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Quantitative online isolation of ^{68}Ge from ^{68}Ge/^{68}Ga generator eluates for purification and immediate quality control of breakthrough

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HIGHLIGHTS

- ^{8}Ga is separated quantitatively and online from co-eluted ^{68}Ge.
- Separation was achieved by the use of a cation exchange resin.
- ^{68}Ge breakthrough in ^{68}Ge/^{68}Ga generators eluate is determined immediately.

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ABSTRACT

The breakthrough of ^{68}Ge from a ^{68}Ge/^{68}Ga-generator is one of the most sensitive parameters in the context of the clinical application of ^{68}Ga-radiopharmaceuticals. The difficulty in its determination lies in the "spectroscopic invisibility" of ^{68}Ge within an excess of ^{68}Ga.

The introduced method for determining the ^{68}Ge content of the ^{68}Ge/^{68}Ga-generator eluate involves the quantitative separation of ^{68}Ga from ^{68}Ge, using a cation-exchanger.

The eluate contains ^{68}Ga free of ^{68}Ge, which can be determined immediately, i.e. prior to the application of the ^{68}Ga-radiopharmaceutical.

1. Introduction

Representative commercially available ^{68}Ge/^{68}Ga-generators provide initial activities of 20 mCi (0.74 GBq), 30 mCi (1.11 GBq), 50 mCi (1.85 GBq) or more of ^{68}Ge and ^{68}Ga. In the first weeks of use, elution produces ^{68}Ga-yields of 60–90%, accompanied by ^{68}Ge-breakthrough between 10^{-2} and 10^{-4}% of the respective initial activities (Roesch and Riss, 2010; Loktionova et al., 2009). This breakthrough of ^{68}Ge from a ^{68}Ge/^{68}Ge-generator is a critical parameter in the context of the routine clinical application of ^{68}Ga-radiopharmaceuticals (Breeman and Verbruggen, 2007; Breeman et al., 2011) Breakthrough refers to the actual ^{68}Ge load of the generator, and is reported as the activity of ^{68}Ge in the eluate relative to that on the generator column. This is the preferred definition, but can also be expressed as the activity of ^{68}Ge co-eluted relative to the ^{68}Ga present in the eluate fraction (Breeman et al. 2011).

^{68}Ge decays exclusively by electron capture and therefore can only be radiochemically detected indirectly through the photon emission of its daughter nuclide, ^{68}Ga. Therefore it is not possible to detect ^{68}Ge within an excess of ^{68}Ga using γ-spectroscopy. For a "fresh" 30 mCi generator, ^{68}Ge activities in the eluate are 3 μCi–30 nCi (for breakthrough levels of 10^{-2}–10^{-4}%), within 18–27 mCi of ^{68}Ga corresponding to 60–90% initial ^{68}Ga elution yield. The decay of the eluted ^{68}Ga, according to its half-life ($t_{1/2}$=67.71 min) follows Eq. (1):

$$A^{t}_{Ga} = A^{o}_{Ga}(e^{-\lambda t}) \tag{1}$$

$$\ln(A^{t}_{Ga}) = \ln(A^{o}_{Ga}) - \frac{\ln 2}{t_{1/2}} t \tag{2}$$

$$\approx \ln(A^{o}_{Ga}) - 0.01t \tag{3}$$

$A_{Ga}{}^{0}$=^{68}Ga activity immediately after elution; $A_{Ga}{}^{t}$=^{68}Ga activity at time points t after elution.

The total activity of ^{68}Ga in the generator eluate, originates from both by the decay of eluted ^{68}Ga and ^{68}Ga generated from the ^{68}Ge co-eluted. Thus, Eq. (4) applies,

$$A^{t}_{Ga} = A^{o}_{Ga}(e^{-\lambda_{Ga} t}) + \frac{\lambda_{Ga}}{\lambda_{Ga} - \lambda_{Ge}} A^{o}_{Ge}(e^{-\lambda_{Ge} t} - e^{-\lambda_{Ga} t}) \tag{4}$$

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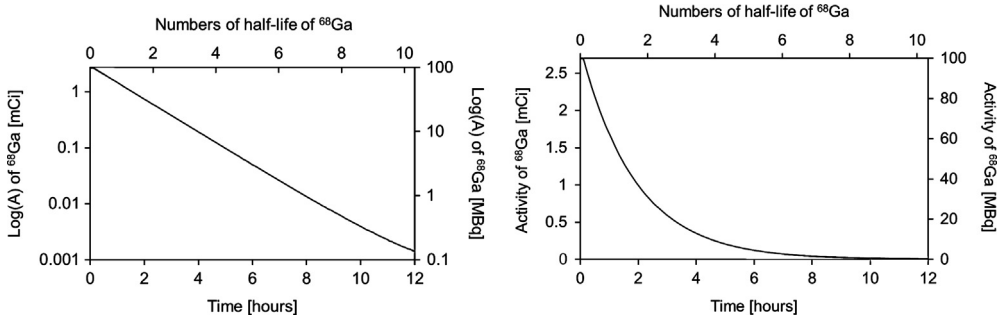

Fig. 1. Decay of initially eluted 27 mCi of ^{68}Ga in terms of real time after elution (lower *x*-axis) and multiples of half-live of ^{68}Ga (upper *x*-axis), with logarithmic (LOG) or linear scale (LIN) of activity.

$A_{Ga}{}^{0}$=^{68}Ge activity co-eluted (=breakthrough); $A_{Ga}{}^{t}$=^{68}Ga activity at time points *t* after elution; $\lambda = \ln 2/t_{1/2}$.

Fig. 1 shows the decay of eluted ^{68}Ga with time (shown in hours, and as the number of ^{68}Ga half-lives) in linear and logarithmic forms. The ^{68}Ga activity eluted is reduced to 10^{-2} and 10^{-3}% of the initial activity present in the eluate at 7.50 and 12.00 h post elution, respectively. At these two time points, the ^{68}Ga activity in the generator eluate equals the ^{68}Ga activity generated by decay of ^{68}Ge-breakthrough levels of 10^{-2} and 10^{-3}%, respectively. That is, the measured activity is constant because the rate of ^{68}Ga decay is equal to the rate of ^{68}Ga generation (from ^{68}Ge decay).

Current methods for the γ-spectroscopic determination of ^{68}Ge involve storing the generator eluate solutions for long periods, to allow for the complete decay of the excess eluted ^{68}Ga down to the ^{68}Ge levels. However, legal regulations governing radiopharmaceutical production require that quality control to be completed prior to the clinical application of the radiopharmaceutical. Given the significantly shorter half-life of ^{68}Ga compared to the time taken for the ^{68}Ge determination by this method, such a determination is not feasible in clinical application. Obviously then, this method of determination does not fit with the standard protocols for the preparation, application and quality control of ^{68}Ga-radiopharmaceuticals.

If the level of ^{68}Ga co-eluted with the ^{68}Ge-breakthrough was zero, then increasing activities of ^{68}Ga would be measured. This would occur according to the secular radionuclide generator equilibrium, Eq. (4), (Roesch and Knapp 2011) which simplifies to Eq. (5) when $A^{o}_{Ga} = 0$

$$A^{t}_{Ga} = A^{o}_{Ge}(1-e^{(\ln 2/t_{1/2})t}) \quad (5)$$

Saturation or equilibrium is obtained when

$$A^{equ}_{Ga} = A^{equ}_{Ge} \quad (6)$$

Eq. (5) is illustrated graphically in Fig. 2 over a period of 10 half-lives, where ^{68}Ge generates ^{68}Ga from a generator eluate with no initial ^{68}Ga present. The activity of ^{68}Ga in an initially pure ^{68}Ge sample registered at 28.10 and 67.71 min will represent ¼ and ½ of the total ^{68}Ge activity initially present. Therefore, under these conditions, it is possible to determine the amount of ^{68}Ge present by multiplying the ^{68}Ga activity measured at 28.10 and 67.71 min by a factor of 4 and 2 respectively.

Currently used methods do not permit determination of the ^{68}Ge level in ^{68}Ga eluates, prior to the application of ^{68}Ga-radiopharmaceuticals.

The procedure described in this paper involves the removal of ^{68}Ga online from the initial generator eluate to obtain a solution completely free of ^{68}Ga, ($A^{o}_{Ga} = 0$), but containing all of the ^{68}Ge-breakthrough. This allows the ^{68}Ge content to be determined free of any activity of eluted ^{68}Ga immediately after the elution by measuring the growing ^{68}Ga activity as generated from ^{68}Ge *in situ*.

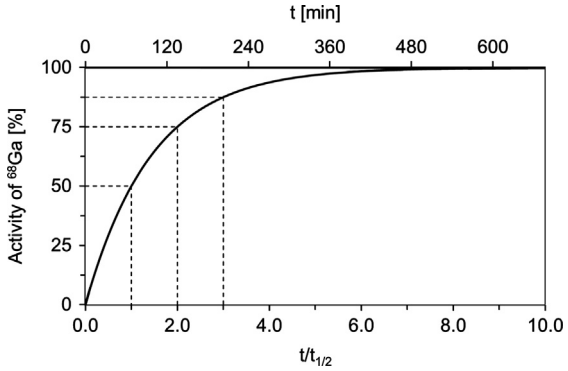

Fig. 2. Kinetics of the secular equilibrium of the ^{68}Ge/^{68}Ga radionuclide generator for $A_{Ga}{}^{0}$=25% and 50% of the saturation (equilibrium) activity of ^{68}Ga are being generated after 28.10 and 67.71 min.

Thus, the experimental concept relies on the quantitative separation of ^{68}Ga from ^{68}Ge and vice versa. Complete separation is achieved by the quantitative online adsorption of ^{68}Ga using strong cation-exchangers (CEXs). The use of CEX adsorbers is derived from the well-established concept used for the post-processing commercial "ionic" ^{68}Ge/^{68}Ga radionuclide generators to separate ^{68}Ga(III) and ^{68}Ge(IV). Zhernosekov et al. (2007) described ~96% online adsorption of available ^{68}Ga from 0.1 N HCl eluate solutions onto 53 mg of AG 50W-X8 (minus 400 mesh), whereas ^{68}Ge was not retained. This is also true for 0.05 N HCl eluting solution as used with organic resin based ^{68}Ge/^{68}Ga-generators, as well as 0.6 N HCl eluting solution as used with SnO_2-based inorganic resin generators (Roesch and Riss, 2010; Loktionova et al., 2009). The aim of this work was to increase the adsorption potential of the cation exchange resin from ~96% to 100% by increasing the amount of resin and/or altering its constitution.

2. Methods

^{68}Ge/^{68}Ga-generator: a two years old ^{68}Ge/^{68}Ga-generator (EZAG, Obninsk) with a yield of ~100 MBq ^{68}Ga and a ^{68}Ge-breakthrough of ~85 kBq was used. The generator was eluted with 5 ml of 0.1 N HCl in all cases.

Two strong acidic CEXs AG 50W-X8 (−400 mesh, Biorad) and SCX (Phenomenex), (cf. Table 1) were investigated for the online separation for ^{68}Ga and ^{68}Ge. The CEXs were used separately in different amounts, and in various combinations. In the case of combined resin columns, the generator was eluted first through the AG 50W-X8 and subsequently the SCX column. Table 1 shows

Table 1
Amount and combination of the used resins for complete partition of ^{68}Ge and ^{68}Ga.

Nr.	AG 50W-X8 (mg)	SCX (mg)
(iii)	50	–
(iv)	50	30
(v)	50	120
(vi)	220	–
	220	120

a list of the different CEX systems investigated. In order to determine the time required to reach equilibrium of $A_{Ga} = A_{Ge}$, the elution was performed without a CEX.

^{68}Ga was adsorbed online by the CEX, whilst ^{68}Ge was not retained and passed through the CEX into a measuring vial. The radioactivity of the CEX eluate was determined in a Curie-Meter (ISOMED 2010, Nuklear-Medizintechnik Dresden GmbH) every 5 min (automated mode) over the course of at least 20 h.

Used CEX were regenerated with 1 mL 4 N HCl followed by 1 mL water.

3. Results and discussion

Fig. 3 shows the variation of the ^{68}Ga activity measured using a Curie-Meter over a period of 20 h post generator elution without and with (ii–v) and without (i) online separation via CEXs.

Plot (i) represents the decay of the complete, non-processed, generator-eluate. The downward curve represents the overall decreasing fraction of ^{68}Ga in the waste vial. Line (ii) indicates the level of ^{68}Ga at ^{68}Ge/^{68}Ga-equilibrium, i.e. generated by the ^{68}Ge-breakthrough. A constant level of activity (or count rate) is indicative of a ^{68}Ge/^{68}Ga-equilibrium. The equilibrium activity depends on concentration of ^{68}Ge eluted (Eq. (4)) are reached after approximately 18 h.

Plots (iii)–(vi) illustrate the effect increasing adsorption of ^{68}Ga online has on the activity profile for the various CEX resins and their mass and/or combination used. Plot (iii) reflects the activity profile of the CEX eluate after using 50 mg of AG 50W-X8 resin to adsorb ^{68}Ga. Separation efficacy of almost two orders of magnitude for ^{68}Ga was achieved, which is in excellent agreement with the findings of Zhernosekov et al. (2007), indicating ~96% of online ^{68}Ga adsorption.

The use of 30 (iv) and 120 mg (v) SCX cartridges in combination with 50 mg of AG 50W-X8 further increased the amount of ^{68}Ga removed from the eluate—note the logarithmic scale used for the activity-axis of Fig. 3. Constant absolute activities of ^{68}Ga are obtained ~4 h after elution for CEX systems (iv) and (v), which is a considerable improvement on ~8 h required for system (iii). Nevertheless, the initial decrease in activity indicates that there is still an excess of ^{68}Ga present compared to that generated by ^{68}Ge.

When 220 mg of AG 50W-X8 (vi) CEX was used, an increasing activity was observed for the purified generator eluate. This increasing activity is indicative of ^{68}Ga generation by the decay of ^{68}Ge-breakthrough. Under these conditions the number of ^{68}Ga atoms present in the processed eluate is lowered by more than one order of magnitude compared to the number of ^{68}Ge atoms reflecting the breakthrough level. Therefore, the activity measured, directly represents the activity of ^{68}Ga generated by ^{68}Ge in the eluate. It was found that the addition of 120 mg SCX cartridge to the 220 mg AG 50W-X8 CEX does not further enhance ^{68}Ga absorption. Therefore, using 220 mg AG 50W-X8 alone (or in combination with 120 mg SCX) produces almost quantitative adsorption.

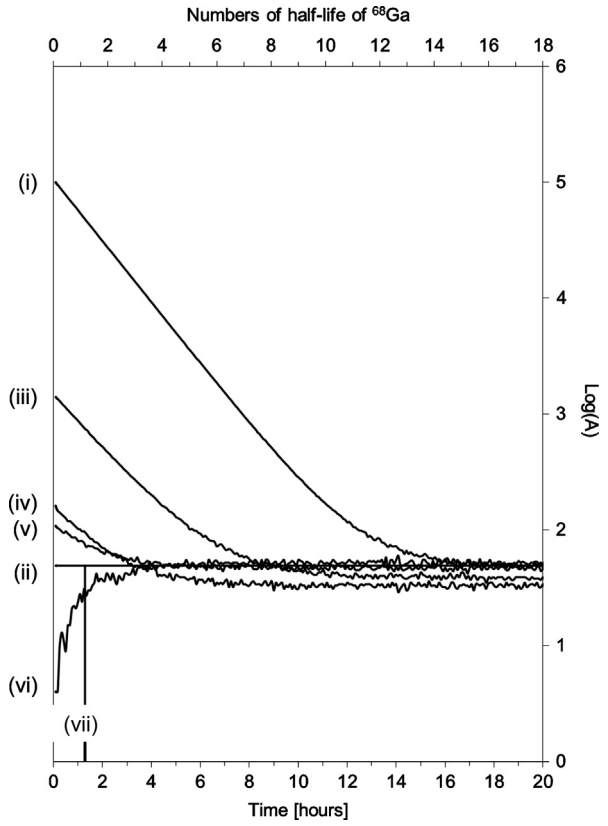

Fig. 3. Results of separation ^{68}Ga from ^{68}Ge-breakthrough for selected cation exchange resins. ^{68}Ga activities measured in kBq. Plot (i) initial, non-processed ^{68}Ge/^{68}Ga radionuclide generator eluate over time, with region (ii) equilibrium reflecting the level of ^{68}Ge activity achieved after about 18 h without purification. Plots (iii), (iv), (v) and (vi) for ^{68}Ge solutions after application of 50 mg AG 50W-X8, 50 mg AG 50W-X8+++30 mg SCX, 50 mg AG 50W-X8+++120 mg SCX and 220 mg AG 50W-X8+++120 mg SCX, respectively. Line (vii) indicates half of the ^{68}Ga equilibrium activity measured after 67.71 min post elution representing 50% of the ^{68}Ge activity present in the case of complete adsorption of ^{68}Ga.

The activity (or count rate) increases for plot (v) according to the secular characteristics of the ^{68}Ge/^{68}Ga radionuclide generator equilibrium (Eq. (4)). A plateau is reached at about 4 half-lives (~5 h post elution), which is~~~95% of equilibrium. However, ^{68}Ge activities are quantifiable within 1 h of generator elution. For example, the activity (or count rate) measured at 67.7 min post elution of the generator (after Curie-Meter background subtraction) can be doubled to give the real ^{68}Ge activity, as at this time point ^{68}Ga is generated to 50% level of equilibrium. This is indicated by line (vii) in Fig. 3.

4. Conclusion

A simple method for the determination of ^{68}Ge content in ^{68}Ge/^{68}Ga-generator eluates immediately following generator elution has been described. Importantly, the protocol is suitable for use as a quality control procedure because it can be carried out in a time-frame which allows for subsequent application of the ^{68}Ga-radiopharmaceutical.

The approach involves quantitative separation ^{68}Ga from co-eluted ^{68}Ge, and vice versa. ^{68}Ga is quantitatively separated from ^{68}Ge using AG 50W-X8 CEX resin on its own, or in combination with SCX CEX resin. The ^{68}Ge content in the generator eluate can be quantified directly after the generator elution, by allowing the ^{68}Ga to “grow” for one (or 1/2) of its half-lives in the CEX purified ^{68}Ge fraction.

The protocol described provides instantaneous information on the level of ^{68}Ge present in ^{68}Ge/^{68}Ga-generator eluates without the use of γ-spectroscopy. In addition to the quality control of radiopharmaceuticals, this protocol may also be used to monitor the ^{68}Ge-breakthrough levels of generator on a daily/weekly/monthly basis. For example, a Curie-Meter might be calibrated to a certain activity of ^{68}Ga defining a "critical" ^{68}Ge-breakthrough. Provided that the activity measured is below the critical level for that time point (e. g. 68 min), the processed eluate is within the maximum allowed ^{68}Ge-content. The European Pharmacopoeia defines a maximum ^{68}Ge-content of 10^{-3}% (European Directorate for the Quality of Medicines and Healthcare, 2013). For a new 30 mCi generator, this corresponds to 50 kBq (or less) at 68 min (one half-life of ^{68}Ga) post elution. In practice, quality control of ^{68}Ge-breakthrough would simply involve validating, that the ^{68}Ga activity in a processed generator eluate (free of eluted ^{68}Ga) at 68 min post elution remains below that level.

This "preparative" protocol could be, for example, established on a weekly basis to monitor the ^{68}Ge content of a given ^{68}Ge/^{68}Ga-generator. It could also be useful to follow the long-term performance of a routinely and/or clinically used generator, as it would indicate when the ^{68}Ge-breakthrough level is no longer acceptable.

The protocol described can be easily implemented with existing methods and modules used for ^{68}Ge/^{68}Ga-generator post-processing. Furthermore, the technology is applicable to all the commercial ^{68}Ge/^{68}Ga-generators, because the CEX resins used are equally effective with a wide range of HCl concentration (0.05–005–0005–0.6 N).

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