PREPARATION OF FLUOTHANE-^{82}Br BY MEANS OF RECOIL LABELING

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Received on April 3, 1974.

SUMMARY

Fluothane (1, 1, 1-trifluoro-2-chloro-2-bromoethane) is a well known anesthetic, and its labeled compound has been much required for the biological investigation. The method was studied to prepare fluothane-^{82}Br by means of recoil labeling. More than 20 organic species labeled with bromine-82 evolved in neutron-irradiated fluothane. From such the complicated system, however, fluothane-^{82}Br was purely obtained by the gas chromatographic separation. Under the optimum conditions, the radiochemical yield of fluothane-^{82}Br contributed to 38 %.

When 200 μl of the target material was irradiated with neutrons to the total neutron dose (nvt) of 3.4 x 10^{16}n/cm^2, the radioactivity of ^{82}Br-labeled fluothane isolated almost quantitatively was about 2.7 mCi.

INTRODUCTION

Fluothane(or halothane, 1,1,1-trifluoro-2-chloro-2-bromoethane)

is a volatile anesthetic and its metabolism has been interested in the flield of biology.[1,2] For the investigation of the behaviors of anesthetics in vivo, fluothane labeled with radioisotopes has been much required. Of many radioisotopes, tritium, carbon-14, chlorine-36 and bromine-82 are suitable to be used as a tracer.

Among them, carbon-14 and chlorine-36 were already referred to the literature,[3] in which various fluorinated anesthetics containing ^{14}C- and ^{36}Cl-labeled compounds were reviewed. In some cases, fluorine-18 is watched with keen interest.

When tritium, carbon-14, and chlorine-36 were used for the preparation ove labeled compounds,[4,5] chemical synthesis takes commonly precedence of other methods. On the contrary, the preparation of ^{18}F- and ^{82}Br-labeled compounds needs more convenient methods rather than chemical synthesis. The nuclear-recoil labeling is one of the convenient methods.

The authors intended to produce ^{82}Br-labeled fluothane by means of nuclear-recoil labeling. The first requisite of this method was that the radiochemical yield was not so low, and the procedure must be simple and completed in a short time. Fortunately, we had been already examined the technique, by which gaseous and liquid compounds were successfully isolated. In this report are described the irradiation and separation conditions, the apparatus for the production, and other additional problems.

EXPERIMENTAL

Irradiation.   1,1,1-Trifluoro-2-chloro-2-bromoethane, fluothane, from Takeda Chemical Industries, containing 0.02 % of thymol was distilled under the reduced pressure before use. In order to determine the optimum conditions of this preparation, the target material (1-10 µl) was sealed in a quartz ampoule in vacuo and then irradiated with neutrons. For the practical preparation of ^{82}Br-labeled fluothane, 100-200 µl of fluothane was irradiated.

These samples were irradiated in T-pipe of the JRR-4 reactor
of the Japan Atomic Energy Research Institute, operated at 2.5 MW.
The thermal neutron flux at the irradiation position was 2.8×10^{13}
n/cm^2·sec with approximate γ-ray dose rate of 1.0×10^8R/hr, and
the temperature was about 50°C. The neutron-irradiated samples
were left in the cooling water of the reactor (~20°C) for more than
20 hr.

Apparatus.   I) Radio-gas chromatograph.   Organic species
labeled with ^{82}Br were separated by the gas chromatographic set-up
consisted of a thermal conductivity detector (TCD) and a glass
column of 2.5 m length with the inside diameter (i.d.) of 5 mm con-
taining the column-packing "DOP" as a stationary phase. The column-
packing "DOP" was 60-80 mesh Chromosolb W coated with dioctyl
phthalate. The ratio of support to column liquid was 100/20.   It
had excellent stability and good selectivity for the separation of
poly-halogenated hydrocarbons, which arose in fluothane irradiated
with neutrons.

The irradiated sample to be analyzed was successfully intro-
duced into the column by means of the specially designed ampoule
breaker.   Inorganic species were adsorbed on the tube filled with
potassium ferrocyanide placed before the gas chromatographic column.
Helium was used as a carrier gas at a flow rate of 120 ml/min.   The
temperature at the TCD-cell was 115°C and that of the glass column
was kept at 60°C, being raised gradually up to 110°C after the
signal due to fluothane was observed.

The radioactivity of the gases leaving the TCD-cell was
detected with the NaI(Tl) detector (well type, 1.75 in x 2 in)
connected to a single-channel pulse height analyzer.   Radioactivity

signals were registered both on a ratemater, feeding a signal to a
two-channel recorder (the other channel giving the mass signal from
the TCD-cell) and on a digital printer.

II)   The apparatus for purification.   For the purpose of the
preparation of the labeled compound of which total radioactivity
varied between 1 and 4 mCi, the apparatus with radiation shielding
consisted of an ampoule breaker, a gas chromatographic set-up, and
an ordinary vacuum line.   The purification apparatus is schematical-
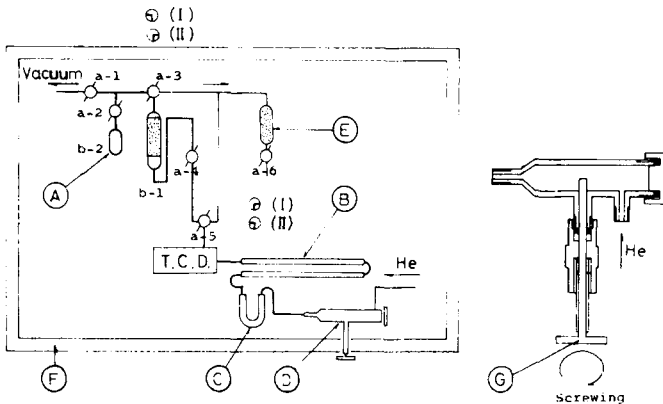ly shown in Fig. 1.   The ampoule breaker, the trap filled with

Fig. 1 Apparatus for the purification of fluothane-[82]Br.

A ; Vacuum manifold,   E ; Trap filled with activated carbon,
B ; Gas chromatograph,   F ; Pb-block with 5 cm thick,
C ; U-trap filled with potassium ferrocyanide, G ; Knob,
D ; Ampoule breaker,   : The irradiated ampoule was placed in the cylinder, and then
                           was broken by screwing down the knob (G).

potassium ferrocyanide, the trap filled with activated carbon, and
the gas chromatographic column were enclosed with Pb-blocks.   The
structure of an ampoule breaker, which is made of stainless steel,
is also shown in Fig. 1.   The ampoule breaker was placed in the
helium line leading to a gas chromatographic column.

The chromatographic column was made of 2 m length and 8 mm i.d. of glass tubing packed with DOP. This column was used at an ambient temperature, and the temperature around the TCD-cell was 100°C. A flow rate of a carrier gas was 100 ml/min. The purification apparatus shown in Fig. 1 was placed in a well ventilated hood.

Measurement. Chemical and radiochemical purity were determined by the radio-gas chromatographic analysis. The radioactivity measurements of the product were carried out with a ionization chamber, which was routinely used as a source calibrator in our laboratory. The observed values were calibrated by the standard source. The radioactivity of ^{82}Br-labeled product shown in this paper is corrected to the time just after the neutron irradiation. In identifying the species separated, a gas chromatograph and mass spectrometer, the GC-MS, from Japan Electron Optics Laboratory, at Miyagi University of Education was used.

Preparation. With the stopcocks in the position shown in Fig. 1, (a-3 and a-5 were fixed to the (II) position and a-4 was open), the helium carring gas flowed through the potassium ferrocyanide phase, the gas chromatographic column, the TCD-cell, the product acceptor, and the activated carbon phase. After the gas chromatographic column was conditioned at a working temperature, the carrier gas was by-passed by turning the stopcocks (a-3 and a-5) anticlockwise by 90°.

The quartz ampoule containing fluothane, which have been irradiated with neutrons and placed in the ampoule breaker, was broken by the manner as shown in Fig. 1. Inorganic species in the volatile components produced in the neutron-irradiated sample were

removed by potassium ferrocyanide in the U-trap (C), and then
organic species were introduced into the gas chromatographic column
with the carrier gas. When the mass signal of fluothane-[82]Br began
to appear, the stopcocks a-3 and a-5 were reversed to the former
position (II) clockwise.

Fluothane-[82]Br isolated was condensed in the trap b-1, which
was filled with glass-grains and cooled with liquid nitrogen. At
the completion of this elution peak, the stopcocks a-3 and a-5 were
turned to the position (I), and the stopcock a-4 was closed.
Subsequently the stopcocks a-1 and a-2 were open, so that the traps
b-1 and b-2 were evacuated. The stopcock a-1 was closed, and the
trap b-2 was cooled with liquid nitrogen instead of the trap b-1.
Through the above mentioned processing, fluothane-[82]Br was collected
successfully in the trap b-2, and the trap was sealed by the upper
end with a hand-burner.

RESULTS AND DISCUSSION

The effectiveness of neuclear-recoil labeling depends on the
nuclear character of the radioisotope and on the radiation resist-
ance of the compound irradiated. In contrast with the fact that the
nuclear character of [82]Br was suitable for the recoil labeling,
there are undesirable facts as to the radiation resistance of fluo-
thane. Our apprehension, that fluothane might be easily decomposed
by radiation during the neutron irradiation, was due to its photo-
sensitivity, because fluothane containing 0.02 % of thymol as a
stabilizer was usually placed in a colored bottle and stored in a
refrigerator.

From the results of the radio-gas chromatographic analysis of
neutron-irradiated fluothane, however, it was found that the chemi-

cal and radiochemical yield of fluothane-^{82}Br was rather higher than expected. Figure 2-a shows the radio-gas chromatogram of fluothane irradiated with neutrons for 30 sec. More than 20 elution peaks relating to the radioactivity of bromine-82 were observed in this chromatogram, and fortunately the peak area of fluothane-^{82}Br was the largest. The radiolysis of the parent molecule is an interesting
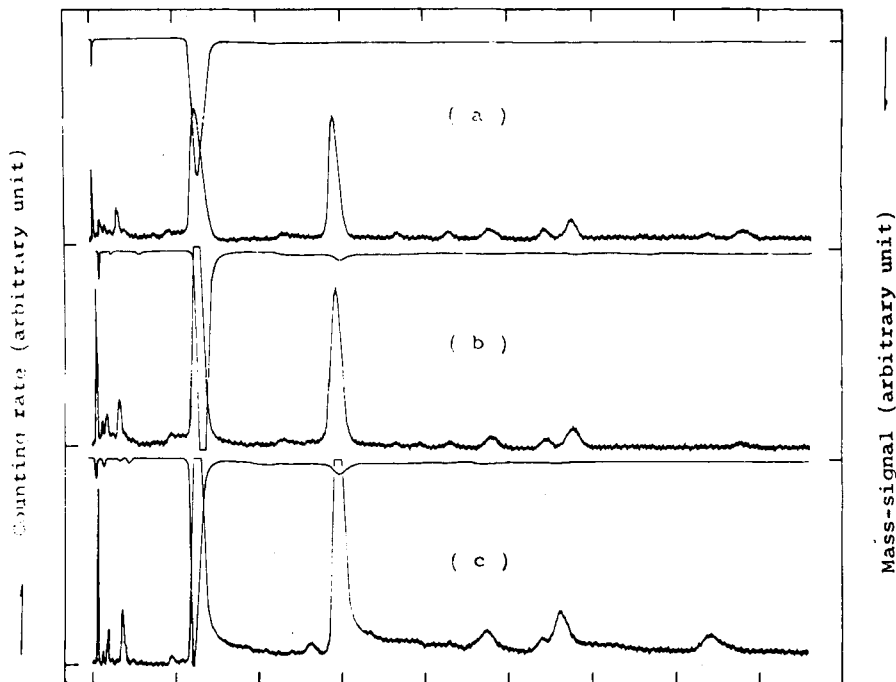

Fig. 2 Radio-gas chromatograms of fluothane irradiated with neutrons in the JRR-4 reactor for 30 sec (a), 20 min (b), and 1 hr (c).

subject, but details on this problem will be reported in the later date.[6]

Figure 2-b shows the radio-gas chromatogram of fluothane irradiated with neutrons for 20 min. The elution pattern of the radioactive fractions is identical with that of Fig. 2-a. In order to find the optimum condition of the neutron irradiation, the radio-chemical yield of fluothane-[82]Br was obtained as a function of the irradiation time in the JRR-4 reactor and is shown in Fig. 3. The yield of [82]Br-labeled fluothane was almost constant (37-38 %) at the irradiation time between 2 min and 3 hr, and decreased gradually above 3 hr. From the radiochemical yield of fluothane-[82]Br, its specific activity was evaluated with reference to each irradiation time. These values are also represented by a broken line in Fig. 3. According to Fig. 3, [82]Br-labeled fluothane with a specific activity of about 15 mCi/mmol will be obtained from target fluothane irradi-ated with neutrons for 3 hr.

The quantity of the complicated products formed by radiolysis and other processes increased in proportion to the irradiation time. As shown in Fig. 2, however, the elution peak of fluothane-[82]Br was isolated enough from the adjacent peaks. Fluothane-[82]Br isolated by the gas chromatographic technique was considered to be pure. The mass spectrum of the product isolated from the target materials, which was irradiated with neutrons for 1 hr and stored for 3 months at about 0°C, agreed well with that of pure fluothane.

Radiation dose became more serious problem for operations of the purification procedure with increasing [82]Br in the neutron-ir-radiated sample. When an ampoule containing 200 μl of the target materials was irradiated with the neutrons for 20 min, the radiation dose rate at the surface of the ampoule was about 2 R/hr even at the
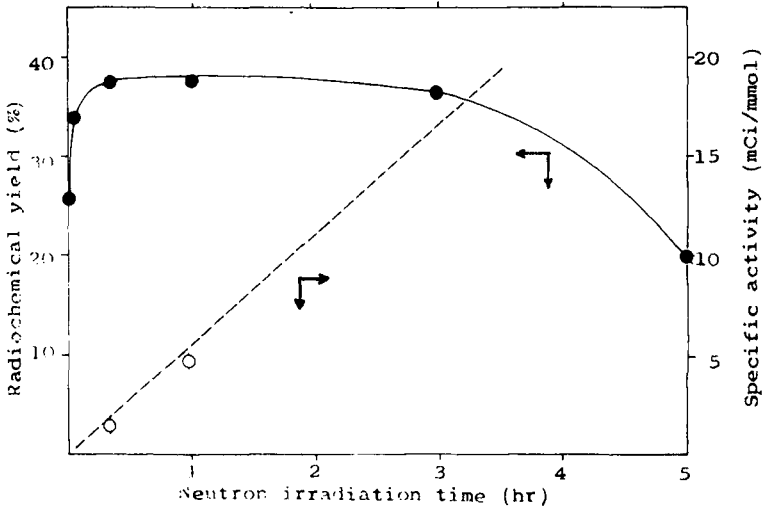
Fig. 3 Plots of the radiochemical yield of fluothane-^{82}Br (-●-)
        and the calculated values of its specific activity (----)
        versus the neutron-irradiation time.

time 24 hr after the irradiation. Since the apparatus used for the
preparation could be operated with the radiation sealding, the
radiation dose rate was lowered below 1 mR/hr at the operating area.
The largest radiation dose rate was observed about the fingers, but
it was 10 mR/hr at the largest. Judging from the fact that the
total time of the operation attended with some radiation dose was
only about ten minutes, the radiation dose during the preparation
needs not be worried about.

On the basis of the experimental results described above, the
preparation of fluothane-^{82}Br was examined for the practical use.
From the results of this experiment as shown in Table 1, it was
found that the radiochemical yield of ^{82}Br-labeled fluothane was
comparable to that shown in Fig. 3 (37-38 %), and that the specific

Kiyoshi Hoizumi, Terutomi Mogi and Hiroshi Kudo

Table 1. Preparation of ^{82}Br-labeled fluothane

Target (μl)	Irradiation time (min)	Total activity of ^{82}Br in neutron-irradiated target (mCi)		Fluothane-^{82}Br		
		Calcd.	Found	Chemical yield (%)	Radiochem. yield (%)	Specific activity (mCi/mmol)
100	10	2.0 ± 0.3	1.7	90	30	0.60
100	10	2.0 ± 0.3	1.6	97	32	0.59
200	0.5	0.15 ± 0.02	---	93	26 *	0.021
200	1	0.3 ± 0.04	---	90	24 *	0.039
200	20	9.7 ± 1.4	9.0	90	30	1.6

* These radiochemical yields were obtained by using the caluculated value as the total activity of ^{82}Br in stead of the observed value.

activity was comparable to the value (broken line) shown in Fig. 3. By the effect of the radiation, the parent molecule might be decomposed more or less, but the loss of fluothane due to the radiolysis within 10 or 20 min of irradiation time was almost negligiblly small and included in the separation loss. In the case of the sample irradiated with neutrons for 1 hr (nvt; $1 \times 10^{17} n/cm^2$) or more, a loss of fluothane due to the radiolysis might not be set aside. However, the radiochemical yield and the purity of fluothane-^{82}Br has a more significant meaning rather than the chemical yield. The chemical and radiochemical purity were determined by the radio-gas chromatographic analysis for the product, which was obtained from the sample irradiated with neutrons for 20 min in the JRR-4 reactor. The radio-gas chromatogram in Fig. 4 shows fluothane-^{82}Br to be of high purity above 99 %.

When 200 μl of the target material was irradiated with neutrons for 20 min, the total activity of fluothane-^{82}Br was 2.7 mCi. When
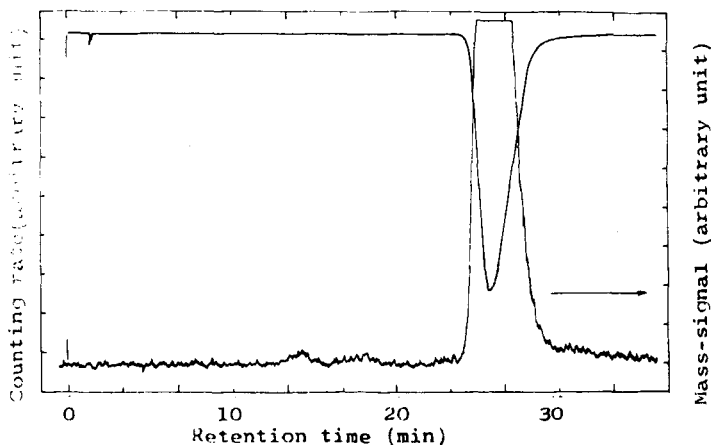

Fig. 4 Radio-gas chromatogram of fluothane-^{82}Br purely separated after being irradiated for 20 min.

1 hr of the irradiation time was applied for this case, the total activity of that might be 8 mCi. The experiment being designed by considering the nuclear character of the short-lived radioisotopes such as bromine-82 and fluorine-18, a close contact with users about their planning of the tracer experiment was very important. Ten millicuries of fluothane-^{82}Br with 0.1 mCi/mmol of specific activity is requested for the successful use in the biological test with tracers. If such a quantity of fluothane-^{82}Br exemplified above (specific activity, 0.1 mCi/mmol; total activity, 10 mCi) is necessary, about two times of this amount of fluothane-^{82}Br must be produced considering the time requiring from the neutron irradiation of the target material to delivery of fluothane-^{82}Br. In general, the labeled compound in small quantity and high specific activity are convenient for the purification. The production of about 20 mCi of fluothane-^{82}Br may be successfully performed by repeating the processing described in this paper two to three times.

Throughout this work we found out the interesting facts as follows. Two extremely large peaks were observed on every chromatogram in Fig. 2. The larger one of the two corresponded to fluothane-^{82}Br and its peak area became smaller than the other over 3 hr of the neutron irradiation. This ^{82}Br-labeled species, which was by-produced from target fluothane during the neutron irradiation, was found to be 1,1,1-trifluoro-2,2-dibromoethane (CF_3CHBr_2-^{82}Br) by means of the mass spectrometric analysis. 1,1,1-Trifluoro-2,2-dibromoethane, which is reported to be a complete anesthetic,[7,8] seemed to be more stable to radiation than fluothane. The specific activity of this ^{82}Br-labeled by-product was far higher than that of fluothane-^{82}Br. Judging from the elution pattern of the radio-gas chromatograms shown in Fig. 2, it was almost certain that

CF_3CHBr_2-^{82}Br might be also purely isolated. This ^{82}Br-labeled compound will be successfully used for the tracer experiment.

The detailed study of recoil labeling of fluothane with ^{82}Br during neutron irradiation will be reported in the near future.[9]

The authors' thanks are due to Professor S. Sasaki and Dr. H. Abe, Miyagi University of Education, for use of the GC-MS and their kind guidance. The authors also wish to thank Mr. T. Moriya of the Japan Atomic Energy Research Institute for his valuable suggestion and discussions.

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