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High-Efficiency Cationic Labeling Algorithm of Macroaggregated Albumin with ^{68}Gallium

Uğur Ayşe[1] · Gültekin Aziz[1] · Yüksel Doğangün[1]

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Abstract

Purpose The generator product radionuclide gallium-68(^{68}Ga) is widely used for PET/CT imaging agents and the ^{68}Ga-labeled MAA is an attractive alternative to ^{99m}Tc-labeled MAA. Using a commercially available MAA labeling kit for ^{99m}Tc, we presented a reliable synthesis protocol with a highly efficient, organic solvent-free cationic method in GMP conditions in the Scintomics automated synthesis unit.

Methods The labeling process was performed by incubating for 7 min at 90 °C in the borax vial containing the generator product $^{68}GaCl_3$ MAA-HEPES eluted from the PSH$^+$ cartridge with 1.5 mL 5 molar NaCl. Quality control of the final product content was examined, and radiopharmaceutical production was carried out in accordance with GMP guidelines.

Results ^{68}Ga eluted from the generator was obtained in more than 99% radiochemical purity and efficiency. In this case, the labeling efficiency was found to be >99%. When the results of SEM-EDX analysis in the final product were examined, it was determined that most of toxic metals were no appreciable in the product content.

Conclusions The radiochemical and chemical purity of the final product allows direct use without purification steps to remove "free ^{68}Ga" or other toxic compounds.

Keywords Gallium-68 · MAA · Macroaggregated albumin · Positron emission tomography · Radiopharmaceutical · Lung perfusion scintigraphy

Introduction

Labeling of radiopharmaceuticals can be defined as the binding of the bioactive component to the radionuclide. Generally, a radiopharmaceutical consists of three components: a vector molecule, a radionuclide for diagnostic or therapeutic applications, and a linker (chelating) there between. The schematic radiopharmaceutical model is shown in Fig. 1. The radionuclide provides the radiation component (radioactivity), while the vector molecule specifically targets biomolecules expressed in tissues or cells [1] (Fig. 2). What is important in labeling reactions is that no change in the structure of the bioactive molecule occurs during binding. Otherwise, the molecule, whose structure is altered or degraded, will not be localized in the desired organ in the body.

Labeling with Gallium-68 (^{68}Ga) consists of complexing the bioactive component and ^{68}Ga metal. The radionuclide ^{68}Ga is a commonly used radionuclide for PET/CT imaging. Germanium-68 (^{68}Ge) decays via pure electron capture (EC) to the ground state of ^{68}Ga with a half-life of 270.95 days [2]. ^{68}Ge is produced from a stable ^{69}Ga isotope (^{69}Ga (p, 2n) ^{68}Ge) in a high-energy cyclotron. The ^{68}Ge is then immobilized in a column filled with inorganic, organic, or mixed matrix, where it spontaneously decays to ^{68}Ga. The most important advantage of ^{68}Ga-labeled radiopharmaceuticals is that the synthesis is based on ^{68}Ga produced by the generator and can be carried out on site and on demand without the need for a medical cyclotron. ^{68}Ga as a short-lived positron emitter is eluted as $^{68}GaCl_3$ by acid treatment from the ^{68}Ge/^{68}Ga generators with different column constructions [3, 4].

✉ Uğur Ayşe
ayseugur@pau.edu.tr

Gültekin Aziz
agultekin@pau.edu.tr

Yüksel Doğangün
dyuksel@pau.edu.tr

[1] Education and Research Hospital, Department of Nuclear Medicine, Pamukkale University, Denizli, Turkey

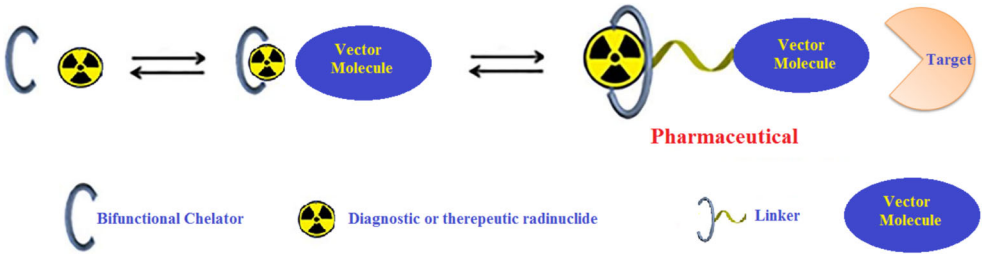

Fig. 1 Formation of a radiopharmaceutical

Earlier, numerous research articles in the 1970s and 1980s describe the use of organic resins as well as inorganic matrices, which selectively adsorb ^{68}Ge and provide ^{68}Ga desorption in weak (0.1–1.0 N) or strong (>1.0 N) hydrochloric acid solutions [5]. The generator elutes based on hydrochloric acid, which provides "cationic" ^{68}Ga instead of "inert" ^{68}Ga-complexes. A high acid concentration increases the risk of impurities from the added solutions while increasing the buffering difficulty. Even if ultra-pure reagents are used, the potential for the presence of impurities in the final product is increased if more reagents need to be added for elution and buffering. Low levels of contaminants in the labeling bottle can potentially reduce labeling efficiency. Other factors affecting labeling efficiency include the amount of bioactive component, pH, temperature, reaction time, and solvent [6].

Gallium oxidation state in aqueous solutions is found in "+3" and is highly affected by pH. Optimal pH conditions range from pH 3 to 5. An extremely acidic medium may protonate donor atoms and thus prevent complex formation, and a neutral or basic medium may cause the formation of non-reactive hydroxide. The stability of the coordination complex between the chelator and the radionuclide is important in the selection of the chelate ligand [7]. Radioisotope selection determines the chelate ligand used. Common chelators for ^{68}Ga include a cyclic ligand family based on heterocyclic *N*-dodecane or *N*-nonane moieties. The most commonly used are 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA); 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA); and 1,4,7-triazacyclononane, 1-glutaric acid-4,7-acetic acid (NODAGA). They have tetra or triacetic acid structure which forms stable coordination complexes with ^{68}Ga. Complex formation with high stability with macrocyclic ligands provides preferential use.

Macroaggregated albumin (MAA) is a sterile and apyrogenic product used for scintigraphy or vascular circulation to detect pulmonary blood supply. MAA belongs to a group of drugs called radiopharmaceutical agents. Human serum albumin spheres labeled with ^{68}Ga is a known use in PET studies of pulmonary perfusion, as PET markers of tissue perfusion. However, Even et al. [8] and Maziere et al. [9] reported a method for the direct labeling of MAA with ^{68}Ga. Similar results have been reported by using commercially available ^{99m}Tc-MAA kit systems [10–12]. The ^{68}Ga-labeled albumin sphere can be effectively obtained by hydrolysis and

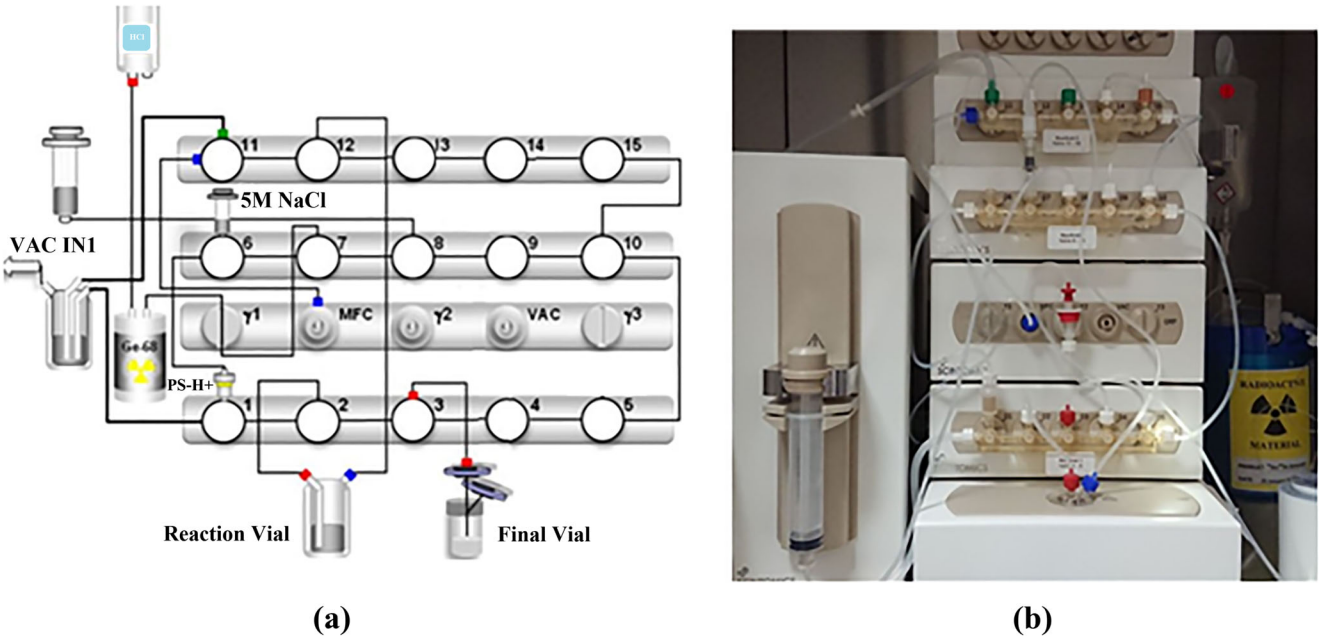

Fig. 2 Software interface module diagram of the automated Scintomics radiosynthesis system used to prepare ^{68}Ga-MAA (**b**). Original Scintomics radiosynthesis module equipped with cassette for the labeling with MAA of ^{68}Ga

precipitation of $^{68}Ga^{+3}$ ion in the presence of albumin particles, or by covalent conjugation of the spheres with a high-affinity gallium chelating ligand.

We report a simple, a reliable, and rapid procedure for labeling in the Scintomics automated synthesis module using without organic solvents by cationic method of ^{68}Ga with MAA using a commercially available MAA labeling kit for ^{99m}Tc. The software has been specially rewritten and adapted to the module for automatic synthesis. In addition, the quality control of the synthesized product and whether the MAA particles undergo a structural degradation are presented.

Materials and Methods

All synthesis and quality control studies were performed in the radiopharmaceutical laboratory of the Nuclear Medicine Department of our University Hospital. Even though the synthesis of radiopharmaceuticals used in nuclear medicine can successfully be carried out manually in the laboratory, the use of automated synthesis systems is increasing day by day. The development of automated synthesis systems has increased the reliability, reproducibility, and radiation safety of radiopharmaceutical productions. The manual labeling procedure was adapted to an automated synthesis module.

This study employed a 1.11 GBq (30 mCi) GalluGEN $^{68}Ge/^{68}Ga$ generator (radionuclide purity ≥98%) that is currently distributed in by the Pars Isotope (Tehran, Iran). The generator age was 6 months at the time these experiments were performed. The fact that the $^{68}Ge/^{68}Ga$ generator is not stationary and eluted with 0.1 N HCl after incubation advises that bacterial growth does not occur in the colon.

A commercial Mallinckrodt MAA Kit (LyoMAA, Technescan, UK) contains approximately 4.4 million particles (vials of 2 mg) of human serum albumin macroaggregates. The other ingredients are sodium chloride, sodium acetate and stannous chloride dehydrate that were suspended in 5 mL sterile saline, isolated by centrifugation (3 min at 3000 rcf); the supernate discarded; and the MAA resuspended in 1 mL sterile saline. These processes were done in MAA sterile vial.

^{68}Ga-MAA synthesis was carried out using the GRP module 4 V automated synthesis system (Scintomics GmbH, Fürstenfeldbruck, Germany) from Scintomics GmbH. The generator was eluted 24 h before labeling to remove the accumulated stable ^{68}Zn from ^{68}Ga decay. For every labeling process, a sterile single-use cassette is installed in the labeling module. Radiolabeling is performed under aseptic conditions in the grade C environment. After installation of the cassette, a leak test is performed with 0.9% NaCl to verify that no leakage occurs. The movement of all liquid reactants is realized; a 10-mL syringe is connected to the syringe-module driven by the Modular-Lab Pharm Tracer® software. The Modular-Lab Pharm Tracer® software has been specially rewritten and adapted to the module for automatic synthesis; the modular software provides a graphical display of the progress of the synthesis and is stored on the computer for control when the synthesis is complete.

7 mL $^{68}GaCl_3$ eluted with 0.1 N HCl (from ABX D-01454 Radeberg, Germany) from the $^{68}Ge/^{68}Ga$ generator will be passed through the PSH^+ cartridge to yield ^{68}Ga of high purity (^{68}Ge residues and toxic metals removed). The PSH^+ cartridge is an original part of the cassette and does not require preconditioning. The ^{68}Ga solution was filtered through a sterile 0.2-μm membrane and aseptically added into the suspension of washed MAA (in 5 mL sterile saline). Reaxion vial was added 1.5 M HEPES buffer solution (*N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid)) (from ABX D-01454). After vigorous mixing with MAA, the ^{68}Ga-MAA suspension was incubated in a heat block at different temperatures and different incubation times. ^{68}Ga-MAA activity was measured in a Comecer VDC-606 touch screen dose calibrator. Only plastic needles were used to prevent the presence of metal traces that could seriously affect the synthesis efficiency.

For ensuring that the final injectable radiopharmaceutical product fulfills regulatory requirements relating to contaminants, suitable production and quality control are crucial [13]. The pH value of the ^{68}Ga-MAA solution in the product vial is checked, and a 5 μL sample is dripped onto Whatman No. 40 paper (8-cm length, 1 cm thick) and carried out with mobile phase to calculate the Rf values. Acetone/glacial acetic acid (9:1) solution was used as mobile phase [14]. Radioactivity measurements will be done with dose calibrator.

The morphological structure of $^{68}GaMAA$, the synthesis product of the study, was analyzed in our University Advanced Technology Application and Research Center in terms of microanalytical aspects. For this purpose, scanning electron microscopy analysis (Quorum Q150R-ES) selected samples were photographed at ×100.00 magnification, and EDX analysis was examined, after the resulting ^{68}Ga-labeled MAA particles were evaluated for structural degradation. In addition, ^{68}Ga degradation, the major radionuclide ^{68}Ge, and other toxic metal content in the final product were investigated. The material was coated with 80% Au and 20% Pd. Au/Pd to ensure conductivity under the electron microscope (SEM). Image analyzes were performed on a secondary electron detector (SE2) on Zeiss Supra 40Vp under a voltage of 30 KV.

A radiopharmacy chemist and a nuclear medicine physician were trained in radiation protection and wear personal optically stimulated luminescence dosimeter (OSLs) (Epsilon, Landauer, USA) which are evaluated monthly. For this study, electronic personal pocket and wrist dosimeters were used (OSL).

Results

Radiolabeling

Since the prewash step was important before adding ^{68}Ga to MAA particles in commercial kits, we carried out the labeling process by washing the MAA particles in our study. We obtained ^{68}Ga-MAA (99% ± 0.1; n = 5) with a higher radiochemical purity than the labeled ^{68}Ga-MAA radiochemical purity (95% ± 0.1; n = 5) without washing. The synthesis time is completed in 16 min in total, and the radiochemical yield after synthesis was 85 ± 3%.

^{68}Ga-MAA-labeled at optimized pH (pH 4–5) is available for clinical application at 80% of total activity 15 min after labeling (quality control time). No radical scavenger was added during the labeling to increase the synthesis yield. The labeling procedure we developed provided maximum ^{68}Ga-MAA activity.

Investigation of the Effect of Temperature and Labeling Time on Labeling Efficiency

Labeling was carried out at 4 different labeling temperatures. The optimum time was determined by studying 4 different labeling times at the determined optimum labeling temperature (90 °C). Increased labeling time and lower temperatures were found to reduce yield (Table 1). The results were evaluated as the average of the radiochemical purity of the repeated 5 times syntheses.

Quality Control

The radiochemical purity of the $^{68}GaCl_3$ solution was checked with Whatman paper in solvent. Other ionic forms of ^{68}Ga such as $^{68}GaCl^{4-}$ (if existed) remained at the origin (Rf:0). Free $^{68}Ga^{3+}$ was coordinated as ^{68}Ga-MAA and migrated to higher Rf. The presence of other ionic species is rare in the presence of a very strong complexing agent (i.e. MAA). Measurements of radioactivity in Rf:0 taken in mobile phase can be considered an insignificant colloidal impurity or ionic impurity.

The structure, morphology, and chemical composition of ^{68}Ga-labeled ^{68}Ga-MAA were analyzed by scanning electron microscopy (SEM)/energy dispersive X-ray (EDX). The resulting ^{68}Ga-labeled MAA particles were examined for structural degradation (Fig. 3). SEM analyses showed the ^{68}Ga-labeled MAA particles to remain within their original size range.

The sterility of the ^{68}Ga final product filtered prior to labeling was not an issue. However, to have a successful kit, the sterility of the final product is very important. To maintain ^{68}Ga-MAA sterility, ^{68}Ga was filtered through a 0.22-μm filter prior to labeling. Likewise, the MAA kit should be produced for ^{68}Ga labeling without albumin and $SnCl_2$, and its sterility should be guaranteed. Due to the size of the MAA particles, final product sterility cannot be guaranteed by filtration. In the human use of ^{68}Ga-MAA, care should be taken to ensure that sterilization is not compromised during all labeling steps and that the synthesis is carried out in a sterile room.

Radiation Safety

The labeling procedure was performed in the lead cabinet in the auto-synthesis module, and withdrawal to the injector took place on a laboratory bench with a lead protector. Personal wrist and collar dosimeter with routine OSL monitoring showed no increase in exposure to body radiation. The instantaneous total personal radioactivity dose during a radioactive labeling process was found to be 3 μSv. However, there is no alternative protective method other than wearing a protective vest during syringe withdrawal and injection.

Discussion

The ^{68}Ge/^{68}Ga generator system used for the study is based on a TiO_2/SnO_2 stationary phase. The generator elution is generally carried out in accordance with the manufacturer's specifications. There is a guarantee that both the initial and permanent ^{68}Ge excretions of the pars generator as specified by the certificate shall be less than 0.0001%. In addition to ^{68}Ge excretion, high acidity of the eluate and presence of further metal ion contaminants such as Zn(II) and Fe(III) are problems addressed by these so-called postprocessing procedures. There is no defined limitation for metal pollutants, but research has shown that trivalent metal cations, in particular, can inhibit effective radioactive labeling with ^{68}Ga. In the presence of metallic impurities from the column material

Table 1 Effect of temperature and labeling time on labeling efficiency (n = 5)

Temperature (°C)	Average yield % ±0.5	Time (min) (in temp. 90 °C)	Average yield % ±0.5
70	90	3	85
80	95	5	97
90	99	7	99
100	99	10	89

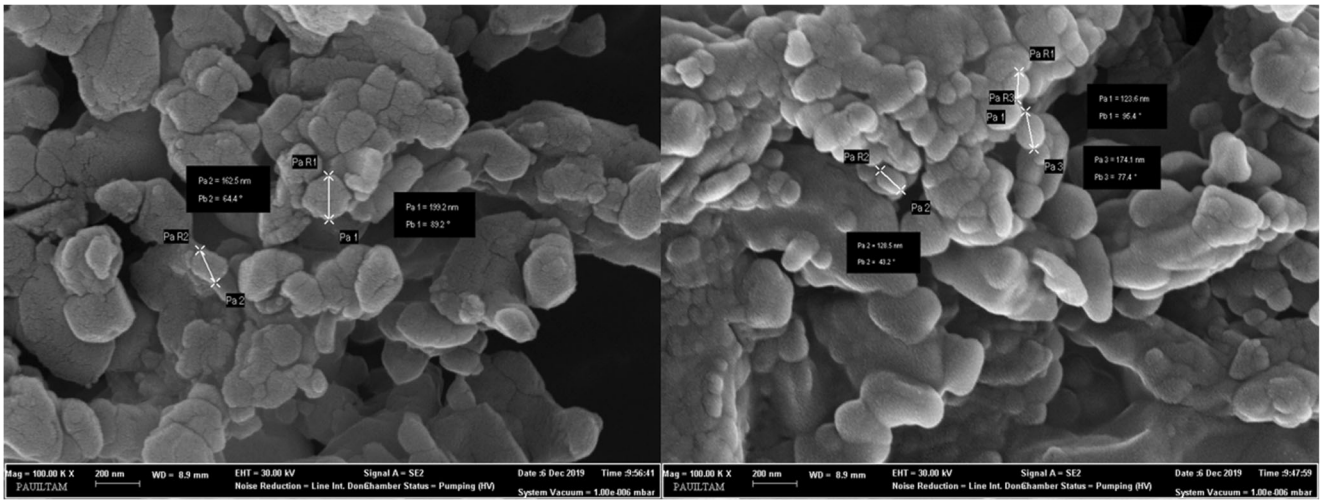

Fig. 3 SEM images of the MAA particles before (left) and after (right) labeling with ^{68}Ga. (200 nm, Mag ×100.00 K, EHT 30.00 KV)

and excess protons from hydrochloric acid used for elution, the generator must be eluted under current good manufacturing practice (cGMP) conditions to ensure safe and sterile production. It provided high-efficiency labeling by allowing rapid purification of ^{68}Ga by adsorption/elution with NaCl from the PSH^{+} cartridge. Higher values of ^{68}Ge excretion

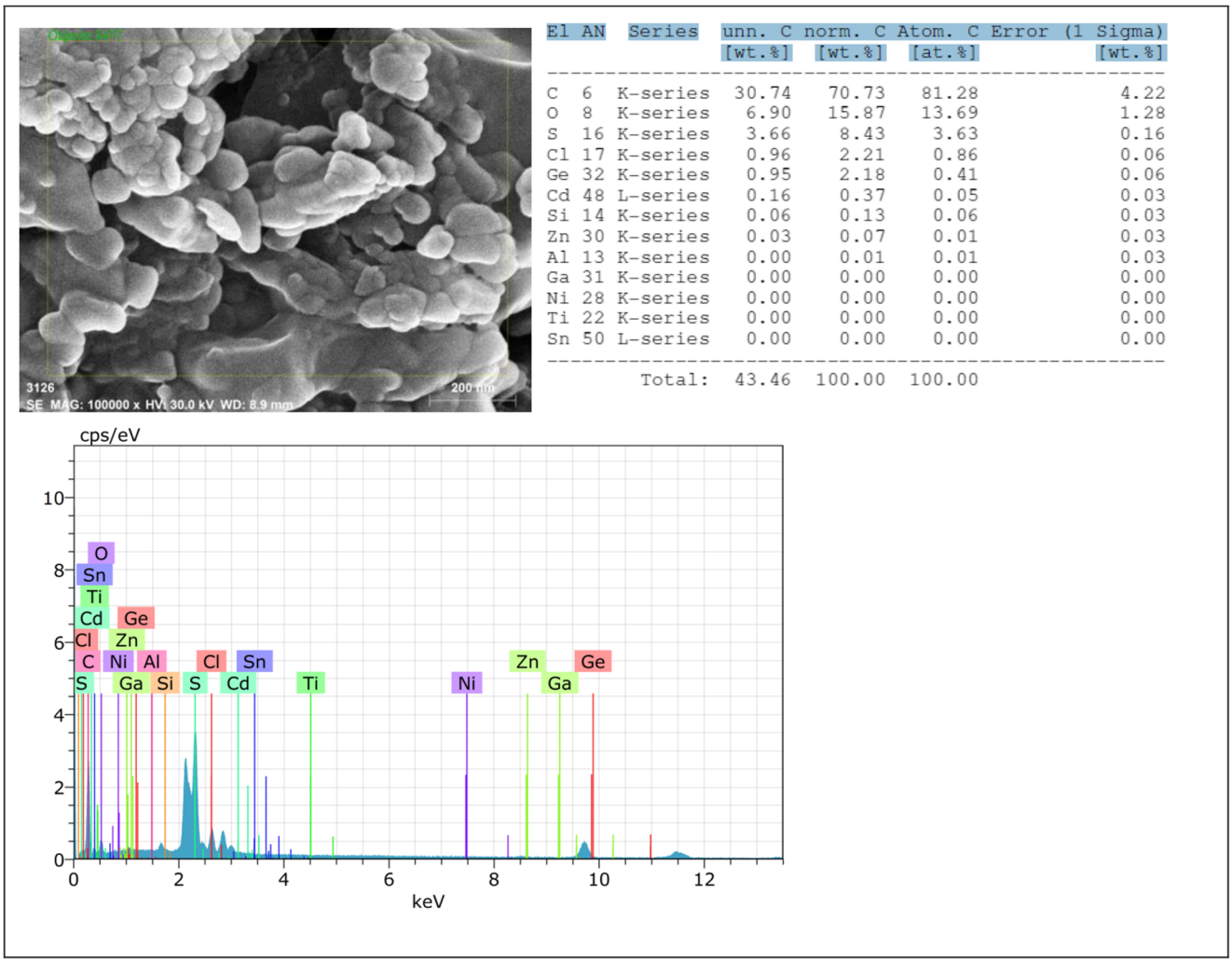

Fig. 4 SEM-EDX analysis results of ^{68}Ga-MAA (SEM images; 200 nm, Mag × 100.00 K, EHT 30.00 KV)

have been reported by other researchers using this generator system [3].

The ^{68}Ga-labeling method for albumin microspheres was adapted for use with the commercial kits available for the preparation of ^{99m}Tc-MAA [8]. Mathias et al. [15] reported higher labeling efficacy using $SnCl_2$-free MAA in ^{68}Ga-MAA labeling. Mueller et al. [12] compared the ^{68}Ga labeling efficacy of radiopharmaceuticals using $SnCl_2$-free MAA and original MAA kits and reported no difference in synthesis yield. The prewash step is important before adding ^{68}Ga to MAA particles in commercial kits. While removing some of the Sn(II) associated with the formulation in the prewash, the increased labeling efficiency with the prewash step is believed to be largely due to the removal of the free albumin adjuvant contained in commercial MAA.

The chemical structure of ^{68}Ga bound to MAA particles is unknown. In reaction mechanism, ^{68}Ga is adsorbed to insoluble gallium hydroxide on the surface of MAA particles; Ga(III) ion was evaluated as a mechanism for capturing specific interactions with proteins on the particle surface.

Increased labeling time and lower temperatures were found to reduce yield (Table 1). Binding was observed at lower temperatures due to the chemical structure of the MAA, but the optimum temperature was found to be 90 °C.

Many studies have reported that the in vitro stability of pharmaceuticals labeled with ^{68}Ga is stable for longer than 60 min [16, 17]. We have shown in our previous study that ^{68}GaMAA that we labeled is stable [18].

As with all radiopharmaceuticals marked with generator-generated radionuclides and intended for human use, careful monitoring of parental excretion is clearly necessary to accurately predict (and control) patient radiation, especially when the final product contains a relatively long-lasting parent. The long-lived radionuclide content of the synthesized ^{68}GaMAA was found to be well below the limit values (Fig. 4). The generator solution comprises a series of metal cations that can compete with the individual ^{68}Ga(III) resulting from the column matrix of the generator, reducing specific radioactivity. Metal cations compete with Ga(III) differently (size, charge, etc.) depending on their chemical and physical properties. 2, 3, and 4 valued cations which can form complexes: zinc (Zn), tin (Sn), nickel (Ni), lead (Pb), aluminum (Al), silicon (Si), and titanium (Ti). Zinc (Zn), the decomposition product of ^{68}Ga from pollutant metals from the generator, is a very strong competitor that can complex with chelators [19]. When the results of SEM-EDX analysis in the final product were examined, it was determined that most of these metals were not present in the product content, and some of them were trace amounts. In addition, zinc (Zn) value was determined below the limits specified in the European Pharmacopoeia Monograph (max. 10 Mg/GBg) [13]. No residues of tin and titanium from the generator column matrix were detected.

The radiation dose received by the staff during the labeling procedure was determined far below the dose that the personnel would receive in the TAEK regulation.

Conclusion

The automated synthesis algorithm with cationic method we developed may label MAA with ^{68}Ga as high efficiency and high purity in a very short time without loss of material in Scintomics automated synthesis module. Quality control of the final product content was examined, and radiopharmaceutical production was carried out in accordance with GMP guidelines. The radiochemical yield is about 99%. When SEM-EDX analyses were performed; no major radionuclide evidence was detected in the final product. It does not use any toxic compound in the procedure. Due to the high chemical and radiochemical purity, a final purification step is not required.

Author Contribution Ayşe Uğur: this author is a radiochemist who synthesized ^{68}Ga-MAA and made quality control. He wrote the article.
Aziz Gültekin: this author organized the research.
Doğangün Yüksel: this author advised the study.

Declarations

Conflict of Interest Uğur A, Gültekin A, and Yüksel D declare that they have no conflict of interest.

Ethics Approval and Consent to Participate This work does not contain any studies with human participants or animals performed by any of the authors.

References

1. Vermeulen K, Vandamme M, Bormans G, Cleeren F. Design and challenges of radiopharmaceuticals. Semin Nucl Med. 2019;49:339–56.
2. Kumar V, Boddeti DK. (68)Ga-radiopharmaceuticals for PET imaging of infection and inflammation. Recent Results Cancer Res. 2013;194:189–219.
3. Meyer GJ, Mäcke H, Schuhmacher J, Knapp WH, Hofmann M. ^{68}Ga-labelled DOTA-derivatised peptide ligands. Eur J Nucl Med Mol Imaging. 2004;31:1097–104.
4. Eppard E, Homann T, de la Fuente A, Essler M, Rösch F. Optimization of labeling PSMAHBED with ethanol-postprocessed ^{68}Ga and its quality control systems. J Nucl Med. 2017;58:432–7.
5. Roesch F. Maturation of a key resource–the Germanium-68/Gallium-68 generator: development and new insights. Curr Radiopharm. 2012;5:202–11.
6. Chao JC, Hong A, Okey RW, Peters RW. Selection of chelating agents for remediation of radionuclide contaminated soil. Proceedings of the Conference on Hazardous Wastes Research. 1 May, Snowbird, Utah; 1998. p. 142–156
7. Fernández-Barahona I, Muñoz-Hernando M, Pellico J, Ruiz-Cabello J, Herranz F. Molecular imaging with ^{68}Ga radio-

nanomaterials: shedding light on nanoparticles. Appl Sci. 2018;8(7):1098.
8. Even GA, Green MA. Gallium-68-labeled macroaggregated human serum albumin, ^{68}Ga-MAA. Nucl Med Biol. 1989;16:319–21.
9. Maziere B, Loc'h C, Steinling M, Comar D. Stable labeling of serum albumin microspheres with gallium-68. Appl Radiat Isot. 1986;37(4):360–1.
10. Amor-Coarasa A, Milera A, Carvajal D, Gulec S, McGoron AJ. Lyophilized kit for the preparation of the PET perfusion agent [^{68}Ga]-MAA. Int J Mol Imaging. 2014;7:269365.
11. Jain A, Subramanian S, Pandey U, Sarma HD, Ram R, Dash A. In-house preparation of macroaggregated albumin (MAA) for ^{68}Ga labeling and its comparison with commercially available MAA. J Radioanal Nucl Chem. 2016;308:817–24.
12. Mueller D, Breeman WAP, Klette I, Gottschaldt M, Odparlik A, Baehre M, et al. Radiolabeling of DOTA-like conjugated peptides with generator-produced ^{68}Ga and using NaCl-based cationic elution method. Nat Protoc. 2016;11:1057–66.
13. European Pharmacopoeia Commission, Council of Europe. European pharmacopoeia. 8th ed. Strasbourg: European Directorate for the Quality of Medicines & Health Care, 2013.
14. Skorupa A, Gierak A. Detection and visualization methods used in thin-layer chromatography. J Chromatogr Sci. 2011;24(4):274–80.
15. Mathias CJ, Green MA. A convenient route to [^{68}Ga]Ga-MAA for use as a particulate PET perfusion tracer. Appl Radiat Isot. 2008;66:1910–2.
16. Ament S, Maus S, Reber H, Buchholz HG, Bausbacher N, et al. PET lung ventilation/perfusion imaging using ^{68}Ga aerosol (Galligas) and ^{68}Ga-labeled macroaggregated albumin. Recent Results Cancer Res. 2013;194:395–423.
17. Breeman WAP, de Jong M, de Blois E, et al. Radiolabelling DOTA-peptides with ^{68}Ga. Eur J Nucl Med Mol Imaging. 2005;32:478–85.
18. Gültekin A, Çayır MÇ, Uğur A, Bir F, Yüksel D. Detection of pulmonary embolism with Gallium-68 macroaggregated albumin perfusion PET/CT: an experimental study in rabbits, contrast Media Mol Imaging. 2020;5607951. https://doi.org/10.1155/2020/5607951.
19. Uğur A, Yaylalı O, Yüksel D. Examination of metallic impurities of ^{68}Ge/^{68}Ga generators used for radioactive labeling of peptides in clinical PET applications. Nucl Med Commun. 2020. https://doi.org/10.1097/MNM.0000000000001307.