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**DOI**

[10.1186/s41181-023-00195-2](https://doi.org/10.1186/s41181-023-00195-2)

**Publication date**

2023

**Document Version**

Final published version

**Published in**

EJNMMI Radiopharmacy and Chemistry

**Citation (APA)**

Trapp, S., Lammers, T., Engudar, G., Hoehr, C., Denkova, A. G., Paulssen, E., & de Kruijff, R. M. (2023). Membrane-based microfluidic solvent extraction of Ga-68 from aqueous Zn solutions: towards an automated cyclotron production loop. *EJNMMI Radiopharmacy and Chemistry*, 8(1), Article 9. <https://doi.org/10.1186/s41181-023-00195-2>

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RESEARCH ARTICLE

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# Membrane-based microfluidic solvent extraction of Ga-68 from aqueous Zn solutions: towards an automated cyclotron production loop

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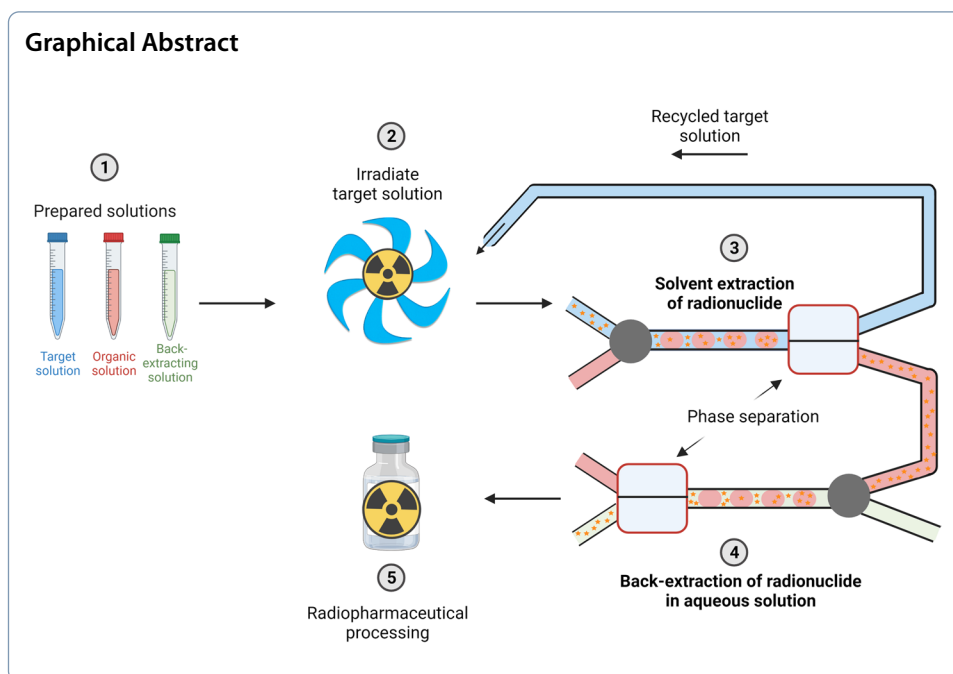
## Abstract

**Background:** The radionuclide Ga-68 is commonly used in nuclear medicine, specifically in positron emission tomography (PET). Recently, the interest in producing Ga-68 by cyclotron irradiation of [<sup>68</sup>Zn]Zn nitrate liquid targets is increasing. However, current purification methods of Ga-68 from the target solution consist of multi-step procedures, thus, leading to a significant loss of activity through natural decay. Additionally, several processing steps are needed to recycle the costly, enriched target material.

**Results:** To eventually allow switching from batch to continuous production, conventional batch extraction and membrane-based microfluidic extraction were compared. In both approaches, Ga-68 was extracted using N-benzoyl-N-phenylhydroxylamine in chloroform as the organic extracting phase. Extraction efficiencies of up to 99.5% ± 0.6% were achieved within 10 min, using the batch approach. Back-extraction of Ga-68 into 2 M HCl was accomplished within 1 min with efficiencies of up to 94.5% ± 0.6%. Membrane-based microfluidic extraction achieved 99.2% ± 0.3% extraction efficiency and 95.8% ± 0.8% back-extraction efficiency into 6 M HCl. When executed on a solution irradiated with a 13 MeV cyclotron at TRIUMF, Canada, comparable efficiencies of 97.0% ± 0.4% were achieved. Zn contamination in the back-extracted Ga-68 solution was found to be below 3 ppm.

**Conclusions:** Microfluidic solvent extraction is a promising method in the production of Ga-68 achieving high efficiencies in a short amount of time, potentially allowing for direct target recycling.

**Keywords:** Microfluidic solvent extraction, Ga-68, Cyclotron production, Medical radionuclide production, Zinc nitrate liquid target, Metal contaminants



## Background

The use of  $^{68}\text{Ga}$ -labelled radiopharmaceuticals has become increasingly popular in nuclear medicine over the last 2 decades. Ga-68 has ideal decay characteristics for high quality PET imaging, such as a positron yield of 89.1% and an average  $\beta^+$  energy of 836 keV (Sanchez-Crespo 2013) while the short half-life of 67.71 min minimizes the total dose to the patient and medical personnel. Since its radiolabelling chemistry is well understood, it can easily be applied in a variety of radiopharmaceuticals (Banerjee and Pomper 2013; Maecke and André 2007; Isabel and Prata 2012). Recently, a new Ga-68 prostate-specific membrane antigen ligand ( $^{68}\text{Ga}$ ][Ga-PSMA-11) for PET imaging of metastasized prostate cancer was approved by US Food & Drug Administration (2022), which can detect significantly more prostate lesions than the previously used  $^{18}\text{F}$ ]fluciclovine (Calais et al. 2019). Furthermore,  $^{68}\text{Ga}$ -labelled fibroblasts activation protein inhibitors (FAPI) showed remarkable results in PET imaging of up to 30 different tumor types (Kratochwil et al. 2019). Currently, over 500 clinical trials are using  $^{68}\text{Ga}$ -labelled compounds as a diagnostic agent (Trials 2022) showing that the applications of  $^{68}\text{Ga}$  in nuclear medicine are steadily expanding. An increase in demand in Ga-68 can therefore be expected. To date, Ga-68 is mostly produced by Ge-68/Ga-68 generators, due to their easy handling and accessibility for hospitals without an irradiation infrastructure (Maecke and André 2007; Rösch 2013). But several limitations of these generators, such as the high costs, an increasing shortage in the target material to produce Ge-68, the limited amounts of Ga-68 that can be eluted from a generator in a day (Rösch 2013; Lambrecht 1983; Dash and Chakravarty 2019), as well as the high amounts of radioactive Ge-68 waste after the shelf-life of a generator is reached, result in the necessity to investigate other Ga-68 production routes. Proton irradiation of Zn-68 could significantly amplify the production of Ga-68 by

the  $^{68}\text{Zn}(p,n)^{68}\text{Ga}$  reaction (Velikyan 2015). Several studies on the cyclotron production of Ga-68 from solid and liquid Zn targets exist to date (Pandey et al. 2014a, 2019; Riga et al. 2018; Alves et al. 2017a; Rodnick et al. 2020; Engle et al. 2012). Although the irradiation of liquid targets results in lower production yields compared to solid targets, they benefit from reduced target preparation steps pre and post irradiation, while still producing higher activities than generators. Most commercial Ge-68/Ga-68 generators produce a maximum of 1.85 GBq per elution, although this has recently increased with the development of a Ge-68/Ga-68 generator producing up to 3.7 GBq per elution (Radiopharma. 2021). These generators can only be eluted between one to three times per working day, dependent on the generator, loaded Ge-68 radioactivity, and the generators age (Velikyan 2015). In comparison, Alves et al. (2017b) reported a yield of 6 GBq Ga-68 in a single 45 min irradiation of a  $^{68}\text{Zn}$ ]Zn liquid target with a 12.9 MeV medical cyclotron and a beam current of 45  $\mu\text{A}$ , which can be upscaled to produce up to 40 GBq per irradiation by increasing the concentration of  $^{68}\text{Zn}$ ]Zn in the liquid target. Therefore, a single irradiation of a  $^{68}\text{Zn}$ ]Zn liquid target could produce a more than 10-times higher yield than the best currently available generators, making it a worthy alternative for hospitals that have a medical cyclotron (up to 12 MeV (Siikanen et al. 2021)) infrastructure available. But at the same time, current extraction methods of Ga-68 from the target solution after irradiation are multistep procedures that can lead to a significant loss of activity due to natural decay and do not enable direct recycling of the costly, enriched target material. Current literature on Ga-68 production using liquid targets focus mostly on the purification of Ga-68 using a combination of anion and cation exchange resins. As a first step cation exchange resins are used (e.g., hydroxamate, DOWEX 50W-X8, AG-50W-X8) to trap Ga-68 and wash most  $^{68}\text{Zn}$ ]Zn. Enriched  $^{68}\text{Zn}$ ]Zn must be recovered and processed from the washing solutions before it can be reused for irradiation. Next, Ga-68 is eluted from the first column in large volumes of highly concentrated HCl solutions and loaded onto an anion exchange resin (e.g., Biorad 1X8, AG-1X-8, DGA, TK200) from which Ga-68 can be eluted in 0.1 M HCl or water, depending on the resin (Pandey et al. 2014a, 2019; Riga et al. 2018; Alves et al. 2017a, b). These methods usually lead to a purified  $^{68}\text{Ga}$ ]GaCl<sub>3</sub> solution in 30–60 min with yields between 78 and 90%. Microfluidic solvent extraction presents a very attractive, fast alternative to column chromatography (Martini et al. 2019) for the separation of Ga-68 from the target solution, with an efficient two-step procedure that potentially enables extraction automatization and direct target recycling. In this approach, the target solution is not changed after irradiation (e.g. change of acid concentration), and can therefore potentially be directly recycled without any further processing steps as shown in Graphical Abstract. While several studies exist on microfluidic solvent extraction on other medical radionuclides (Trapp et al. 2023; Martini et al. 2021; Dalmázio and Oehlke 2017), Pedersen et al. (2019) were the first to develop a microfluidic solvent extraction method to separate Ga-68 from a ZnCl<sub>2</sub> in HCl target solution. However, the rate of radiolysis of water in hydrochloric acid-based target solutions is known to lead to rapid pressure increase upon cyclotron irradiation, potentially forcing the abortion of irradiations (Pandey et al. 2014a). It can also lead to strong corrosion of the Havar<sup>®</sup> foil used in the liquid target body (Oehlke et al. 2015). Hence, irradiation

of zinc liquid targets focusses almost exclusively on zinc nitrate solutions in dilute nitric acid target (do Carmo et al. 2020; Pandey et al. 2019; Riga et al. 2018; 2017a, b; Oehlke et al. 2015) due to the ability of nitrates to scavenge free radicals produced during the irradiation of water, thereby reducing the target pressure (Pandey et al. 2014b). Zhuravlev et al. (2022) took the first steps in the development of a membrane-based microfluidic solvent extraction of Ga-68 from zinc nitrate solutions using an arylamino phosphonate leading to an extraction efficiency of 80% in flow. However, this compound is not commercially available and was newly synthesized for their approach. In our study, we present a highly efficient microfluidic solvent extraction method for the selective extraction of Ga-68 from zinc nitrate liquid target solutions using a commercially available chelator, therefore avoiding the need for complex synthesis. Batch experiments are conducted to determine equilibrium extraction efficiencies for different concentrations of potential target solutions, followed by microfluidic experiments including a proof-of-principle run with a irradiated target solution.

## Materials and methods

### Materials and analytical methods

N-benzoyl-N-phenylhydroxylamine (BPHA; reagent grade <98%; CAS: 304-88-1) and zinc nitrate hexahydrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ; reagent grade <98%) were purchased from ACROS ORGANICS (VWR, Amsterdam, the Netherlands), nitric acid ( $\text{HNO}_3$ ; Reag. ISO, Reag. Ph. Eur.), hydrochloric acid (HCl; technical grade) and chloroform (Reag. Ph.Eur., ACS; stabilized with 0.6% ethanol) and were purchased from Sigma Aldrich (Merck Sigma, Zwijndrecht, the Netherlands). Acid dilution were performed in ultrapure water, obtained from a Milli-Q Advantage system. An Eckert & Ziegler IGG100 GMP Ge-68/Ga-68 generator was generously supplied by Erasmus MC, the Netherlands.  $\text{Zn-69m}$  ( $t_{1/2} = 13.7$  h, gamma energy = 438 keV (95%)) was produced by neutron irradiation of 6 mg  $[\text{natZn}]\text{Zn}$  foil at the Hoge Onderwijs Reactor (HOR) of the Reactor Institute Delft (the Netherlands). After subsequent cooling for 10 h, the foil was dissolved in 0.1 mL 8 M  $\text{HNO}_3$  and diluted to 10 mL with distilled water. Microfluidic extractions were executed with a SEP-10 membrane separator with hydrophobic membranes (pore size: 0.45  $\mu\text{m}$ ) from Zaiput Flow Technologies (Waltham, Massachusetts, USA). AL-1000 Programmable Syringe pumps (941-371-1003) were purchased from World Precision Instruments Inc. and used to deliver solutions to the membrane separator via PTFE tubing (100 cm length, 0.03 inch inner diameter). A slug flow was achieved by using a Microfluidic Y Connector PEEK from IDEX Health & Science (Oak Harbor, Washington, USA). The Vortex-Genie 2 used for batch extractions was purchased from Scientific Industries, Inc (Bohemia, New York, USA). Metal contaminations were measured with an ICP-OES Optima 8000 from Perkin Elmer (Groningen, The Netherlands). The Wallac Wizard<sup>2</sup> 3" 2480 Automatic Gamma Counter from Perkin Elmer (Groningen, The Netherlands) was used for gamma-radiation measurements.

### Experimental methods

#### Batch extraction

To optimize the Ga-68 extraction, aqueous solution concentrations ranging from 1 to 5 M  $[\text{natZn}]\text{Zn}(\text{NO}_3)_2$  in 0.01 M–1 M  $\text{HNO}_3$  were tested, according to reported

target concentrations in the literature (do Carmo et al. 2020; Pandey et al. 2019; 2017a, b). 10–15 kBq of Ga-68 from a Ge-68/Ga-68 generator was added to each aqueous solution to trace Ga-68 extraction and 3–5 kBq Zn-69m were added to trace Zn-coextraction. BPHA was chosen as the extractant due to its capability to extract  $\text{Ga}^{3+}$  from highly acidic media and known kinetics of the BPHA-Ga complex (Riedel 1973; Lyle and Shendrikar 1965; Morroni et al. 2004). The organic solution used consisted of 0.2 M BPHA in chloroform. Additionally, dithizone was selected as an extractant to selectively extract Cu contaminations from the target solution. Conventional batch extraction experiments were executed in Eppendorf vials with a 1:1 volumetric ratio of aqueous and organic phase with volumes of 0.5 mL each. The Eppendorf vials were shaken with the Vortex for 10 min or 1 min with BPHA or Dithizone, respectively, to ensure extraction equilibrium was reached. Afterwards, the organic phase was separated by pipetting. The Ga-68 and Zn-69 m radioactivity in the aqueous solution before extraction ( $A_{\text{initial}}$ ) and organic phase after extraction ( $A_{\text{organic}}$ ) was measured with the Wallac Wizard<sup>2</sup> 3" 2480 Automatic Gamma Counter and corrected for decay. The extraction efficiencies (EE%) were calculated according to:

$$\text{EE\%} = \frac{A_{\text{organic}}}{A_{\text{initial}}} \cdot 100\% \quad (1)$$

To evaluate back-extraction of Ga-68 from the organic phase into an aqueous phase, different concentrations of 0.1 M to 6 M HCl were added to the organic phase, again with a 1:1 volumetric ratio. The Ga-68 activity of the HCl solutions was measured separately after back-extraction ( $A_{\text{HCl}}$ ), corrected for decay, and back-extraction efficiencies (BEE%) were calculated according to:

$$\text{BEE\%} = \frac{A_{\text{HCl}}}{A_{\text{organic}}} \cdot 100\% \quad (2)$$

Each extraction experiment was executed in triplicate and errors are given as one standard deviation of the mean. Since Fe, Cu, Ni, Co and Mn are often found as non-isotopic impurities after cyclotron irradiation of  $[^{68}\text{Zn}]\text{Zn}(\text{NO}_3)_2$  solutions (Riga et al. 2018), their co-extraction in the developed extraction system was investigated following the same procedure. Therefore, 0.1 mM stable Fe(III), Cu, Ni, Co and Mn were added to the zinc solution. Their co-extraction was measured using ICP-OES.

### **Microfluidic solvent extraction**

To achieve complete phase separation after the extraction, a membrane-based separation device (Zaiput Membrane Separator (Zaiput Flow technologies 2022)) was used. Solvent extraction was executed in microfluidic PTFE tubing of 100 cm length and an inner diameter of 0.03 inch. The tubing was connected to two syringes, containing the aqueous and organic solutions (Fig. 1). A constant flow of the aqueous and organic solutions was achieved using syringe pumps and a slug flow was created with a microfluidic Y-connector, to increase the surface-to-volume ratio of the aqueous and organic solution, maximizing extraction. All microfluidic solvent extraction experiments were performed at a 1:1 volumetric ratio with a flow rate of 40  $\mu\text{L}/\text{min}$ . The radioactivity of Ga-68 was again measured with the Wallac Wizard<sup>2</sup> 3" 2480 Automatic Gamma Counter in the aqueous ( $A_{\text{aqueous}}$ ) and



**Fig. 1** Microfluidic solvent extraction set-up using a Zaiput Sep-10 membrane separator. The slug flow is created by the fluidic y-connector

organic solution ( $A_{\text{organic}}$ ) after the extraction. Extraction efficiencies (EE%) were calculated according to:

$$\text{EE\%} = \frac{A_{\text{organic}}}{A_{\text{organic}} + A_{\text{aqueous}}} \cdot 100\% \quad (3)$$

Additionally, back-extraction was investigated in the microfluidic set-up with 2 M and 6 M HCl and varying flow rates from 50 to 150  $\mu\text{L}/\text{min}$ . Back-extraction efficiency (BEE%) was calculated according to:

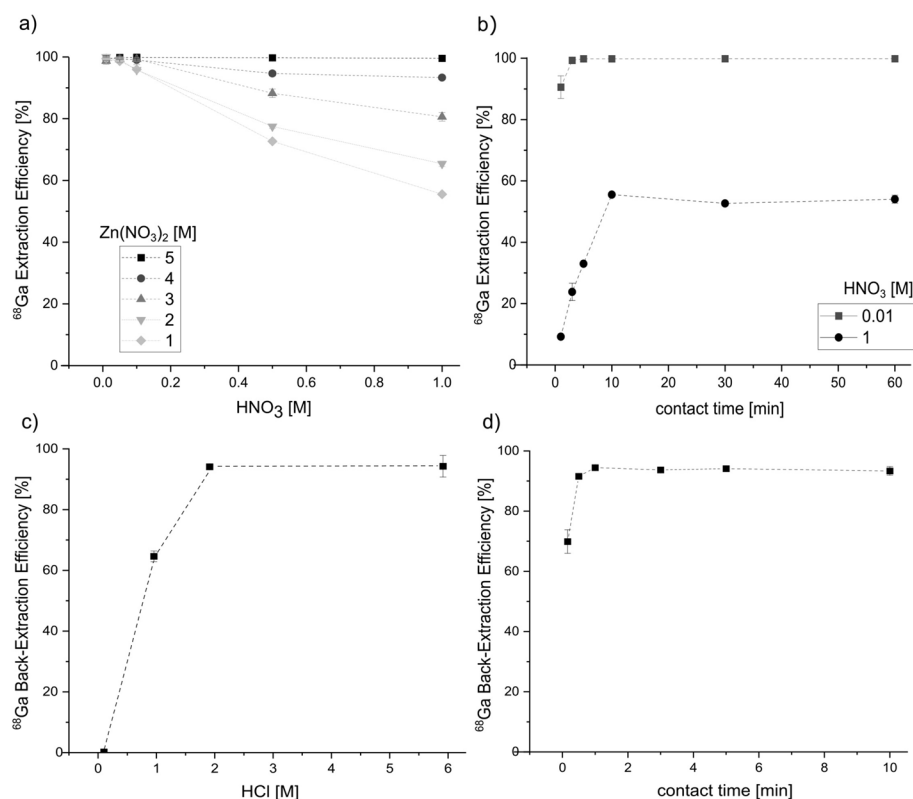
$$\text{BEE\%} = \frac{A_{\text{HCl}}}{A_{\text{organic}} + A_{\text{HCl}}} \cdot 100\% \quad (4)$$

with  $A_{\text{HCl}}$  = Ga-68 activity in the aqueous HCl solution after back-extraction and  $A_{\text{organic}}$  = Ga-68 activity in the organic solution after back-extraction. Solutions used for back-extraction were collected to determine Zn, Cu, Ni, Co, Fe and Mn contamination by ICP-OES. All experiments were executed in triplicate and errors are given as one standard deviation of the mean. All measured activities were corrected for decay.

#### **Cyclotron targetry and irradiation**

Three irradiations on 2 M  $[\text{natZn}]\text{Zn}(\text{NO}_3)_2$  solutions in 0.01 M  $\text{HNO}_3$  were executed on TRIUMF's TR13 cyclotron, a 13 MeV self-shielded, negative hydrogen ion cyclotron. This target concentration was selected due to its common use in literature (Pandey et al. 2014a, 2019; Riga et al. 2018; Alves et al. 2017a; Rodnick et al. 2020) as well as extraction performance. The target solutions were irradiated in a siphon-style niobium body target with an internal expansion chamber as described by Hoeher et al. (2012) and Lowis et al. (2021). The target chamber has a volume of 1.48 mL and is separated from the cyclotron vacuum by a double foil window with a water-cooling jacket on the back and a helium jet cooling on the front site of the target. These two foils (25  $\mu\text{m}$  thick aluminium outside of





**Fig. 2** **a** Extraction efficiencies for batch extraction of  $^{68}\text{Ga}$  from various  $\text{Zn}(\text{NO}_3)_2$  solutions in  $\text{HNO}_3$  for a contact time of 10 min. **b** Extraction efficiencies over time for batch extraction of  $^{68}\text{Ga}$  from 1 M  $\text{Zn}(\text{NO}_3)_2$  in 0.01 and 1 M  $\text{HNO}_3$  solutions. **c** Back-extraction efficiencies for the batch extraction of  $^{68}\text{Ga}$  into different HCl concentrations within 1 min contact time. **d** Back-extraction efficiencies obtained for different contact times for the back-extraction of  $^{68}\text{Ga}$  into 2 M HCl. Error bars represent one standard deviation of the mean

target and 38  $\mu\text{m}$  HAVAR<sup>®</sup> foil inside of target) cause the proton beam to get degraded to 12 MeV. Before the start of the irradiation the internal expansion chamber was pressurized to 200 psi. A 10  $\mu\text{A}$  proton beam was applied for 30 min ( $n=3$ ). After irradiation and unloading, 5 mL of radioactive solution was obtained. 2.5 mL was loaded into a syringe and microfluidic extractions were executed as described above with a flow rate of 40  $\mu\text{L}/\text{min}$ . The extraction efficiency of  $^{68}\text{Ga}$  was calculated according to Eq. 3 using the characteristic peak at 1077.34 keV.

## Results

### Batch extraction

Batch extraction of different concentrations of target solutions, ranging from 1 to 5 M  $\text{Zn}(\text{NO}_3)_2$  in 0.01–1 M  $\text{HNO}_3$ , were performed to investigate optimal target concentrations to optimize  $^{68}\text{Ga}$  extraction efficiencies. 5 M  $\text{Zn}(\text{NO}_3)_2$  solutions all showed maximum extraction efficiencies of over 99.3%. However, a trend of decreasing extraction efficiencies can be observed with increasing  $\text{HNO}_3$  concentrations for lower  $\text{Zn}(\text{NO}_3)_2$  concentrations (Fig. 2a). Extraction efficiencies in the solutions containing 1 M  $\text{Zn}(\text{NO}_3)_2$  range from  $99.5\% \pm 1.2\%$  to  $55.5\% \pm 0.6\%$  in 0.01 M and 1 M  $\text{HNO}_3$ , respectively. Increasing the contact time to up to 60 min did not increase extraction



efficiencies (Fig. 2b), indicating that the results in Graphical Abstract represent equilibrium. Therefore, lower  $\text{HNO}_3$  concentrations of 0.01 M  $\text{HNO}_3$  are required to achieve high extraction efficiencies, exceeding 99% for all studied  $\text{Zn}(\text{NO}_3)_2$  concentrations. Zinc co-extraction was measured to be  $0.09\% \pm 0.06\%$  from a 2 M  $\text{Zn}(\text{NO}_3)_2$  solution in 0.01 M  $\text{HNO}_3$ . Back-extraction efficiencies of  $94.5\% \pm 0.6\%$  could be achieved within 1 min by using 2 M HCl as back-extracting agent. Higher HCl concentrations showed to achieve similar results, while lower HCl concentrations led to decreasing back-extraction efficiencies (Fig. 2c).

Increasing the contact time to more than 1 min did not show increased back-extraction efficiencies (Fig. 2d) indicating equilibrium was reached within such a short time.

#### Co-extraction of other metal impurities

Co-extraction of non-isotopic metal impurities from a 2 M  $^{\text{nat}}\text{Zn}(\text{NO}_3)_2$  solution in 0.01 M  $\text{HNO}_3$  into BPHA and dithizone was investigated in batch (Table 1). Cu and Fe are co-extracted by BPHA with  $99.3 \pm 0.4\%$  and  $99.45 \pm 0.03\%$  efficiency respectively, using a contact time of 5 min. Mn, Co and Ni are not extracted in significant amounts by BPHA ( $< 5\%$ ). Using 10 mM dithizone in chloroform, Cu impurities could be extracted from the target solution within 1 min of vortexing time with an efficiency of  $99.91 \pm 0.06\%$  without co-extracting Ga-68, giving the opportunity to extract Cu-61, which is simultaneously produced by proton irradiation of  $^{\text{nat}}\text{Zn}$  (Council of Europe 2019; International Atomic Energy Agency 2018). All other tested impurities showed very little to no extraction using dithizone ( $< 10\%$ ).

#### Metal contamination in final solutions

Metal contamination in the back-extracted 2 M and 6 M HCl solutions after batch extraction from 2 M  $^{\text{nat}}\text{Zn}(\text{NO}_3)_2$  in 0.01 M  $\text{HNO}_3$  was measured by ICP-OES

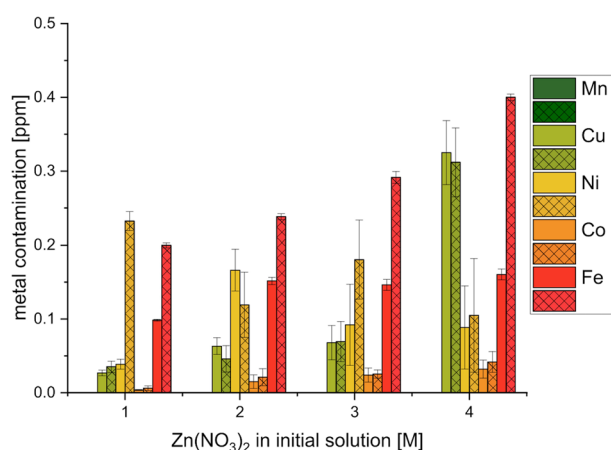
**Table 1** Extraction efficiencies for different metals using BPHA (5 min contact time) and dithizone (1 min contact time) from 2 M  $\text{Zn}(\text{NO}_3)_2$  in 0.01 M  $\text{HNO}_3$ . Fe(III), Cu, Mn, Co and Ni ions were added to the  $\text{Zn}(\text{NO}_3)_2$  solution at concentrations of 0.1 mM. The reported uncertainty is given as one standard deviation of the mean ( $n=3$ )

	Zn [%]	Ga [%]	Fe [%]	Cu [%]	Mn [%]	Co [%]	Ni [%]
BPHA	$0.09 \pm 0.06$	$99.6 \pm 0.3$	$99.45 \pm 0.03$	$99.3 \pm 0.4$	$0.8 \pm 0.3$	$4.1 \pm 1.6$	$1.9 \pm 1.2$
Dithizone	$0.31 \pm 0.19$	$0.03 \pm 0.02$	$9.3 \pm 3.0$	$99.91 \pm 0.06$	$3.3 \pm 2.3$	$5.1 \pm 1.5$	$7.3 \pm 1.9$

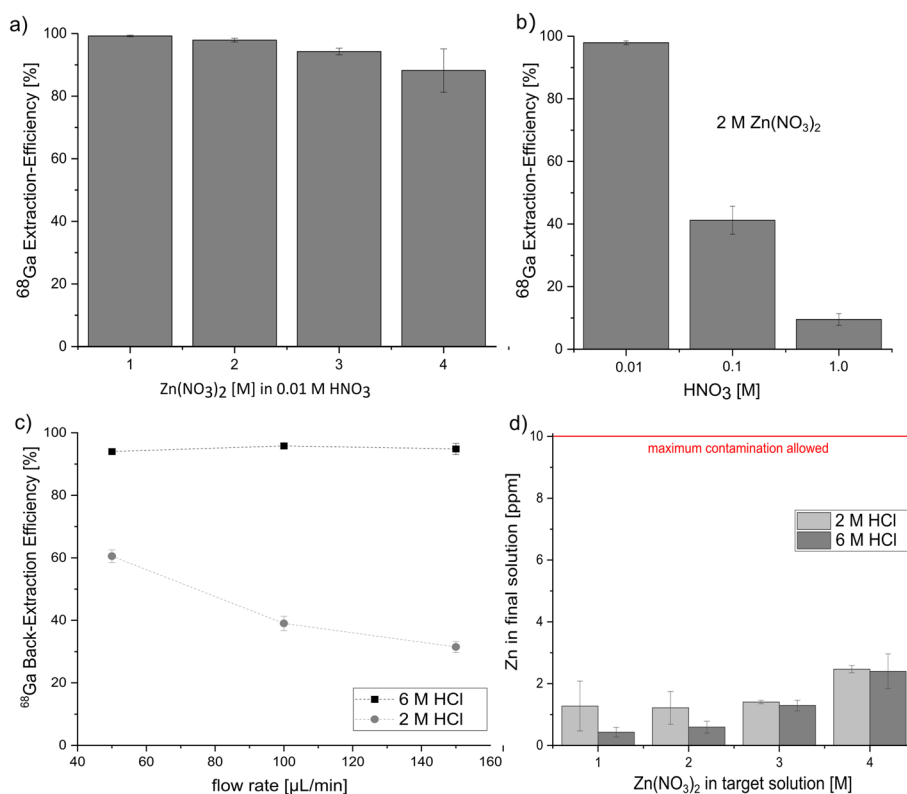
(Fig. 3). All measured metal contaminations, including Cu, Ni, Co, Fe, and Mn were below 0.5 ppm, with Mn being below the detection limit ( $< 10$  ppb).

#### Microfluidic extraction

Microfluidic extraction experiments were performed with varying  $\text{Zn}(\text{NO}_3)_2$  and nitric acid concentrations (Fig. 4). The results show a decreasing extraction efficiency with increasing  $\text{Zn}(\text{NO}_3)_2$  concentrations (Fig. 4a). While 1 M  $\text{Zn}(\text{NO}_3)_2$  resulted in an extraction efficiency of  $99.2\% \pm 0.3\%$ , the 4 M  $\text{Zn}(\text{NO}_3)_2$  solution only showed an efficiency of  $88.2\% \pm 6.9\%$  when the same flow rate of 40  $\mu\text{L}/\text{min}$  was applied. Increasing the  $\text{HNO}_3$  concentration significantly impacted the extraction efficiency (Fig. 4b),



**Fig. 3** Mn, Cu, Ni, Co and Fe contaminations in back-extracted solutions (plain = into 2 M HCl; crossed = into 6 M HCl) after extraction from 2 M Zn(NO<sub>3</sub>)<sub>2</sub> solution in 0.01 M HNO<sub>3</sub>. Mn was below the detection limit of 10 ppb. Error bars represent one standard deviation of the mean (n = 3)



**Fig. 4** **a** Extraction efficiencies for microfluidic extraction of Ga-68 from various Zn(NO<sub>3</sub>)<sub>2</sub> solutions in 0.01 M HNO<sub>3</sub>. The flow rate was 40 µL/min for both solutions. **b** Extraction efficiencies for microfluidic extraction of Ga-68 from 2 M Zn(NO<sub>3</sub>)<sub>2</sub> solutions with varying nitric acid concentrations. The flow rate was 40 µL/min. **c** Back-extraction efficiencies for microfluidic back-extraction of Ga-68 into 2 M and 6 M HCl with varying flow rates. **d** Zn contamination in final HCl solutions after back-extraction. Error bars represent one standard deviation of the mean (n = 3)

decreasing it, for instance, for 2 M  $\text{Zn}(\text{NO}_3)_2$  from  $97.9\% \pm 0.6\%$  in 0.01 M  $\text{HNO}_3$  to  $41.2\% \pm 4.5\%$  and  $9.5\% \pm 1.8\%$  in 0.1 M and 1 M  $\text{HNO}_3$ , respectively. Very different back-extraction efficiencies were found using either 2 M or 6 M HCl as back-extraction solutions. While 6 M HCl consistently showed efficiencies exceeding 94% for varying flow rates between 50 and 150  $\mu\text{L}/\text{min}$ , 2 M HCl achieved only  $60.5\% \pm 2.0\%$  decreasing to  $31.5\% \pm 1.8\%$ , for 50 and 150  $\mu\text{L}/\text{min}$ , respectively (Fig. 4c). Zn contaminations did not exceed 3 ppm for all tested target solutions concentrations (Fig. 4d), conforming to IAEA TecDoc standards (International Atomic Energy Agency 2018). To estimate the contamination according to the European Pharmacopoeia (Council of Europe 2019), where a maximum of 10  $\mu\text{g}/\text{GBq}$  is allowed, we compared our results to a production of Ga-68 from enriched  $[^{68}\text{Zn}]\text{Zn}$  by Alves et al. (2017a). They irradiated a 2.2 M  $\text{Zn}(\text{NO}_3)_2$  solution, which resulted in an Ga-68 activity of 25 GBq at EOB. Taking 2 h of decay into consideration for processing of the target solution and production of radiopharmaceuticals, while considering a Zn contamination of 3 ppm after the presented separation methods, the resulting contamination would equal to 2.5  $\mu\text{g}/\text{GBq}$ . While this is just a approximation and results highly depend on irradiation parameters and target concentrations (Pandey et al. 2019), the results look promising to fulfil the European Pharmacopoeia standards (Council of Europe 2019).

Additionally, a microfluidic extraction was performed on a cyclotron irradiated 2 M  $\text{Zn}(\text{NO}_3)_2$  solution in 0.01 M  $\text{HNO}_3$ . Extraction efficiencies of  $97.0\% \pm 0.4\%$  ( $n=3$ ) were obtained and are within uncertainty identical to the results observed with low activity solutions of  $97.9\% \pm 0.6\%$ . However, it was observed that the membranes used in these experiments were sensitive to radiolysis and started to break down after being in contact with a target solution containing between 500 and 600 MBq of Ga-68 for more than 20 min.

## Discussion

### Extraction behaviour

During cyclotron irradiation of liquid targets high amounts of gases can be produced, leading to a drastic pressure increase inside the target body. Adding nitric acid to Zn target solutions can reduce the pressure build up by scavenging free radicals. Several different concentrations of nitric acid in target solutions have been used successfully in the literature (Pandey et al. 2019). Therefore, in this study multiple nitric acid concentrations (0.01–1 M) in the target solutions were studied. These target solutions have a pH between – 1.3 and 1.7, which is generally not preferred for solvent extraction purposes. Most conventional extractants only efficiently extract ions at higher pH ( $> \text{pH } 2$ ) (Vadasdi 1969; Dhond and Khopkar 1976; Bhattacharya et al. 2007; Inoue et al. 1988; Lee et al. 2002; De and Asit 1967) or from highly acidic chloride solutions that will lead to the formation of chloride species (Ahmed et al. 2013; Song et al. 2020; Katsuta et al. 2012; Maljkovic et al. 1990). However, in the solutions tested in this study, the dominant species is  $\text{Ga}^{3+}$  with minor amounts of  $\text{Ga}(\text{OH})^{2+}$  (calculated with *CHEAQS Next* software (Verweij Wi. *CHEAQS Next* (0.2.1.8))). Therefore, most commercial extractants are not applicable. BPHA on the other hand has the ability to bind to  $\text{Ga}^{3+}$  and  $\text{Ga}(\text{OH})^{2+}$  according to reactions in Eqs. 5 and 6 (adapted

from Morroni et al. (2004) with L representing the ligand BPHA and M the metal Ga), making it a suitable extractant for extracting  $^{68}\text{Ga}$  from  $\text{Zn}(\text{NO}_3)_2$  liquid targets.



In the presented experiments, a clear effect of the amount of  $\text{H}^+$  ions in the solution can be observed, indicating that lower acid concentration in the target solution (0.01–0.1 M  $\text{HNO}_3$ ) are generally preferable to achieve the highest equilibrium extraction efficiency in the shortest contact time. Intramolecular hydrogen bonding was proposed as a reason for this reduction in extraction efficiencies (Morroni et al. 2004). However, increasing the  $\text{Zn}(\text{NO}_3)_2$  concentrations in the target solutions leads to an increase in Ga-68 extraction efficiency even when higher acid concentrations are used, probably due to the ‘salting-out effect’. This effect describes the positive effect of salt concentration on the partition coefficient and the separation factor of the extractant during the extraction process, consequently improving the extraction efficiency of Ga-68. The high effectiveness of  $\text{Zn}(\text{NO}_3)_2$  as a salting-out agent in solvent extraction has been described before (Qi 2018). We also observed that higher levels of radioactivity, and the corresponding increase in radiolysis of the target solution after cyclotron irradiation, did not affect the extraction. Most likely the BPHA has sufficient radiation resistance to withstand breakdown during the short contact time, enabling Ga-68 extraction with the same efficiency as at low levels of radioactivity. The membrane on the other hand, seemed to be affected by the radiation dose and the corresponding radiolysis resulting in the formation of radicals in the target solution as well as the chloroform solution. Therefore, future research about the radiation stability of the membrane is necessary in order to ensure a continuous separation of radioactive nuclides.

Martini et al. (2021) reported a maximum of 89.6% EE in batch, and 80% EE in membrane separator experiments from a 1 M  $\text{Zn}(\text{NO}_3)_2$  in 0.01 M  $\text{HNO}_3$ . They used a comparable set-up for the phase separation utilizing slug flow in a 100 cm long tubing with 0.03 inch inner diameter connected to the Zaiput membrane separator for subsequent phase separation. But the flowrates used in their study were approximately 6 times faster at 250  $\mu\text{L}/\text{min}$ . An additional mixing step was also included in their set-up with simultaneous heating of the solution to 50 °C. In comparison, the results from our study show maximum EEs as high as 99.9% in batch, and  $99.2\% \pm 0.3\%$  in membrane separator experiments for the same zinc nitrate solution at room temperature without additional mixing, but with a slower flowrate of 40  $\mu\text{L}/\text{min}$ .

Finally, the measured zinc concentration in the back-extracted solutions in our study was below 3 ppm, while Martini et al. (2021) reported 11 ppm in their study, which exceeds regulations of maximum 10 ppm, posed by the International Atomic Energy Agency TecDoc Quality Control in the Production of Radiopharmaceuticals (International Atomic Energy Agency 2018).

Our results present a robust, highly efficient strategy for the rapid extraction of Ga-68 from cyclotron-irradiated  $\text{Zn}(\text{NO}_3)_2$  liquid target solutions, based on a commercially

available chelator allowing for quick implementation in  $^{68}\text{Ga}$ -producing cyclotron facilities.

#### **Applicability in clinical setting**

The developed method, presented in this study could increase the production of Ga-68 in the future. The total purification time after cyclotron irradiation can be as quick as 10 min if applied to a module of multiple microfluidic devices, since one device only has a throughput of 40  $\mu\text{L}/\text{min}$  it would take significantly longer otherwise. This method can increase the amount of extracted Ga-68 by almost 25% compared to other purification methods, for the same irradiation time while potentially allowing for direct target recycling and re-irradiation. With just a single irradiation, produced activities could be tenfold higher compared to an elution of the best currently available Ge-68/Ga-68 generator. The recyclability of the target solution after the extraction could lead to more irradiations per day, potentially increasing Ga-68 production tremendously. However, before this method can be readily used in clinical settings, some issues have to be overcome. Since the radiolysis in the target solution after irradiation leads to damage in the membrane used for the separation, and to the best of our knowledge no membrane for this specific set-up exists that might have higher radiation stability, further development should focus on a different microfluidic set-up without a radiation-sensitive membrane or on the improvement of the stability of the membranes.

#### **Conclusion**

A new two-step microfluidic extraction method for the efficient extraction of Ga-68 from liquid target solutions was developed. By using BPHA in chloroform as the extracting phase, extraction efficiencies of over 99% and back-extraction efficiencies of up to 95% could be achieved, leading to a total Ga-68 recovery of up to 94% within 5–15 min. Best results were achieved when 0.01 M  $\text{HNO}_3$  was used as the target solution, while only co-extracting  $0.09 \pm 0.06\%$  Zn.

This method presents a very promising new approach to selectively extract Ga-68 from  $[^{68}\text{Zn}]\text{Zn}(\text{NO}_3)_2$  liquid targets, which decreases the time required for Ga-68 extraction, and subsequently increasing production yield, while potentially allowing for direct target recycling.

#### **Acknowledgements**

We thankfully acknowledge TR13 operators Spencer Staiger, Toni Epp and Ryley Morgan for all the help they provided during loading and unloading of the liquid target.

#### **Author contributions**

ST and TL: data collection, analysis and interpretation of results. ST: writing of manuscript. GE: data collection and analysis. RMK, EP, AGD, CH: review of the article and revision for significant intellectual content. All authors read and approved the final manuscript.

#### **Funding**

This study was financed by the Dutch Research Council (NWO) under the Project Number 16913. The project is supported by NRG, Urenco, UMCG, Fluidomica and TRIUMF. TRIUMF receives funding via a contribution agreement with the National Research Council of Canada (NRC).

#### **Availability of data and materials**

The datasets generated or analyzed during the current study are included in this published article. Supplementary information is available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

Received: 15 March 2023 Accepted: 25 April 2023

Published online: 05 May 2023

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