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Ion beam induced ^{18}F -radiofluorination: straightforward synthesis of gaseous radiotracers for the assessment of regional lung ventilation using positron emission tomography†

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A simple, straightforward and efficient method for the synthesis of ^{18}F CF₄ and ^{18}F SF₆ based on an ion beam-induced isotopic exchange reaction is presented. Positron emission tomography ventilation studies in rodents using ^{18}F CF₄ showed a uniform distribution of the radiofluorinated gas within the lungs and rapid elimination after discontinuation of the administration.

Imaging methods visualizing areas of impaired ventilation may become a powerful tool in the early/differential diagnoses of lung diseases. Currently, clinical ventilation studies are mainly performed using planar gamma camera imaging or single photon emission computerized tomography (SPECT). Gases such as $^{81\text{m}}\text{Kr}^1$ and more often radiolabelled aerosols generated either from water-soluble agents ($^{99\text{m}}\text{Tc}$ -DTPA)² or solid particles (Technegas)³ are used as contrast agents. After inhalation, $^{81\text{m}}\text{Kr}$ is distributed according to the regional ventilation. However, limited access and short half-life ($T_{1/2} = 13$ s) restrict its use. Liquid radio-aerosols and Technegas show central airway deposition and peripheral hotspot formation in patients with obstructive lung diseases. Moreover, both planar gamma camera imaging and SPECT have limitations in terms of sensitivity, spatio-temporal resolution and quantification.

The high sensitivity and quantitative nature of Positron Emission Tomography (PET) may result in a valuable alternative to assess lung ventilation. Positron emitter-labelled gases such as neon-19 (^{19}Ne) and [^{13}N] N_2 have been successfully applied to ventilation studies.⁴ Unfortunately, the short half-lives of both isotopes ($T_{1/2} = 17.4$ s and 9.97 min for ^{19}Ne and ^{13}N , respectively) have restricted routine application in the clinical setting. Indeed, the use of PET in the context of lung ventilation studies has been historically thwarted by the lack of appropriate radiotracers.

Previous promising investigations using MRI with fluorinated gases as contrast agents in both animal models⁵ and human

subjects⁶ inspired us in the potential use of ^{18}F -labelled gases for the non-invasive assessment of regional lung ventilation using PET. Contrary to aerosols and suspensions, which often separate and clump upon settling, gases distribute uniformly at equilibrium. Hence, the preparation of inert, non-toxic, and water-insoluble fluorinated gases radiolabelled with ^{18}F ($T_{1/2} = 109.7$ min) may result in an extremely useful tool for the ultra-sensitive, *in vivo* and non-invasive assessment of regional lung ventilation.

Our first target compound was [^{18}F]CF₄, whose non-radioactive analogue (CF₄) is non-toxic after inhalation (lethal concentration is low in rat = 890 000 ppm/15 minutes),[‡] has very low solubility in water and is chemically inert. The production of this radioactive gas was first envisaged by following a well-established procedure under non-radioactive conditions, based on the reaction of silicon carbide (SiC) with F₂. However, the fact that F₂ is used in large excess in this synthetic approach, which is difficult to implement under radioactive conditions, together with the unease around producing and manipulating [^{18}F]F₂, encouraged us in the pursuit of simple and straightforward alternative synthetic strategies, which may ultimately facilitate the future application of the novel radiotracers in the clinical setting. Taking into account that the specific radioactivity (amount of radioactivity per unit mass) of the labelled compounds should not be an issue in the assessment of lung ventilation (ventilation studies with fluorinated gases performed with MRI require an extremely high concentration of the fluorinated agent, see for example ref. 6), isotopic exchange reactions appeared appropriate.

Isotopic exchange reactions of fluorocarbons with ^{18}F were already attempted in the early 50s using conventional chemical methods with very poor results.⁷ Despite the exchange of ^{18}F with CF₄ could be observed in the presence of metallic salts acting as catalysts, the fraction exchanged was extremely low even at high temperatures.⁸ No exchange could be observed for SF₆ even in the presence of metal catalysts.⁹

During the 70s and the 80s, recoil ^{18}F atoms were used in a variety of gas-phase kinetic investigations.¹⁰ Recoil ^{18}F atoms, generated *via* different nuclear reactions, proved to undergo

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Table 1 Amount of radioactivity (Act., mean \pm standard deviation, $n = 3$) decay corrected to the end of irradiation, obtained under different experimental conditions for production methods A and B; NA: not applicable

Entry	P_1 (bar)	C_1 (μ A h)	C_2 (μ A h)	C_3 (μ A h)	Act. (GBq)
1 ^a	2	4	NA	NA	0.27 \pm 0.03
2 ^a	2	8	NA	NA	0.49 \pm 0.04
3 ^a	4	4	NA	NA	0.58 \pm 0.04
4 ^a	4	8	NA	NA	0.80 \pm 0.06
5 ^b	NA	NA	1	1	2.30 \pm 0.08
6 ^b	NA	NA	1	2	2.47 \pm 0.08
7 ^b	NA	NA	1	4	2.75 \pm 0.13
8 ^b	NA	NA	4	4	8.43 \pm 0.59

^a Production method A. ^b Production method B; P_1 : CF_4 filling pressure under method A; C_1 : integrated current, method A; C_2 : integrated current, first irradiation, method B; C_3 : integrated current, second irradiation, method B.

isotopic exchange reactions and the formation of $[\text{F}^{18}]\text{CF}_4$ and $[\text{F}^{18}]\text{SF}_6$ was reported.¹¹

In this context, we first anticipated that the irradiation of CF_4 with protons with an appropriate energy should produce *in situ* recoil F^{18} atoms *via* the $^{19}\text{F}(\text{p,pn})^{18}\text{F}$ nuclear reaction, capable of undergoing an isotopic exchange reaction with surrounding CF_4 molecules to form $[\text{F}^{18}]\text{CF}_4$. With this aim, cyclotron (IBA Cyclone 18/9)-accelerated protons with a nominal energy of 18 MeV were used to irradiate a mixture of CF_4 /neon (method A; see Fig. S1 (ESI[†])) for the general configuration of the experimental set-up). In brief, the target was filled with CF_4 to a final pressure of $P_1 = 2\text{--}4$ bar, topped with neon to 20 bar and subjected to proton irradiation (integrated current $C_1 = 4\text{--}8 \mu\text{A h}$). The target gas was finally collected in a liquid nitrogen cooled cryogenic trap. Higher concentrations of CF_4 during irradiation and increased integrated current values (C_1) lead to higher amounts of $[\text{F}^{18}]\text{CF}_4$ (Table 1, entries 1–4); however, the production yields were relatively low and only 0.80 ± 0.06 GBq of the radiotracer could be produced with an integrated current of $8 \mu\text{A h}$. Cross-sectional values for the nuclear reaction $^{19}\text{F}(\text{p,pn})^{18}\text{F}$, which has an energy threshold of approximately 10 MeV and reaches a maximum at cross-sectional values of around 150 mb between 20 and 30 MeV,¹² suggest that the limiting step in the production of $[\text{F}^{18}]\text{CF}_4$ using method A is the generation of the radionuclide, which is limited due to the maximum energy available in our cyclotron (18 MeV).

Gas chromatography analysis of the radioactive gases showed the presence of two radioactive species at the end of the cryogenic trapping step (Fig. 1a) identified as $[\text{F}^{18}]\text{CF}_4$ and $[\text{C}^{11}]\text{CO}_2$. Although the source of ^{11}C has not been demonstrated, this radionuclide may be generated due to the presence of N_2 in the target during irradiation *via* the $^{14}\text{N}(\text{p},\alpha)^{11}\text{C}$ nuclear reaction, as N_2 is present as an impurity in the CF_4 gas bottle. *In situ* reaction with O_2 absorbed in the target walls may result in the formation of $[\text{C}^{11}]\text{CO}_2$. Importantly, $[\text{C}^{11}]\text{CO}_2$ could be easily removed by passing the irradiated gases through a soda lime trap before cryogenic trapping (Fig. 1b). Mass spectrometry analysis confirmed the presence of 5 major species, corresponding to N_2 , CF_4 , CO_2 , C_2F_6 , and $\text{C}_2\text{F}_6\text{O}_3$, respectively (Fig. 1c). One very minor unidentified peak was also observed (Fig. 1c, inset).

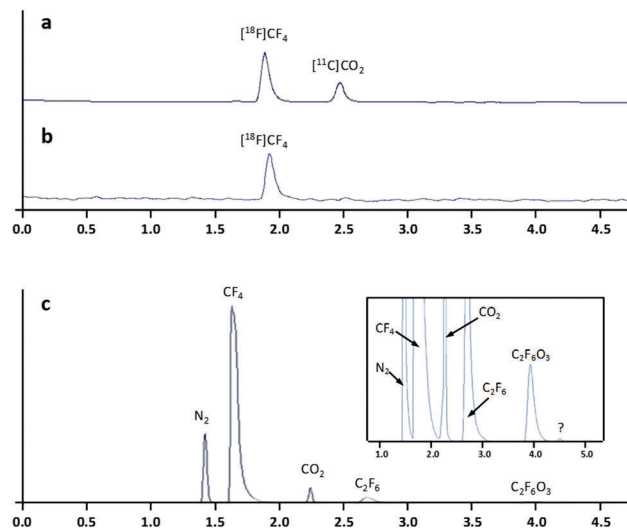


Fig. 1 (a and b) Chromatograms (radioactivity detector) corresponding to the analysis of $[\text{F}^{18}]\text{CF}_4$ before (a) and after (b) passing through a soda lime trap, and (c) chromatogram (MS detector) corresponding to the analysis of $[\text{F}^{18}]\text{CF}_4$. Inset shows magnification of the region between 1 and 5 min.

The determination of the amount of activity present in the collection reservoir at 30 min intervals for 4 hours confirmed the radionuclidic purity of the radiofluorinated gas (calculated $T_{1/2} = 109.5$ min).

In order to improve the production yields, we envisioned the synthesis of the labelled species using a dual beam approach (method B). In brief, the process consisted of four steps: (i) the target was filled with $[\text{O}^{18}]\text{O}_2$ at $P = 20$ bar and was irradiated with protons (integrated current $C_2 = 1\text{--}4 \mu\text{A h}$); (ii) the target gas was removed by cryogenic retrieval; (iii) the target was filled with CF_4 to 4 bar, topped with neon to 20 bar and irradiated with protons (integrated current $C_3 = 1\text{--}4 \mu\text{A h}$); and (iv) the target gas was cryogenically trapped as above.

In this process, during the first irradiation of pure $[\text{O}^{18}]\text{O}_2$, F^{18} is generated in a high yield, because the cross-sectional values of the nuclear reaction $^{18}\text{O}(\text{p,n})^{18}\text{F}$ have an energy threshold of around 2 MeV and reach a maximum at energies of around 6 MeV (cross-sectional values at this energy is around 500 mb).¹³ During the cryogenic recovery of the ^{18}O -enriched oxygen, fluorine-18 remains absorbed on the walls of the target chamber, and is ready to undergo isotopic exchange reactions in the following step. Indeed, the subsequent irradiation of CF_4/Ne resulted in the formation of $[\text{F}^{18}]\text{CF}_4$. Similar chromatographic profiles to those obtained under method A were found (see Fig. S2, ESI[†]). A clear correlation between the amount of $[\text{F}^{18}]\text{CF}_4$ and the integrated current used for the first irradiation was observed, as expected (entries 7 and 8, Table 1). Interestingly, the integrated current for the second irradiation had a minor effect on the amount of $[\text{F}^{18}]\text{CF}_4$, suggesting that the isotopic exchange reaction is relatively fast (Table 1, entries 5–7).

In previous works, the mechanism of formation of $[\text{F}^{18}]\text{CF}_4$ was described as a result of a substitution reaction of a “hot” F^{18} atom with CF_4 , leading to the vibrationally excited molecule $\text{CF}_3^{18}\text{F}^*$, which ultimately deactivates *via* collision with the



surrounding molecules.¹⁴ In our case, the reaction mechanism should differ from that previously described. Because the incident protons impact majorly in the gas, hot ^{18}F atoms are only produced by the $^{19}\text{F}(\text{p,pn})^{18}\text{F}$ nuclear reaction, which has a minor contribution to the final amount of $[^{18}\text{F}]\text{CF}_4$. Hence, we hypothesize that the ionic species CF_3^+ , which is unreactive towards CF_4 , is formed during the irradiation of the CF_4/Ne mixture, and reacts with an ^{18}F atom absorbed on the walls of the target chamber, leading to the rapid formation of $[^{18}\text{F}]\text{CF}_4$. With this in our hands, 8.43 ± 0.59 GBq of $[^{18}\text{F}]\text{CF}_4$ could be produced in short irradiation times. These results are extraordinary, especially considering that full optimization of the experimental conditions was not carried out. An increase in the CF_4 filling pressure or application of higher integrated current values in the first irradiation may lead to higher production yields.

With the aim of proving the suitability of our method for the preparation of other fluorinated gases, the synthesis of $[^{18}\text{F}]\text{SF}_6$ was approached using the same experimental conditions (methods A and B) but replacing the CF_4 gas bottle by an SF_6 gas bottle. Under method A, an integrated current of $4 \mu\text{A h}$ resulted in the formation of 0.36 ± 0.05 GBq of $[^{18}\text{F}]\text{SF}_6$, while under method B, an integrated current of $4 \mu\text{A h}$ in both irradiations resulted in 6.77 ± 0.21 GBq of pure $[^{18}\text{F}]\text{SF}_6$. The analysis of the radioactive gas by radio GC-MS after purification using a soda lime trap confirmed the presence of only one radioactive species identified as $[^{18}\text{F}]\text{SF}_6$. MS analysis confirmed the presence of 4 species that were identified as N_2 , CF_4 , F_3N , and SF_6 . These results are extremely positive, as they suggest that the strategy reported here might be extrapolated to the preparation of other radiolabelled fluorinated gases.

To prove the suitability of the radiofluorinated gases for the assessment of lung ventilation, *in vivo* PET studies combined with Computerised Tomography (CT) imaging were conducted using $[^{18}\text{F}]\text{CF}_4$ and a simple administration protocol based on dilution of the radioactive gas in the carrier oxygen (Fig. S3, ESI†). The time-activity curve in the lungs showed a sharp increase just after the onset of the administration of $[^{18}\text{F}]\text{CF}_4$ (Fig. 2a). When the delivery was discontinued, almost complete elimination from the lungs was achieved in a few seconds. The time-averaged PET images clearly show uniform distribution of the gas within the lungs (Fig. 2b). The use of $[^{18}\text{F}]\text{CF}_4$ as a ventilation marker has two major advantages: (i) the non-radioactive analogue is chemically inert, non-toxic, and has poor solubility in water; hence, a low translocation to the blood and remote organs is expected. Additionally, the gas is rapidly exhaled. Altogether, these factors contribute to a minimisation of the radiation dose posed on the subject; and (ii) the relatively long half-life of ^{18}F and the efficient production method reported here should enable centralized production and distribution to nearby imaging centres, facilitating a potential translation into the clinical setting. Of note, such a translation may require the development of tailored gas administration systems capable of collecting the radioactive gases exhausted by the patients.

In conclusion, we report here the unprecedented, highly efficient, simple and easy-to-automate preparation of $[^{18}\text{F}]\text{CF}_4$ and $[^{18}\text{F}]\text{SF}_6$ following an ion beam induced chemical reaction

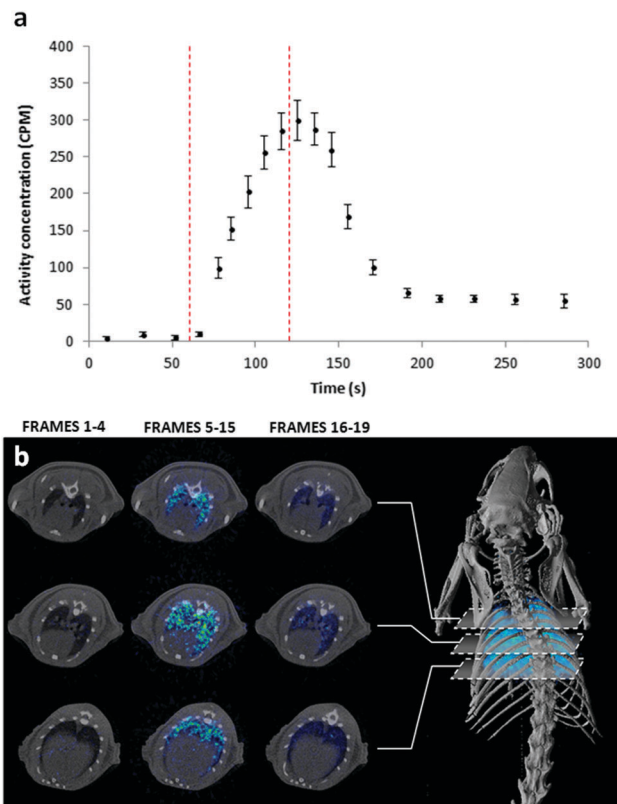


Fig. 2 (a) Concentration of radioactivity (arbitrary units, counts per minute, CPM) in the lungs as a function of time (mean \pm standard deviation, $n = 2$). (b) Left: Axial PET-CT slices of the thoracic cavity, before (frames 1–4), during (frames 5–15) and after (frames 16–19) administration of $[^{18}\text{F}]\text{CF}_4$; right: surface-rendered 3D-CT images of the skeleton coregistered with volume-rendered 3D-PET images of the lungs (frames 5–15).

based on a double proton irradiation approach. The methodology might be extended to other fluorinated gases. $[^{18}\text{F}]\text{CF}_4$ has proven suitable for the determination of regional lung ventilation using PET-CT. The radiofluorinated gases reported here may become powerful tools in the diagnostic, prognostic or evaluation of response to treatment for a wide variety of lung diseases. Future works will focus on the refinement of the experimental set-up and evaluation of $[^{18}\text{F}]\text{CF}_4$ as a ventilation marker using animal models of impaired lung ventilation.

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Notes and references

† Data obtained from Material Safety Data Sheet from Air Liquide, prepared to U.S. OSHA, CMA, ANSI and Canadian WHMIS Standards. As stated in this document, exposures to high concentrations of this gas may cause sensitization of the heart to adrenaline and nor-adrenaline. The compound is not considered nor suspected to be a cancer-causing agent. It is not irritating, and it is not known to cause sensitization. The most significant hazard associated with this gas is inhalation of oxygen-deficient atmospheres.

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