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Development of microwave-based automated nucleophilic [^{18}F]fluorination system and its application to the production of [^{18}F]flumazenil

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Abstract

Introduction: This study presents the development of an automated radiosynthesis system integrating a microwave reactor and its subsequent application in the synthesis of [^{18}F]flumazenil, a potentially useful compound in the evaluation of central benzodiazepine receptor density.
Methods: Preparation of dry $[K/K_{222}]^{+18}F^-$ complex and radiofluorination of the nitro-flumazenil precursor were achieved using the developed microwave-based radiosynthesis system. The crude product was prepurified in a C18 cartridge followed by reversed-phase preparative high-performance liquid chromatography. The isolated [^{18}F]flumazenil was evaporated in vacuo and reconstituted in an ethanol-free solution.
Results: Optimum incorporation of $^{18}F^-$ in the nitro-precursor was achieved in 5 min time utilizing 2 mg of precursor in *N*,*N*-dimethylformamide reacted at 160°C which gave an incorporation yield of 40±5%. The radiochemical yield obtained at the end of synthesis was 26±4% (EOB) with a radiochemical purity of >99% and a total synthesis time of about 55–60 min. The produced [^{18}F]flumazenil was observed to be stable for at least 8 h.
Conclusion: The developed [^{18}F]flumazenil radiosynthesis system offers shorter reaction time, simplicity in operation and applicability for use in routine clinical practice.

Keywords: [^{18}F]flumazenil; Microwave; Radiofluorination; Central benzodiazepine receptors

1. Introduction

One of the important aspects in positron emission tomography (PET) research is to label the compound of interest in the least possible time. Previous reports have shown that the utilization of microwaves poses the advantage over conventional heating of performing the labeling reaction in a shorter time [1–4]. The increase in reaction rate could be attributed to the ability of the microwave to couple directly with the sample mixture possessing polar properties [5]. Furthermore, the radiochemical yield using the microwave method has been reported to be similar to or higher than that of the conventional method in the synthesis of 2-[^{18}F]fluoro-2-deoxyglucose [4] and ^{18}F-radiofluorination of selected aromatic and aliphatic model compounds [1–3]. However, the complexity of the microwave system hinders the development of an automated radiosynthesis system, and only results obtained by manual operation have been reported. In this study, we developed an automatic system integrating a single-mode microwave reactor, which provides practicable routine production and less radiation exposure to the operator, and optimized it for the synthesis of [^{18}F]flumazenil as well as formulation of the ethanol-free injectable solution.

Radiolabeled flumazenil is an important radiopharmaceutical for the assessment of the central benzodiazepine

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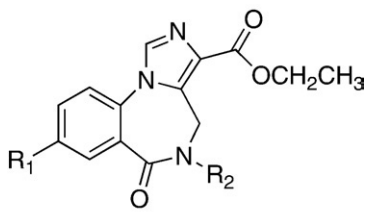
receptor (cBZR) concentration [6]. Its carbon-11-labeled analog ([^{11}C]flumazenil, Fig. 1, I) has been well studied for cBZR evaluation in epilepsy [7], panic disorder [8], Huntington's disease [9] and identification of irreversibly damaged tissue after acute stroke [10,11] using PET imaging. However, due to the short half-life of carbon-11 (20.4 min), usage of this radiopharmaceutical is limited to cyclotron-equipped centers. On the other hand, the fluorine-18 analog, [^{18}F]flumazenil (Fig. 1, II), presents an advantage with a longer half-life of 109.8 min. Moreover, a PET study conducted on the brain of a cynomolgus monkey showed similar uptake patterns of [^{18}F]flumazenil and [^{11}C]flumazenil [12]. Also, [^{18}F]flumazenil in human brain showed more stable binding potential values and a less noisy image than [^{11}C]flumazenil [13], thus substantiating the potential of [^{18}F]flumazenil as a substitute to the widely used [^{11}C]flumazenil.

Earlier reports on [^{18}F]flumazenil synthesis were conducted via isotopic exchange of ^{19}F for ^{18}F using flumazenil (Fig. 1, III) as the precursor in a synthesis time of 45 min [14,15]. However, in isotopic exchange, specific activity could be low due to the presence of a carrier (the precursor flumazenil). Hence, further development on [^{18}F]flumazenil synthesis was reported by the same research group wherein the utilization of nitro-flumazenil (Fig. 1, IV) as a precursor resulted in high specific activity which is essential in brain receptor imaging studies. However, a longer synthesis time of about 75 min was reported [12].

This study presents an advancement in the radiosynthesis of [^{18}F]flumazenil with the aim of improving the radiolabeling process via microwave dielectric heating. Then, using the opportunity of the shorter synthesis process, we added the solvent exchange system to obtain ethanol-free [^{18}F] flumazenil injectable solution. Ethanol is a common additive to enhance the solubility of lipophilic drugs such as flumazenil, but it can cause an anaphylactoid reaction with rather high incidence, especially in Asian races. Finally, a small animal PET study in rats was performed to confirm the availability of the new formula of [^{18}F]flumazenil.



I R_1=F R_2=$^{11}CH_3$
II R_1=^{18}F R_2=CH_3
III R_1=F R_2=CH_3
IV R_1=NO_2 R_2=CH_3

Fig. 1. Basic structure of flumazenil and its analogs.

2. Materials and methods

2.1. Reagents

All reagents utilized were of at least analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Anhydrous *N*,*N*-dimethylformamide (DMF) was obtained from Kanto Chemical (Tokyo, Japan) and Aldrich (St. Louis, MO, USA). The nitro-flumazenil precursor (ethyl5,6-dihydro-5-methyl-8-nitro-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate) and standard flumazenil (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a] [1,4]benzo-diazepine-3-carboxylate) were purchased from Pharmasynth AS (Tartu, Estonia) and utilized as supplied. For the animal experiments, flumazenil obtained from Sigma (St. Louis, MO, USA) was used. Dimethyldichlorosilane (DMDCS) was purchased from WAKO Pure Chemical Industries (Osaka, Japan), and high-performance liquid chromatography (HPLC)-grade solvents were from Kanto Chemical.

2.2. Microwave reactor system

The radiosynthesis was performed using a CEM Discover (Matthews, NC, USA) single-mode microwave reactor system. This system is capable of continuous microwave power delivery with selectable power output (0–300 W) and a programmable temperature from 25°C to 250°C. It is equipped with an infrared temperature control system located below the microwave cavity floor which monitors and controls the temperature conditions of the reaction vessel. The temperature sensor is used in a feedback loop with the magnetron to regulate the power output which maintains the temperature set-point. The 10-ml-thick walled (length=9 cm) Pyrex reaction vessel supplied with the instrument was utilized for the radiolabeling reactions. The reaction vessel was precoated prior to its use with DMDCS, an alkyl-substituted silicon tetrahydride reagent, to minimize the product adsorption on the inside wall.

The reaction vessel cap was modified to automate the radiosynthesis process. It was especially fabricated using polyetheretherketone (PEEK) polymer with an aluminum base having an access port of 1.65 cm which can exactly hold the mouth of the 10-ml reaction vessel. The PEEK reaction vessel cap can be tightly screwed to the aluminum base, and by using a Teflon spacer disc, it can be completely sealed, making pressurized reactions achievable. The PEEK reaction vessel cap has access ports for inserting PEEK or Teflon tubings of 1/16″ outer diameter which can be utilized for transfer of reagents in and out of the reaction vessel and also serve as inlet/outlet for Argon (Ar) gas and vacuum. To monitor the pressure inside the reaction vessel, a pressure gauge was connected. Further developments were made by attaching a series of six-way and three-way valves to the system, and with the aid of a Program Logic Controller (PLC, Mitsubishi, Tokyo, Japan) and the Wizcon software (version 7.61, Elutions, France), radiosynthesis could be

controlled using a computer (Fig. 2). The developed system is able to perform open or closed system reactions and is capable of stop and flow operation that facilitates the transfer of reagents in/out of the reaction vessel and the end-product for purification.

2.3. Radiosynthesis

No carrier added $^{18}F^-$ was produced via $^{18}O(p,n)^{18}F$ reaction using the RDS eclipse RD/HP cyclotron (Siemens/CTI, Knoxville, TN, USA). The $^{18}F^-$ was first trapped into a SepPak Light QMA cartridge (WATERS, Milford, MA, USA; preconditioned with 1 ml of 0.2 M K_2CO_3 and 10 ml of water) followed by elution with K_2CO_3/K_{222} solution in MeCN/H_2O (96:4). The K_2CO_3/K_{222} solution utilized was based on a previous study [16] with a slight modification wherein only 1 ml of the recommended solution was used. The eluted $[K/K_{222}]^{+18}F^-$ complex was then dried at a temperature of 110°C with an average microwave power of about 150 W under vacuum and Ar flow (20 ml/min). The dried complex was used either directly or azeotropic drying was conducted by adding 1 ml of anhydrous acetonitrile (3×).

Nitro-flumazenil precursor (1–8 mg) dissolved in anhydrous solvent [acetonitrile, DMF or dimethyl sulfoxide (DMSO); (0.5–2.0 ml)] was added to the dried $[K/K_{222}]^{+18}F^-$ complex, and experiments were conducted (under closed system reaction) to optimize different variables including the solvent system, labeling temperature, reaction time, nitro-precursor amount and reaction vessel pressure. The crude product was then cooled using compressed air (option provided in the microwave system) followed by dilution with 4.5 ml water and adsorption on a SepPak Light C18 cartridge (WATERS). During optimization of the radiofluorination, samples from the crude product were measured for incorporation yield using radioTLC, and the result obtained was corrected based on the percent distribution of the radioactivity obtained in the reaction mixture and in the reaction vial. After washing the SepPak Light C18 cartridge with 5 ml water, the trapped [^{18}F] flumazenil was then eluted with 0.7 ml of acetonitrile/water (7:3) and diluted with 0.7 ml of water prior to purification in the HPLC system.

2.4. HPLC purification and reformulation of end-product

The HPLC system utilized for purification consisted of a Shimadzu LC-10AD pump (Kyoto, Japan), Shimadzu SPD-10AVP UV detector and Ludlum 44-10 NaI(Tl) radioactive detector (Ludlum Measurements, Sweetwater,

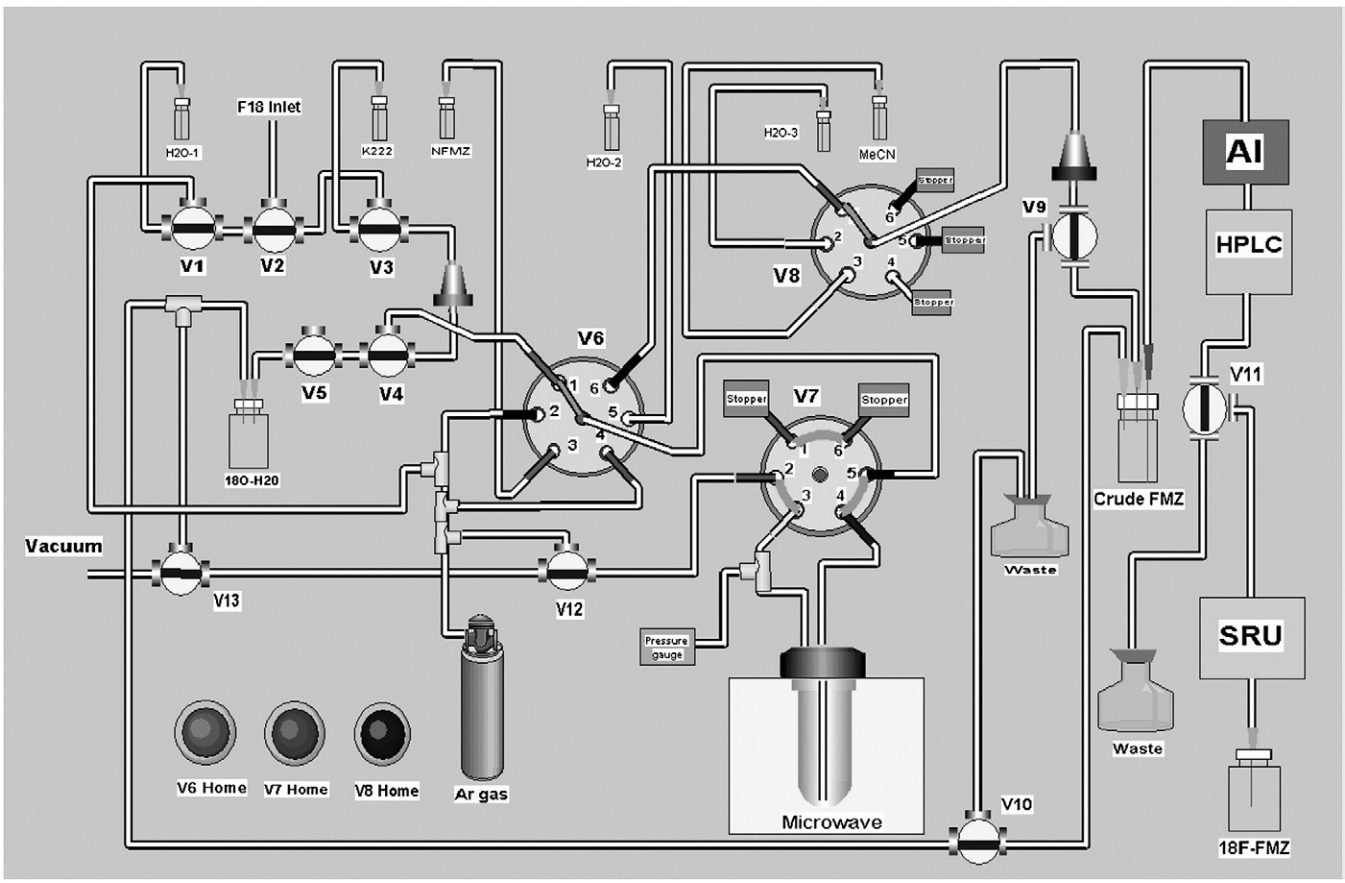


Fig. 2. Control panel screen of the developed radiosynthesis system (AI, automatic injector; SRU, solvent replacement unit).

TX, USA). Purification was achieved using μBondapak C18 (7.8×300 mm) column (WATERS) with acetonitrile/water (25:75) as mobile phase at a flow rate of 3 ml/min. The isolated [^{18}F]flumazenil was then directly transferred to a solvent replacement unit (JFE Holdings, Tokyo, Japan) wherein removal of the solvent system was performed in vacuo. The residue was then redissolved in 0.01% Tween 80 in normal saline solution and passed through a sterile filter (Millipore-Millex LG, Billerica, MA, USA).

2.5. End-product evaluation

The radiochemical purity and stability of [^{18}F]flumazenil were determined by radioTLC performed using RP_{18} F_{254} TLC plate (Merck and Co., Whitehouse Station, NJ, USA) with ethyl acetate/ethanol (95:5) as mobile phase. Utilizing the AR2000 TLC scanner (Bioscan, Washington, DC, USA), the R_f value of [^{18}F]flumazenil was determined at around 0.60 and confirmed by coelution with standard flumazenil (visualized under ultraviolet light). Specific activity and radiochemical purity were determined by analytical HPLC equipped with a Shimadzu LC-20AD pump, Shimadzu SPD-20A UV spectrophotometer and Ludlum 44-10 NaI(Tl) radioactive detector and using a Cosmosil $5C_{18}$-MS-II (4.6×150 mm, Nacalai Tesque, Kyoto, Japan) column with acetonitrile/water (3:7) as mobile phase at a flow rate of 1 ml/min and a column oven temperature of 27°C. The [^{18}F] flumazenil peak detected at 4.70 min was verified by coinjection with standard flumazenil.

2.6. Small animal PET imaging

All animal studies were conducted in observance of the Guidelines of the Institutional Animal Care and Use Committee of the Faculty of Medical Sciences at the University of Fukui, Fukui, Japan, which conforms to the

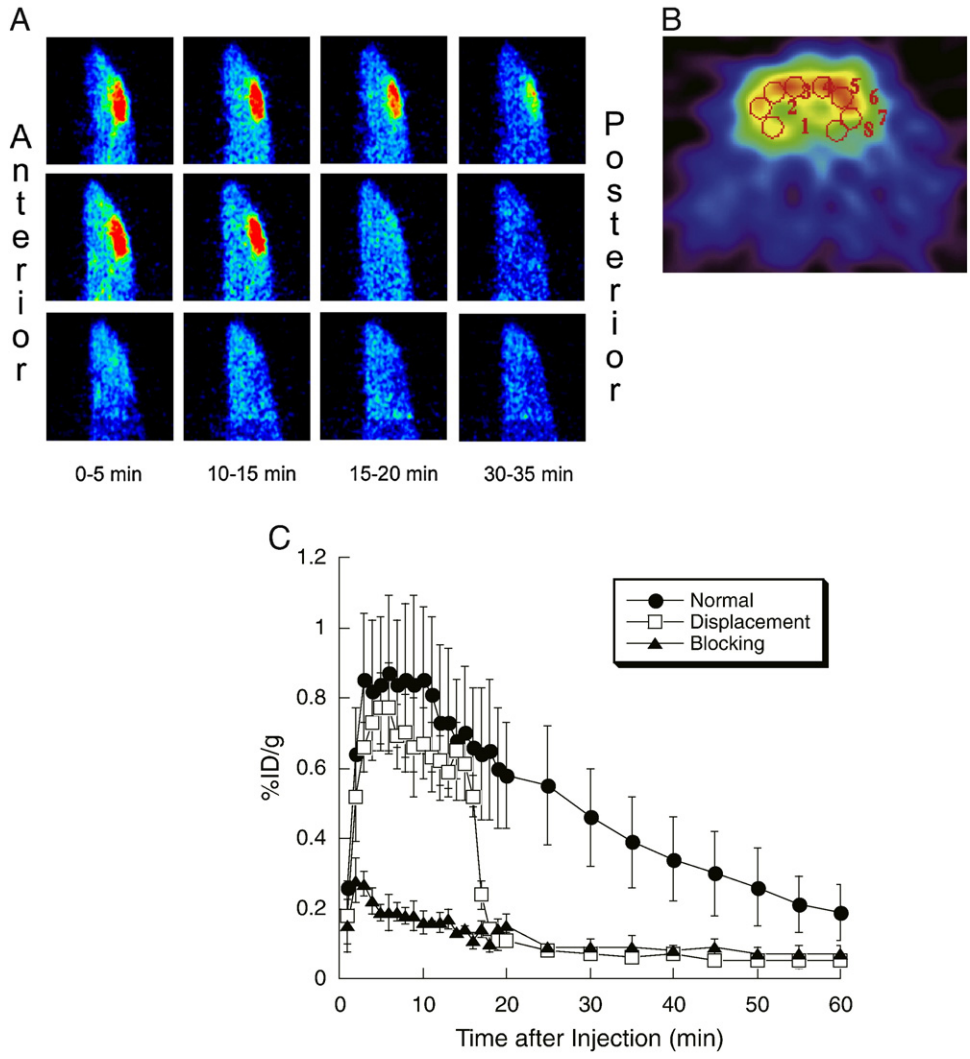


Fig. 3. (A) Representative PET images of a rat from each study at 0–5, 10–15,15–20 and 30–35 min acquisition time (top: normal study, middle: displacement study, bottom: blocking study). (B) Representative circular ROIs drawn on rat brain cortex image. (C) Time course of radioactivity in the rat brain (n=3 per study) after injection of 18.5 MBq [^{18}F]flumazenil. In the blocking and displacement experiments, 100 μg of cold flumazenil was injected 15 min before and after the injection of [^{18}F]flumazenil, respectively. Anesthesia was maintained by intermittent iv injection of chloral hydrate.

Guide for the Care and Use of Laboratory Animals (US National Institute of Health Publication No. 85-23, revised in 1996). The male Sprague–Dawley (SD) rats (9–10 weeks old, 300–360 g) were obtained from Japan Slc (Shizuoka, Japan).

PET brain imaging on SD rats (n=3 per study) was performed using a dedicated small animal PET scanner (Hamamatsu SHR-41000). The scanner has a transaxial resolution of 2.0 mm FWHM in the center of the field of view and an axial resolution of about 2.8 mm FWHM on the axis of the ring [17]. The SD rats (300–360 g) were first anesthetized by intraperitoneal injection of chloral hydrate (400 mg/kg body weight) and then maintained by intermittent intravenous (iv) injection. Transmission scan was then acquired for 60 min with an axial field of view of 100 mm covering the head and proximal part of the trunk. This was followed by iv bolus injection of about 18.5 MBq of [^{18}F]flumazenil. Dynamic emission scan was then acquired for 60 min and the coincidence data were stored in list mode. The list mode data were edited to a 3D data set and then converted to 213 slices of 2D sinograms by Fourier rebinning algorithm. Reconstruction of 2D sinograms was performed using a standard 2D filtered back projection algorithm at selected time frames. The blocking or displacement studies were performed by iv injection of 100 μg of cold flumazenil 15 min before or after [^{18}F]flumazenil administration, respectively.

Circular regions of interest (ROIs) were drawn on the rat brain cortex (Fig. 3B) for all the time frames and their mean counts were obtained. The counts per pixel per minute from the ROIs were converted to tracer activity (Bq/ml) using a cross-calibration factor obtained by scanning a cylindrical phantom with known ^{18}F activity concentration. Assuming a tissue density of 1 g/ml, tracer activity was divided by the injected dose (Bq) to obtain an image ROI-derived tissue uptake index in % ID/g.

3. Results and discussion

3.1. Radiosynthesis with microwave reactor system

The utilization of microwave for radiopharmaceutical synthesis is one of the techniques that can be used to reduce radiolabeling time. However, to date, a radiosynthesis system that integrates microwave dielectric heating is still lacking for widespread use. In this study, a radiosynthesis system incorporating a microwave reactor was developed and employed for the synthesis of [^{18}F]flumazenil. The developed system can be remotely operated which then minimizes the operator's exposure to radiation, simplifies the radiopharmaceutical production and facilitate the optimization of the radiosynthesis procedure in a closed environment. The microwave reactor setup was initially utilized for the preparation of dry $[K/K_{222}]^{+}{}^{18}F^{-}$ complex. One milliliter of the prepared K_2CO_3/K_{222} solution was enough to elute the $[K/K_{222}]^{+}{}^{18}F^{-}$ complex from the SepPak Light QMA cartridge with >96% elution yield. Dry $[K/K_{222}]^{+}{}^{18}F^{-}$ complex was obtained at a temperature of 110°C with an average microwave power of 150 W for a period of 110 s. Moreover, no significant difference in [^{18}F]flumazenil yield was observed for runs conducted with or without additional azeotropic drying (Table 1, Series 6 and 7) which is in accordance with the previous report using other model compounds [16]. Dry $[K/K_{222}]^{+}{}^{18}F^{-}$ complex could thus be prepared in less than 2 min.

In order to optimize the $^{18}F^{-}$ incorporation rate into the nitro-flumazenil precursor, the solvent system, nitro-

Table 1
Summary of [^{18}F]flumazenil labeling parameters optimization

Series no.	NFMZ (mg)	Molar ratio NFMZ/ $[K_{222}/K_2CO_3]$	Reaction temperature (°C)	Solvent	Pressure (kPa)/ time (min)	No. of runs	%[^{18}F]FMZ yield (RTLC)
1	2	0.5:1	90	Acetonitrile	0/10	1	<1
2	2	0.5:1	160	Acetonitrile	0/10	1	8
3	5	1.25:1	160	Acetonitrile	0/10	1	9
4	5	1.25:1	200	DMSO	0/10	1	27
5	2	0.5:1	160	DMSO	0/10	2	25±1
6	2	0.5:1	160	DMF	0/10	3	31±4[a]
7	2	0.5:1	160	DMF	0/10	7	30±6
8	1	0.25:1	160	DMF	0/10	1	<3
9	4	1:1	160	DMF	0/10	3	27±6
10	5	1.25:1	160	DMF	0/10	3	30±8
11	8	2:1	160	DMF	0/10	3	29±9
12	2	0.5:1	140	DMF	0/10	3	15±2
13	2	0.5:1	180	DMF	0/10	3	21±4
14	2	0.5:1	200	DMF	0/10	3	14±6
15	2	0.5:1	160	DMF	100/10	1	39
16	2	0.5:1	160	DMF	100/5	3	33±3
17	2	0.5:1	160	DMF	200/5	7	40±5
18	2	0.5:1	160	DMF	200/2	3	21±2
19	2	0.5:1	160	DMF	200/10	3	34±6

[a] With additional azeotropic drying.

flumazenil precursor amount, labeling temperature, labeling time and reaction vessel pressure were varied (Table 1). For solvent system optimization, three commonly used solvent systems for radiolabeling reaction were tested, that is, acetonitrile, DMF and DMSO. These solvents have medium to high dielectric loss and hence are likely to couple efficiently with the microwave energy leading to rapid heating [5]. Radiolabeling with acetonitrile resulted in a poor incorporation yield while DMSO resulted in a better yield than acetonitrile but was slightly less compared to DMF at equivalent conditions (Table 1, Series 1–7). Furthermore, the reaction with DMSO showed several ^{18}F-labeled by-products besides [^{18}F]flumazenil as determined by the HPLC of the crude reaction mixture while the DMF reaction gave only one minor ^{18}F-labeled by-product besides [^{18}F]flumazenil. DMF was then determined as the most appropriate solvent due to its higher yield and lesser ^{18}F-labeled by-product.

The incorporation yield by varying the amount of nitro-flumazenil keeping the K_2CO_3/K_{222} constant showed no significant increase in incorporation yield from 2 mg of nitro-flumazenil up to 8 mg, while 1 mg of nitro-flumazenil (nitro-flumazenil/[K_2CO_3/K_{222}] ratio of 0.25:1) gave less than 3% yield (Table 1, Series 7–11) which was perhaps due to the degradation of the produced [^{18}F]flumazenil in a more basic environment [14,15]. In this study, a nitro-flumazenil/[K_2CO_3/K_{222}] ratio of 0.5:1 was enough to obtain the optimum yield as compared to the 1:1 ratio recommended in the previous study [12]. This result shows that a minimal amount of nitro-flumazenil precursor was required for the radiosynthesis, thereby saving on the production cost and facilitating the purification process (Table 2). The reduction in the amount of precursor is one of the special features observed in microwave reactions [3].

Utilizing DMF as a solvent, the optimum labeling temperature was determined to be at 160°C with an average microwave power of around 30 W (Table 1, Series 7, 12–14). The lower yield observed at higher temperatures may be attributed to degradation of [^{18}F]flumazenil [15]. Furthermore, a better yield with a shorter reaction time (5 min) was observed as a result of pressurizing the reaction vessel with 200 kPa of Ar gas prior to labeling (Table 1, Series 7, 15–19). Utilizing 2 mg of precursor in DMF reacted at 160°C (with 200 kPa reaction vessel pressure) and employing an average microwave power of 30 W gave an incorporation yield of 40±5% in 5 min time.

Table 2
Comparison of [^{18}F]flumazenil radiosynthesis parameters and end-product specification obtained in this study with those in the literature

Properties	Conductive heating		Microwave
	Ref. [12]	Ref. [15]	This study
Precursor	Nitro-flumazenil	Flumazenil	Nitro-flumazenil
Precursor amount (mg)	8	2	2
$[K/K_{222}]^{+}{}^{18}F^{-}$ complex drying time (min)	–	–	2
Radiofluorination time (min)	30	15	5
HPLC purification time (min)	22–24	–	17–19
RCY (EOB)	30%	47%	26±4%
RCY (EOS)	19%		18±3%
RCP	–	>97%	>99%
Specific activity (GBq/μmol)	185	0.37	295±54
Total synthesis time (min)	75–80	45	55–60

The crude product was then diluted with water and pre-purified in a SepPak Light C18 cartridge to remove the DMF solvent prior to HPLC purification. [^{18}F]Flumazenil was then isolated between 17 and 19 min. Reformulation of the final product in ethanol/saline solution can be achieved with the SepPak C_{18} cartridge, which is convenient and requires less time. However, this might present untoward effects on patients with alcohol intolerance, so the isolated [^{18}F]flumazenil was subjected to evaporation in order to remove the HPLC solvent and was then redissolved in 0.01% Tween 80, a surfactant approved for use in parenteral products [18], in normal saline solution. The radiochemical yield obtained at the end of synthesis was 26±4% (EOB) with a radiochemical purity of >99% and a total synthesis time of about 55–60 min (Table 2). The specific activity was recorded at around 295 GBq/μmol (EOS). Furthermore, the obtained [^{18}F]flumazenil was observed to be stable for at least 8 h.

The developed radiosynthesis system which uses microwave dielectric heating is robust, flexible and was easily manipulated to produce [^{18}F]flumazenil. In Table 2, the results of [^{18}F]flumazenil radiosynthesis obtained in this study based on microwave dielectric heating are compared to the results of previously reported syntheses based on conductive heating. With the microwave heating system, the total synthesis time including improved injectable formulation was shortened to 55–60 min as compared to the conductive heating system which took 75–80 min using the nitro-flumazenil as the precursor. The nitro-group to ^{18}F exchange reaction increased the specific activity to about 185 GBq/μmol [12] in contrast to 0.37 GBq/μmol reported for the isotopic exchange reaction [15]. In this study, it was noted from the HPLC chromatogram of the [^{18}F]flumazenil product (data not shown) that the cold flumazenil which was synthesized by F-19 from Teflon tubes and cyclotron lines, and/or nonlabeled organic impurity which corresponds to the UV absorption of flumazenil tend to be lower with smaller amounts of the nitro-precursor. Further tests will however be conducted to clarify this observation. The conditions involved in this radiosynthesis (taking care to reduce possible contamination with fluoride) along with the minimal amount of nitro-precursor gave a calculated high specific activity of 295 GBq/μmol. Although the radiochemical yield at EOB was observed to be slightly low, this was compensated for by the shorter production time leading to a comparable radiochemical yield at EOS. Using the developed radiosynthesis system that incorporates a microwave reactor, the [^{18}F]flumazenil can thus be produced at a

shorter time, with reproducible yield, higher specific activity and at a sufficient amount for use in PET receptor imaging studies. Furthermore, it is simple to operate and hence was easily modified for radiofluorination of other model compounds (to be published elsewhere).

3.2. Small animal PET imaging

Using dynamic PET imaging, the initial high uptake and rapid decline in retention of [^{18}F]flumazenil in the brain was clearly visualized (Fig. 3C). The time course of radioactivity is similar to the results of [^{3}H]flumazenil in vivo binding in the mouse brain [19]. After injection of [^{18}F]flumazenil, there was a rapid and high level of accumulation of radioactivity in the brains of normal rats. The high uptake in the cortical region was observed at 3–15 min after injection followed by quick elimination over 60 min (Fig. 3C). Fig. 3A shows representative PET images of rats. Pretreatment with cold flumazenil suppressed the accumulation of [^{18}F]flumazenil by around 75% (at 20 min acquisition time) as compared with normal rat study. Furthermore, in the displacement study, a marked decrease in brain radioactivity of about 80% (at 20 min acquisition time) as compared to the normal rat study was observed 5 min after injection of the cold flumazenil. The in vivo blocking and displacement studies performed further confirmed the specific binding of [^{18}F]flumazenil. Gathering these data, similar bioavailability and characteristics of the ethanol-free [^{18}F]flumazenil injectable solution with those of radiolabeled flumazenil could be visualized.

4. Conclusion

Utilizing the developed radiosynthesis system, [^{18}F] flumazenil was produced with ease in a minimal time and with higher specific activity. The capability of the ethanol-free [^{18}F]flumazenil injectable solution to clearly visualize the cBZR distribution in rodents makes it a potential radiopharmaceutical for molecular imaging experiments. Several other evaluations are being conducted in order to fully establish the synthesized [^{18}F]flumazenil's suitability for clinical application.

The system presented is a first online microwave-based radiosynthesis system which, in contrast to a batch system, minimizes possible contamination from outside environment and facilitates transfer of reagent/product. Furthermore, the system is capable of carrying out reactions in a completely sealed condition, which then enables it to perform chemical reactions requiring high pressure. The developed system also offers simplicity in operation and applicability for use in radiopharmaceutical production.

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References

[1] Stone-Elander S, Elander N. Fast chemistry in microwave fields: nucleophilic ^{18}F-radiofluorinations of aromatic molecules. Appl Radiat Isot 1993;44(5):889–93.
[2] Elander N, Jones JR, Lu SY, Stone-Elander S. Microwave-enhanced radiochemistry. Chem Soc Rev 2000;29:239–49.
[3] Stone-Elander S, Elander N. Microwave applications in radiolabelling with short-lived positron emitting radionuclides. J Label Compd Radiopharm 2002;45:715–46.
[4] Taylor MD, Roberts AD, Nickles RJ. Improving the yield of 2-[^{18}F] fluoro-2-deoxyglucose using a microwave cavity. Nucl Med Biol 1996;23:605–9.
[5] Hayes BL. Microwave synthesis (chemistry at the speed of light). North Carolina: CEM publishing; 2002.
[6] Pike VW, Halldin C, Crouzel C, Barré L, Nutt DJ, Osman S, et al. Radioligands for PET studies of central benzodiazepine receptors and PK (peripheral benzodiazepine) binding sites — current status. Nucl Med Biol 1993;20:503–25.
[7] Ryvlin P, Bouvard S, Le Bars D, De Lamérie G, Grégoire C, Kahane P, et al. Clinical utility of flumazenil-PET versus [^{18}F]fluorodeoxyglucose-PET and MRI in refractory partial epilepsy. Brain 1998;121: 2067–81.
[8] Malizia AL, Cunningham VJ, Bell CJ, Liddle PF, Jones T, Nutt DJ. Decreased brain $GABA_A$-benzodiazepine receptor binding in panic disorder. Arch Gen Psychiatry 1998;55:715–20.
[9] Holthoff VA, Koeppe RA, Frey KA, Penney JB, Markel DS, Kuhl DE, et al. Positron emission tomography measures of benzodiazepine receptors in Huntington's disease. Ann Neurol 1993;34(1):76–81.
[10] Heiss WD, Grond M, Thiel A, Ghaemi M, Sobesky J, Rudolf J, et al. Permanent cortical damage detected by flumazenil positron emission tomography in acute stroke. Stroke 1998;29(2):454–61.
[11] Heiss WD, Kracht L, Grond M, Rudolf J, Bauer B, Wienhard K, et al. Early [^{11}C]flumazenil/H2O positron emission tomography predicts irreversible ischemic cortical damage in stroke patients receiving acute thrombolytic therapy. Stroke 2000;31(2):366–9.
[12] Ryzhikov NN, Seneca N, Krasikova RN, Gomzina NA, Shchukin E, Fedorova OS, et al. Preparation of highly specific radioactivity [^{18}F]flumazenil and its evaluation in cynomolgus monkey by positron emission tomography. Nucl Med Biol 2005;32:109–16.
[13] Odano I, Farde L, Karlsson P, Varrone A, Krasikova R, Airaksinen A, et al. [^{18}F]Flumazenil for central benzodiazepine receptor imaging and its comparison with [^{11}C] in human brain. J Nucl Med 2007;48:61P.
[14] Krasikova RN, Ryzhikov NN, Gomzina NA, Vassiliev DA, Kostikov AP, Fedorova OS. Isotopic ^{18}F/^{19}F exchange in the flumazenil molecule using K^{18}F/Kryptofix complex. J Label Compd Radiopharm 2003;46:S213.
[15] Ryzhikov NN, Gomzina NA, Fedorova OS, Vasil'ev DA, Kostikov AP, Krasikova RN. Preparation of [^{18}F]flumazenil, a potential radioligand for PET imaging of central benzodiazepine receptors by isotope exchange. Radiochemistry 2004;46(3):290–4.
[16] Gomzina NA, Zaitsev VV, Krasikova RN. Optimization of nucleophilic fluorination step in the synthesis of various compounds labeled with fluorine-18 for their use as PET radiotracers. J Label Compd Radiopharm 2001;44:S895–7.
[17] Yamada R, Watanabe M, Omura T, Sato N, Shimizu K, Takahashi M, et al. Development of a small animal PET scanner using DOI detectors. IEEE Trans Nucl Sci 2008;55(3):906–11.
[18] Liu R. Water-insoluble drug formulation. 2nd ed. Florida: CRC Press; 2008.
[19] Goeders NE, Kuhar MJ. Benzodiazepine receptor binding in vivo with [^{3}H]-Ro 15-1788. Life Sci 1985;37:345–55.