An enticing characteristic of the positron-emitting radionuclide $^{68}$Ga is its cyclotron-independent availability via $^{68}$Ge/$^{68}$Ga generators. Also attractive is the high positron yield ($89\%$ positron branching) and accompanying low photon emission ($1.077$ keV, $3.22\%$). It ensures the removal of $^{68}$Ge before $^{68}$Ga-radiopharmaceutical preparation and high labeling yields of $^{68}$Ga-labeled radiopharmaceuticals for routine medical application. Methods: In an effort to overcome the problem associated with acetone in the currently applied method, we have investigated the feasibility of replacing it with ethanol. The purification of $^{68}$Ga from coeluted metallic impurities ($^{68}$Ge$^{4+}$, Fe$^{3+}$, Zn$^{2+}$, and Tl$^{4+}$) on various cation-exchange columns has been investigated with a variety of post-processing solutions. As a proof of principle, the post-processed $^{68}$Ga was used to radiolabel DOTATOC in combination with high-purity water and various buffer solutions. Results: An effective protocol for the processing of generator-produced $^{68}$Ga on the basis of cation-exchange chromatography using EtOH/HCl medium has been developed. Up to $95\%$ of the initially eluted $^{68}$Ga activity can be collected in a $1\text{mL}$ fraction of $90\%$ EtOH/0.9N HCl after removal of $^{68}$Ge-breakthrough in a washing step. The post-processed eluate has been used to radiolabel DOTATOC in combination with high-purity water and various buffer solutions. Conclusion: The described novel protocol improves the radio-labeling efficiency and efficacy of DOTATOC, providing yields of greater than $99\%$ (decay-corrected). As a result, further purification to separate the desired product from uncomplexed $^{68}$Ga is not necessary. The developed post-processing and labeling protocols permit reliable and high-yield preparation of injectable $^{68}$Ga-DOTATOC (or other $^{68}$Ga-labeled radiopharmaceuticals) that are suitable for routine application. It is possible to incorporate this protocol into existing automated modules.

Key Words: $^{68}$Ga; post-processing; ethanol; DOTATOC; $^{68}$Ge

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MATERIALS AND METHODS

Metals Used for Ion-Exchange Distribution Measurements

A commercial 1,100-MBq generator with 68Ge4+ adsorbed on a TiO2 support was obtained from Cyclotron Co. Ltd. 

An 85-MBq quantity of 68Ga in 5 mL of 0.1N HCl was obtained from an 18-month-old 1,100-MBq generator, which had been eluted more than 200 times previously. The activity of 68Ge breakthrough in the eluted 68Ga fraction was about 7 kBq.

59Fe was produced by a neutron-capture nuclear reaction of naturally occurring metallic iron. Iron (200 mg) was irradiated for 21 d at the Hahn-Meitner-Institute Berlin neutron source at 1.6 × 1014 n cm−2 s−1, yielding 456 MBq of 59Fe. The iron was dissolved in concentrated HCl solution and then diluted to give a solution of 0.1N HCl. TiCl4 was obtained from Sigma-Aldrich as 0.09 M titanium solution in H2O. The distributions of 68Ga3+ and 59Fe3+ were measured using 0.1N HCl and (v) H2O. The distributions of 68Ga3+ and 59Fe3+ in the different fractions were investigated to determine the most suitable setup. To analyze the behavior of relevant impurities, the distributions of 59Fe3+ and Ti4+ in 0.1N HCl were determined using the described protocol.

68Ga Radiolabeling

The 68Ga eluate obtained after elution with 1 mL of 90% EtOH/0.9N HCl (9 × 10−4 mol HCl) was used for radiolabeling experiments. Labeling was performed in water (4 mL) or suitably prepared buffer solutions (NaOAc, NH4OAc, or N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid [HEPES]) in open standard reagent vials containing DOTATOC (21 nmol). The volume and concentrations of the buffer were varied to establish the optimal conditions. Solutions were preheated to the desired temperature (80°C or 95°C depending on the setup used) and reacted for either 10 or 15 min. For comparison, the labeling kinetics of DOTATOC were determined using 68Ga purified by the standard acetone post-processing method. In this case, labeling was performed in water (4.6 mL) at 80°C and 95°C for 15 min.

Purification of 68Ga-Labeled Peptide and Quality Control

68Ga-DOTATOC was separated from unreacted 68Ga species by reverse-phase chromatography. The reaction mixture was cooled to about 50°C and passed through a C-18 cartridge. The peptide was with a second solution of varied EtOH/HCl composition and allowed to stand for 2 min. Subsequently, the column was eluted with varying amounts (200–800 μL) of the same solution and collected in an Eppendorf vial (2 mL). The protocol is designed such that this eluent should contain the purified and concentrated 68Ga3+. The column was reconditioned with 4N HCl (1 mL) and then H2O (1 mL). In each experimental setup, 5 fractions were analyzed for their 68Ga and 68Ge contents: (i) 0.1N HCl, (ii) washing solution (80% EtOH/0.1–0.2N HCl), (iii) elution mixture (EtOH/HCl), (iv) 4N HCl, and (v) H2O. The distributions of 68Ga3+ and 68Ge4+ in the different fractions were investigated to determine the most suitable setup.

### TABLE 1

<table>
<thead>
<tr>
<th>Volume</th>
<th>Fraction</th>
<th>Eluent</th>
<th>AG 50W-X8</th>
<th>AG 50W-X4</th>
<th>Strata X-C</th>
<th>Varian SCX</th>
<th>Merck SCX</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mL</td>
<td>i</td>
<td>0.1N HCl</td>
<td>0.13</td>
<td>1.79</td>
<td>5.44</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>1 mL</td>
<td>ii</td>
<td>80% EtOH/0.15N HCl</td>
<td>0.57</td>
<td>2.65</td>
<td>0.05</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>0.4 mL</td>
<td>iii</td>
<td>90% EtOH/0.9N HCl</td>
<td>80.47</td>
<td>87.38</td>
<td>23.90</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>1 mL</td>
<td>iv</td>
<td>4N HCl</td>
<td>17.21</td>
<td>5.48</td>
<td>8.34</td>
<td>98.32</td>
<td>95.94</td>
</tr>
<tr>
<td>1 mL</td>
<td>v</td>
<td>H2O</td>
<td>1.65</td>
<td>3.17</td>
<td>5.53</td>
<td>4.48</td>
<td>5.28</td>
</tr>
</tbody>
</table>

FIGURE 1. Dependence of 68Ga-desorption yield on HCl concentration for AG 50W-X8 resin eluted with 0.4 mL of 90% EtOH/0.05–0.95N HCl solutions (n = 3).
Quantitatively retained on the reverse phase. After the cartridge had been washed with 1 mL of H2O, the 68Ga-DOTATOC was eluted using 0.4 mL of ethanol.

Thin-layer chromatography was used to analyze the reaction yields. The reaction solution (2 μL) was spotted on thin-layer chromatography plates and developed with 0.1 M Na3-citrate water solution (pH 4), and the activity population distribution was measured using a flat-bed scanner.

Each experiment was performed in triplicate to accurately determine the SD. All reported yields are decay-corrected.

**RESULTS**

**Distribution of 68Ga**

The initial 68Ga adsorption from the generator eluate onto the CEX resins was about 99%. Subsequent washing using an 80% EtOH/0.15N HCl solution caused less than 3% of the 68Ga to be removed from the CEX and lost to the waste fraction in each case. In fact, this percentage loss remained constant for varying consti-tutions (70%–90% ethanol and 0.2–0.1N HCl) of the washing solution. Subsequent desorption of the 68Ga from the resin was achieved using a 90% EtOH/0.9N HCl CEX eluting solution with desorption yields that varied depending on the CEX used. Desorption efficiencies were greater than 75% (mean) for the AG 50W-X8/AG 50W-X4 columns, approximately 23% (maximum) for the Bond Elut Strata-X-C columns, and less than 1% (maximum) for the LiChrolut Strata-X-C columns. The distribution of 68Ga between the different fractions (i–v), using 80% EtOH/0.15N HCl as the washing solution (ii) and 90% EtOH/0.9N HCl as the CEX eluting solution (iii), is shown in Table 1 for each CEX investigated.

Desorption was optimal using the AG 50W-X8 and AG 50W-X4 resins, and these became the focus of subsequent investigations. Desorption of 68Ga from the resin was also found to be influenced by the acidity, ethanol content, and volume of solution iii used. Figures 1, 2, and 3 show the dependency of the desorption yield on the acidity, ethanol content, and eluting volume, respectively (with all other parameters kept constant). For a constant ethanol content and volume, the desorption yield increases with increasing acidity up to 0.90N HCl (Fig. 1). Maximum desorption was obtained when solution iii consisted of 90% ethanol (Fig. 2).

It is clear that the AG 50W-X8 and AG 50W-X4 CEX resins are suitable for the adsorption and desorption of 68Ga. A further important consideration is the efficacy of the washing step at separating 68Ga from metallic impurities. This efficacy was evaluated in terms of the distribution of 68Ge4+, 59Fe3+, and Ti4+ after post-processing.

**Distribution of 58Ga for 1-mL Mixtures of 90% EtOH/0.9N HCl**

A profound effect was observed when the volume of the solution was increased (Fig. 3), with the yield reaching a maximum (~95%) at 1 mL.

The relative distributions of 68Ga3+, 68Ge4+, Fe3+, and Ti4+ on CEX columns AG 50W-X8 (~400 mesh) and AG 50W-X4 (200–400 mesh) (50 mg), using 1 mL of the 90% EtOH/0.9N HCl as the CEX eluting solution, are summarized in Table 2. In a setup that does not include the washing step, such as those used within automated modular systems, the elution with the 90% EtOH/0.9N HCl solution would produce 68Ga desorption yields of about 97%.

**Distribution of 68Ge4+**

For both CEX resins, approximately 97% of 68Ge4+ passes through the column with the initial 0.1N HCl solution (i). The remaining traces are further reduced (~3%) by the washing step with solution ii. After solution v, less than 0.25% of the initially eluted 68Ge remains trapped on the resin. There was no detectable 68Ge in fractions iii, iv, or v within 8 h of measurement. Because the 68Ge detection limit for the γ spectrometer was 12 Bq, this equates to a purification factor of more than 400.

**Distribution of 59Fe3+**

The relative distribution of Fe3+ varied considerably between the AG 50W-X8 and AG 50W-X4 CEX columns. The amount of Fe3+ in fraction iii could be reduced to approximately 14% and 33% of the initial content with AG 50W-X8 and AG 50W-X4, respectively. Purification factors for the respective CEX columns are therefore about 7 and 3.

**Distribution of Ti4+**

Ti4+ is almost entirely adsorbed on the CEX columns from solution i. Up to 11% of the initial Ti4+ content is removed with solution iii, with the AG 50W-X8 CEX performing slightly better in this regard (~7% in purified 68Ga fraction). The largest tita-nium content is observed for solutions iv and v, indicating that the column regeneration is effective.
After dilution to 5 mL using H₂O. When this solution was used significantly. However, only at certain pH and buffer concentrations did specific activities, provided that the yields did not differ significantly in Figure 4 along with the analogous labeling experiments generally, but not always, gave rise to slightly lower yields. This result is not surprising given that the reactants are more dilute and thus have a detrimental effect on the rate of the reaction. It was possible to achieve yields of greater than 89% of the reaction. It was possible to achieve yields of greater than 89% at a pH of 4 with NaOAc (0.5 M and 0.1 M), NH₄OAc (0.1–1.0 M), and HEPES (0.25–1.0 M) with 4 mL of the bufer, albeit with low specific activities (9 MBq/nmol). There was only a single example of a similar yield at the same pH when the buffer volume was reduced to 1 mL of buffer result in a reliable yield of greater than 89% (specific activity of 22–23 MBq/mol). Specifically, these conditions were NaOAc, pH 5, 0.5–1.0 M; NH₄OAc, pH 5, 0.1–1.0 M; and HEPES, pH 5, 0.1–1.0 M. The equivalent higher-volume (4 mL) analogs of these experiments generally, but not always, gave rise to slightly lower yields. This result is not surprising given that the reactants are more dilute and thus have a detrimental effect on the rate of the reaction. It was possible to achieve yields of greater than 89% at a pH of 4 with NaOAc (0.5 M and 0.1 M), NH₄OAc (0.1–1.0 M), and HEPES (0.25–1.0 M) with 4 mL of the bufer, albeit with low specific activities (9 MBq/nmol). There was only a single example of a similar yield at the same pH when the buffer volume was reduced to 1 mL of buffer result in a reliable yield of greater than 89% (specific activity of 22–23 MBq/mol). Specifically, these conditions were NaOAc, pH 5, 0.5–1.0 M; NH₄OAc, pH 5, 0.1–1.0 M; and HEPES, pH 5, 0.1–1.0 M. 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Further improvements in the processing method were sought through changes to the constitution and volume of the eluting solution (iii). On the basis of these changes, the optimal combination for the post-processing was found to be resin AG 50W-X4, 1 mL of 80% EtOH/0.15 M HCl as the washing solution (ii), and 1 mL of 90% EtOH/0.9 M HCl as the eluting solution (iii). The steps involved are similar to those of the acetone-based method, which has been incorporated into the successful EZAG modules (Eckert and Ziegler Radiopharma Inc.). Therefore, it is feasible that this new ethanol-based protocol could be easily implemented within the same module and others.

On the basis of these findings, the 2 AG 50W resins were evaluated further for ability to separate $^{68}$Ga from unwanted metal ion impurities. Given that both resins provide an acceptable desorption yield of $^{68}$Ga, the next important consideration is the purity of the fraction used for labeling. Both resins were effective for the removal of $^{68}$Ge$^{4+}$ and lowered the Ti$^{3+}$ and Fe$^{3+}$ content to an acceptable level. The $^{68}$Ge$^{4+}$ content of the purified $^{68}$Ga-eluate is below the detection limit of γ spectroscopy, equating to a purification factor of less than 400. Significantly, the reduction of the initial $^{68}$Ge breakthrough on the cation-exchangers using the ethanol-based protocol fulfills legal requirements for the routine use of the $^{68}$Ge/$^{68}$Ga generator in nuclear medicine. It is also possible to envision further applications such as the use of 2 or more $^{68}$Ge/$^{68}$Ga generators connected in 1 line eluting onto a single CEX resin. Resin AG 50W-X8 provided a more than 2-fold greater reduction in $^{59}$Fe$^{3+}$ removal than AG 50W-X4. Iron content is dictated by the HCl used, and therefore the resin with the largest $^{68}$Ga desorption yield is preferred. Thus, AG 50W-X4 is the preferred resin for post-processing of $^{68}$Ga by the ethanol-based method. Significantly, the reduction in Fe$^{3+}$ and $^{68}$Ge content was more than 3-fold greater for the ethanol-based method than for the acetone-based method (3).

The processed $^{68}$Ga eluate facilitates high labeling yields and specific activities of $^{68}$Ga-DOTATOC because of the increased chemical and radiochemical purity. In contrast to the acetone-based protocol, the purified eluate resulting from the ethanol method is more acidic and of little practical use for radiolabeling after simple dilution. However, through the inclusion of a simple buffer formulation it is possible to produce nearly quantitative

**DISCUSSION**

Five CEX resins were evaluated in terms of their ability to adsorb $^{68}$Ga from the initial generator eluate and to subsequently release the purified $^{68}$Ga when solutions in which the acetone is substituted for ethanol were used. Each of the resins was effective for the initial trapping of $^{68}$Ga from solution I; however, only AG 50W-X4 and AG 50W-X8 resins permitted desorption with solution iii in an acceptable yield. In comparison to AG 50W-X4, AG 50W-X8 was more effective for the initial $^{68}$Ga adsorption but produced a slightly smaller desorption yield with eluting solution iii.

**Purification of $^{68}$Ga-DOTATOC and Quality Control**

Purification on Strata-X (C18 cartridge) did not significantly improve the radiochemical purity when the initial labeling yield was greater than 99%. Quality control was performed by thin-layer chromatography or high-performance liquid chromatography.

**FIGURE 4.** Comparison of $^{68}$Ga-DOTATOC radiolabeling yield obtained at 80°C and 95°C for 3 different buffer solutions (NaOAc, NH$_4$OAc, and HEPES, all at 0.5 M) at pH 5, using 1 and 4 mL of buffer solution ($n = 3$).

**FIGURE 5.** Radiolabeling kinetics (0–15 min) of $^{68}$Ga-DOTATOC obtained in 1 M HEPES buffer (80°C, 2 mL) after ethanol-based post-processing and in pure water (5 mL, 80°C and 95°C) after acetone-based post-processing (80°C and 95°C) ($n = 3$).
yields of $^{68}$Ga-DOTATOC within 10 min. Significantly, the labeling efficiency using ethanol-processed $^{68}$Ga was better than that using acetone-processed $^{68}$Ga at a lower temperature. The rate of radiolabeling was such that it is possible to obtain the radiolabeled product ready for injection 5 min after initial generator elution. Recently, significant increases in radiochemical yields of a trivalent radiometal–ligand complex formation were reported in the presence of ethanol in the aqueous labeling solutions (6). It is likely that the presence of ethanol in the labeling solution is in part responsible, but the differences in labeling pH and volume between the 2 methods should not be ignored.

Certain commercial synthesis modules routinely added 1 vol% ethanol to act as a radiolytic stabilizing agent. With the ethanol-based procedure, the addition of ethanol is already incorporated, thus reducing the number of overall steps. Depending on the volume of the labeling solution used, ethanol makes up between 18 and 45 vol% of the final preparation.

CONCLUSION

An appropriate and efficient protocol for the post-processing of generator-produced $^{68}$Ga, based on cation-exchange chromatography with ethanol/hydrochloric acid medium, has been described. Like the acetone-based protocol, the ethanol-based protocol allows for concentration of $^{68}$Ga generator eluate with only small losses of eluted $^{68}$Ga. Quantitative removal of $^{68}$Ge-breakthrough is routinely possible and ensures that the final injectable radiopharmaceutical fulfills legal requirements relating to the $^{68}$Ge content of the injectable solution. A significant and improved reduction in the $\text{Ti}^{4+}$ and $\text{Fe}^{3+}$ content was also demonstrated, which promotes the synthesis of radiopharmaceuticals with higher specific activities. Furthermore, the use of ethanol facilitates more efficient radiolabeling and enhances the radiolytic stability of the radiolabeled compound. It has been shown that it is possible to prepare $^{68}$Ga-DOTATOC suitable for medical application with a radiochemical yield of more than 99% at a lower temperature than that of the standard acetone-based procedure. The entire post-processing protocol, including generator elution, can be completed within 5 min and may easily be incorporated into commercially available automated modules. The absence of acetone, $^{68}$Ge, and unchelated $^{68}$Ga in the final formulation using ethanol-based post-processing is an important step toward the kit-type synthesis of $^{68}$Ga radiopharmaceuticals.

DISCLOSURE

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Ethanol-Based Post-processing of Generator-Derived $^{68}$Ga Toward Kit-Type Preparation of $^{68}$Ga-Radiopharmaceuticals

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