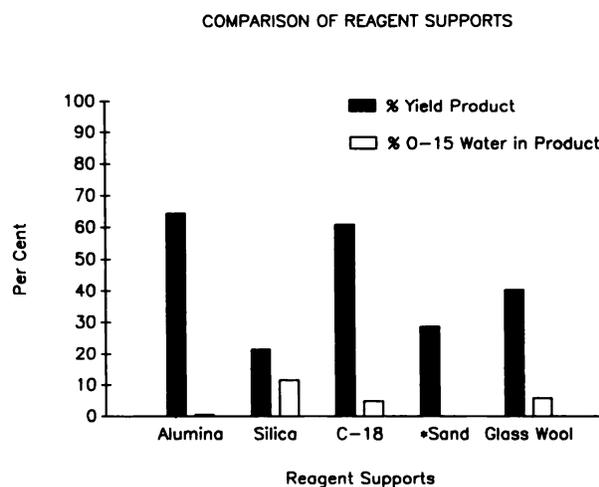
  

Analysis	Range	Best
% Water in product (labeled)	0-0.20	
% Sec-Butanol in product	4-6	NA
Total butanol in product (mg)	19-30	8.5
Solid residue (boron compounds) (μg)	40-200	0

\* For each parameter measured, the normal range experienced in over 200 preparations is given as well as the best result obtained. In each case, the bombardment used was 15 μA for 10 min. Maximum practical beam current is 40 μA.

was prepared within 3 min. This product contains acceptably low levels of boric acid and carrier butanol and is sterile and pyrogen-free (30 tests). Our maximum routine beam current (40 μA) gives a maximum yield of ~250 mCi of injectable butanol.

An alumina bed containing 0.075 ml tri-n-butylborane provided the highest yield and purity of <sup>15</sup>O-butanol. The cartridge could be stored several weeks under argon but only a few days in room air. Silica, C-18 bonded silica, glass, wool, and sand were not as effective as reagent supports (Fig. 2) Alumina dried at 200°C



**FIGURE 2**  
Performance of various reagent supports. For each support (n > 20; except sand, glass, wool, n = 3), the chemical yield of injectable butanol based on starting oxygen, and the percentage of water impurity in that product is shown. The described optimum procedure (age, load, etc.) was used in each case with the exception of the reagent support. \*In the case of sand, the purity was not determined.

under vacuum trapped as much as 40% less oxygen and gave a lower conversion to butanol. Water content on the alumina between 5% and 20% gives optimum performance, with no clear preference within that range. Untreated chromatographic alumina ( $\gamma$ ) falls within this range and can be used as delivered. Neutral alpha alumina performed in a similar fashion, but an additional 25%–30% of the activity was lost as labeled water waste. Similarly, acidic  $\gamma$ -alumina sent more activity to waste, and basic  $\gamma$ -alumina was less efficient at trapping oxygen.

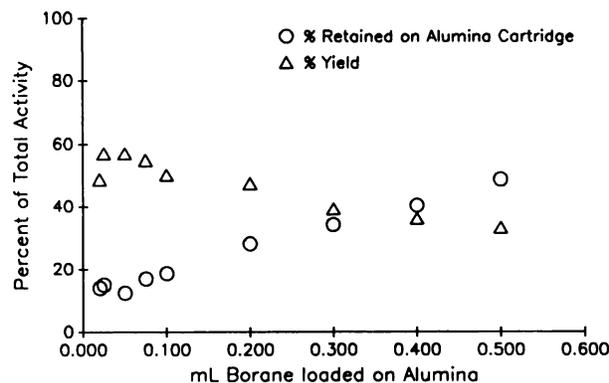
In order to reduce the carrier butanol level, we studied the effects of the oxygen content of the target gas. A maximum yield of 50% of butanol was obtainable from 2% oxygen in the target, with the remainder found as labeled water. One percent oxygen gave up to 90% butanol yields. The minimum concentration tested was 0.2% oxygen which, like 0.5% and 1% oxygen, gave excellent yields. The entire target yield was consistently trapped on the reagent even at the highest gas flows obtainable. This allowed the  $^{15}\text{O}$  to be collected in as little as 20 sec.

Figure 3 shows the effects of the volume of tri-*n*-butylborane loaded on the alumina, using 0.2% oxygen gas. Clearly, additional reagent causes additional activity to remain on the cartridge. Above 75  $\mu\text{l}$  (a small excess over a stoichiometric ratio with carrier oxygen), the yield decreases. Below this amount, the yield drops rapidly due to a deficiency of reagent relative to oxygen.

The age of the loaded alumina cartridges (time since loading) clearly affected several parameters. Yield, activity trapped at EOB, and conversion of trapped activity to butanol were all maximized at 24–72 hr after loading the reagent onto the cartridge. Loaded cartridges have remained usable 21 days after loading. Freshly prepared cartridges (aged 15 min up to 15 hr) do not show any other differences, aside from a significantly lower yield.

A significant amount of labeled butanol was consistently retained on the reagent cartridge. Only solutions with a basic pH removed it slightly better than the pure water reference, but the slight gain in yield was insufficient to offset the additional processing requirements imposed. Use of solutions containing oxidants, acids, or organic solvents and temperature variations from 0 to 70°C had no desirable effects on the results.

The optimum removal of butanol from the alumina occurs at a total wash volume of 2.0–2.5 ml. It was necessary to allow several seconds during the initial wash to hydrolyze the intermediate and release butanol. The optimal wash scheme, therefore, is a 1-ml wash, which is loaded onto the cartridge and allowed to stand several seconds, followed by a 3-ml water wash. The 10% of the total activity remaining on the reagent cartridge is high in labeled butanol (95%), but this was only removable with an organic solvent.



**FIGURE 3**  
Effect of borane load on the chemical yield (triangles) of injectable butanol (based on starting oxygen), and on the amount of radioactivity retained on the alumina support (circles). The optimum wash procedure (see text) was used in each case. Each point,  $n = 10$ , averages shown.

The function of the C-18 cartridges is to hold the labeled butanol so it can be washed free of labeled water. Two cartridges were necessary to avoid premature removal of the butanol while washing it. Butanol was retained on a double cartridge while labeled water was reduced to <0.4%, and often to zero. Two 0.5-ml portions of water, each followed by an air bolus gave an acceptable level of labeled water (1.5%) in the product. We preferred to use three portions, which reduced labeled water to 0%–0.3% at a cost of an additional 7% of the product being lost to waste. It was necessary to prevent the C-18 cartridge washes from passing through the reagent cartridge. This was due to residual water on that cartridge which slowly elutes, raising product water content to a constant 2%.

## DISCUSSION

### Role of the Alumina Support

The use of alumina as a solid support for tri-*n*-butylborane is a major modification over our previous method, raising the chemical yield of butanol from 50% to 80%–90%. Therefore, the alumina is acting not only as a support but is also taking part in the chemistry. This is supported by the observation that optimum performance is reached 15 hr after reagent loading. Modifications of borane reactivity by alumina, silica, and florisil supports has been attributed to Lewis acid properties of the supports (13).

The need for an aged reagent cartridge of hydrated gamma alumina requires that alumina is directing the hydrolysis step of the reaction. This is consistent with the accepted concept (14–16) that a somewhat random distribution of ionic sites is present on the surface of gamma alumina. This distribution allows groups of anionic acid and cationic clusters to form catalytic sites. Adsorption of water modifies the arrangement of sites by addition of hydroxyl ions, which explains the need

for a certain amount of water. This also explains the observation that only gamma alumina is active in directing the butanol synthesis. Alpha alumina, silica, and the other supports which were tested either do not have such sites or have them distributed in a more regular pattern which would preclude the formation of catalytically active clusters. While we have not tested this hypothesis, this alumina model does provide a basis for understanding the otherwise peculiar set of observations.

Very little boron is found in the crude product, which indicates it is not readily washed off of the alumina. Also, the only boron compound isolated from the system was boric acid,  $B(OH)_3$ . The  $BuB(OH)_2$ , which was observed in abundance during previous work (9), either was not stable using this method or was not removed from the alumina.

Reduction of the oxygen content of the target gas (from 2% to 0.2% in nitrogen) is another significant improvement over the original method. Although alumina directs the reaction toward butanol, this does not occur when more than 1% oxygen (1.5 mmol) is used with a corresponding amount of borane reagent. Reduction of oxygen also allowed the borane quantity to be reduced (Fig. 3), and thereby reduced the boron and carrier butanol in the product.

#### Clinical Utility of the Method

A clinical method must be fast, easily repeated within 10 min, and give over 100 mCi of butanol. The use of an alumina support, the low oxygen content in the target gas, and a rapid purification were very important in achieving that goal. In applying this procedure to clinical production, it is important to take advantage of each of these factors. In our hands, the maximum production yield of 250 mCi is then comfortably in excess of the clinical requirement. The synthesis requires as little as 2 min, and cleaning and set-up of the manual procedure can be performed within the preferred 10-min delay between blood flow measurements. The automation of the procedure speeds both of these aspects of the synthesis. All cartridges and solutions can be prepared in advance, and as noted above, reagent cartridges prepared well in advance give significantly higher product yields.

#### Product Composition

Analyses by radio-HPLC of the crude product showed three labeled compounds, water, n-butanol, and sec-butanol (Table 1). Water was removed during the procedure. Sec-butanol comprised 4%–6% of the total product butanol activity. It originated from sec-butyls present in the tri-n-butylborane and was not separable from the n-butanol without HPLC. Since the utility of the radiopharmaceutical as a blood flow tracer is dependent primarily on its lipophilicity (5,7,17), the presence of sec-butanol as a minor impurity would not be

expected to significantly alter the measurements and was therefore considered to be acceptable.

The yield and the product composition are significantly affected by small changes in the procedure. The optimum reagent and wash volumes may vary with the target gas, apparatus size, and flow rates. The data given here can be used, however, to adjust the conditions for optimum performance with any particular apparatus. Minimum wash volumes, optimum reverse-phase material load, and the optimum compromise between yield and purity are easily determined. In this procedure, we have opted for very high radiochemical purity, allowing nearly 40% of the total butanol to be wasted.

The quantities of the nonradioactive impurities present in the product carrier (butanol and boric acid, Table 1) are well below any physiologically active dose. For comparative purposes, the toxic doses of butanol and of boric acid are in the range of grams to tens of grams, and normal serum contains 0.1–0.2 mg of boron per deciliter (18–22).

#### Automation

This synthesis was originally performed, and most of the data obtained, with a manual system in which additions were made by syringe though the two addition lines (Fig. 1). However, it was a simple matter to automate the system as shown. A regulated gas supply is used to drive water and the 10% ETOH/saline solution through the apparatus, as well as provide the necessary bursts of air. Solenoid valves are used to dispense air and fluids and direct their flow through the apparatus. Air and fluid volumes are measured by timing the period that the dispensing valve is open. The exact timing is determined empirically, and depends on the gas pressure, and tubing lengths and diameters. Aside from the obvious advantages of automation, this approach also speeds the set-up between clinical studies by eliminating the need to measure the fluid injections.

#### CONCLUSION

Oxygen-15-labeled butanol can be produced from tri-n-butylborane in amounts exceeding that necessary for routine PET use. A 10-min, 8 MeV deuteron bombardment at 15  $\mu$ amps can be expected to produce 100 mCi consistently. The maximum routinely producible quantity using our cyclotron is 250 mCi. The synthesis is sufficiently rapid and simple that sequential doses may be prepared at 8-min intervals with a manual or automated system. The actual synthesis and purification requires up to 3 min. The purity and toxicity of the product are well within prudent limits. This method represents a significant advance in simplicity, speed, and reliability over our previously published approach.

#### ACKNOWLEDGMENTS

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