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Efficient post-processing of aqueous generator eluates facilitates ^{68}Ga-labelling under anhydrous conditions

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Summary. The present study reports a convenient method for ^{68}Ga-labelling under anhydrous conditions using solid-phase derived gallium-68-acetylacetonate ([^{68}Ga]Ga(acac)$_3$) in a microwave-enhanced radiosynthesis. Commercial ^{68}Ge/^{68}Ga-radionuclide generators utilizing TiO_2 to adsorb ^{68}Ge(IV) (Obninsk-generators) were used. The initial aqueous generator eluate was transferred online onto a cation exchange resin and ^{68}Ga was absorbed quantitatively. From this resin, ^{68}Ga was eluted with different acetone-based, non-aqueous solvent systems. More than 95% of the generator-eluted ^{68}Ga was obtained from the cation exchange resin with 600 µL of a 98% acetone/2% acetylacetone mixture providing n.c.a. [^{68}Ga]Ga(acac)$_3$ as labelling agent. Water-insoluble macrocyclic polypyrrole derivatives were chosen as model compounds for a proof-of-principle labelling of lipophilic compounds with ^{68}Ga. Labelling of two different porphyrin derivatives, *meso*-tetraphenyl-porphyrin (Tpp) and (3-(1-hydroxyheptyl)deuteroporphyrin dimethylester (HHDPD) was performed in chloroform in a focused microwave synthesis system in yields of up to 90% within 5 min using phenol as co-ligand. Moreover, new co-ligands were investigated to be more effective and significantly less toxic than phenol. Among the phenol alternatives, gentisic acid (2,5-dihydroxy benzoic acid, DHB, 5 mg) emerged as the most useful, non-toxic phenol substitute. It facilitates reducing the load of co-ligand by 95%, while providing an increased labeling yield of 97%. ^{68}Ga-labelled porphyrins may facilitate the medical application for molecular imaging *via* positron emission tomography.

1. Introduction

Recent improvements in the performance of commercially available ^{68}Ge/^{68}Ga-radionuclide generator systems have led to an elevated interest in the development of ^{68}Ga^{III} based imaging strategies [1]. Gallium-68 displays excellent decay properties, with a half-life of $T_{1/2} = 67.71$ min and 89% positron abundance accompanied by low photon emission (1.077 MeV, 3.22%) and a decay to stable ^{68}Zn [2]. It is thus a cost-effective, highly available alternative to the cyclotron produced radiolabels ^{11}C and ^{18}F. Consequently, the development of Ga-based radiopharmaceuticals is of high importance for the further improvement of the potential of clinical positron emission tomography (PET).

The use of ^{68}Ga has often been complicated by the low chemical purity of the generator-derived ^{68}Ga^{III} which necessitated further purification [3–5]. Recently, a convenient post-processing method of generator-produced no carrier added (n.c.a.) gallium-68 has been reported [6]. It is the most widely accepted method for concentration and purification of the generator eluate for instant labeling chemistry. However, this method is primarily applicable to hydrophilic compounds in aqueous solutions, such as ^{68}Ga-DOTATOC.

In contrast, Ga(acac)$_3$ is a powerful reagent for handling Ga^{III} under non-aqueous, even anhydrous conditions. The properties of acetylacetone (acac) as extraction agent for trivalent metals was reported in the fifties [7, 8]. The formation of organic acac complexes with stable Ga^{III} has been reported as well [9]. Additionally, the potential of [^{67}Ga]Ga(acac)$_3$ as labelling agent was adopted [10, 11] and is also used for ^{68}Ga-labelling [12]. In earlier reports, Ga(acac)$_3$ had been formed *via* evaporation of the generator eluate followed by the addition of acetylacetonate to the residue [12]. An initial synthesis of gallium porphyrins using gallium^{III}-acetylacetonate (Ga(acac)$_3$) has been reported [13].

The present study is concerned with a novel generator post-processing method for the online formation of anhydrous n.c.a. [^{68}Ga]Ga(acac)$_3$. The method comprises concentration of the ^{68}Ga^{III} on a cation exchange resin, separation of any metal contaminants and drying of the resin-bound radioactivity followed by the convenient release of trivalent ^{68}Ga as [^{68}Ga]Ga(acac)$_3$. The product complex is n.c.a. [^{68}Ga]Ga(acac)$_3$ which does neither contain significant traces of ^{68}Ge, ^{68}Zn nor non-radioactive metal ions. This new method shall facilitate ^{68}Ga-labelling with pre-purified ^{68}Ga^{III} under anhydrous conditions, complementary to the known protocol for labelling under aqueous conditions [6]. As a proof-of-principle the method is applied in the present work for the ^{68}Ga-labelling of lipophilic macrocyclic tetrapyrrole derivatives in anhydrous chloroform using cation-exchange refined [^{68}Ga]Ga(acac)$_3$.

Macrocyclic pyrroles, such as porphyrins, chlorins, bacteriochlorins and their metal-complexes are prevalent nat-

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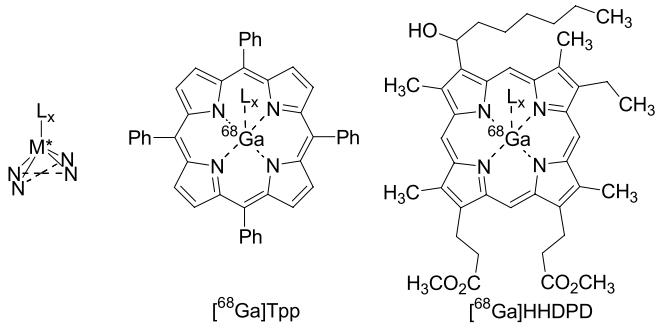

Scheme 1. Tetragonal pyramidal coordination of the five-coordinate metalloporphyrin (L_x: axial co-ligand, M*: trivalent metal ion) [13, 24] and postulated structure of [^{68}Ga]Tpp and [^{68}Ga]HHDPD (L_{x1-7} see Table 1).

ural products. These compounds selectively accumulate in tumour tissue and artheromatous plaques [14–17]. Their photochemical properties have found application in photodynamic tumour therapy (PDT) [18] and fluorescence reflectance imaging (FRI) [19]. The biomedical application of radiolabelled porphyrins, including PET, has been described too [19–21]. In addition, their tumour-localising properties have been considered for drug delivery in boron neutron capture therapy [22].

Ga^{III} shows a significant analogy to Fe^{III} (van der Waals radii: $Ga^{III} = 62$ pm, $Fe^{III} = 65$ pm; electron configuration: Ga^{III}: [Ar]$3d^{10}$; Fe^{III}: [Ar]$3d^5$) [23]. The Fe-complexes of various porphyrins and chlorins are functional parts of biological systems and known to be stable *in vivo*. Therefore, a labelling approach based on the Fe/Ga-analogy was used. For ^{68}Ga-labelled porphyrin derivatives, we assume the coordination geometry illustrated in Scheme 1.

2. Materials and methods

2.1 General

All chemicals were obtained from Sigma-Aldrich and used without further purification. Commercial ^{68}Ge/^{68}Ga-radionuclide generators were obtained from Cyclotron Co. Ltd., Russia. A focussed microwave reactor (CEM GmbH, Kamp-Lintfort) was used for labelling. Waters Sep-Pak® light Accell QMA plus cartridges were applied for solid phase extraction. Radio thin layer chromatography (radio-TLC) was performed on reverse phase TLC-sheets (RP18 F_{254}, 5 × 7.5 cm, Merck) or silica TLC-sheets (silica 60 F_{254}, 5 × 7.5 cm, Merck) and analyzed using an imaging scanner (Instant Imager, Canberra Packard). Radio-HPLC was executed using a solvent delivery system (S1121, Skykam) connected to a UV/vis-detector (UVIS 200, Linear, $\lambda =$ 405 nm) and a radioactivity detector at 511 keV (ISOMED 110, Nuklear-Medizintechnik Dresden GmbH). An analytical RP-HPLC column (LiChroCART® 250-4, LiCroSorb® RP-18 (7 μm), Merck) was used for quality control.

2.2 Generator post-processing

The generator was eluted with 0.1 M aqueous hydrochloric acid (10 mL) and the eluent was passed through a self-built Bio-Rad AG50 W×8 cation exchange resin (50 mg, > 400 mesh, loose packing, 3 mm column diameter) preconditioned with 4 M HCl (1 mL) and washed with water (1 mL). The resin was dried with air (5 mL). Subsequently, the resin was washed with a solution of HCl (0.15 M, 20% in acetone, 1 mL). The resin was dried in a stream of argon for 2 min, followed by the elution of the trapped radioactivity as a [^{68}Ga]Ga(acac)$_3$ complex using 2% acetylacetone in acetone in volumes ranging from 200 to 1600 μL. For comparison, the cation exchange resin was also eluted with 97.6% acetone/0.05 M HCl or 100% acetone in the same manner. The identity and purity of the labelling agent were verified *via* radio-TLC using 1 μL sample aliquots on reverse phase TLC-sheets. Radio-TLC were developed in ethyl acetate : ethanol 1 : 1 (v/v). ([^{68}Ga]Ga(acac)$_3$: $R_f =$ 0.85; $^{68}Ga^{3+}$: $R_f = 0.0$).

2.3 Labelling procedure

20 μL Tpp (33 nmol, 1 mg/mL in $CHCl_3$) were added to 280 μL chloroform and an appropriate amount of co-ligand L_{x1}–L_{x7} (ranging from 1 mg to 100 mg, see Table 1). For labelling of HHDPD, 20 μL HHDPD (34 nmol, 1 mg/mL in $CHCl_3$) were added to 280 μL chloroform and 5 mg (32 μmol) 2,5-dihydroxy benzoic acid (DHB; L_{x2}) or 100 mg (1.06 mmol) phenol (L_{x1}). Subsequently, 20–260 MBq of [^{68}Ga]Ga(acac)$_3$ in 100 μL $CHCl_3$ were added and the mixture was heated in a sealed Duran® vessel *via* microwave irradiation at 300 W for time periods of 1 to 15 min. The reaction mixture was cooled to 50 °C prior to concentration to dryness in a stream of argon. The residue was taken up in ethanol (400 μL) and the clear violet solution was passed through a QMA® anion exchange cartridge. The resin bound radioactivity was washed with water (3 mL) and eluted with Dulbecco's phosphate buffered saline (DPBS) solution (0.1 M, 2 mL). In parallel, non-chelated amounts of 15 ± 9% ^{68}Ga were retained on the resin, depending on the co-ligand.

2.4 Quality control

Labelling yields and radiochemical purity were verified *via* radio-TLC and radio-HPLC. For radio-TLC, 1 μL aliquots of the appropriate compound solution were spotted on TLC sheets and developed in different solvent systems (a: silica; $CHCl_3$: MeOH 9 : 1 (v/v); b: silica; $CHCl_3$: MeOH

Table 1. Co-ligand concentrations used for the synthesis of [^{68}Ga]-GaTpp-complexes.

Entry x	Co-ligand L_x	Amount/mg	LY/%
1	Phenol	100	86 ± 5
		25	86 ± 11
		5	90 ± 4
		1	71 ± 5
2	2,5-dihydroxy benzoic acid	5	97 ± 2
3	Salicylic acid	5	41 ± 18
4	L-ascorbic acid	5	14 ± 6
5	L-serine	5	< 1
6	Imidazole	5	< 1
7	Chloride	−/−[a]	< 1

a: no co-ligand added; LY: labelling yield.

7 : 2 (v/v); c: RP18, ethyl acetate : ethanol 1 : 1 (v/v)). Corresponding R_f values were: [^{68}Ga]GaTpp: $R_f(a) = 1.0$, $R_f(b) = 0.8$; [^{68}Ga]GaHHDPD: $R_f(a) = 1.0$, $R_f(c) = 0.9$; $^{68}Ga^{3+}$: $R_f(a, b, c) = 0.0$. Radio-HPLC was performed under isocratic conditions (acetonitrile : H_2O 95 : 5, 0.1% TFA; 0.5 mL/min). The products were eluted after the following retention times (t_R): Tpp: $t_R = 13{:}54$ min; [^{68}Ga]GaTpp-DHB, $t_R = 11{:}18$ min; HHDPD: $t_R = 28{:}30$ min; [^{68}Ga]-GaHHDPD-DHB, $t_R = 13{:}30$ min; ^{68}Ga: $t_R = 7{:}52$ min.

3. Results

3.1 Generator post-processing

A ^{68}Ge/^{68}Ga-generator post-processing setup was used as published previously [6]. The desorption of $^{68}Ga^{III}$ from the cation exchange resin in the form of [^{68}Ga]$Ga(acac)_3$ was optimised as illustrated in Fig. 1 for three different solvent systems. In this procedure, already 85 ± 5% of the radioactivity initially eluted from the generator was obtained as [^{68}Ga]$Ga(acac)_3$ complex using 400 µL of 2% acetylacetone in acetone. This yield could be increased using 600 µL of that eluate system. The whole procedure including generator-setup, elution, post-processing and reconstitution in anhydrous chloroform was accomplished within 10 min.

3.2 Labelling of lipophilic porphyrins

The initial labelling experiments of *meso*-tetraphenylporphyrin (Tpp) and (3-(1-hydroxyheptyl)deuteroporphyrin dimethylester (HHDPD) [24, 25] were conducted with phenol as co-ligand. The significance of the axial co-ligand to achieve a tetragonal pyramidal coordination of the five-coordinate metal core (scheme 1) has already been discussed in the literature [26, 27]. Using phenol (100 mg) as co-ligand, labelling yields of 86 ± 5% of the [^{68}Ga]GaTpp-phenol-complex and 87 ± 6% of the [^{68}Ga]GaHHDPD-phenol-complex were achieved within 5 min, respectively (Fig. 2). Using the pure acetone eluate, the labelling reaction did not proceed.

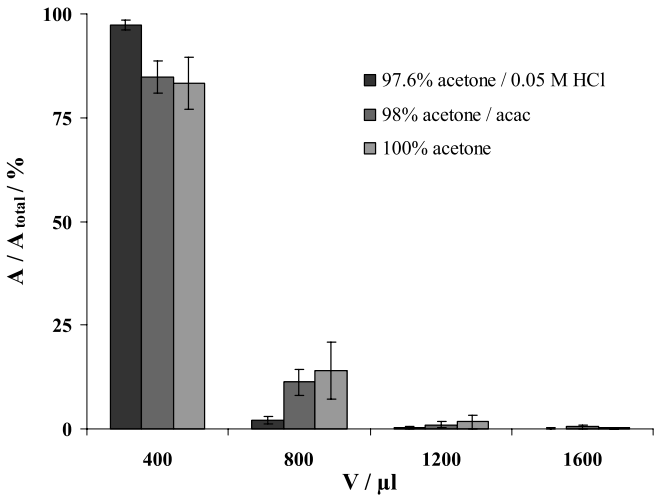

Fig. 1. Elution profile of gallium-68 from the cation exchange resin (SCX) using different acetone systems ($n = 4$). The SCX column was loaded with 150 µL of these solutions first and the resin bound ^{68}Ga was allowed to equilibrate with the eluent for 2 min.

Due to the toxicity of phenol (LD_{50}(rat) (*intra peritoneal*, *i.p.*) = 127 mg/kg), a substantial reduction of the phenol concentration or an appropriate substitution was necessary prior to biomedical application. Therefore, a variety of alternative co-ligands was screened for [^{68}Ga]GaTpp-complex formation (Table 1).

Among the phenol alternatives, gentisic acid (2,5-dihydroxy benzoic acid, DHB, 5 mg) emerged as the most useful, non-toxic phenol substitute. In this case, labelling yields of 97 ± 2% for [^{68}Ga]GaTpp and 95 ± 2% for

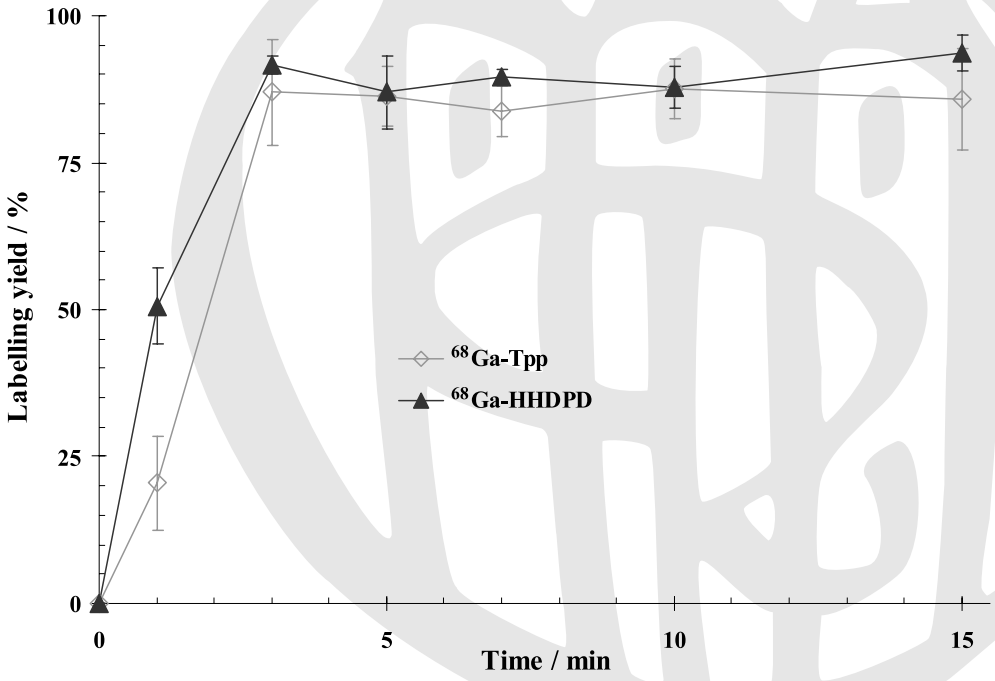

Fig. 2. Time dependence of labelling reactions for [^{68}Ga]GaTpp and [^{68}Ga]GaHHDPD using phenol as co-ligand (100 mg L_{x1}, 33 nmol Tpp, 34 nmol HHDPD; microwave setup: 220 °C, 300 W).

[^{68}Ga]GaHHDPD were achieved within 5 min prior to purification.

Purification of the labelled ^{68}Ga porphyrin derivatives was performed *via* solid phase extraction resulting in a radiochemical purity of 98 ± 1% for [^{68}Ga]GaTpp and 95 ± 2% for [^{68}Ga]GaHHDPD. Overall, non-decay corrected radiochemical yields of 73 ± 6% for [^{68}Ga]GaTpp and 50 ± 9% for [^{68}Ga]GaHHDPD were achieved. Total time of synthesis, consisting of generator elution, post-processing, labelling and purification, was 17 min for both compounds.

4. Conclusion

In conclusion, n.c.a. [^{68}Ga]Ga(acac)$_3$ as labelling synthon for synthesis in non-aqueous media was obtained in high yield adopting the cation exchange-based post-processing of ^{68}Ge/^{68}Ga radionuclide generators [6]. Subsequent radiolabelling of water-insoluble macrocyclic tetrapyrroles was conducted in a focused microwave synthesis system. Labelling yields of 97 ± 2% for [^{68}Ga]GaTpp and 95 ± 2% for [^{68}Ga]GaHHDPD were achieved within 5 min in chloroform. Compound purification was achieved by solid phase extraction, resulting in radiochemical purities of 98 ± 1% for [^{68}Ga]GaTpp and 95%2 for [^{68}Ga]GaHHDPD within 17 min post elution. Based on these results, the novel procedure providing n.c.a. [^{68}Ga]Ga(acac)$_3$ offers a wide scope of applications for this labelling agent.

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