

AN EVALUATION OF THE INTRODUCTION OF STABLE NUCLIDES OF BROMINE INTO HIGH SPECIFIC ACTIVITY RADIOBROMINATIONS*

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SUMMARY

An investigation has been carried out to evaluate the quantity of stable nuclides of bromine that might be introduced into the reaction mixtures of high specific activity radiobrominations from the reagents used. In the investigation neutron activation analyses were performed on the radionuclide solutions, solvents, oxidizing agents, and substrates that have been used in radiobromination reactions to determine the concentrations of bromine present. All of the reagents examined had detectable quantities of bromine. Among the reagents, a large variation in the concentrations of bromine was found. Due to the quantities of bromine found and the variability of bromine concentrations in the reagents, it is suggested that the reagents used in high specific activity radiobrominations be analyzed for bromine.

Key Words: Radiobromination, Specific Activity, Bromine Analysis, Neutron Activation.

INTRODUCTION

Radiobrominated compounds are of interest as receptor-binding radiopharmaceuticals.¹ However, obtaining the radiobrominated compounds in the specific activity required for non-saturation of the specific receptors has been a difficult task. For example, it has been estimated that radiobrominated estrogens will need to have a specific activity of at least 1000 Ci/mmol to be useful in detecting estrogen receptors in hormone-dependent tumors.² Many of the radiobrominations of estrogens that have been reported have yielded labeled estrogens which had specific activities of less than (or equal to) the desired 1000 Ci/mmol, even though the initial specific activity of the bromine-77 was >2000 Ci/mmol.³⁻⁷

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The radionuclide of bromine that has been used most often for high specific activity radiobrominations is bromine-77 ($T_{1/2} = 57$ h, $E_{\gamma} = 239$ and 521 keV). Production of this nuclide by irradiation of a molybdenum target with 800-MeV protons at the Los Alamos Meson Physics Facility has yielded curie quantities of very high specific activity radiobromine.⁸ In every radiobromination in which the specific activity of the product has been measured, the specific activity has decreased considerably from the radiobromide to the radiobromine-labeled compound. This decrease in specific activity has most likely been brought about by the introduction of stable nuclides of bromine from the various reaction components. It is important to know the amounts of bromine being introduced by the reagents that are used in a radiobromination so that the specific activities of the radiobrominated compounds can be optimized. Therefore, several of the reagents that have been used in radiobrominations in these laboratories were analyzed by neutron activation analysis to determine the concentration of bromine present in them. Neutron activation analysis was chosen since this method has been shown to be very sensitive for determining the quantity of bromine present in a variety of samples.⁹ The results of the analyses and a discussion of their significance are reported here.

MATERIALS AND METHODS

General. Reagents were obtained from commercial sources and were analytical reagent grade unless otherwise noted. The commercial sources and lot numbers of the reagents are listed in the tables within the text for each of the reagents examined. The water used in the radiobromination studies was obtained either by passing in-house deionized water through a Barnstead NANOpure apparatus (Sybron/Barnstead, Boston, MA) or by single distillation (Corning Megapure Still). The tert-butylhypochlorite was prepared by a literature procedure.¹⁰ The organosilanes and organostannanes were synthesized as previously reported.^{11,12,13} The quartz and polyethylene vials used in the study were fabricated specifically for use at the Omega West Reactor (OWR) at Los Alamos and the NOVA TRACK facility at TRIUMF.¹⁴

Sample Preparation.¹⁵ All samples were enclosed in flame sealed quartz vials for neutron irradiations. Solid samples were accurately weighed (50-250 mg) in the vials prior to sealing. Liquid samples were pipeted (100-1000 μ L) into the vials. An empty vial was sealed and irradiated to give a value for the amount of bromine from the quartz. The liquid samples were cooled in a Dry Ice/EtOH bath prior to sealing the vials. All sample vials were tested for leaks by immersion in a boiling water bath for 10 minutes prior to irradiation.

Irradiations of 1 mL aliquots of some solvents demonstrated that the quantity of bromine present was below the detection limits of the activation analysis, therefore 500 mL of each of the solvents examined was reduced in volume to near dryness under vacuum. The remaining solvent was removed from the round-bottomed flask by a Pasteur pipet and was placed in a quartz vial. A 1 mL aliquot of water was added to the flask and the sides of the flask were rinsed. After the water had collected in the bottom, it was removed and placed in a second vial. The amount of bromine in the two vials was totaled and divided by 500 mL to obtain the values in Table II.

Irradiations.¹⁵ The sealed vials were placed in polyethylene "rabbit" vials, and were pneumatically injected into a thermal port of the OWR at Los Alamos. The samples were irradiated for 30 minutes in a port that had a nominal thermal flux of 6.5×10^{12} n/sec/cm². The flux was monitored during each irradiation by a fission ion chamber, although the flux has been quite constant (generally less than 5% variation) during normal reactor operation.

Counting. The irradiated samples were allowed to decay over a five-day period,¹⁶ after which they were transferred to clean polyethylene vials for the gamma-ray counting. Each sample was counted for 30 minutes with a large Ge(Li) detector which had a resolution of 1.85 keV at the 1332 keV gamma-ray of cobalt-60. Data acquisition was accomplished with a Canberra Series 80 Multi-channel Analyzer (MCA). The MCA was interfaced to a DEC LSI-11 stand-alone computer that utilized a RSX11M operating system and a DSD-880 Winchester peripheral device for disc storage. All of the acquired gamma-ray spectra were stored on tape and were subsequently analyzed with the computer program RAYGUN.¹⁷

Calculations and Standardization. An absolute method was utilized for the calculations of the bromine concentration in each sample. Initially, detector energy calibration, resolution, and ADC linearity were measured with a National Bureau of Standards (NBS) ^{152}Eu gamma-ray source. The resolution and characteristic peak shape (as a function of energy) were also determined for the detector used. Nuclide characteristics, including neutron capture cross section, isotopic abundance, half-life, and gamma-ray branching ratios were used to calculate the bromine concentrations from the measured flux, irradiation time, decay time, and gamma-ray intensities. The 554.3 keV and 776.5 keV gamma-ray emitted by bromine-82 were used for the bromine assay. NBS 1571 Orchard Leaves¹⁸ and NBS 1632a Coal,¹⁹ which have a bromine concentration of 10 ppm and 43 ppm respectively, were used to obtain the "effective" bromine-81 neutron cross section.²⁰ The influence of water on the effective neutron cross section was measured by preparing several 1 mL samples of NaBr in water in the range of 1000 to 0.1 μg . The lower limit of detection (i.e., sensitivity) for bromine in most of the samples irradiated at the OWR was found to be 0.01-0.05 μg , depending on the concentrations of the other radioactive species in the samples. The detection limits at the NOVA TRACK facility was 0.5 μg . Based on the measurements made on the NBS certified materials, and on in-house pure-element standards, the overall error in the bromine assays is estimated to be less than 5% for the solid samples. Due to the additional errors introduced with aliquoting the liquids, and the matrix effects noted for the water samples,²¹ overall errors for the bromine concentrations in the liquid samples ranged from 10 to 30%. The error analyses are not included in the discussions of the results or tables as the absolute values for the bromine concentrations do not impact upon the conclusions of this study. However, it is interesting to note that identical samples analyzed at both Los Alamos and TRIUMF are in close agreement with one another (Tables III and IV).

RESULTS AND DISCUSSION

Evaluation of the amount of stable bromine present in radiobromination reaction mixtures could not be accomplished by analyzing the reaction mixtures themselves due to the small amounts of bromine present. Furthermore, such information would not reveal how much bromine each of the reagents had introduced into the reaction mixture, making optimization of the specific activity impossible. Therefore, analyses were carried out on single reagents. This also permitted using much larger quantities of the reagents so that more reliable values could be obtained for the concentrations of bromine present. To aid in understanding which reagents were most likely to contribute significant quantities of bromine to the reaction mixtures, the various reaction components were grouped into four categories. The results of the neutron activation analyses of the reagents in the four categories; the radionuclide solution, the solvents, the reagents, and the substrates, are described separately below.

Radionuclide Solution. Due to the very high specific activities of the bromine-77 radionuclide solutions as produced at Los Alamos, hundred-millicurie quantities of radiobromine had to be set aside to decay in order to have sufficient quantities of the stable nuclides of bromine present to detect by neutron activation analyses. Unlike the other reagents, activation analyses of the bromine-77 samples were carried out two separate times to determine the quantities of bromine-81 and bromine-79, respectively. This was necessary because the ratio of the two nuclides produced in the spallation reactions of the 800-MeV protons with the molybdenum target material was not the same as found in natural bromine.²² In the analyses the quantity of bromine-81 was determined by the abundance of bromine-82 as previously described.²³ However, to determine the quantity of bromine-79 in the sample the short-lived bromine-80 ($T_{1/2} = 17.4$ minutes) was examined.²³ In these irradiations the samples (same as examined for bromine-81) were irradiated for 100 seconds and were counted for 5 minutes after a decay period of 5 minutes. In these irradiations the 616.2 keV gamma-ray emitted by bromine-80 was used for the assay.

The derived isotopic abundance for the bromine nuclides are shown in Table I. It is apparent that the use of bromine-81 as an assay for total bromine would lead to a considerable underestimation. Also, the bromine-77 content cannot be ignored as it is present in nearly the same quantities as bromine-81. Considering these results, the value of 5920 Ci/mmol reported by Senderoff et.al.²⁴ for one of the bromine-77 shipments from Los Alamos may be higher than it actually was.

The error analyses of the quantities of bromine are not shown in Table I, however, cumulative errors of about 25% were estimated, and the errors for the specific activities are similar.

Table I: Specific Activity Determinations of Bromine-77

Sample Date	⁷⁷ Br mCi	Bromine μg	Specific Activity (Ci/mmol)
1-11-83	104	0.79(Br-81) 2.66(Br-79) 0.15(Br-77)* 3.60(total)	2300
9-16-83	240	0.35(Br-81) 4.33(Br-79) 0.34(Br-77)* 5.02(total)	3800
9-26-83	143	0.25(Br-81) 4.45(Br-79) 0.20(Br-77)* 4.90(total)	2300

*Based on theoretical specific activity of ⁷⁷Br (1 mCi = 1.4 ng)

Solvents. The solvent is the reaction component that is present in the greatest quantity. Even though only 50-100 μL of a solvent is used in most high specific activity radiobrominations, based on the quantity alone, the solvent

used has the highest potential for introducing significant quantities of bromine. Activation analyses results of several different solvents used in radiobrominations are given in Table II. It is interesting to note the large variation in the amounts of bromine present in the different methanol samples.

From the data in Table II it is clear that, generally, insignificant quantities of bromine (<1 ng) will be introduced into high specific activity radiobrominations if volumes of 100 μ L or less are used. However, it is also clear that large amounts of bromine (i.e., 29 ng) might be added if the reactions were run with MeOH from the bottle that contained the highest concentration of bromine.

Table II: Concentrations of Bromine in Solvents

Solvent	Concentration (ng/mL)
MeOH (MCB, lot 2025)	154
MeOH (MCB, lot 7P09)	293
MeOH (Baker, lot 246607)	12
HOAc (Mallincrodt, lot WDCJ)	8
H ₂ O (NANOpure)	4
H ₂ O (MegaPure)	40*
EtOH (US Indust. Chem., USP)	8
EtOH (Standard Chem., Canada, USP)	40*

*Analyses performed at TRIUMF-NOVA TRACK facility.

Reagents. Most of the high specific activity radiobrominations that have been carried out thus far have been electrophilic substitution reactions. These reactions have been chosen because rapid reaction rates are needed when working with the very small quantities of bromine present in no-carrier-added radiobromine solutions. In the reactions the electrophilic radiobrominating agent is generally prepared in situ by oxidation of the bromide ion. The neutron activation analyses of some of the commonly employed oxidizing reagents are given in

Table III. Multiple analyses were carried out on some of the reagents to compare different lots and samples from different manufacturers.

Table III: Concentrations of Bromine in Reagents

Reagent	Concentration ($\mu\text{g/g}$)
N-Chlorosuccinimide (Alfa, lot 031979)	3 (3*)
N-Chlorosuccinimide (Aldrich, lot 6405EE)	20
N-Chlorosuccinimide (Aldrich, lot JD071097)	189
N-Chlorosuccinimide (Sigma, lot 29C-D112)	139
Chloramine-T (Eastman, lot A4X)	24
Chloramine-T (MCB, lot C3M24)	5 (4*)
tert-Butylhypochlorite	0.3
HCl (Allied Chem. Co.)	0.4
HCl (Anachem 208123)	1.3*

*Analyses performed at TRIUMF-NOVA TRACK facility.

Analysis of the tert-butylhypochlorite was accomplished by reacting it with phenol (to produce a non-explosive mixture) and irradiating the resultant mixture. An equal amount of phenol was irradiated, and the amount of bromine present in the phenol (Table IV) was subtracted. Analysis of the concentrated HCl was obtained directly. The latter analyses were obtained because HCl is often used to acidify reaction mixtures (e.g., reactions of chloramine-T) and has been used in 100 μL quantities for distilling BrCl when inorganic oxidants are used.²⁵ The results show that HCl used in quantities of 100 μL will add significant amounts of bromine (i.e., 40 to 130 ng). Even though only a few μg to a few mg of oxidizing reagent are used in the radiobrominations, some of the reagents tested would certainly contribute sufficient quantities of bromine to make a significant difference in the specific activity of the radiobrominated compound. For example, if 1 mg of N-chlorosuccinimide were used with 1 mCi of bromine-77 having a specific activity of 2300 Ci/mmol, 3 to 190 ng of bromine would be added to the 35 ng of bromine present in the radionuclide solution. Considering only the oxidizing agents' dilution of bromine, the specific activity would decrease

from 2300 Ci/mmol to 2080 Ci/mmol or 360 Ci/mmol depending on which bottle of NCS was used.

Substrates. To distinguish the compounds that are to be (or have been) radiobrominated from the other reagents, they have been classified as substrates. Of the many possible compounds that might be radiobrominated, estradiol and ethynylestradiol were chosen for activation analyses because the specific activities of the bromine-77 labeled compounds have been reported.⁴ The results of the analyses are shown in Table IV.

The results obtained for phenol and the estrogens indicate that in reactions carried out with 1 mg or less of substrate, the amount of bromine added from the substrate will not significantly alter the specific activity of the product. Phenol and the estrogens had never contained a bromine substituent, but many of the organometallic substrates that are currently under investigation for use in high specific activity radiobrominations were synthesized from the corresponding bromides. Evaluation of several organosilanes and organostannanes (Table IV) confirmed that significant quantities of bromine were present in the compounds, even after purification. To decrease the amount of stable brominated compound present, where possible direct metallation or use of the corresponding chlorides or iodides in the metal-halogen interconversions might be warranted. This fact is exemplified by the low bromine concentrations found in *p*-*n*-Bu₃Sn-anisole which was synthesized from *p*-iodoanisole. Additionally, neutron irradiation might be used to assist in evaluating the results of conventional purification procedures when the bromides are used in metal-halogen exchange reactions.

Since aryltrimethylsilanes are not affected by most reaction conditions, the trimethylsilyl group can be introduced early in a synthetic scheme. An interest in how the quantity of bromine might change as aryltrimethylsilanes are reacted to form new aryltrimethylsilanes led to the activation analyses of some aryltrimethylsilanes used in the synthesis of *p*-trimethylsilylhippuric acid.²⁶ Thus, conversion of *p*-bromobenzaldehyde to *p*-trimethylsilylbenzaldehyde after purification of the product by distillation had about 0.01% (by weight) of bromine present. Conversion of this compound mixture to the benzoic acid by oxidation of

the aldehyde, followed by purification led to a decrease of bromine present. However, reaction of the benzoic acid to yield the silylhippuric acid, followed by recrystallization to purify the product, led to an increase in the amount of bromine present. These results indicate that the bromine can be carried through several conversions and purifications, so the end product must be analyzed.

Table IV: Concentration of Bromine in Substrates

Substrates	Concentration ($\mu\text{g/g}$)
Phenol (Mallincrodt, lot KJHR2)	0.1
Estradiol (Sigma, lot 99C-0324)	2.5
Ethynylestradiol (Sigma, lot 120F-0071)	0.3
p-Me ₃ Si-toluene	650
p-K ₂ SiF ₅ -toluene	170
p-n-Bu ₃ Sn-toluene	65 (58)*
p-n-Bu ₃ Sn-Anisole	1*
p-n-Bu ₃ Sn-fluorobenzene	2850*
p-Me ₃ Si-benzaldehyde	99
p-Me ₃ Si-benzoic acid	31
p-Me ₃ Si-hippuric acid	90

*Analyses performed at TRIUMF-NOVA TRACK facility.

CONCLUSIONS

This investigation was carried out to determine to what extent the reagents contribute to the dilution of specific activity when compounds are radiobrominated with high specific activity bromine-77. There was no attempt to use neutron activation to determine the specific activity of the various radiobrominated compounds. The primary reason for this is that relatively small amounts of radioactivity were used in most radiobromination reactions (few μCi to a few mCi) so the amount of bromine present would be below the limit of detection. The most direct method of determining the specific activity of the labeled compound is by

a non-destructive detection system such as a UV detector on a HPLC.^{4,27} Another method that has been used to estimate the specific activity of the radiobrominated product is the use of a biological assay.⁷

It should be pointed out that the neutron activation analysis results can not be used to determine the maximum specific activity that might be expected for the radiobrominated compound as there is no indication of whether the bromine present is free to react. However, it is reasonable to assume that some (if not all) of the bromine present could contribute to the dilution of the specific activity. Therefore, if an optimization of the specific activity of the radiobrominated compound is desired, knowledge of the amounts of bromine in the various reagents is essential.

While the absolute quantities of bromine found in the specific lots of reagents in this investigation are only important in our studies, the data does point out the variability of the bromine concentrations that might be expected in different reagents and in different lots of the same reagent. The fact that bromine was present in a measurable amount in all of the reagents examined suggests that every reagent added to the reaction mixture may contribute to a decrease in the specific activity. Whether the decrease is significant or not will depend on the specific activity of the radionuclide that was used, the amount of reagent used, and the amount of bromine present in each reagent.

The results of this investigation have exemplified the need to analyze all of the components of radiobromination reactions if high specific activity radiobrominated compounds are desired. Furthermore, the variability of concentrations of bromine in the reagents has led these investigators to set aside the reagents with the lowest concentrations of bromine to be used exclusively in high specific activity radiobrominations.

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The lower detectable limit was 0.5 $\mu\text{g/g}$ and the error estimated for the analyses is comparable to that obtained with the OWR reactor at Los Alamos.

16. Short-lived ^{24}Na , which has a half-life of 15 h, is the predominant activity in this matrix. A 5-day decay period decreases the background₈₂ from the decay of ^{24}Na and gives an increased sensitivity for the 35-h ^{82}Br .
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