Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.
This journal is © The Royal Society of Chemistry 2016

Monolith-based ^{68}Ga processing: a new strategy for purification to facilitate direct radiolabelling methods

Ping He, Benjamin P. Burke, Gonçalo S. Clemente, Nathan Brown, Nicole Pamme and Stephen J. Archibald.

Department of Chemistry, Positron Emission Tomography Research Centre and School of Engineering, University of Hull, Hull HU6 7RX, UK.

Email: S.J.Archibald@hull.ac.uk

Electronic Supporting Information

S1: Materials and Methods

All reagents and solvents, poly(ethylene oxide) (PEO) with average relative molar mass of 100 kDa, tetraethoxysilane (TEOS), nitric acid aqueous solutions (1 N), ammonium hydroxide (5 N), ethanol, 3-mercaptopropyltrimethoxysilane (MPTMS), hydrochloridric acid (30%) and hydrogen peroxide (30%) aqueous solution were purchased from Sigma Aldrich (UK). DOTA and NOTA were purchased from CheMatech (France) and DOTATOC was purchased from ABX (Germany). Polymeric cation-exchange column (SXC) was purchased from Phenomenex. All reagents were used as obtained without further purification. Gallium-68 was obtained in the form of $^{68}GaCl_3$ from a 14 month old commercial $^{68}Ge/^{68}Ga$ generator (Eckert & Ziegler, 740 MBq) based on a TiO_2 phase adsorbing $^{68}Ge(IV)$ with a negligible ^{68}Ge-breakthrough. The generator was eluted with 5 mL of 0.1 N HCl at a flow-rate of 1 mL/min, resulting in a final activity of around 150 MBq.

Scanning electron microscopy (SEM) images were obtained using a Carl Zeiss SMT EVO 60 scanning electron microscope operated at 20 kV. Each sample was sputter-coated with a thin layer of gold-platinum (~2 nm of thickness) using a SEMPREP 2 Sputter Coater (Nanotech Ltd.). Elemental analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Optima 5300 DV) for metals and EA1108 (Carlo Erba) for sulfur.

S2: Preparation of silica-monolith

Silica-based monoliths were prepared using the sol–gel method described in our previous study.[1] Briefly, the desired amount of PEO was added to an aqueous solution of nitric acid and the mixture was cooled in an ice bath and stirred until a homogeneous solution was formed. The required amount of TEOS was added and the mixture was stirred vigorously in an ice bath for 30 min to form a transparent solution. Subsequently, the solution was poured into a plastic tube (diameter 7.4 mm and length 70 mm), both ends were closed and the tube was incubated in an oven at 40^{o}C for 3 days, during which time a wet, semi-solid gel monolith was formed. The wet gel monoliths were then washed with copious amounts of water to remove any possible residues and transferred to a 10 times volume of 1 M NH_4OH aqueous solution in an autoclave where it was incubated at 80^{o}C for 24 h. The monoliths were again washed with plenty of water before drying in an oven at 85^{o}C for 24 h.

S3: Preparation of cation-exchange silica-monolith

For thiol–functionalisation of silica-monolith, 250 mg of silica-monolith rods were added to a solution containing MPTMS (1 mL) in ethanol (10 mL) and water (10 mL) and the mixture was heated at 80°C for 24 h. The silica–monolith supported thiols (SiO_2Monolith–SH) were washed with methanol (2 × 10 mL) and water (800 mL) to remove any residues. The obtained SiO_2Monolith-SH was then oxidized by reaction with 30% hydrogen peroxide (10 mL) in methanol (20 mL) at 60°C overnight to convert the –SH groups into –SO_3H groups. The monolith rods were washed with methanol (2 × 10 mL) and water (800 mL) followed by acidification with H_2SO_4 (30 mL, 0.1 M) at 60°C for 6 h. The obtained sulfonic acid–functionalized silica–monolith (SiO_2Monolith–SO_3H) was washed three times with water (100 mL) and dried at 60°C overnight. To form the cation–exchange monolith columns, the SiO_2Monolith–SO_3H rod (diameter 5.6 mm) was fixed into an empty Sep-Pak Vac tube (diameter 5.8 mm and length 65 mm) and the void volume between the monolith rod and tube wall was firmly filled up with the powdered SiO_2Monolith–SO_3H (ca. 10% by weight of total monolith column mass) and plastic frits were placed at both ends.

S4. Determination of cation-exchange capacity (CEC) by a breakthrough method

Cation exchange capacity (CEC) of the SiO_2Monolith–SO_3H was determined through a breakthrough method. A KCl aqueous solution was continuously pumped through the cation–exchange silica–monolith column. Due to ion exchange between K^+ and H^+, the potassium content in the effluent changed with the volume of the KCl solution passed through the column. The CEC was calculated by summing the product of potassium contents and volumes of the samples collected and subtracting this from the product of potassium concentration of the feed and the total volume of the effluent passed through the monolith, as given by equation (1).

$$CEC = \frac{C_{feed}V_{total} - \sum C_i V_{i,effluent}}{M_K W_{monolith}} (\mu eq / g) \tag{1}$$

Where C_{feed} and C_i are potassium concentration (ppm) of the feed and of the effluent sample collected, V_{total} and $V_{i,eluent}$ are the total volume (mL) of effluent passed through the monolith and the volume (mL) of the effluent sample collected, $W_{monolith}$ is the weight (g) of the monolith and M_K is the molecular mass of potassium.

Table S1 Summary of CEC measurements for SiO_2Monolith–SO_3H[a].

Entry	Solvent for functionalisation reaction	MPTMS (mL)	KCl[b] (ppm)	Flow rate (mL/min)	CEC (μeq/g)
1	Toluene (20 mL)	0.2	22	0.1	7
2	Ethanol (10 mL) and water (10 mL)	0.2	250	0.1	90
3	Ethanol (10 mL) and water (10 mL)	0.2	500	0.1	90
4	Ethanol (10 mL) and water (10 mL)	0.2	500	0.5	90
5[c]	Ethanol (10 mL) and water (10 mL)	0.2	500	0.5	90
6	Ethanol (10 mL) and water (10 mL)	1.0	500	0.5	181

[a] Mass and volume of SiO_2Monolith−SO_3H used for CEC measurements were about 0.18 g and 0.66 mL. [b] 0.3 mL of sample was collected each time and the potassium concentration was analyzed by flame photometer. [c] Reused SiMonolith-SO_3H presented in Entry 2.

The CEC breakthrough method is based upon the replacement of exchangeable protons by potassium ions. Due to ion exchange between K^+ and H^+, the potassium content in the effluent changed with the volume of the KCl solution passed through the column. The breakthrough of the column was always relatively sharp and the potassium content in the effluent rose rapidly to its input value. When this stage was reached the exchange process was considered complete. The area above the breakthrough curve is equal to the amount of potassium adsorbed inside the column and the CEC can be calculated by equation (1). The CEC of SiMonolith-SO_3H prepared by using different solvents and MPTMS volume is summarized in **Table S1** and a typical breakthrough curve for the SiO_2Monolith–SO_3H column presented in Entry 4 is shown in **Figure S1**.

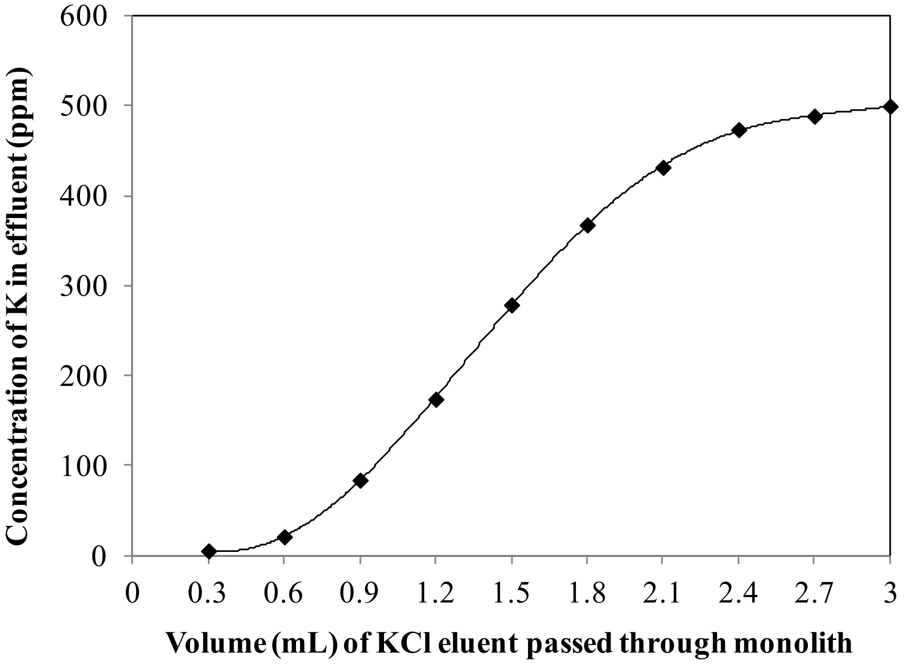

Figure S1: Typical breakthrough curve obtained with the SiO_2Monolith–SO_3H column (Entry 4 in Table S1).

As mentioned previously, the area above breakthrough curve is equal to the amount of potassium adsorbed inside the monolith column. The shape of the breakthrough curve actually depends on many factors. For example, slower flow rates shorten the mass transfer zone while an increase in the concentration of eluent lengthens the mass transfer zone. Also, any channeling in the column will flatten the curve. However, none of these effects has any influence on the area above the curve. In other words, these parameters have no effect on the CEC of cation-exchange column. Indeed, from Entry 2-4 in **Table S1** we can see that the changes in the concentration and flow rate of KCl have no influence on the CEC. The solvent used for the thiol-functionalisation have a great influence on the CEC. The best solvent was a mixture of ethanol and water at a ratio of 1 to 1, which gave a CEC of 90 µeq/g (see Entry 2-4 of **Table S1**) while only 7 µeq/g were obtained for SiO_2Monolith–SO_3H functionalized using toluene solvent (see Entry 1 of **Table S1**). This is due to the fact that the hydrophilic co-solvents EtOH and water greatly favor the formation of thiol-functionalities on the hydrophilic surface of silica-monolith compared to using the hydrophobic solvent toluene. After the SiO_2Monolith–SO_3H was exchanged with K^+ into SiO_2Monolith–SO_3K, the potassium–monolith was eluted with a solution of 0.2 N HNO_3 at a flow rate of 0.1 mL/min. The elution profiles (see **Figure S2**) show that the amount of potassium in the monolith column can be totally eluted within 1.2 mL of eluent, just the double volume of the monolith column, and the SiO_2Monolith–SO_3H column was completely recovered. As a result, the same CEC was obtained after reconditioning of the used SiO_2Monolith–SO_3K (see Entry 5 of **Table S1**). The MPTMS concentration also has significant effect on the CEC. As shown in **Table S1**, 1 mL of MPTMS used for preparation of SiO_2Monolith–SO_3H gave a CEC of 180 µeq/g being 30 times higher compared to a commercial cation–exchange CM column (6 µeq/g). Sulfur element analysis of SiO_2Monolith–SO_3H showed an S content of 174 µmol/g. Comparison of S content from CEC data indicates the complete oxidation of the anchored thiols to sulfonic acids. This monolith was used to evaluate the performance of ^{68}Ga isolation from the generator eluate.

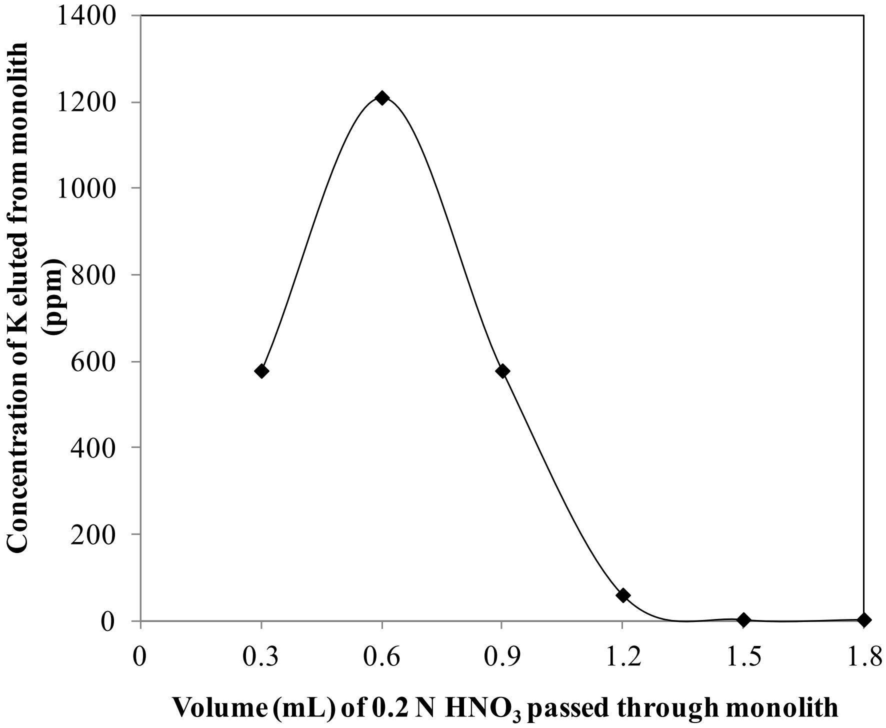

Figure S2: The elution profiles obtained by treating the SiO_2Monolith–SO_3K sample with 0.2 N HNO_3 at flow rate of 0.2 mL/min.

S5: BET Nitrogen adsorption and desorption isotherms of SiO_2Monolith–SO_3H.

Nitrogen adsorption and desorption isotherms were measured at 77 K using a Micromeritics Surface Area and Porosity Analyzer. The isotherms were analyzed to obtain the specific surface area according to the Brunauer–Emmett–Teller (BET) model. **Figure S3** shows a representative nitrogen adsorption/desorption isotherm of the SiO_2Monolith–SO_3H. We can see significant hysteresis between adsorption and desorption indicating a type 4 nitrogen adsorption isotherm, which is consistent with the disordered mesoporous structure displayed in the SEM giving specific surface area of 237.6 m^2/g and nanopore volume of 0.75 cm^3/g, as well as nanopore diameter of 12.6 nm.

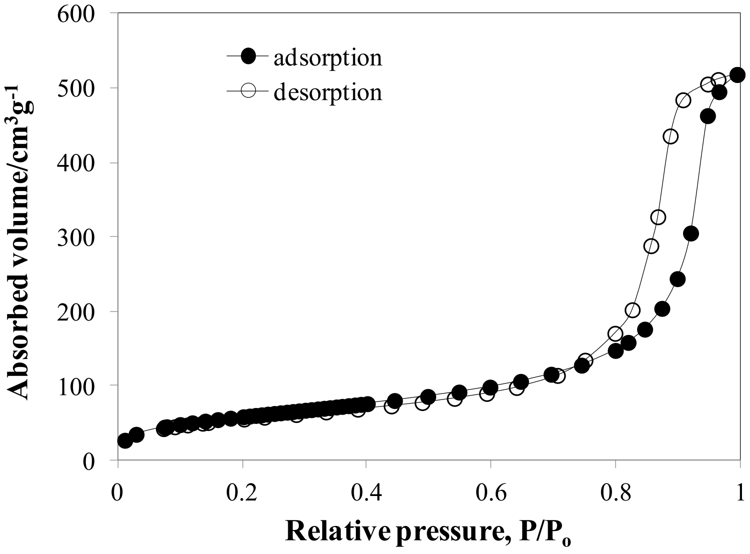

Figure S3. BET Nitrogen adsorption and desorption isotherms of SiO_2Monolith–SO_3H.

S6: ^{68}Ga radiolabeling of chelators

After post-processing of ^{68}Ga, the obtained ^{68}Ga solution was directly used for radiolabeling by adding the chelating agent (DOTA 1-20 µM and NOTA 0.5-5 µM in the reaction solution) in NH_4OAc (0.5 mL, 0.5 M, pH 5) buffer. The reaction mixture was incubated for 10 min at 95°C for DOTA and 10 min at room temperature for NOTA. The radiolabeling yields for ^{68}Ga-DOTA and ^{68}Ga-NOTA were determined by using thin layer silica gel chromatography aluminum plates (TLC-SG, 1×10 cm strip) eluted with 0.4 M sodium phosphate buffer (pH 4). The TLC strips were scanned using a TLC scanner (Scan-RAM, Radio-TLC from LabLogic).

S7: ^{68}Ga radiolabeling of DOTATOC

The ^{68}Ga eluate obtained after elution with 0.5 mL of 90%EtOH/0.5 N HCl was used for radiolabeling of DOTATOC. Labeling was carried out in an open standard vial. 0.5 mL of 1 M NH_4OAc buffer containing 14 nmol of DOTATOC preheated to 95°C, 0.5 mL of the ^{68}Ga eluate was then added to the buffer solution. The reaction was maintained for either 10 or 15 min. The radiochemical yield was determined by TLC using the same conditions that were used for the DOTA reaction analysis. Specific activity calculated by theoretically using all gallium-68 eluate of a new generator (740 MBq) and amount of DOTATOC added in the reaction (14 nmol), non-decay corrected.

Reference

1. P. D. I. Fletcher, S. J. Haswell, P. He, S. M. Kelly and A. Mansfield, *J Porous Mater*, 2011, **18**, 501-508.