A New Concept for the Production of ^{11}C -labelled Radiotracers

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Abstract

Background: GMP-compliant production of radiopharmaceuticals has been performed using disposable units (cassettes) with a dedicated synthesis module. To expand this "plug 'n' synthesize" principle also to a broader scope of modules we developed a pressure controlled setup that offers an alternative to the usual rotary valves, which are controlled by stepper motors. The new concept was successfully applied to the synthesis of N-methyl-[^{11}C]choline, *L*-S-methyl-[^{11}C]methionine and [^{11}C]acetate.

Results: The target gas purification cyclotron produced [^{11}C]CO₂ and conversion to [^{11}C]MeI were carried out on a Tracerlab FxCPro module. The labelling reactions were controlled with a Tracerlab FxFE module. With the presented modular principle we were able to produce N-methyl-[^{11}C]choline and *L*-S-methyl-[^{11}C]methionine by loading a reaction loop with neat *N,N'* dimethylaminoethanol (DMAE) or an ethanol/water mixture of NaOH and *L*-homocysteine (*L*-HC) and subsequent reaction with [^{11}C]MeI. After 18 minutes N-methyl-[^{11}C]choline could be isolated in 52 % decay corrected yield and a radiochemical purity of >99 %. For *L*-S-methyl-[^{11}C]methionine the total reaction time was 19 min reaction yielding 25 % of pure product (>97%, *n* = 1). The reactor design was used as an exemplary model for technically demanding [^{11}C]acetate synthesis. The disposable unit was filled with 1 ml 0.75 M MeMgCl in tetrahydrofuran (THF). After complete release of [^{11}C]CO₂ the reaction mixture was quenched with water and guided through a series of ion exchangers (H⁺, Ag⁺ and OH⁻). The product was retained on a strong anion exchanger, washed with water and finally extracted with saline. The product mixture was acidified and degassed to separate [^{11}C]CO₂ before dispensing. Under these conditions the total reaction time was 17 ± 2 minutes and decay corrected 51 ± 5% pure [^{11}C]acetate (*n* = 10) could be isolated.

Conclusion: Herein, we described a novel single use unit for an synthesis of carbon-11 labelled tracers for preclinical and clinical applications of N-methyl-[^{11}C]choline, *L*-S-methyl-[^{11}C]methionine and [^{11}C]acetate.

Background

The first production of carbon-11 (^{11}C) was reported almost 100 years ago. (Crane et al. 1934) The ability to label and metabolically track almost any biologically relevant carbon-containing compound by ^{11}C makes this radionuclide an indispensable marker in molecular imaging by positron emission tomography (PET) and modern nuclear medicine. Nevertheless, due to its short half-life of only 20 minutes and its complicated chemistry, potent tracers find fewer clinical applications than ^{18}F -labelled tracers.

A common access to ^{11}C -labelled radiopharmaceuticals is the direct activation of [^{11}C]CO₂ with organometallic reagents. It can also be converted into [^{11}C]CH₃I (Larsen et al. 1997) or [^{11}C]CH₃OTf (Jewett 1992) and then used for radiolabelling. In any case, the radioactive building block is used in gaseous form with an inert carrier gas (usually helium). The actual labelling step occurs by bubbling gas into a reactor or a reaction loop. Tracer specific work-up by solid phase extraction (SPE) and cartridge

purification has become widely accepted which often eliminates the need for time-consuming HPLC separation. A schematic overview is depicted in Figure 1.

It is obvious that the used materials can have a significant influence on the success or failure of the radio synthesis. To minimize problems, cGMP-compliant production of radiopharmaceuticals is preferably performed using very expensive disposable units (cassettes). The synthesis then proceeds according to a "plug 'n' synthesize" principle and delivers reliable results. In addition, cross-contamination with other in-house produced pharmaceuticals and handling errors caused by personnel are minimized.

The major clinical benefit of N-methyl-[^{11}C]choline PET however has been in the evaluation of patients with biochemically recurrent prostate cancer (Hara et al. 1998; Scattoni et al. 2007; Reske et al. 2008; Rinnab et al. 2008; Mitchell et al. 2012), and currently, for localization of parathyroid adenoma (Orevi et al. 2014; Liu et al. 2020; Noltes et al. 2021). *L*-S-methyl-[^{11}C]methionine is a ^{11}C -labelled analogue of the essential amino acid methionine. It enters several metabolic pathways and has been mainly used clinically for imaging brain tumours (Sato et al. 1992), hyperparathyroidism (Sundin et al. 1996; Rubello et al. 2006), neck and head tumours (Lindholm et al. 1993) and currently in multiple myeloma including staging, prognostication and assessment of therapy response (Lapa et al. 2017; Morales-Lozano et al. 2020; Lückcrath et al. 2015). The synthesis of both N-methyl-[^{11}C]choline and *L*-S-methyl-[^{11}C]methionine is quite robust and for routine use in many institutions with a cyclotron.

[^{11}C]acetate has been used as imaging agent for studying myocardial oxidative metabolism (Nesterov et al. 2015; Grassi et al. 2012) and regional myocardial blood flow (Gropler et al. 1991) as well as for imaging multiple sclerosis (Takata et al. 2014) and prostate cancers (Oyama et al. 2002).

The most common method for the production of [^{11}C]CO₂ is the irradiation of a $^{14}\text{N}_2$ target with protons following the reaction [$^{11}\text{N}(\text{p},\alpha)^{11}\text{C}$]. The unavoidable addition of traces of [^{16}O]oxygen ($^{16}\text{O}_2$) to target gas leads to the formation of undesired [^{13}N]nitrogen during irradiation, which is present as nitrogen oxides (NO_x) and has to be removed before further conversions (Ache et al. 1966). Modern synthesis units use molecular sieves for this purpose, as they could be regenerated and have a long shelf life. It is possible to bind [^{11}C]CO₂ selectively on the solid matrix and by purging with an inert carrier gas, the unwanted by-products could be flushed out and separated. At elevated temperatures (>250°C), the purified carbon dioxide is released. This can either be used directly for the synthesis (CO₂ Bypass) or converted to [^{11}C]methyl iodide. In the so-called "gas phase" conversion it is reduced with hydrogen at 360°C on a heterogeneous nickel catalyst to methane and then reacted with iodine at 720°C to produce [^{11}C]CH₃I (Larsen et al. 1997) (figure 2 left). The individual syntheses for N-methyl-[^{11}C]choline (Pascali et al. 2000; Jinming et al. 2006; Kuznetsova et al. 2003), *L*-S-methyl-[^{11}C]methionine (Lodi et al. 2008), and [^{11}C]acetate (Berridge et al. 1995; Kruijer et al. 1995; Roeda et al. 2002; Soloviev et al. 2006; Kang Se et al. 2016; Maurer et al. 2019; Mitterhauser et al. 2004) have been optimised in the last decades and are reviewed in detail (Oleksiy et al. 2013; Gomzina et al. 2015; Dahl et al. 2017). A general overview is shown in Figure 2.

The requirements towards materials and equipment for ^{11}C methylation reactions are undemanding, as no aggressive solvents or sensitive reagents need to be used. The conditions for carboxylation reactions, however, are quite different. The reaction of organometallic compounds requires organic solvents such as tetrahydrofuran or diethyl ether. These are not compatible with many polymers used in regular disposable cassettes. Furthermore, the reagents are sensitive to air and moisture, which also leads to difficulties in handling. Despite these important applications, there are no currently none cassette-like systems that can handle all these requirements. This motivated us to developed novel flexible disposable setup for the synthesis of tracers according to the synthesis principle shown in figure 1.

Methods

General

$[^{11}\text{C}]\text{CO}_2$ was prepared by bombardment of $^{14}\text{N}_2$ target gas containing 1% O_2 with 10-60 μA of 16.5 MeV protons using a PETtrace 860 cyclotron (GE Healthcare). For the production of ^{11}C -labeled radioprecursors from $[^{11}\text{C}]\text{CO}_2$ a TRACERlab FXC Pro (GE Healthcare) system was used. To be able to use $[^{11}\text{C}]\text{CO}_2$ directly valve (Vx) was installed (CO_2 bypass, see Figure 3). This does not affect the Mel circuit. No further modifications were made.

Primary Precursor $[^{11}\text{C}]\text{CO}_2$: Before each synthesis, the CH_4 oven was conditioned at 350 °C with helium for 20 min and then cooled to <35 °C before end of bombardment (EOB). The cyclotron produced $^{11}\text{CO}_2$ is trapped in a 4 Å MS at room temperature a released by heating the oven to 380 °C. To achieve a complete activity transfer, a helium gradient was chosen. The total duration is about 8 minutes.

Secondary Precursor $[^{11}\text{C}]\text{CH}_3\text{I}$: The general synthesis process of labeling with $[^{11}\text{C}]\text{methyl iodide}$ can be described as follows (see service Manual TRACERLab FX_C): Preparation: Before each synthesis, the CH_4 oven was conditioned at 350 °C with hydrogen for 20 min and then cooled to <45 °C before end of bombardment (EOB). The cyclotron produced $[^{11}\text{C}]\text{CO}_2$ is trapped in a 4 Å MS and online converted by Ni-catalyst (Shimalite-Ni) support, under continuous addition of Hydrogen at 360°C into $[^{11}\text{C}]\text{CH}_4$. Both formed H_2O and unconverted $[^{11}\text{C}]\text{CO}_2$ are held back by an Ascarite-trap (immobilized NaOH). The produced $[^{11}\text{C}]\text{Methane}$ is trapped on a carbosphere (60-80 mesh) under N_2 liq. cooling at -75°C for further purification and concentration. Afterwards the purified $[^{11}\text{C}]\text{CH}_4$ is allowed to react with iodine at 720°C to form $[^{11}\text{C}]\text{methyl iodide}$ in a gas circulating process system. Formed HI is removed by a separate ascarite trap. $[^{11}\text{C}]\text{Methyl iodide}$ is separated out of this circulation process by a trap containing Porapak Q (50-80 mesh) as adsorbens at room temperature. Unconverted $[^{11}\text{C}]\text{CH}_4$ returns back into the circulation process for reaction with Iodine. The collected $[^{11}\text{C}]\text{methyl iodide}$ is released in a moderate helium flow off the adsorbens (190°C) and used for further labeling. The total time is about 15 minutes.

General modifications to the Tracerlab FX FE Pro:

The synthesis module was cleaned and dried cleaned before each synthesis and used as a control unit for the disposable setup. The 2-way valve at V4 was replaced by a 3-way valve and connected to an ascatite trap. This allows pressure exhaust trapping of unreacted [^{11}C]CO₂ during carboxylation reactions. The V18 and V19 lines (normally connected to the module reactor) are mounted on the waste vessel. The product vessel is filled via the (normally closed) 2nd neck with a tube or cannula reaching the bottom of the vial (See figure 4). The activity detector of the reactor was dismantled and could be placed close to the disposable unit to monitor the activity during the reaction.

Radiolabelling

N-methyl-[^{11}C]choline: For the production 2-Dimethylamino-ethanol (Sigma Aldrich, # 471453 was used without purification. Reagents for reaction workup were water (Milli-Q; obtained in house from a Milli-Q Ultrapure water system), 0.9 % Saline (USP, B.Braun, 10 ml, #2737756) and Ethanol (Supelco, ACS,ISO,Reag. Ph Eur., Merck, # 1.00983) Ion exchange cadridge: weak cation exchanger, Sep-Pak Accell Plus CM Plus Short Cartridge, 360 mg Sorbent per Cartridge, 37-55 μm , was used without conditioning.

The solvent reservoirs of the FxFe module were filled as follows: Vial 1: 4 ml 0.9% Saline (Extract), Vial 2: EMPTY, Vial 3: EMPTY, Vial 4: EMPTY, Vial 5: EMPTY, Vial 6: 5 ml Milli-Q® Water (Wash), Vial 7: 5 ml Ethanol, Product vessel: Empty, Reaction Loop: 100 μl DMAE

For the construction of the disposable synthesis unit following materials were used: 3 x Check valves (AMT Medica, Germany, Ref: 0079), 2 x Luer Lock T Shape Adapter, FFM, PP (Cole Parmer Instruments, Germany, 45508-85), 1 x Luer Lock T Shape Adapter, FMM, PP (Cole Parmer Instruments, Germany, 45508-75), 1x Loop: PE LECTROcath extension 1.5 x 2.5 mm, length 150 cm (Vygon)

Process Description: The disposable unit was assembled as described in figure 5 and the loop was filled with DMAE. The SPE cartridge was placed in front of the reactor activity detector and the reaction loop was positioned close to the product detector. The connection lines to the solvent reservoirs were connected and the synthesis program was started. Monitoring and semi-automatic control was performed by using the pressure and activity detectors (for details see Table 1).

Table 1 Description, time list and in process control of N-methyl-[^{11}C]choline Synthesis at FxFe Module.

Step	Time	Device	Value	Comment (CholinV1.3)
Radiolabelling	0	V11	b (right)	Check valve B (CV _B) was open and CV _C was closed. After complete release of [¹¹ C]CH ₃ I the operator started the workup.
	0	V15	b (left)	
	0	V16	Open	
	0	V19	Open	
	5	Activity Product		
	5	Activity Reactor		
	10	Process Control	Show message and wait	

Ethanol Wash	t1+5	V19	Close	To remove unreacted starting material, the system was washed with ethanol. Meanwhile, an increase in activity on the cartridge was observed. A pressure increase in the waste container indicates that the washing step has been completed.
	t1+7	V07 Vial7	b (up)	
	t1+20	Process Control	Show message and wait	
	t2+1	V07 Vial7	a (down)	
	t2+1	V19	open	
	t2+10	V19	close	
Water Wash	t2+11	V06 Vial6	b (up)	To remove the ethanol residues from the cartridge, the unit was then washed with water. The activity on the cartridge remains constant during this process. A pressure increase indicated the end of this step.
	t2+35	Process Control	Show message and wait	
He Purge	t3+1	V06 Vial6	a (down)	Further purging with helium closes check valve B (CV _B)
	t3+10	V19	Close	
	t3+11	V02 Vial2	Open	
	t3+20	Process Control	Show message and wait	
Product Extraction	t4+1	V02 Vial2	Close	By releasing the pressure from the product vial, the check valve C was opened and the product was extracted by ion exchange with saline.
	t4+2	V15	a (right)	
	t4+3	V01 Vial1	Open	
	t4+1'0	Process Control	Show message and wait	
Filling	t5+1	V15	b (left)	Filling
	t5+2	V01 Vial1	Close	
	t5+3	V13	Open	
	t5+1'0	Process Control	Show message and wait	
End	t6+1	Process	Reset	

Control

The total reaction time was 22 minutes and 898 mBq (decay corrected 52%) pure ^{11}C -could be isolated. Irradiation Parameters (2 min, 20 μA , $A_{\text{EOB}}([^{11}\text{C}]\text{CO}_2) = 3700 \text{ mBq}$).

Quality control: The radiochemical purity was determined by HPLC (Dionex (Ion Pac CS12A, $l = 25 \text{ cm}$, $d = 4 \text{ mm}$, cation exchange resin), mobile phase 0.02 M Methanesulfonic acid, flow 1.0 ml/min, $R_f = 7.5 \text{ min}$) and was >99%. The residual DMAE content was also determined by HPLC as well and was less than 0.07 mg/ml.

L-S-methyl- ^{11}C methionine: Precursor solution: 1.5 mg L-homocysteine (*L*-HC) (Sigma Aldrich, # 69453 was used without purification), 10 μl 50% v.v. NaOH/ H_2O), 200 μl H_2O (Milli-Q; obtained in house from a Milli-Q Ultrapure Water system) and 200 μl Ethanol (Supelco, ACS,ISO,Reag. Ph Eur., Merck, # 1.00983) were mixed in an Eppendorf vial. Extraction medium: 0.9 % Saline (USP, B.Braun, 10 ml, #2737756). SPE cadridge (Sep-Pak C18 Plus Short Cartridge, 360 mg Sorbent per Cartridge, 55-105 μm) was used without conditioning. Disposable synthesis unit: 3 x Check valves (AMT Medica, Germany, Ref: 0079), 1 x Luer Lock T Shape Adapter, FFM, PP (Cole Parmer Instruments, Germany, 45508-85), 1 x Luer Lock T Shape Adapter, FMM, PP (Cole Parmer Instruments, Germany, 45508-75), 1x Loop: PE Lectrocath extension 1.5 x 2.5 mm, length 150 cm (Vygon).

The solvent reservoirs of the FxFe module were filled as follows: Vial 1: 4 ml 0.9% Saline (Extract), Vial 2: EMPTY, Vial 3: EMPTY, Vial 4: EMPTY, Vial 5: EMPTY, Vial 6: EMPTY, Vial 7: EMPTY Product vessel: Empty, Reaction Loop: total precursor solution.

Process Description: The disposable unit was assembled as described in Figure 6 and the loop was filled with the precursor solution. The connection lines to the solvent reservoirs were connected and the synthesis program was started (for details see table 2). Neither a pressure nor an activity profile were recorded during this reaction.

Table 2 Description and time list of L-S-methyl- ^{11}C methionine synthesis at FxFe Module.

Step	Time	Device	Value	Comment (MethionineV1.03)
Radiolabelling	15	Process Control	Show message and wait	After complete release of [^{11}C]CH ₃ I the operator started the workup.
He Purge	t1+5	V02 Vial2	open	After the reaction was completed, the aperture was purged with helium to remove traces of unreacted methyl iodide.
	t1+1'30	V02 Vial2	close	
Product Extraction	t1+1'31	V01 Vial1	b (up)	Product extraction
	t1+2'30	Process Control	Show message and wait	
Filling	t2+1	V15	b (left)	Filling
	t2+2	V01 Vial1	Close	
	t2+3	V13	Open	
	t2+1'0	Process Control	Show message and wait	
End	t3+1	Process Control	Reset	

Under these conditions the total reaction time was 23 minutes (19 min reaction + 3 min filling) and 3000 MBq (decay corrected 25%) pure *L*-S-methyl-[^{11}C]methionine could be isolated.

Quality control: Irradiation Parameters (5 min, 60 μA , A_{EOB} ([^{11}C]CO₂) = 25000 mBq). The radiochemical purity was determined by HPLC (Phenomenex Gemini 5 μ C18 110Å, l = 15 cm, d = 4.6 mm, C-18 Resin), mobile 0.05 M NaH₂PO₄ / 2 % Ethanol, pH 6.2 – 6.3, flow 0.75 ml/min, R_f = 3.4 min) and was radiochemical purity was >97%.

[^{11}C]acetate: A 0.75 M grignard stock solution was prepared using the Schlenk technique and stored in a 25 ml Schlenk flask (Glasgerätebau Ochs, Germany, # 143514) under argon. This solution is stable for at least 3 months at room temperature: 3 M MeMgCl in THF (Sigma Aldrich, #189901) and THF (ExtraDry, stored over molsieve, stabilized AcroSeal™, Acros, # 10292182) were used without purification. For solution transfer, Injekt®-F Tuberculin 1 ml Syringe B Braun, Ref 9166017V) were used. Reagents for reaction workup: Water (Milli-Q; obtained in house from a Milli-Q Ultrapure Water system), 0.9 % Saline (USP, B.Braun, 10 ml, #2737756) and 139 mM sodium citrate pH 4.7 (for 50 ml: 1.33 g anhydrous citric acid, 6.1 ml 2 M NaOH) (Maurer et al. 2019). Ion exchange cadtridges: PS-OH[−] (200 mg), PS-H⁺ (230 mg), and PS-Ag⁺ (240 mg) cartridges (Macherey-Nagel, Düren, Germany) were conditioned with 10 ml of

ethanol and 10 ml of water and blown dry with 10 ml air. NOTE: 3 x Ps-Ag⁺ cartridges were used to ensure a the complete removal of chloride.

Disposable synthesis unit: 4 x Check valves (AMT Medica, Germany, Ref: 0079), 2 x Luer Lock T Shape Adapter, FFM, PP (Cole Parmer Instruments, Germany, 45508-85), 1 X Luer Lock T Shape Adapter, FMM, PP (Cole Parmer Instruments, Germany, 45508-75), 1 x Reactor: 10 ml Luer lock PP Syringe Body without plunger (DB Plastic, Ref 300912) sealed with a rubber septum or crimped with a butylrubber plug with cap R 20B-L (CS chromatographie, Art. Nr. 300242), 1 x Exhaust cannula: Sterican® 1.20 x 40 mm, orange (B.Braun), 2x Quench and wash cannula: Sterican® 0.6 x 60 mm, blue BL/LB), Optional: PE LECTROcath extension lines 1.5 x 2.5 mm, length 30 – 150 cm available (Vygon)

The solvent reservoirs were filled as follows Vial 1: EMPTY, Vial 2: 2ml Milli-Q® Water (to Quench the reaction), Vial 3: EMPTY, Vial 4: 15 ml Milli-Q® Water (for transport and Washing) Vial 5: EMPTY. Vial 6: 15 ml Milli-Q® Water (Washing), Vial 7: 4 ml 0.9% Saline (Extraction of anion exchange cartridge), Product vessel: 1 ml 139 mM citric buffer pH = 4.7 (for removal of [¹¹C]CO₃²⁻)

Process Description: The ion exchangers were conditioned and the parts were assembled as described in Fig 7. The connection lines to the solvent reservoirs were connected and the synthesis program was started. A helium overpressure was generated via V5 and CV_A was closed. The reactor was purged with dry argon for 5 minutes via the "normally CO₂" input. Then the grignard solution was carefully added to the reactor via the septum. Then the argon line was exchanged for the [¹¹C]CO₂ line. The anion exchange cartridge was placed in front of the reactor activity detector and the reaction vessel was positioned close to the product detector (for details see Table 3).

Table 3 Description, time list and in process control of [¹¹C]acetate Synthesis at FxFe Module.

Step	Time	Device	Value	Comment (AcFull210909.3)
Presynthesis	0	V05 Vial5	open	Check valve A (CV _a) was closed by helium overpressure from V5 to fill Grignard reagent. After purging with helium the [¹¹ C]CO ₂ was connected.
	0	V11	b (right)	
	0	V15	b (left)	
	0	V16	Open	
	10	Process Control	Show message and wait	
Radiolabelling	t1+5	Activity		

		Product		The [¹¹ C]CO ₂ line was close to the product detector so temporary an increase in activity was observed. After the reaction, the activity is located in the reaction vessel.
	t1+6	Activity Reactor		
	t1+10	Process Control	Show message and wait	
Quench and Wash	t2+10	V02 Vial2	Open	The reaction mixture was quenched and washed with water. The check valves A and B were opened by applying a vacuum. An increase in activity on the anion exchange cartridge was observed. A pressure increase in the waste vessel indicates that the washing step has been completed.
	t2+20	V02 Vial2	Close	
	t2+20	Power	On	
	t2+20.1	V05 Vial5	Close	Note: This step can take from 2 to about 4 minutes, as the reaction mixture must be passed through four ion exchangers.
	t2+25	V18	Open	
	t2+25.1	V04 Vial4	Open	
	t2+30	Process Control	Show message and wait	
Wash wash anion exchanger	t3+5	V04 Vial4	Close	To ensure that all by-products are separated, the anion exchanger cartridge was washed again with water. A pressure increase in the waste vessel indicates that the washing step has been completed.
	t3+10	Vial06 Vial6	b (up)	
	t3+20	Process Control	Show message and wait	
Helium Purge	t4+5	V06 Vial6	a(down)	Further purging with helium closes check valve B (CV _B).
	t4+5.1	V05 Vial5	Open	
	t4+5.2	V18	Close	
	t4+5.3	Power	Off	
	t4+10	Process Control	Show message and wait	
Product extraction and degassing	t5+1	V05 Vial5	Close	By releasing the pressure from the product vial, the check valve C was opened and the product was extracted by ion exchange with saline. The product is transferred into the citrate buffer solution and [¹¹ C]carbonate was removed by helium bubbling.
	t5+5	V05 Vial5	Close	

	t5+5.1	V15	a (right)	
	t5+5.2	V07 Vial7	b (up)	
	t5+1'30	Process Control	Show message and wait	
Filling	t6+5	V07 Vial7	a (down)	Filling
	t6+10	Process Control	Show message and wait	
	t7+5	V15	b (left)	
	t7+5.1	V13	Open	
	t7+30	Process Control	Show message and wait	
End	t8+1	Process Control	Reset	

This reaction was optimised under the following conditions: Irradiation Parameters (2 min, 10 μ A, $A_{\text{EOB}}([^{11}\text{C}]\text{CO}_2) = 2000 \text{ mBq}$) yielding $52 \pm 5\%$ product ($n = 9$) after $18 \pm 2 \text{ min}$.

To investigate clinically relevant conditions, higher starting activities were used as well. The diagrams shown are from this experiment. Irradiation Parameters (5 min, 60 μ A, $A_{\text{EOB}}([^{11}\text{C}]\text{CO}_2) = 25000 \text{ mBq}$). The total reaction time was 17 minutes and 6600 mBq (decay corrected 51%) pure product could be isolated.

Quality Control: The radiochemical purity was determined by HPLC (CarboPac PA10 (Dionex, Thermofisher Scientific, $l = 25 \text{ cm}$, $d = 4 \text{ mm}$, particle size $10 \mu\text{m}$) mobile phase: 0.1 M NaOH, flow 1.0 ml/min, $[^{11}\text{C}]\text{Acetate } R_f 8 - 10 \text{ min}$) and TLC (0.5 μL of the product solution on NaOH impregnated silica plates with methanol as the mobile phase as described before (Berridge et al. 1995) (Maurer et al. 2019). Product had R_f values of 0.8–0.9, while the contaminant near the baseline (R_f from 0.0 to 0.1). Neither carbonate nor other impurities could be detected.

Results

With the present modular principle, we were able to establish successfully the production of N-methyl- $[^{11}\text{C}]\text{choline}$, *L*-S-methyl- $[^{11}\text{C}]\text{methionine}$ and $[^{11}\text{C}]\text{acetate}$ in a high radiochemical yield and purity for research and potential clinical applications. The target gas purification (CO_2 Bypass) and conversion to $[^{11}\text{C}]\text{MeI}$ were carried out on a Tracerlab FxCPro module. The labelling reactions were controlled with a Tracerlab FxFE module.

We chose the loop configuration for the synthesis of N-methyl-[¹¹C]choline and L-S-methyl-[¹¹C]methionine. Neat N,N' dimethylaminoethanol (DMAE) or an ethanol/water mixture of NaOH and L-homocysteine (L-HC) were loaded to the loop and reacted with [¹¹C]MeI. After a total time of less than 20 minutes, both tracers were obtained in good yields. Since clogging during hydrolysis of the grignard solution is a major problem in [¹¹C]acetate synthesis, we have chosen a reactor design that prevents this problem. Reliable results were obtained using quite similar conditions to Kang Se *et al.* (Kang Se et al. 2016). 1 ml of 0.75 M solution of methyl magnesium chloride in tetrahydrofuran was used as an alkylation reagent. The ion exchangers were adjusted to the corresponding amount of substance. Total reaction time was about 18 minutes. Quality control was performed according to the literature and was in accordance with monograph of Pham Eu. for [¹¹C]acetate and L-S-methyl-[¹¹C]methionine injection solutions. The preliminary isolated yields and beam parameters are summarized in Table 4.

Table 4 Results for the ¹¹C-tracer production.

¹¹ C-Tracer	N-methyl-[¹¹ C]choline	L-S-methyl-[¹¹ C]methionine	[¹¹ C]acetate
Reaction Type	Methylation		Carboxylation
Time ^[a]	18 min	19 min	18 ± 2 min
Isolated Yield	900 MBq ^[b]	3000 MBq ^[c]	6600 MBq ^[c]
Yield ^[d] on ¹¹ CH ₃ I ^[e]	85 %	42 %	-
Yield ^[d] on ¹¹ CO ₂	52 % (n=1)	25 % (n=1)	52 ± 5%, (n = 10)

[a] Total reaction time including radioprecursor synthesis: ¹¹CH₃I (14 min) or ¹¹CO₂ Bypass (8 min); [b] Irradiation parameters 20 µA, 2 min ([¹¹C]CO₂ A(EOB)_{cal} = 3.7 GBq); [c] 60 µA, 5 min ([¹¹C]CO₂ A(EOB)_{cal} = 25 GBq); [d] Decay corrected at EOB, [e] Gas phase conversion to ¹¹CH₃I 60 %. [

Discussion

The main objective was to use disposable, commercially available materials "out of the box" without complicated modifications. A summarized general setup is shown Figure 8. The individual reaction paths were controlled externally by pressure regulation. The special feature is the use of check valves as control units or even 3 way valves.

The basic principle of this construction consists of pluggable elements that allows customisation. T-shaped luer lock adapters, which are available in various designs serve as the framework. A "loop" or "reactor" setup is possible in either case. For the latter, a disposable syringe body with septum can be used. Luer lock check valves are used to attach the desired SPE cartridges for cleaning and a T-shaped valve is connected at the end to separate wash and extraction solution. Solvent and gas supply was

provided by the synthesis module. A typical reaction proceeds as follows. The check valves (CV) A, B and C are closed by a gas overpressure (orange adapter). The gaseous radio-precursor can then be passed through a check valve into the apparatus. The gas flow is automatically directed into the reactor or reaction loop where the chemical reaction takes place. Pressure equalisation is permitted via the connected exhaust line. The gas supply is then terminated and the waste vessel

evacuated. This opens valves A and B and the reaction mixture can be passed through the SPE cartridge and be washed. Once all washing steps have been completed, purging with gas continues until the generated overpressure closes CV B again. By opening the exhaust valve at the product vessel CV C is opened. The product can then be extracted, sterile filtered and dispensed.

Conclusion

Each material is available as a sterile, affordable and single-use product. Furthermore, key materials are available in inert materials, such as Polyethylene (PE) or Polypropylene (PP) which is necessary when using sensitive chemicals and organic solvents. The used materials are listed in the methods section. As pressure/vacuum control is possible with a variety of modules the adaption of this design should be easy. This concept may not only help in the establishment of new ^{11}C -tracers for research and routine clinical applications but also to improve the synthesis of established ^{11}C -labelled PET-tracers in general. Especially when no cassette based modules are available or reactive chemicals have to be used. We are convinced that this setup system can also be adopted to other tracers. The extension of this concept to routine tracers was part of our current research. The first experience shows that the new unit can successfully be applied to the synthesis of $[^{11}\text{C}]$ choline, $[^{11}\text{C}]$ methionine and $[^{11}\text{C}]$ acetate with good radiochemical yields in less than 20 min synthesis time.

Abbreviations

GMP: Good Manufacturing Practice; DMAE: N,N' dimethylaminoethanol; d.c.: decay corrected; THF: tetrahydrofuran; PET: positron emission tomography; SPE: solid phase extraction; HPLC: High Performance Liquid Chromatography; L-HC: L-homocysteine; L-HCTL: L-homocysteinethiolactone; EOB: End of Bombardment; PE: Polyethylene; PP: Polypropylene.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reason-able request.

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Authors' contributions

JW conducted the experiments, performed the data analysis and wrote the manuscript. FA assisted in the experiments. SS was the supervisors of this study. All authors contributed to the writing of the manuscript and approved the final version

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Competing interests

The authors declare no potential conflicts of interest relevant to this article.

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Figures

Figure 1

General description of ^{11}C -tracer synthesis. 1. Reaction Step, 2 Workup and transport of the product mixture to SPE purification cartridge(s), 3. Washing step and 4. Product extraction.

Figure 2

Radiochemistry overview. Left: Gas chemistry for the preparation of ^{11}C Precursors. Purple: Methylation reaction using the example of [N-methyl- ^{11}C]choline starting from N,N'- Dimethylaminoethanol (DMAE) and conversion of L-homocystein (HC) or L-homocysteinthiolactone (HCTL) for the production of L-[S-methyl- ^{11}C]methionine. Green: Carboxylation reaction for the production of ^{11}C acetate with Methyl Grignard compound.

Figure 3

Schematic structure of the FxCPro module. The installation of V_x valve allows a simple CO₂ bypass.

Figure 4

Configuration of the FxFe module. The modifications compared to the standard setup are highlighted in red.

Figure 5

Setup for N-methyl-[^{11}C]choline.

Figure 6

Setup for L-S-methyl-[^{11}C]methionine.

Figure 7

Setup for [^{11}C]acetate.

Figure 8

General Setup for radiotracer synthesis. left to right: Gas inlet check valve for the [^{11}C]Precursor. T-Adapter with reaction vessel (loop or reactor). Check valve A and T-adapter for SPE cleaning. Separation valve with CV B and C for product isolation. Valve configuration: Reaction (all CVs closed), wash (CV C closed) and extraction (CV B closed). Opening and closing of the valves is controlled by gas pressure (here helium marked red).

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