Processing of Generator-Produced ^{68}Ga for Medical Application

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The ^{68}Ge/^{68}Ga generator provides an excellent source of positron-emitting ^{68}Ga. However, newly available "ionic" ^{68}Ge/^{68}Ga radionuclide generators are not necessarily optimized for the synthesis of ^{68}Ga-labeled radiopharmaceuticals. The eluates have rather large volumes, a high concentration of H^+ (pH of 1), a breakthrough of ^{68}Ge, increasing with time or frequency of use, and impurities such as stable Zn(II) generated by the decay of ^{68}Ga, Ti(IV) as a constituent of the column material, and Fe(III) as a general impurity. **Methods:** We have developed an efficient route for the processing of generator-derived ^{68}Ga eluates, including the labeling and purification of biomolecules. Preconcentration and purification of the initial generator eluate are performed using a miniaturized column with organic cation-exchanger resin and hydrochloric acid/acetone eluent. The purified fraction was used for the labeling of nanomolar amounts of octreotide derivatives either in pure aqueous solution or in buffers. **Results:** Using the generator post-eluate processing system, >97% of the initially eluated ^{68}Ga activity was obtained within 4 min as a 0.4-mL volume of a hydrochloric acid/acetone fraction. The initial amount of ^{68}Ge(IV) was decreased by a factor of 10^4, whereas initial amounts of Zn(II), Ti(IV), and Fe(III) were reduced by factors of 10^5, 10^2, and 10, respectively. The processed ^{68}Ga fraction was directly transferred to solutions containing labeling precursors—for example, DOTA-DPhe^1-Tyr^3-octreotide (DOTATOC) (DOTA = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid). Labeling yields of >95% were achieved within 10 min. Overall yields reached 70% at 20 min after generator elution relative to the eluted ^{68}Ga activity, not corrected for decay. Specific activities of ^{68}Ga-DOTATOC were 50 MBq/nmol using a standard protocol, reaching 450 MBq/nmol under optimized conditions. **Conclusion:** Processing on a cation-exchanger in hydrochloric acid/acetone media represents an efficient strategy for the concentration and purification of generator-derived ^{68}Ga(III) eluates. The developed scheme guarantees high yields and safe preparation of injectable ^{68}Ga-labeled radiopharmaceuticals for routine application and is easy to automate. Thus, it is being successfully used in clinical environments and might contribute to a new direction for clinical PET, which could benefit significantly from the easy and safe availability of the radionuclide generator-derived metallic positron-emitter ^{68}Ga.

Key Words: ^{68}Ge/^{68}Ga radionuclide generator; post-processing of eluates; cation-exchange chromatography; molecular imaging; PET/CT

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An important characteristic of positron-emitting ^{68}Ga is its cyclotron-independent availability via the ^{68}Ge/^{68}Ga radionuclide generator system. The relatively long-lived ^{68}Ge (half-life [$t_{1/2}$] = 270.95 d) produces a short-lived ^{68}Ga ($t_{1/2}$ = 67.71 min), which subsequently decays to stable ^{68}Zn. ^{68}Ga is an excellent positron emitter, with 89% positron branching accompanied by low photon emission (1,077 keV, 3.22%) (*1*,*2*). ^{68}Ge/^{68}Ga radionuclide generators have been the object of development and investigation for almost 50 y. For a recent review on this and other PET radionuclide generator systems see Rösch et al. (*3*).

Today, the most common commercially available ^{68}Ge/^{68}Ga radionuclide generator is based on a TiO_2 solid phase (Cyclotron Co. Ltd.) (*4*). The generators are produced with ^{68}Ge activities of up to 3.7 GBq. "Ionic" $^{68}Ga^{3+}$ is eluted in 0.1N HCl solution. The ^{68}Ga yield is >60% in 5 mL of the eluate; the ^{68}Ge breakthrough usually does not exceed $5 \cdot 10^{-3}$%.

The ^{68}Ge/^{68}Ga radionuclide generator systems known today are not necessarily optimally designed for direct application in a medical context. The eluate from the commercial generator still contains measurable activities of long-lived ^{68}Ge. In addition, the rather large volume and the relatively high concentration of hydrochloric acid in many cases prevent the direct use for labeling reactions. Furthermore, labeling yields and specific activities might not reach maximum values due to the presence of metallic impurities. For example, significant amounts of Zn(II) are generated from the decay of ^{68}Ga. For a "fresh" 1,110-MBq generator, the number of stable ^{68}Zn atoms generated within 1 d after an

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elution amounts to $8.93 \cdot 10^{13}$ atoms (i.e., 10 ng of Zn(II)) compared with $4.69 \cdot 10^{12}$ atoms of ^{68}Ga for 800 MBq ^{68}Ga eluted. In addition, Ti(IV) or other residuals resulting from the generator column material and Fe(III) are present in the eluate. All of this will prevent achieving sufficient ^{68}Ga-labeling yields and specific activities if low amounts of the labeling precursor are mandatory. Thus, dedicated procedures to process the initial radionuclide generator eluate—ideally including labeling and purification of ^{68}Ga radiopharmaceuticals—need to be developed. Several approaches for processing generator-derived ^{68}Ga(III) were described recently (*5–8*).

Anion-Exchange Chromatography. In these cases (*5,6*), the initial volume of 10 mL of the 0.1N HCl eluate is transferred to a vial containing 15 mL of 9.5N HCl to obtain a final hydrochloric acid concentration of 5.5 M. Under these conditions, ^{68}Ga can be adsorbed on a strong anion- exchanger as anionic chloro complexes of ^{68}Ga(III). After a washing step with 1 mL of 5.5N HCl, the resin is flushed with a stream of nitrogen and then eluted with H_2O in small volumes. This strategy separates ^{68}Ge but does not allow direct loading of ^{68}Ga(III) on the anion-exchange resin from 0.1N HCl and it does not provide sufficient purification of Ga(III) from, for example, Zn(II) and Fe(III). The time needed to process the generator eluate, to synthesize and to purify, for example, ^{68}Ga-labeled DOTA-conjugated peptides (DOTA = 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid), and the efficiency related to each of the individual steps define an overall practical yield of generator eluate processing before subsequent synthesis of >94% and of about 46% ± 5% for the ^{68}Ga-labeled DOTA-conjugated octreotide, both referred to the initially eluted activity of ^{68}Ga (*9*).

Fractionation. Another approach to overcome problems such as eluate volume, acidic pH, and content of ^{68}Ge and chemical impurities is to fractionate the initial generator eluate (*8*). The concept uses the fact that the eluted ^{68}Ga activity peaks within ~1–2 mL, representing about two thirds of the total activity. In the context of synthesizing ^{68}Ga-labeled compounds, decay-corrected yields of ^{68}Ga radiopharmaceuticals thus cannot exceed 60%–70%. Due to processing times and labeling efficacies, in practice, effective yields of labeled DOTA-conjugated peptides such as ^{68}Ga-DOTATOC amount to about 50% (*10*). Contents of ^{68}Ge and metallic impurities are lowered because of the lower eluate volume used but principally not chemically removed before the ^{68}Ga-labeling steps.

Strategy of the Present Approach. The aim of this work was to develop an efficient and simplified system for processing ^{68}Ge/^{68}Ga generator–produced ^{68}Ga eluates adequate for clinical requirements. It combines volume reduction with chemical and radiochemical purification. This leads to almost complete removal of metallic impurities and ^{68}Ge breakthrough, thus providing the purified ^{68}Ga in a form useful for direct labeling (acceptable pH, volume, purity). It allows for preparation of injectable ^{68}Ga-labeled radiopharmaceuticals using a generator-associated, combined processing/labeling/purification module for easy routine use in a clinical environment.

The key step consists in the direct transfer of the initial 0.1N HCl ^{68}Ga eluate to a cation-exchanger. Due to high distribution coefficients, generator-produced ^{68}Ga can be quantitatively adsorbed on the resin directly from the generator eluate. Literature data indicate that, with the increase of the content of acetone in the HCl solutions of the same acid concentration, the affinity of ^{68}Ga for the organic cation-exchange resin decreases (*11,12*). Consequently, systematic experiments have been performed in the present study to adapt this approach for processing generator-produced ^{68}Ga. In addition, literature data do not necessarily provide separation conditions for tetravalent germanium and titanium, divalent zinc, and trivalent iron. Thus, it was another aim of this work to investigate the composition of hydrochloric acid/acetone solutions under which trivalent gallium can not only be concentrated but also be purified from Ge(IV), Ti(IV), Zn(II), and Fe(III).

MATERIALS AND METHODS

^{68}Ge/^{68}Ga Radionuclide Generator

Commercial generators based on a TiO_2 phase adsorbing ^{68}Ge(IV) were obtained from Cyclotron Co. Ltd. In the present study, 740- and 1,110-MBq devices were used.

Radiometals Used for Ion-Exchange Distribution Measurements

Ga(III): 110 MBq of ^{68}Ga in 7 mL of 0.1N HCl were obtained from a 1-y-old 740-MBq generator after >200 elutions.

Ge(IV): The activity of ^{68}Ge in the ^{68}Ga eluate used was about 170 kBq.

Fe(III): ^{59}Fe was produced in a neutron-capture reaction on natural iron. One hundred ninety-eight milligrams of iron oxide Fe_2O_3 were irradiated for 50 d at the Hahn-Meitner-Institut (HMI) neutron source at 1.6×10^{14} n cm^{-2} s^{-1}, yielding 440 MBq ^{59}Fe. The iron oxide was dissolved in HNO_3 solution and, after evaporation, transferred into appropriate solutions.

Zn(II): ^{69}Zn was produced with a specific activity of ~700 kBq/mg by irradiation of 380 μg of ^{68}Zn (as $Zn(NO_3)_2$, >98% isotopically enriched ^{68}Zn) for 6 h at the TRIGA II reactor Mainz at a neutron flux of 4×10^{12} n cm^{-2} s^{-1}. The target was subsequently dissolved in 0.1N HCl.

The absolute activity of the radionuclides was analyzed by γ-spectrometry using a high-purity germanium (HPGe) detector.

Chemicals and Equipment

Analytic-reagent grade chemicals and Milli-Q water (18.2 MΩ·cm; Millipore) were used. Bio-Rad AG 50W-X8 cation-exchanger <400 mesh was used to prepare microchromatography columns (50 mg resin, ~2-mm inner diameter, ~5-mm length). For labeling reactions, 11-mL glass vials (Mallinckrodt) were used. A heating block with 1.5-cm lead thickness for radiation adsorption was built using 24-V Peletier Thermal Cycler heating elements. For processing of the labeling product, C-18 cartridges, Phenomenex Strata-X Tubes, 30 mg were used. Sterile filtration was done on 0.22-μm membrane filters (Millex GV).

For labeling of octreotide derivatives, DOTA-D-Phe[1]-Tyr[3]-conjugated octreotide (DOTATOC) was used.

Experiments

Distribution of Metal Cations. A microchromatography column was prepared using 50 mg of the resin. The distribution of ^{68}Ge(IV), Fe(III), Zn(II), and Ti(IV) on this cation-exchange column was investigated to quantitatively evaluate the possibility of purification of the generator produced ^{68}Ga from contaminants.

The ^{68}Ga eluted with 5−10 mL of 0.1N HCl was transferred on line within 1–2 min on the chromatographic column. This represents the basic step to recover radiogallium from the generator eluate and to remove the main parts of the chemical and radiochemical impurities.

In a second step, the column was eluted with hydrochloric acid/acetone solutions of 80% acetone and HCl concentrations of 0.10N, 0.15N, or 0.20N. The volumes of the investigated mixtures ranged from 0.6 to 5.0 mL. The eluant remaining on the cation-exchanger was removed by passage of air. These 2 processes aimed to remove most of the remaining chemical and radiochemical impurities from the resin, whereas ^{68}Ga(III) should quantitatively remain on the column.

Third, the column was filled with 150 μL of a 97.6% acetone/0.05N HCl solution. About 2 min standing appeared to be best for complete desorption of the ^{68}Ga(III) from the resin into the liquid phase. An additional 250 μL of this mixture were applied, and the purified ^{68}Ga(III) was obtained in 400 μL of this eluent overall. The column was reconditioned with 1 mL of 4N HCl and 1 mL of H_2O.

Thus, 5 fractions were obtained and analyzed for their ^{68}Ga and ^{68}Ge contents (Table 1): (i) 7 mL 0.1N HCl; (ii) 80% acetone/0.10–0.20N HCl solutions; (iii) 97.6% acetone/0.05N HCl solution; (iv) 4N HCl (this fraction contains the metals remaining after the elution with the 97.6% acetone/0.05N HCl solution); (v) H_2O.

To analyze the behavior of the relevant impurities, the distributions of ^{59}Fe in 83 μg Fe(III) and of ^{69}Zn in 130 μg Zn(II) were determined using the same protocol. In addition, the behavior of stable Ti(IV) was investigated to estimate the distribution of Ti(IV) coeluted in the ^{68}Ga fraction. Thus, 20 μg Ti(IV) in 5 mL 0.lN HCl was processed the same way. The distribution of Ti(IV) in the different fractions was studied by an Elan 5000 ICP-MS (Perkin-Elmer).

Labeling. After preconcentration and purification of the initial generator eluates on the microchromatography column, ^{68}Ga(III) was eluted with the 400-μL 97.6% acetone/0.05N HCl solution ($2\cdot10^{-5}$ mol HCl). This fraction was used directly for the labeling of DOTATOC—that is, the processed activity was added to 4.5 mL pure H_2O in a standard glass reagent vial (11 mL; Mallinckrodt) containing 7–14 nmol of DOTATOC. No buffer solution was added.

Parallel experiments, however, were done with additional buffer solutions using 0.5–0.7 mL of 1 M *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), pH 4.0–4.1, in 2-mL reaction vessels (polypropylene brand equipped with a vent) containing 2–5 nmol of DOTATOC only.

All labeling reactions were performed at temperatures of ~98°C. The kinetics of the complexation were recorded by taking aliquots of 1 μL at 1, 2, 5, and 10 min.

Determination of Labeling Yields/Quality Control. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (Machery Nagel column, Nucleosil 5 C18-AB, 250 × 4 mm) were used for analyzing reaction yields. For TLC,

TABLE 1
Relative Distribution of Ga(III), Ge(IV), Zn(II), Ti(IV), and Fe(III) on Microchromatographic Column (50 mg of Bio-Rad AG 50W-X8 resin, 400 Mesh) in Hydrochloric Acid/Acetone Media

Volume	Step	Eluent (concentration)	Relative distribution (%)				
			Ge(III)	Ge(IV)	Zn(II)	Ti(IV)	Fe(III)
Set 1							
7 mL	Generator elution	0.10N HCl	0.19	98.14	0.61	5.34	0.45
5 mL	Purification	80% acetone/0.10N HCl	0.58	1.83	99.39	2.68	53.75
0.4 mL	Ga(III) elution	97.6% acetone/0.05N HCl	98.50	$2\cdot10^{-2}$	$5\cdot10^{-2}$	$5\cdot10^{-2}$	43.54
1 mL	Washing	4N HCl	0.53	$6\cdot10^{-3}$	$<10^{-3}$	90.92	2.01
1 mL	Washing	H_2O	0.20	$5\cdot10^{-3}$	$<10^{-3}$	1.01	0.29
Set 2							
7 mL	Generator elution	0.10N HCl	0.16	97.08	0.77	7.30	0.13
0.6 mL	Purification	80% acetone/0.15N HCl	1.43	2.92	98.15	0.68	37.86
0.4 mL	Ga(III) elution	97.6% acetone/0.05N HCl	97.82	$3\cdot10^{-2}$	1.08	$7\cdot10^{-2}$	49.78
1 mL	Washing	4N HCl	0.41	$5\cdot10^{-3}$	$5\cdot10^{-3}$	72.15	11.61
1 mL	Washing	H_2O	0.18	$3\cdot10^{-3}$	$<10^{-3}$	19.80	0.62
Set 3							
7 mL	Generator elution	0.10N HCl	0.11	97.68	0.43	7.18	0.73
5 mL	Purification	80% acetone/0.15N HCl	6.29	2.32	99.57	3.99	87.37
0.4 mL	Ga(III) elution	97.6% acetone/0.05N HCl	92.73	$5\cdot10^{-3}$	$<10^{-3}$	0.11	11.10
1 mL	Washing	4N HCl	0.77	$2\cdot10^{-3}$	$<10^{-3}$	86.54	0.71
1 mL	Washing	H_2O	0.10	$2\cdot10^{-3}$	$<10^{-3}$	2.18	0.09
Set 4							
7 mL	Generator elution	0.10N HCl	0.29	98.08	0.92	6.33	0.4
0.6 mL	Purification	80% acetone/0.20N HCl	6.34	1.86	96.58	1.11	54.0
0.4 mL	Ga(III) elution	97.6% acetone/0.05N HCl	92.96	$5\cdot10^{-2}$	3.70	0.11	42.6
1 mL	Washing	4N HCl	0.28	$6\cdot10^{-3}$	$<10^{-3}$	87.37	2.9
1 mL	Washing	H_2O	0.13	$6\cdot10^{-3}$	$<10^{-3}$	5.08	0.1

aluminum-backed silica gel 60 and 0.1 M Na_3citrate water solution as a mobile phase were selected. A pH of ~5 of the eluent was found to be optimum, providing R_f = 0.9 for free ^{68}Ga(III) while ^{68}Ga-DOTATOC remains at the origin. For HPLC, a Machery Nagel column (Nucleosil 5 C18-AB, 250 × 4 mm) and 20% AcCN, 80% trifluoroacetic acid/0.01% H_2O (flow rate, 1 mL/min under isocratic conditions) were used. Retention times were about 2 and 9 min for free ^{68}Ga(III) and ^{68}Ga-DOTATOC, respectively.

Purification of ^{68}Ga-Labeled Peptide. ^{68}Ga-Labeled DOTA-octreotide was purified from unreacted ^{68}Ga species by reversed-phase chromatography. The reaction mixture was passed through a small C18 cartridge (Phenomenex Strata-X tubes, 30 mg), providing quantitative recovery of the peptide on reverse phase. After washing the cartridge with 5 mL H_2O, the ^{68}Ga-labeled peptide was recovered with 200–400 μL of pure ethanol.

Determination of Acetone Contents. The acetone contents in the reaction mixture and in the final product were analyzed by gas chromatography (Hewlett Packard 6890 series gas chromatography system). According to the eluate processing and labeling protocols, 400 μL of a 97.6% acetone/0.05N HCl solution were added to 4.5 mL of preheated (~98°C) water in an open reaction vial (11 mL; Mallinckrodt). The acetone content in this solution was measured at different time points during incubation. In addition, the acetone concentration in the solutions after processing on the C18 cartridge was determined. The cartridge was washed with 5 mL of water and with 0.5 mL of ethanol. Finally, the ethanol fraction containing the labeled product was analyzed.

RESULTS

Eluate Purification: Chemical and Radiochemical Purities

Relative distributions of ^{68}Ga(III), ^{68}Ge(IV), Zn(II), Ti(IV), and Fe(III) on a microchromatography column (50 mg AG 50W-X8, 400 mesh) in hydrochloric acid/acetone media are summarized in Table 1.

^{68}Ga: The microchromatographic column provides quantitative adsorption of >99% of ^{68}Ga from the initial generator eluate. An additional purification step with 80% acetone/HCl solutions causes some loss of ^{68}Ga activity. The part of ^{68}Ga, lost per milliliter volume of this eluent, increased with increasing acid concentration and was <7% in 0.6 mL (~12% ^{68}Ga/mL) with 0.20N HCl. The same activity was obtained in 5 mL with 0.15N HCl (~1.4% ^{68}Ga/mL) and only about 0.6% in 5 mL with 0.10N HCl (~0.1% ^{68}Ga/mL) (Table 1). Under those conditions (set 2), >99% of the ^{68}Ga initially remaining on the cation-exchanger could be eluted in 400 μL of the 97.6% acetone/0.05N HCl solution.

^{68}Ge: ^{68}Ge(IV) passes through the column in the original 0.10N HCl solutions. The amount remaining in the free volume of the cation-exchanger column is further reduced by washing with the 80% acetone/HCl solutions. As a result, the final ^{68}Ga fraction contains <0.01% of ^{68}Ge relative to the initial eluate, independent of the HCl concentration of the 80% acetone solutions. Thus, by post-processing, an additional decontamination factor of $\sim 10^4$ could be achieved for ^{68}Ge. Relative to an ~0.005% original ^{68}Ge generator breakthrough, for example, a very low overall content of ^{68}Ge in the processed ^{68}Ga fraction of $\sim 5 \times 10^{-7}$% is achieved. This represents a significant step toward a medical radionuclide generator approvable for direct clinical use.

Zn: Divalent zinc is quantitatively adsorbed (>99%) from the 0.10N HCl eluate along with trivalent gallium. However, Zn(II) is desorbed by the 80% acetone/HCl purification step, whereas ^{68}Ga(III) remains on the resin. The highest reduction of the zinc amount could be achieved applying 5 mL of an 80% acetone/0.15N HCl solution. The final content of Zn(II) in the processed ^{68}Ga(III) fraction of 400 μL of the 97.6% acetone/0.05N HCl solution is $<10^{-3}$%.

Ti: Ti(IV) is almost completely adsorbed on the cation-exchange resin directly from the 0.1N HCl solution. Approximately 0.1% of Ti(IV) is eluted in 400 μL of the 97.6% acetone/0.05N HCl solution. The remaining Ti(IV) is eluted from the cation-exchange resin almost completely in 4N HCl, which allows the column to be cleaned.

Fe: The best separation factors are provided at an HCl concentration of 0.10N, but a large volume of the eluent must be applied because the distribution coefficient of iron is still high. Only about 54% of Fe(III) could be obtained in 5 mL of the 80% acetone/0.10N HCl solution (set 1), accompanied by ~0.6% of ^{68}Ga. At an HCl concentration of 0.20N (set 4), the mixture has evidently a higher elution capability for both cations. Here, 54% of iron and 6% of ^{68}Ga were eluted already in 0.6 mL. Application of an 80% acetone/0.15N HCl solution (set 2) seems to be an optimum, providing the best ratio of eluted amounts of iron relative to the loss of gallium. It was 38% of Fe(III) compared with 1.4% of Ga in 0.6 mL of the eluate. Five milliliters of the solution allowed washing down almost 90% of Fe(III) and about 6% of ^{68}Ga(III), resulting in the best decontamination factor for iron.

Labeling

The preconcentrated and purified ^{68}Ga(III) eluted from the resin with 400 μL of the 97.6% acetone/0.05N HCl mixture was used for labeling reactions. For labeling in pure water, up to 700 MBq ^{68}Ga were added to 4–4.5 mL (preheated) H_2O in open standard reagent vials containing 7–14 nmol of DOTATOC. The amount of HCl contained in the final eluate solution (400 μL 97.6% acetone/0.05N HCl ≡ $2 \cdot 10^{-5}$ mol H^+) provided an overall pH of 2.30 ± 0.05. At ~98°C and under these acidic conditions, radiolabeling yield was >95% within 10 min. Unstable radiochemical yields and increased adsorption on the glass surface were detected if <14 nmol of DOTATOC was used. Incorporation also dropped with decreasing reaction temperature.

Specific activities of up to 50 MBq/nmol could be achieved. To increase the specific activity, additional experiments were performed in HEPES buffer systems using lower amounts of DOTATOC. The processed ^{68}Ga eluate of 2 combined generators ($2 \cdot 10^{-5}$ mol HCl; up to 1,400 MBq ^{68}Ga) was added to 0.5–0.7 mL 1 molal HEPES solutions

of pH 4.0–4.1 in 2-mL reaction vessels containing 2–4 nmol of DOTATOC, resulting in the mixture pH of 3.7–3.9. The radiochemical yield was up to 88% at ~99°C within 10 min, and the specific activity was up to 450 MBq/nmol.

Post-processing and Syntheses Using a Prototype of a Module for Clinical Application

The developed eluate processing and labeling protocol were used to design a prototype of a module system of generator post-processing (Fig. 1). A microchromatography column with about 50 mg of the cation-exchanger was prepared using two 3-way valves (I and II). The ^{68}Ge/^{68}Ga radionuclide generator was connected to the first valve (line 1). PEEK capillary tubing (line 4) was directed to the reagent vials in a heating block. The column could be eluted using a standard single-use syringe (position 3) and was connected to the waste vial (line 2). A small C18 cartridge (Phenomenex Strata-X tubes, 30 mg) was attached to the system (line 5) and was also operated using standard single-use syringes.

The processing of the generator eluate included several steps.

Elution of Generator with 0.1N HCl. As there is no need to limit the elution volume and, thereby, lose some of the ^{68}Ga activity available at the radionuclide generator, 10 mL of 0.1N HCl were always injected into the generator. The eluate was passed directly through the microchromatography column (line 1) and transferred into the waste vial (line 2). This step provided recovery of >99% of ^{68}Ga from the eluate on the cation-exchanger resin.

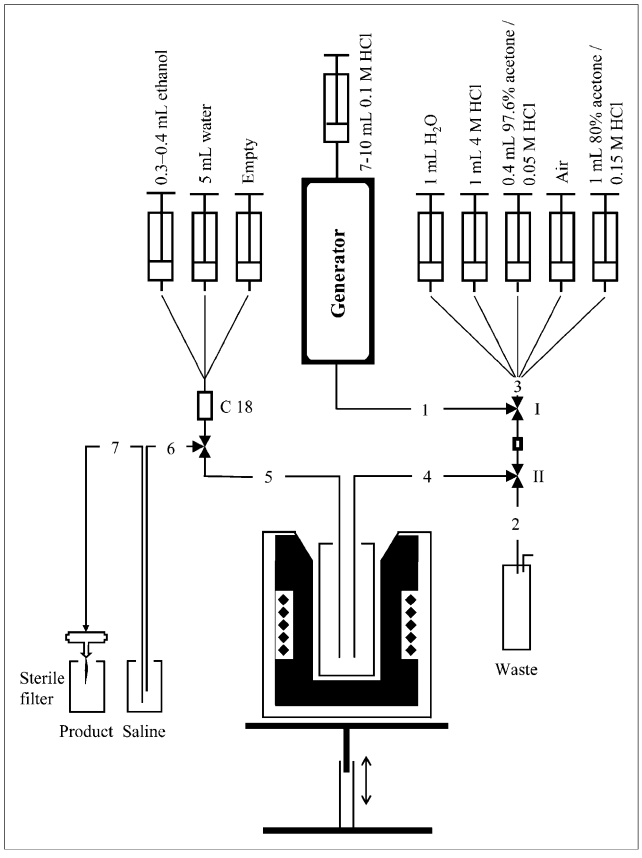

FIGURE 1. Scheme (top) and post-processing of ^{68}Ga-eluates and generator-associated syntheses of ^{68}Ga-labeled compounds (e.g., labeling and product purification for DOTATOC as a proof-of-principle precursor).

Purification Step. After switching the 3-way valve (I), the column was washed, using a single-use syringe (line 3) with 1 mL of the 80% acetone/0.15N HCl solution. The solution removed ^{68}Ge(IV), which remained in the dead volume of the column, Zn(II), and Fe(III) and was collected in the waste vial (line 2). The remaining 0.80% acetone/0.15N HCl solution was removed from the cation-exchange resin by blowing air using a single-use syringe. The loss of ^{68}Ga activity was <3%.

Elution of Purified ^{68}Ga Activity into Reaction Vial. After switching the 3-way valve (II), the column was eluted using a single-use syringe filled with 400 μL 97.6% acetone/0.05N HCl solution into the reaction vial (line 4). The column was first filled with 150 μL of the solution and, after 2 min, ^{68}Ga was washed off with the rest of the mixture. The column was freed of the remaining solution again using air from a single-use syringe. ^{68}Ga was recovered from the resin with >99% yield—that is, <1% of the ^{68}Ga adsorbed on the resin was lost.

After transfer of ^{68}Ga into the labeling vial, the microchromatography column is reconditioned with 1 mL 4N HCl and 1 mL H_2O (lines 2 and 3).

Complexation. Complexation is performed in unbuffered water as described earlier. The activity was eluted directly into the reaction vial (11 mL; Mallinckrodt) containing 14 nmol of DOTATOC in 4.5 mL of preheated (~98°C) water. Radiochemical yields of >95% could be achieved for ^{68}Ga-DOTATOC syntheses within 10 min. The reaction mixture was passed through a small C18 cartridge (Phenomenex Strata-X tubes, 30 mg), using an empty syringe (line 5) providing quantitative absorption of the peptide on the cartridge. After washing the cartridge with 5 mL H_2O (line 5), the ^{68}Ga-labeled peptide was recovered from the cartridge with 200–400 μL of pure ethanol (line 6). Less than 0.5% of the activity in the ethanol eluate corresponded to free gallium. A radiochemical purity of >99.5% was obtained by processing the labeling mixture on a RP 18 cartridge. Thus, a radiochemically pure product, which did not depend on the initial radiolabeling yields, was guaranteed.

The ethanol eluate containing pure ^{68}Ga-DOTATOC was dissolved in an appropriate volume of sterile 0.9% saline for medical application (line 6) and sterilized by filtration (line 7) through a 0.22-μm membrane filter. The developed scheme allowed the preparation of injectable ^{68}Ga-labeled DOTA-peptides within 20 min, with overall yields up to 70% in reference to the initially eluted gallium activity, not corrected for decay (80% as corrected for decay). For a fresh 1,110-MBq generator, the batch activity of ^{68}Ga-DOTATOC was 700 MBq before sterile filtration. The specific activity depended on the actual generator activity

(as the peptide amount remains constant) and was up to 40 MBq/nmol for the unbuffered water labeling system compared with ~450 MBq/nmol for HEPES buffer systems with low peptide amounts.

Routine Quality Control

A radiochemical purity of >99.5% was guaranteed by processing the labeling solution on RP18 cartridges independent of the initial radiolabeling yield. However, routine quality control for the radiochemical purity is recommended in addition. It can be performed rapidly by TLC or HPLC.

Content of Acetone

The total amount of acetone within the 400 μL 97.6% acetone/0.05N HCl mixture transferred to the labeling vial is ~200 mg. Acetone evaporates while the labeling mixture is kept at ~95°C in the open vial. After 10 min, about 4.5 μg acetone remained in the reaction mixture. After purification of the ^{68}Ga-labeled peptides on the RP18 cartridge, the overall acetone content in the final, injectable solution was <0.5 μg.

Those numbers are far below the intravenous toxicity of acetone in rats or mice (median lethal dose [LD_{50}] > 1 g/kg (*13,14*)). Acetone is classified as a solution with a negligible intravenous toxicity of 5,500 mg/kg (rat) (*14*).

DISCUSSION

Radiolabeled DTPA- and DOTA-conjugated peptides of high affinity and selectivity for tumor membrane receptors applied to SPECT of various tumors clearly have indicated the great scientific and diagnostic potential of this particular molecular targeting. Recently, these applications have been expanded by the availability of new ^{68}Ga-labeled analogs. The favorable nuclear decay parameters of ^{68}Ga ($t_{1/2}$ = 67.71 min, 89% β^+ branching) allow the application of the most potent nuclear medical imaging technology—that is, PET—for the routine diagnosis of neuroendocrine and other tumors. The identification of solid tumors and disseminated soft-tissue and bone metastases has reached a completely new level of highly accurate tumor diagnosis, especially if PET/CT is used. Consequently, there is an increasing demand for commercially available ^{68}Ge/^{68}Ga radionuclide generators, ready for routine clinical application.

A commercial ^{68}Ge/^{68}Ga radionuclide generator system based on a TiO_2 matrix is available from Cyclotron Co., Ltd. and provides high and stable elution yields. Because of the large volume of the eluate and its high acidity, its contents of chemical impurities such as Zn(II), as generated from the decay of ^{68}Ga, Ti(IV) or other residuals representing the generator column material, and Fe(III), as well as its breakthrough representing long-lived ^{68}Ge, the initial generator eluate might fail to provide sufficient ^{68}Ga-labeling yields and specific activities if low amounts of the labeling precursor are mandatory. Moreover, it might limit its wide application for routine patient diagnosis not only because of the chemical aspects but also because of requirements related to legal considerations for radiopharmaceutical registration.

As demonstrated in this study, efficient and rapid concentration and purification of the generator-produced ^{68}Ga can be performed on cation-exchangers if hydrochloric acid/acetone media are used. The procedure requires only 4 min and guarantees yields of 97% ± 1% of the initially eluted ^{68}Ga, purified from ^{68}Ge, Zn(II), Ti(IV), and even Fe(III).

The eluate processed according to the new protocol described in this study allows high labeling yields because of the increased chemical and radiochemical purities.

In addition, the significant removal of almost the entire ^{68}Ge breakthrough on the cation-exchanger using the HCl/acetone systems fullfils the basic requirements relevant to the medical use of the radionuclide generator: Analyses of a possibly unexpected and critical breakthrough of ^{68}Ge in each ^{68}Ga fraction eluted (or in the final ^{68}Ga-labeled pharmaceutical fraction) before the application of the ^{68}Ga-labeled tracer do not belong to today's routine procedures and are difficult to establish. The cation-exchange resin represents a guarantee for avoiding the transfer of this breakthrough to the labeling system or to the patient.

Furthermore, this procedure extends the useful life of the ^{68}Ge/^{68}Ga radionuclide generator systems. Even in cases in which the initial ^{68}Ge breakthrough of a newly installed generator might be acceptable, this breakthrough might increase by an order of magnitude as a consequence of frequent elutions and definitely limits its long-term or high-volume application.

Labeling

Processing of the generator eluate provides excellent ^{68}Ga purification, pH optimization ($[H^+] = 2 \cdot 10^{-5}$ mol H^+), as well as volume reduction, which guarantees high labeling yields and high specific activities for subsequent ^{68}Ga-labeling reactions. The ~400 μL of acetone do not interfere. The procedure might even be modified, for example, by first evaporating the 400 μL of the 97.6% acetone/0.05N HCl fraction to dryness before adding the labeling precursor or by eluting ^{68}Ga from the cation-exchange cartridge with a solution containing the labeling precursor.

Specific Activity

DOTA forms complexes with a ligand-to-metal ratio of 1:1. Because 1 nmol of ^{68}Ga corresponds to 102.6 GBq activity, the theoretic (maximum) specific activity of ^{68}Ga-DOTATOC is 102.6 GBq/nmol. Labeling in pure water provided specific activities of up to 50 MBq/nmol. Application of a buffer system allowed the optimization of reaction conditions—that is, decrease of volume (which in parallel increases concentration of the reagents). A specific activity of up to 450 MBq/nmol could be achieved. Nevertheless, this specific activity is still much lower than

the theoretic value. Only about 0.5% of the DOTA-conjugated ligands are involved in complex formation with ^{68}Ga(III). A further increase in the specific activity requires decreasing the amount of ligand. However, handling of very small peptide quantities can be a critical factor for the labeling performance. Similar to Breeman et al. (*8*), we observed that the use of <1–3 nmol of the DOTA-conjugated peptide leads to its loss in the system. It decreases the reproducibility of the labeling procedure, which is especially problematic for routine production. Thus, increasing the initial ^{68}Ga activity rather than decreasing the amount of ligand should be used as a strategy for preparation of labeled compounds with high specific activity. This again requires high efficacies of ^{68}Ga transfer from the generator to the labeling precursor.

Routine Clinical Application

The developed generator-associated eluate processing and labeling protocol were used to design a system for routine synthesis of ^{68}Ga-DOTATOC, providing a safe kit-type labeling of DOTA-peptides even under conditions when an equipped radiochemical laboratory is not available. The time needed to process the generator eluate, to synthesize and to purify ^{68}Ga labeled DOTA-conjugated peptides, is <20 min. The chemical efficacies related to each of the individual steps define a total yield of about 70% of ^{68}Ga-DOTATOC in reference to the initially eluted ^{68}Ga activity. This overall yield is higher than the yields from the other approaches to process the generator eluate—namely, using anion-exchange chromatography (*6,9*) and fractionation (*8,10*) with 46% ± 5% and 50%, respectively.

The generator eluate post-processing is combined directly with the synthesis of ^{68}Ga-labeled compounds. In the case of ^{68}Ga-labeled peptides, final purification of the labeled product is included. This represents another essential aspect for routine syntheses, as no breakthrough of non-complexed ^{68}Ga or hydrolyzed ^{68}Ga species into the final product occurs. Independent of the individual labeling yields of different batches, this process-inherent purification provides complete radiochemical purity. Additional quality control before the injection of the ^{68}Ga-labeled compounds might be excluded in this case.

Content of Acetone

Acetone is classified as a solution with a negligible intravenous toxicity of 5,500 mg/kg (rat) (*14*). After 10 min, about 4.5 μg acetone remained in the reaction mixture. After purification of the ^{68}Ga-labeled peptides on the RP18 cartridge, the overall acetone content in the final, injectable solution was <0.5 μg. Those numbers are far below the intravenous toxicity of acetone in rats or mice (LD_{50} > 1 g/kg (*14*)).

Simultaneous Use of Several Generators

Due to the highly efficient purification and concentration performance of the post-processing protocol and due to the low hydrodynamic resistance of the generator system, it was possible to connect several generators in a cascade scheme. The eluent was piped through the first generator, which was connected to the eluent line of the next one. The second generator was connected directly to the microchromatography column. Alternatively, 2 generators can be eluted separately to one and the same cation-exchange column, which subsequently is processed as described.

A possible optimization scheme is presented graphically in Figure 2. It supposes that one half-life of ^{68}Ge might be considered as a lower limit of the ^{68}Ga activity required for radiopharmaceutical syntheses (solid line 1). To prolong the period of clinical generator use, its initial activity can be increased by a factor of 2 (i.e., 50-mCi vs. 25-mCi generators). Thus, 200% (dotted line 1″) provides routine use for $2 \cdot t_{1/2}$. Alternatively, a cascade scheme supplements the amount of ^{68}Ge, providing logistic advantages. An "old" 50% generator coupled with a fresh 100% generator results in 150% of overall ^{68}Ge content (solid line 1 + 2). This 150% will provide enough ^{68}Ga activity within $1.58 \cdot t_{1/2}$. Shelf-life of the first 100% generator in this case is approaching $1.3 \cdot t_{1/2}$. Administration of a third generator (solid line 1 + 2 + 3) increases this time up to $1.4 \cdot t_{1/2}$. This might be relevant in a context of routine clinical use, as it increases the efficacy of generator availability. Thus, application of several generators in a cascade scheme can be used to optimize the shelf life of the generators and to reduce costs.

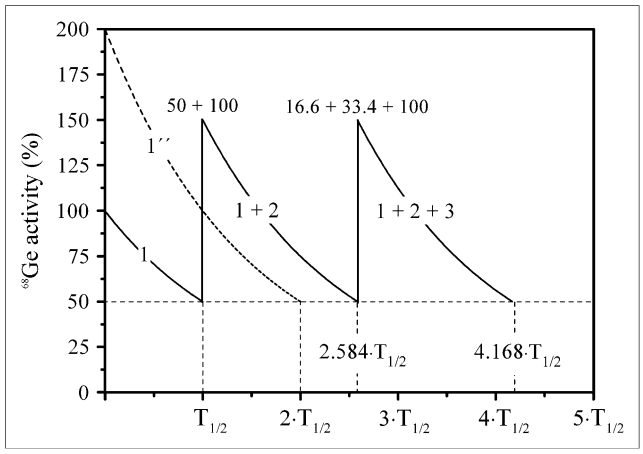

FIGURE 2. Cascade use of generators: Solid line (1) gives decay of a first, initial generator, which drops from 100% to 50% within 1 half-life of ^{68}Ge. The 50% is considered here to determine the end of a generator usage. If, after this first half-life of 270 d, a second "fresh" generator of the same initial activity is added to the first, used one, the total activity of ^{68}Ge achieved is 150%. With this activity, another working period of $1.584 \cdot t_{1/2}$ of ^{68}Ge is created, until the activity available again is dropped to 50% relative to a single fresh generator. Instead of 2 successive times of $1 + 1\ t_{1/2}$ of ^{68}Ge working periods, in this case, $2.584 \cdot t_{1/2}$; is achieved. This can easily be extended adding a third "fresh" generator to the 2 "old" ones. With this approach, a period of $4.168\ t_{1/2}$ is provided, instead of $3.0 \cdot t_{1/2}$. This saves more than 1 generator.

CONCLUSION

A rapid, simple, and chemically efficient processing of generator-produced ^{68}Ga was developed on the basis of cation-exchange chromatography in hydrochloric acid/acetone media. It successfully allows the purification and concentration of ^{68}Ga. The procedure is described for a TiO_2-based commercial ^{68}Ge/^{68}Ga radionuclide generator using 0.1N HCl as eluent. It might be adapted to other radionuclide generator types as well, whenever volume minimization and chemical and radiochemical purification are required before subsequent labeling reactions. For example, one of the initial attempts to design a ^{68}Ge/^{68}Ga radionuclide generator was an anion-exchange resin eluted with 0.01N hydrofluoric acid solutions. It allowed high-purity separations due to the significant difference in the distribution coefficients of Ge(IV) and Ga(III) in the form of fluoro complexes (*15*). Its use, however, seemed to be rather complicated due to the problems arising from the amounts of HF in the ^{68}Ga fraction eluted. The post-processing described in the present work would be perfectly compatible with this radiochemical concept as it absorbs ^{68}Ga from 0.05N HF on the cation-exchange resin and provides, finally, an HF-free, 97.6% acetone/0.05N HCl fraction containing almost all of the ^{68}Ga.

The post-processing of volumes and impurities is easily compatible with the synthesis of ^{68}Ga-labeled compounds and their purification. The whole process guarantees safe preparation of injectable ^{68}Ga-DOTATOC (or other ^{68}Ga-labeled radiopharmaceuticals) for routine application and can be successfully used in the clinical environment. Automated versions of the processing are currently being developed.

Application of several generators in a cascade scheme can be used to optimize the shelf life of the generators and to reduce costs. The post-processing described here also provides a guarantee for chemical and radiochemical purification in this case.

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Processing of Generator-Produced ^{68}Ga for Medical Application

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