DEVELOPMENT AND EVALUATION OF REMOTELY OPERATED SYNTHESIZER FOR [^{18}F]-FLUOROCHOLINE SYNTHESIS

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ABSTRACT

Among the cancer imaging modality that is utilized by Malaysia today is the Positron Emission Tomography (PET). Even though PET studies are usually done with [^{18}F]-fluoro-2-dioxyglucose (FDG) as the tracer, [^{18}F]FDG exhibits several weakness in detecting certain kind of tumours such as brain tumour and prostate metastasis. A new tracer namely [^{18}F]-fluorocholine (FCH) has been identified as an alternative to [^{18}F]FDG. The absence of specific synthesizer for FCH production hampers the application of this tracer in PET studies at local premises. This study will discuss about the development of the [^{18}F]FCH synthesizer prototype and its challenges. The design would emphasized on its simplicity, relatively low cost, semi-automated during synthesis and purification, reliable and safe to be use. The [^{18}F]FCH was synthesized in two step approaches; reacting [^{18}F]-fluoride with dibromomethane into [^{18}F]-fluorobromethane before purification using Sep-Pak silica cartridges and finally converted into [^{18}F]FCH by reacting with N,N-dimethylaminoethanol. We have successfully developed the synthesizer and are able to achieve decay-corrected radiochemical yield of 1.45 % in under 100 min. Optimization of the radiochemical yield is still underway.

ABSTRAK

Antara kanser mengimejkan modus yang digunakan oleh Malaysia hari ini ialahPositron Emission Tomography (PET). Walaupun kajian-kajian PET biasanya merupakan habis selesai [^{18}F]-fluoro-2-dioxyglucose (FDG) sebagai penyurih, [^{18}F]FDG mempamerkan beberapa kelemahan dalam mengesan tertentu agak tumorseperti metastasis barah otak dan prostat. Seorang penyurih baru iaitu [^{18}F]-fluorocholine (FCH) telah dikenal pasti sebagai satu alternatif untuk [^{18}F]FDG.Ketiadaan alat sintesis khusus untuk pengeluaran FCH menghalang permohonanpenyurih ini di kajian-kajian PET di premis-premis tempatan. Kajian ini akanmembincangkan mengenai pembangunan [^{18}F]Prototaip alat sintesis FCH dancabaran-

cabarannya. Reka bentuk akan menekankan tentang kesederhanaannya,kos agak rendah, semi automatik semasa sintesis dan penulenan, boleh dipercayaidan selamat guna. [18F]FCH disintesiskan di dua pendekatan langkah; bertindak balas [18F]-fluorida dengan dibromomethane ke dalam [18F]-fluorobromethane sebelum penulenan menggunakan Sep Pak silika catridges dan akhirnya bertukar ke dalam [18F]FCH dengan bertindak balas dengan N, N-dimethylaminoethanol. Kami telah berjaya membangunkan alat sintesis dan mampu mencapai hasil radiokimiadibetulkan reputan 1.45 % di di bawah 100 min. Pengoptimuman hasil radiokimiamasih sedang dijalankan.

Keywords: Positron Emission Tomography (PET), cancer imaging modality, [18F]-fluoro-2-dioxyglucose

INTRODUCTION

Cancer has becomes an increasing health problem in Malaysia. It is estimated that one out of four people in Malaysia will develop cancer at certain age of one's life (Lim et al., 2003; Lim et al., 2004). In order provide improved quality of life for the cancer patient and ultimately to reduce the mortality, emphasis has been given to detect and delivers effective treatment at early stage of cancer development. In recent years, positron emission tomography (PET) has gained considerable interest as a mean of cancer imaging tool in Malaysia. PET studies can provide functional information using radio-labelled tracer, especially using [F]-fluoro-2- dioxyglucose ([18F]FDG) (Fass 2008).

It has been proven that, while [18F]FDG is quite competent to be used as PET tracer, it still has several weakness. Reports such as by Price et al. (2002), Picchio et al. (2003), and Reske et al. (2006) has shown that [18F]FCH is more capable than [18F]FDG to detect both primary and metastatic prostate cancer. Other reports also suggest the clinical usefulness of [18F]FCH compared to [18F]FDG, especially in brain metastases (Pieterman et al. 2002).

It is undeniable that [18F]-Fluoro-2-deoxyglucocose ([18F]FDG) alone could not tackle all oncological problems face by mankind. There are other PET radiopharmaceuticals with large potential that can be use as a tool to explore various metabolic pathways (Bombardieri et al, 2008; Pantaleo et al, 2008). Among them is [18F]FCH. Even though the usefulness of the radiopharmaceutical has been proven considerably (see for example Hara, 2001), if not beyond doubt, but because there is no specific synthesizer to produce [18F]FCH available in local hospitals and institutes, the production of the pharmaceutical in Malaysia has been hampered.

The [18F]FCH was first successfully synthesized by DeGrado et al. (2000) by reacting N, N-dimethylaminoethanol with 1-[18F]fluoro-2-bromomethane ([18F]FBM). To the best of our knowledge, there are two methods of [18F]FCH synthesis reported so far. The first method (DeGrado et al, 2000;2001) involves the reaction between [F]-fluoride with dibromomethane, at temperature 100 °C to produce [18F]FBM. The precursor is then purified by Gas Chromatography (GC) before it is allowed to react with 2-dimethyl aminoethanol (DMAE), thus producing [F]FCH. The radiochemical yield was reported to be 20 -40 % (not decay-corrected) under 40 min.

The second method employs the use of [18F]-fluoromethyl triflate. Iwata et al. (2002) has reported a method to purified [18F]FBM simply by just using disposable Solid Phase Extraction (SPE) cartridges

before the [^{18}F]FBM is conveniently converted to more reactive [F-18]fluoromethyl triflate, in order to synthesize [^{18}F]FCH. The [^{18}F]FBM is converted to [^{18}F]fluoromethyl triflate by passing the freshly purified [^{18}F]FBM through a preheated (200 °C) column impregnated with silver triflate (AgOTf). The radiochemical yield at the end of synthesis is within 54-67 %. the time of synthesis is less than 30 minute.

Other choline-analogues such as [^{18}F]fluoroethylcholine ([^{18}F]FEC) and [^{18}F]propylcholine have been synthesized by using ethyl bromide and propyl bromide respectively (DeGrado et al., 2001). Another method involves the use of ethyl tosylate as precursor has been used to synthesize [^{18}F]FEC (Hara, Noboru and Hiroichi, 2002; Bauman et al., 2003; Piel et al., 2007). However, [^{18}F]FEC has so far seemed holds no significant clinical advantages over [^{18}F]FCH (Degrado et al., 2001; Hara et al., 2002). DeGrado et al. (2001) reported that the uptake of FCH and choline in cultured prostate cancer cells were comparable, whereas uptake of FEC was approximately one fifth of FCH. Therefore, [^{18}F]FCH has potential to be use in PET technique.

According to Zheng and Berridge (2000), [F-18]fluoroiodomethane ([F-18]FIM) can be synthesized at lower temperature, which is at room temperature, compare to [F-18]FBM, with radiochemical yield (not corrected) 40±8 %. However, Bergman et al. (2001) reported that the yield of [F-18]FIM is around 5.7±5.5 % (decay corrected). They argued that the difference might has been triggered by the high reactivity of [F-18]FIM, hence making the yield too sensitive for various approaches in synthetic strategy and manipulation.

As of June 2011, cyclotron-PET facilities in Malaysia have only produce [^{18}F]FDG for their routine use. In order to address this defieciency even though there is effort to synthesis other tracer such as 3,4-dihydroxy-6- F-fluoro-L-phenylalanine ([^{18}F]FDOPA) by Hospital Putrajaya. From the practical points of view, it is hoped that with a home-made synthesizer that is competitively simple and low cost, that the best solution for the problem could be paved.

MATERIALS AND METHOD

Components of the synthesizer

The [^{18}F]FCH synthesizer module consists of two separate plant; namely processing plant and reagent plant. The processing plant is installed inside the hot-cell, where all the process that involves contact with radioactive material are being done, while the reagent plant is use to introduce non-radioactive chemicals into the processing plant. The reagent plant is connected to the processing plant via tygon silicon tubing through special ports at the side of the hot-cell.

Two-way (PN: EW-98302-12) and three-way (PN: EW-98302-12) solenoid operated pinch valves, tygon silicone tubing (PN: EW-95702-01), and needles with non-coring deflected tips (PN: EW-25701-32) were purchased from Cole-Palmer. Stainless steel straight barbed connectors (PN: EW-31208-00), y barbed connectors (PN: EW-31209-55) and tee barbed connectors (PN: EW-31208-31) from Cole-Palmer are use to connect between tubing wherever tolerance to chemical and high temperature are required. We use stainless steel female luer lock with hose barb (PN: EW-31507-29) and male luer lock with hose barb (PN: EW-31507-26) from Cole Palmer to connect Sep-Pak cartridge and needle to tubing. In the same manner, we use polypropylene straight barbed connectors (PN: P-06365-11), y barbed connectors (PN: EW-30726-41) and male luer lock with hose barb (PN: P-45503-00) in less demanding area.

The electrical parts of the synthesizer consist of toggle switches (PN: 320-922), control panel (PN: 580-411), junction box (PN: 580-398), LED indicator (PN: 250-106); all purchased from RS Component, and DC power supply (PN: RS-75-24) from Mean Well. The air process heaters (PN: 200-2496), digital temperature controllers (PN: 461-206) and thermocouple type J (PN: 455-4270) were obtained from RS Component to form the heating system for the synthesizer. The pinch valves were connected to the power supply, toggle switches, LED indicators and manually operated by opening the switches. By applying pressure and vacuum through the tubing, and opening the desired valves, we can control the movement of the fluid inside the synthesizer from one point to another.

Reagent for synthesis

Anhydrous acetonitrile, dibromomethane, N,N-dimethylaminoethanol, potassium carbonate, ethanol, 0.85% saline solution, Kryptofix 2.2.2, and ethanol were purchased from Sigma- Aldrich. All chemical were used without any further purification. Sep-Pak plus tC18, Sep-Pak plus Silica, Sep-Pak plus Accell plus CM, and Sep-Pak light QMA Carbonate cartridges were obtained from Waters. The Sep-Pak Accell plus CM cartridge was conditioned with 5 mL HCl 0.5N and were rinsed with 10 mL ethanol prior to synthesis. The Sep-Pak Silica and QMA Carbonate were use without preconditioning.

[^{18}F]-Fluoride production

The no carrier added [^{18}F]-fluoride was produced by using a PETtrace cyclotron (GE, Uppsala) at Hospital Putrajaya. A beam of 16.5 MeV proton was allowed to bombard the target material, [O]-water; thus producing the [^{18}F]-fluoride via O(p,n) F nuclear reaction. The [^{18}F]-fluoride was then delivered into the GE TRACERlab MX FDG synthesizer and collected into a 10 mL sealed vial, readily put inside a lead pot. The lead pot was then place inside a transport box before transported to Nuclear Malaysia Agency. Upon arrival (transit time ~ 30 min), the radioactivity of [F]-fluoride will be determined using radioisotope calibrator (Capintec CRC-712MH) before being transferred into the hot-cell prior to synthesis.

RESULTS AND DISCUSSION

3.1 Development of the synthesizer

Before the synthesizer could be develop, the most important thing to do is to set up several criteria on which the synthesizer would be developed. The [^{18}F]FCH synthesizer are based from several criteria as following: a) relatively easy to develop, b) user friendly, c) high radiochemical yield and purity, d) relatively low cost, as well as e) safe and reliable.

The basic functions of the synthesizer are: a) introduction of [^{18}O]-water into the system, b) separation of [^{18}F]-fluoride from [^{18}O]-water; the most commonly method to do it is by using an anion exchanger resin, c) elimination of water traces by using azeotropic distillation, d) nucleophilic substitution between [^{18}F]-fluoride and precursor forming radiolabeled intermediate, e) intermediate purification using silica cartridges, f) methylation process of N,N-dimethylaminoethanol (DMAE) with intermediate, g) product purification by using cation-exchange resin, and g) purification of final product.

Based from the literature review conducted earlier in the study, several models of the synthesizer specific for the [^{18}F]FCH would be designed and considered. From these models, a final model would be

carefully selected and further modified. Materials needed for the development of the synthesizer were identified and purchased and materialization of the synthesizer was based on the final design.

The synthesizer is consists of two major parts. The first part is the processing plant, where all processes that involved radioactivity were done in a hotcell (Figure 1). The other part is the reagent plant (Figure 2), where all the chemicals, stored in conical vials were transferred into the hotcell via tygon silicon tubing through special ports at the side of the hot- cell. This design has some advantages over single, non-separated design especially during synthesis. It allows reagents to be freshly prepared or changed even when the synthesis process has started. It also contributes to smaller dimension, allowing it to fit into smaller hotcell, since almost all the reagents were located outside of the hotcell.

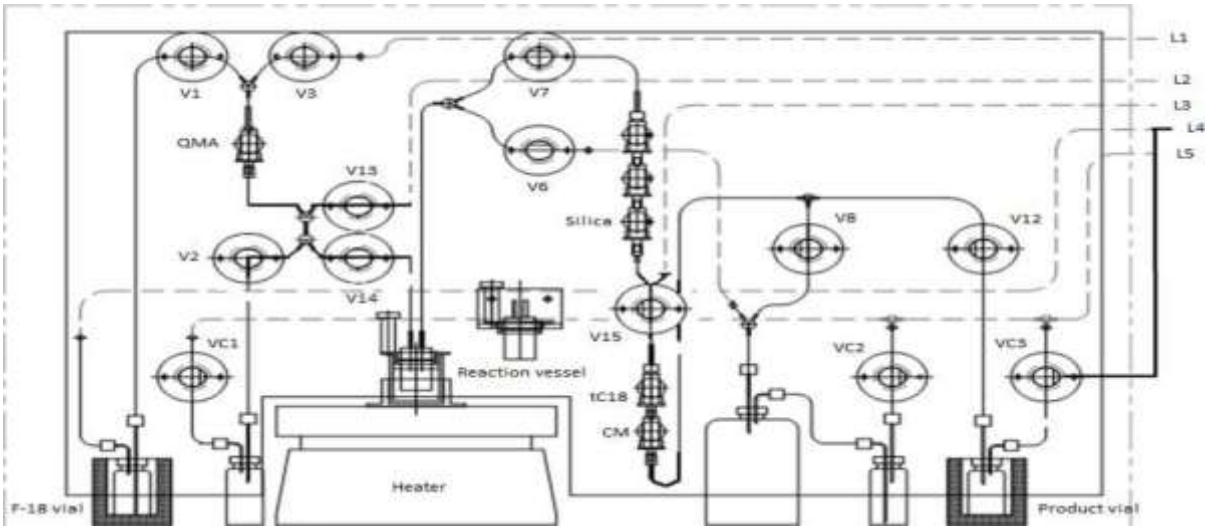


Figure 1. The processing plant of the synthesizer.

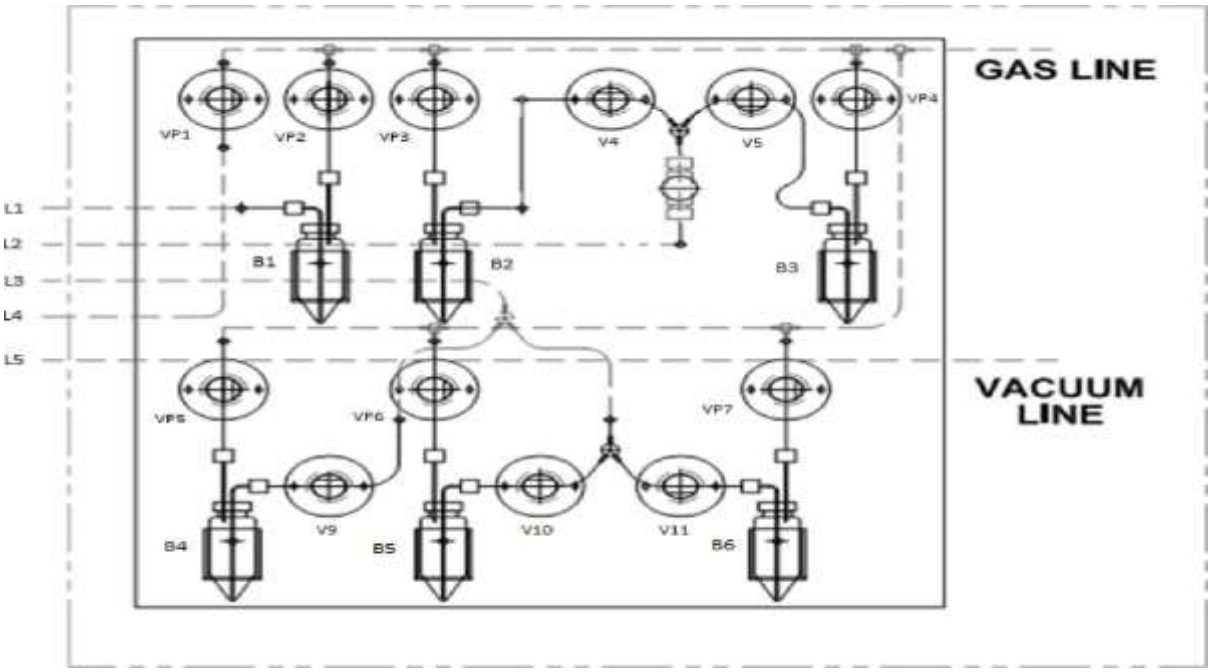


Figure 2. The reagent plant of the synthesizer.

All the reagents except DMAE were put into disposable 20 mL glass vials and sealed using butyl rubber septums and aluminium crimp. In order to maximum extraction from the flat bottom vials, we later

modified them into conical vials with the help of our glassblower team. B1 vial contains K_2CO_3 solution, B2 contains acetonitrile, B3 contains primary precursor, B4 contains ethanol, B5 contains purified water, and B6 contains saline solution. Each vial was punctured with a pair of needles prior to synthesis, which one of the needle will penetrate until it reach the bottom of the vial and the other will penetrate just deep enough for the gas to flow.

The fluids are transferred into the processing plant through tygon tubes (internal diameter 1/16 inch) by blowing carrier gas (in our case, oxygen-free nitrogen) and under suction of vacuum, created using a mini venturi pump, connected to L5 line. When VP1 valve is opened, the nitrogen gas will flow through the tubing and creating positive pressure at [^{18}F]-fluoride vial. By opening V1, V2, VC1 valves, the positive pressure inside the vial and the negative pressure created by vacuum would force [^{18}F]-fluoride into tubing. The [^{18}F]-fluoride would be trapped at Sep-Pak QMA cartridge (anion exchange resin) while [^{18}O]-water would passed through the cartridge and finally collected in the water recovery vial. In the same manner, by controlling which valves to be opened, the direction of the fluid flow can be precisely manipulated for any desired process.

Simulations were run using sterile water to validate that the synthesizer; a) have proper tubing connections and ensure no leakage occurred. b) showing the intended flow direction. Correction actions were taken to rectify the problems founds and as the result, two additional two-way valves (V13 and V14) and 1 three-way valve (V15) were introduced to the synthesizer. The finished prototype is shown in Figure 1. The dimension of the synthesizer is 0.46m (length) x 0.5m (height) x 0.14m (wide) for the reagent plant and 0.74m (length) x 0.50m (height) x 0.5m (wide). The cost of the synthesizer is estimated around RM 28, 000.

Figure 3 The remotely operated synthesizer; left picture- the processing plant and right- the reagent plant.

Calibration of heating system

One of the critical components of the synthesizer is the heating system. In the [^{18}F]FCH synthesizer, there are at least two occasions where heating are required. The first heating event happened during the azeotropic distillation, where the mixture of [^{18}F]-fluoride, solution of 33 mM K_2CO_3 and acetonitrile are heated. The second heating are required for reaction between anhydrous [^{18}F]-fluoride with dibromomethane. Therefore, the heating system must be able to achieve accurate and consistent required temperature in order for the synthesizer to function as it should be.

There is a wide range of suggested temperature for the first heating process (evaporation process) from 110 °C (Iwata et al., 2002), 100 °C (Hara et al., 2002), 95 °C (Kryza et al., 2008) and 90 °C (Zuhayra et

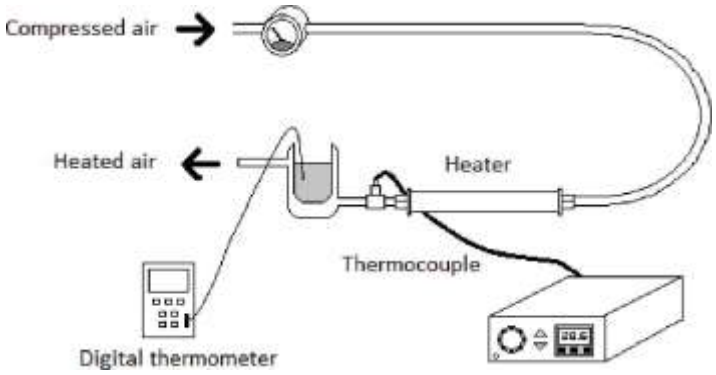
al., 2008). The second heating process did not have specific temperature, as the reaction temperature is the manipulated variable for the synthesis. Based from Kryza et al. (2008) report, we set the temperature range to be within 70-90 °C.

It is worth mentioning that since both heating process were not done simultaneously and both processes were done at the same vessel, normally only one heating system is required to do both jobs. Among the heating methods use for synthesis PET fluoro-compound are oil bath for example use by Iwata et al. (2002), stream of heated air such as showed by Cheung and Ho (2009) and heating tape for example use by Ahmed et al. (2007).

We have tested oil bath method where we used 20 mL silicone oil as heating medium in a custom-made glass cup. We used temperature controller connected to a hotplate and the controller receives input from a thermocouple soaked in the medium, where once the targeted temperature have been achieved, the controller would cut off power supply to the hotplate. Unfortunately, reliable temperature could not be achieved by using this configuration since temperature fluctuations were high (data not shown). Thus we decided to try the air process heater as alternative, since our team have experienced some success when using the system before.

The new heating system using the air process heater worked principally the same as the hotplate system, instead of using hotplate, air process heater was used where hot air passed through a custom-made inner glass tube (open at the top) surrounded by a glass jacket. The heated air is circulated within the jacket and the heat is transfer from the glass tube to the silicone oil (Figure 4). Once this heating concept has been proved successfully, the glass tube and the jacket were replaced with stainless steel tube and jacket.

Pressure regulator



Temperature Controller

Figure 4. Simplified diagram of the air process heater.

The heating system was tested and calibrated using a digital thermometer. The heating system showed good accuracy, consistent performance and stable temperature. However, it is found that the heater required about 30 minute to achieve designated temperatures from ambient temperature (Figure 5), thus two set of heaters are used, one is for evaporation process (Heater A) and the other is for the fluorination process (Heater B). Nevertheless the results showed that the temperature has to be set higher than the required temperature, as a result of heat loss. Therefore, through a series of try and errors, a balance of temperature set and actual temperature of the heating medium is obtained (Figure 6). The calibration curve is then tested and the results are showed in Table 1.

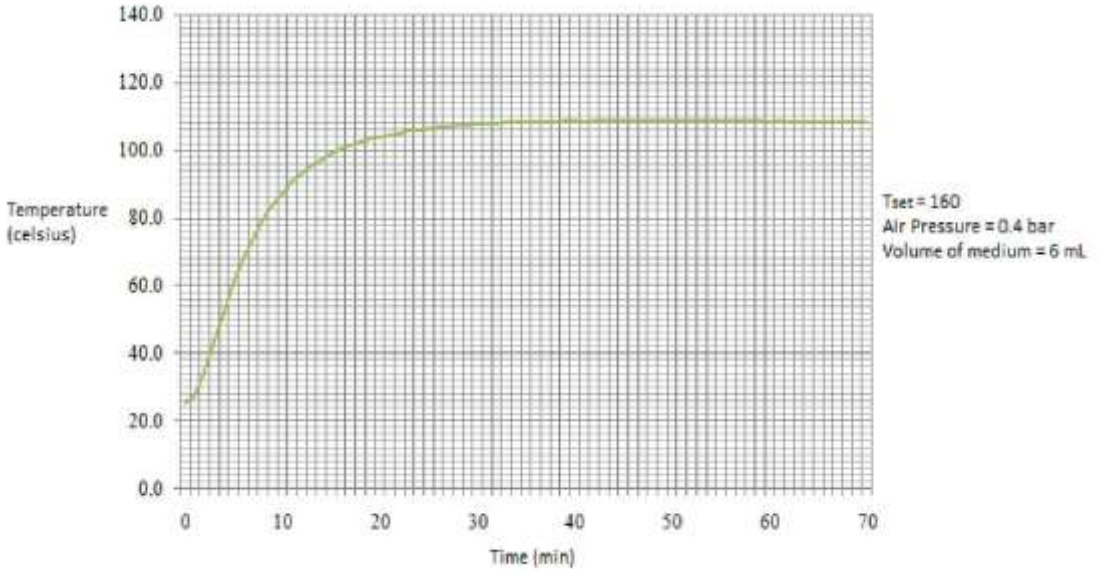


Figure 5. Temperature profile of heating system of the synthesizer.

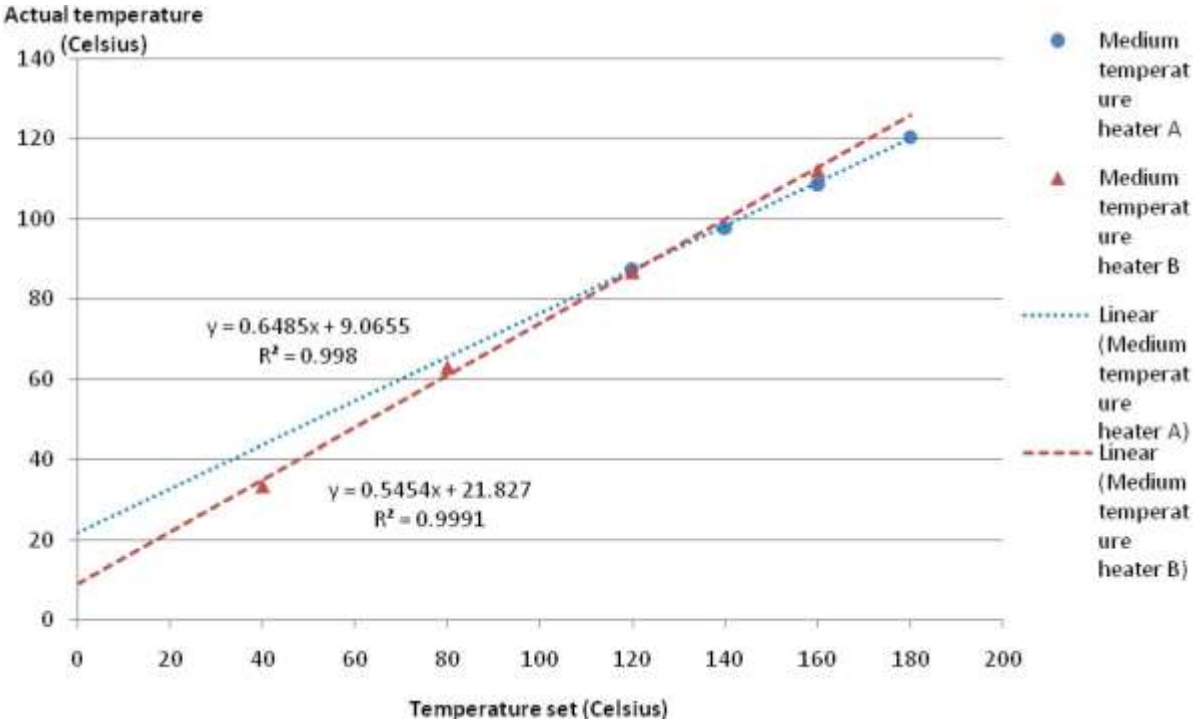


Figure 6. Calibration of heating system of the synthesizer.

Table 1. Validation of the heating system based on value calculated from calibration

$T_{require}$	T_{set}	$T_{measure}$
	(°C)	
110	161[a]	111.342 ± 0.149
90	125	90.630 ± 0.333
80	109	80.822 ± 0.263
70	94	72.498 ± 0.272

a - Data obtained from heater A. The rest are performed on heater B. b - Measurements were made from 30^{th} to 90^{th} min after the heater is on.

The trapped [^{18}F]-fluoride was eluted from the cartridge by using 1 mL K_2CO_3 solution (33 mM) at B1 vial into the reaction vessel. Then 1 mL acetonitrile was added inside the vessel and the mixture was heated at 80-90 °C until dried. The process is repeated three times to ensure no residual water was left for the next process. Primary precursor (CH2Br2) was added to reaction vessel and allowed to react for 6 minute at temperature of 80-90 °C.

The labelled intermediate was then transferred to triple Sep-Pak silica cartridges for separation of labelled and unlabelled compounds. The labelled compound were directed to tC18 cartridge containing 400 pL DMAE and the product is trapped at Sep-Pak CM cartridge by 10 mL ethanol elution. The cartridges were rinsed with 15 mL purified water. Final step is involving the elution of the product into the product vial by 3 mL 0.85 saline solutions. Data of the synthesis is shown in Table 2.

The resulted radiochemical was disappointingly low. The highest yield achieved so far is only 1.446 %. In contrast to Kyrza et al. (2008), they reported non-corrected yield of 15-25 %. Further study is required to find solution for the low yield. Since manipulating the amount of precursor and solvent, reaction temperature and time, as well as evaporation temperature and time did not give significant increase in radiochemical yield, we suspect the problem lie in QMA carbonate cartridge that we used, compare to ordinary QMA cartridge used by other groups. However, further study proved that was not the case as more than 80% of the activities were collected at the reaction vessel and less than 0.2 % was trapped at the cartridge after elution (Table 3). Another study showed that there is still a significant activity left at the reaction vessel, 78.923 % after a complete synthesis was done (Table 4). The result suggested that the low yield was probably contributed by incomplete reaction between [^{18}F]-fluoride and the precursor. Currently we are still trying to overcome this problem and the result will be reported elsewhere.

Table 2. Effect of quantity of precursor, reaction temperature, evaporation time on radiochemical yield of [^{18}F]FCH

Quantity of CH_2Br_2 (P^L)	Reaction Temperature (°C)	Evaporation Temperature (°C)	Evaporation time (min)	Corrected Yield (%)
300	80	90	10x3 (+ 15 mg K222)	0.089
700	80	90	10x3 (+ 15 mg K222)	0.193
700	80	90	15x3 (+ 15 mg K222)	0.100
700	80	80	15x3 (+ 15 mg K222)	0.291
700	90	80	15x3 (+ 15 mg K222)	0.334
100/1 mL asetonitrile	90	80	15x3 (+ 15 mg K222)	0.024
200/0.5 mL asetonitrile	90	80	15x3 (+ 15 mg K222)	0.206
1000	90	80	15x3 (+ 15 mg K222)	1.446

Table 3. Activity distribution of [^{18}F]F vial, Sep-Pak light QMA Carbonate cartridge, collection vial and reaction vessel after elution.

Component	**Radioactivity (decay corrected), mCi**		
	Sample 1	Sample 2	Sample 3
[^{18}F]F vial before elution	7.610	5.550	7.080
[^{18}F]F vial after elution	0.154	0.030	0.076
Cartridge after elution	0.008	0.004	0.007
Collection vial	0.033	0.023	0.025

Reaction vessel	6.750	5.000	5.890

The product was analyzed using HPLC system (column C18 4.6 x 150 mm, acetonitrile:ammonium formiate 0.1 mol/L at 79:2). From the radiochromatograph, it is clear that the radiochemical purity of the [F-18]-fluorocholine produced by using our synthesizer is high; >99% (Figure 7). No other peaks were found. Retention time is 4.32 min. When we injected a pure [^{18}F]-fluoride into the HPLC system, we find a good separation between [F-18]-fluoride and [^{18}F]-fluorocholine, with retention time = 1.56 min.

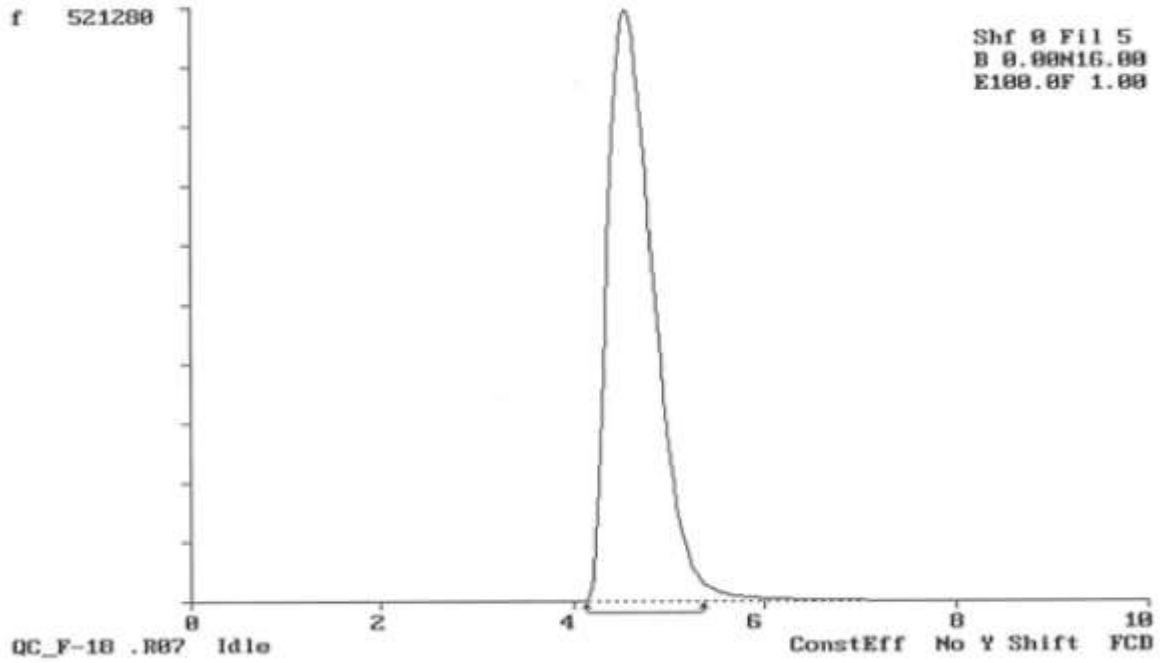


Figure 7. Radiochromatogram of the the [^{18}F]FCH synthesized.

CONCLUSION

We have managed to develop a lab-scale [^{18}F]FCH synthesizer and are able to achieve decay- corrected radiochemical yield of 1.45% under 100 min. Optimization of the radiochemical yield is still underway.

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