UTILIZATION OF 30 MEV DAE MEDICAL CYCLOTRON FOR PRODUCTION OF MEDICALLY USEFUL RADIOISOTOPES AND CORRESPONDING RADIOPHARMACEUTICALS

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Abstract

Cyclotrons are extensively used to produce radioisotopes for diagnostic and therapeutic use for cancer care. In India, the IBA Cyclone-30, 30MeV, 350μA proton cyclotron has been commissioned and made operational in September, 2018 for the production of radioisotopes/radiopharmaceuticals for medical application. The cyclotron has five beamlines, out of which three beamlines are dedicated for the production of radioisotopes for medical use. This cyclotron has the potential to produce SPECT (Single-Photon Emission Computed Tomography) Isotopes (^{67}Ga, ^{111}In, ^{123}I, ^{201}Tl etc.), PET (Positron Emission Tomography) isotopes (F-18, Ge-68/Ga-68 generator for in situ production of Ga-68, Ga-68, Cu-64, Zr-89, I-124 etc.) and therapeutic isotope like Pd-103. Herein, the production of ^{18}F-FDG, ^{68}Ga-PSMA-11 and ^{201}TlCl radiopharmaceuticals using Cyclone-30 has been reported. The specification of the radiopharmaceuticals complies with norms of the regulatory bodies in India. Presently, India is importing long lived SPECT Isotopes. The high cost of imported isotopes makes the treatment expensive. Indigenous production is going to be a boon to make the treatment cost more affordable.

1. INTRODUCTION

Currently, ^{18}F-FDG is the most successful PET radiopharmaceutical so far. The advancement in synthesis and quality control of ^{18}F-FDG, together with its approval by the US FDA and the availability of reimbursement, are probably the main reasons for the flourish of clinical PET. The labelled ^{18}F-FDG compound has a relatively short shelf life, which is dominated by the physical decay of ^{18}F with a half-life of 109.8 minutes, or slightly less than 2 hours. Still, this half-life is sufficiently long to allow shipping the compound to remote PET scanning facilities, in contrast to other medical radioisotopes like ^{11}C. In PET imaging, ^{18}F-FDG can be used for the assessment of glucose metabolism in the heart, lungs [1], and the brain. It is also used for imaging tumors in oncology, where a static ^{18}F-FDG PET scan is performed and the tumor ^{18}F-FDG uptake is analyzed in terms of Standardized Uptake Value (SUV). ^{18}F-FDG is taken up by cells, phosphorylated by hexokinase (whose mitochondrial form is greatly elevated in rapidly growing malignant tumours), [2] and retained by tissues with high metabolic activity, such as most types of malignant tumours. As a result, FDG-PET can be used for diagnosis, staging, and monitoring treatment of cancers, particularly in Hodgkin's disease, non-Hodgkin lymphoma, colorectal cancer, breast cancer, melanoma, and lung cancer. It has also been approved for use in diagnosing Alzheimer's disease.

The SPECT isotope ^{201}Tl ($t_{1/2}$ = 73.06 hours) in the form of ^{201}TlCl is a diagnostic myocardial flow tracer to detect coronary artery disease and to assess myocardial viability, with an accuracy comparable to that of positron emission tomography. Other medical applications of the same include possible assessment of physiology, as a renal medullary imaging agent, and for tumor detection [3]. ^{201}TlCl has higher myocardial extraction fraction (85%) compared to ^{99m}Tc-MIBI (65%) and ^{99m}Tc-Tetrofosmin (60%). The lower myocardial extraction fraction of ^{99m}Tc-MIBI and ^{99m}Tc-Tetrofosmin results in underestimation of blood flow at high flow compared to ^{201}TlCl [4]. Clearance half-life is faster in case of ^{201}TlCl compared to ^{99m}Tc-MIBI and ^{99m}Tc-Tetrofosmin [5]. ^{201}Tl decays to stable Mercury-201 (^{201}Hg) nuclide via electron capture with the emission of mercury K-X-rays of 69 - 83 keV (90%) along with γ-rays of 135 keV and 167 keV in total abundance of 10%. ^{201}Tl is produced in Cyclone-30 using solid target via ^{203}Tl(p,3n)^{201}Pb → ^{201}Tl nuclear reaction utilizing a proton (energy: 28MeV) beam current of 50μA for up to 6-8h. The potential radionuclidic impurities in Thallium-201 produced with during the above nuclear reaction are Thallium-200 (^{200}Tl, t1/2 = 26 h), Thallium-202 (^{202}Tl, t1/2 = 12.2 d) and Lead-203 (^{203}Pb,

t1/2 = 52 h). However, the percentage for formation of ^{200}Tl, ^{202}Tl and ^{203}Pb can be controlled by optimizing the incident proton energy (28MeV) on the target during irradiation and giving an optimum decay time of 32 h for ^{201}Pb to ^{201}Tl [6]. The allowed limits for ^{200}Tl, ^{202}Tl and ^{203}Pb were 0.6%, 1.2% and 0.2% expressed as a percentage of ^{201}Tl injection activity at calibration date and time [7]. There are many approaches that address the wet separation of ^{201}Pb from ^{203}Tl and ^{201}Tl from ^{201}Pb from a dissolved solid target, typically ending with ^{201}TlCl as the product. Such approaches include ion exchange resin chromatography and solvent/solvent extraction [8]. Ion exchange column chromatography and solvent extraction methods have been employed by us for radiochemical separation and purification of ^{201}Tl from dissolved solid target. We herein report a semi-automated production of curie level, pharmaceutical grade ^{201}TlCl using IBA Chemistry module.

Gallium-68 (^{68}Ga, $t_{1/2}$ = 67.8 min) possesses great potential in nuclear medicine [9,10] being extensively used in labelling of biomolecules like somatostatin and PSMA inhibitor analogues [11,12,13,14]. ^{68}Ga decays to stable Zinc-68 (^{68}Zn) nuclide via electron capture (11%) and positron decay (89%) and is generally produced via ^{68}Ge/^{68}Ga generators [15,9]. An alternative method to produce ^{68}Ga is by cyclotron using high enriched ^{68}Zn via the ^{68}Zn(p,n)^{68}Ga reaction [16,17,18]. There are many approaches that address the wet separation of 68Ga and ^{68}Zn from a dissolved solid target, typically ending with [^{68}Ga]$GaCl_3$ as the product. Such approaches include solid phase extraction, solvent extraction, and precipitation [19,20]. Ion Exchange column chromatography and solvent extraction methods have been employed by us for radiochemical separation and purification of ^{68}Ga from dissolved solid target. Due to the increasing demand for various ^{68}Ga based radiopharmaceuticals production and applications entering clinical trials worldwide, there is a need to produce large quantity of ^{68}Ga. Hence, $^{68}GaCl_3$ is produced on medium energy cyclotron via the ^{68}Zn(p,n)^{68}Ga reaction which is useful for production of large quantity of ^{68}Ga. The starting material is a solid target in the form of a target plate, since solid target will always have a higher concentration of zinc, which leads to significantly higher yields. Furthermore, the ^{68}Ga must be separated from the bulk parent ^{68}Zn isotope and purified to remove any unwanted metal contaminants. The end product obtained is $^{68}GaCl_3$, which is similar to the eluate obtained from the ^{68}Ge/^{68}Ga generator, is then used as a solution for radiolabelling to prepare ^{68}Ga-based diagnostic radiopharmaceuticals like ^{68}Ga-PSMA-11, ^{68}Ga-DOTA-TATE. Currently, [^{68}Ga]Ga-PSMA-11 (Glu-NH-CO-Lys-(Ahx)-[[^{68}Ga]Ga-HBED-CC] (HBED CC: N,N'-Bis(2-hydroxy-5-(ethylene-be-tacarboxy)benzyl)ethylenediamine N,N'-diacetic acid) is among the most widely used agents for prostate cancer PET/CT imaging. Prostate cancer is one of the leading causes of morbidity and death in men in the western world, and the second most common cancer in men worldwide [21]. We herein report a semi-automated production of curie level, pharmaceutical grade [^{68}Ga]$GaCl_3$ radiochemical and [^{68}Ga]Ga-PSMA-11 radiopharmaceutical using IBA Chemistry module.

2. PRODUCTION OF DIFFERENT RADIOISOTOPES AND RADIOPHARMACEUTICALS

2.1. ^{18}F-FDG Radiopharmaceutical using IBA-SYNTHERA Module

2.1.1. Production of ^{18}F from O-18-Water and Synthesis of ^{18}F-FDG

[^{18}F]fluoride ion/[^{18}O]water was transferred from target to chemistry module following which the synthesis of ^{18}F-FDG (Fig. 1) was carried out using automated, closed loop and computer-controlled IBA synthera module (Fig. 2) inside Comecer make Hotcells (75 mm Pb thickness wall). ABX, Germany reagents and ancillary kits along with IFP (Integrated Fluidic Processor) are utilized in the IBA Synthera module for the synthesis and purification of ^{18}F-FDG (Fig. 3). The F-18 is produced in the cyclotron by irradiation of $H_2{}^{18}O$ (97% enriched) [^{18}O(p,n)^{18}F] using 18 MeV proton beam (35-45 μA current) for 30 min to 2 hours (Fig. 4 and Fig. 5). The dispensing of the product is carried out using TIMOTHEO-LT dispensing module inside Comecer dispensing Hotcell having ISO Class A environment. The final ^{18}F-FDG product obtained from IBA Synthera synthesis module is collected in 30 ml sterile glass vial (supplied by ABX Germany) containing 0.68 ml of 14.6% sodium chloride (inactive ingredient) to make the final solution isotonic, in the dispensing hotcells. The production yield of ^{18}F-FDG varied from 65-70 % (without decay correction). A 0.5 ml of sample from each FDG batch was taken in a sterile vial for Q. C. analysis. The physico-chemical and bio quality control tests were performed as per USP specifications with satisfactory results.

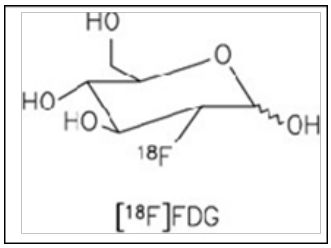

FIG. 1. Structure of ^{18}F-FDG

FIG. 2. IBA-SYNTHERA Module

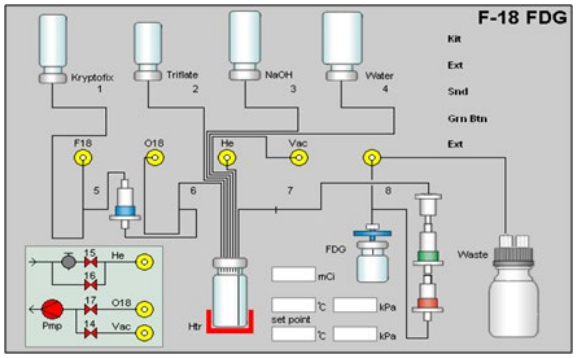

FIG. 3. ^{18}F-FDG Synthesis Flow Diagram

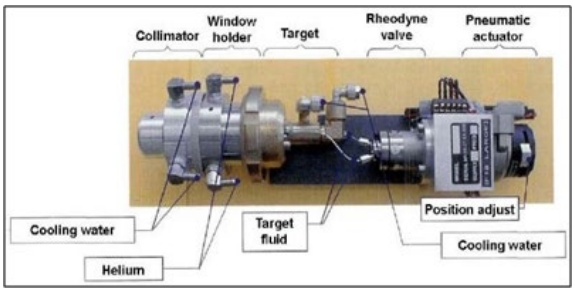

FIG. 4. F-18 water target

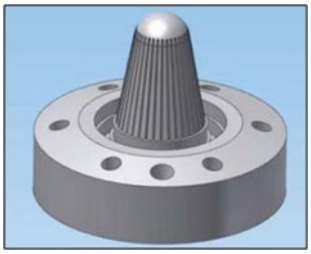

FIG. 5. Conical shaped Niobium cavity for O-18 water

2.1.2. *Quality Control Results*

- The physicochemical quality control tests of ^{18}F-FDG were performed by its checking appearance, pH, radiochemical purity by either method A (HPLC) or method B (TLC). The HPLC system is more expensive and elaborate than the TLC system, radionuclides purity by HPGe method.
- The radioactivity assay i.e. yields determination and half-life estimation were performed in dose calibrator.
- The presence of bacterial endotoxin in the ^{18}F-FDG were assayed by Charles River's Endosafe PTS (Portable Endotoxin Testing System).
- The sterility testing for every individual batch of ^{18}F-FDG has been inoculated on both fluid thioglycolate medium (FTM) and soybean casein digest medium (SCDM) within 30 hours of production at 37°C and 25°C respectively.
- The residual solvent in ^{18}F-FDG i.e., ethanol and acetonitrile were estimated in Gas chromatography (GC).
- The radiochemical purity of the ^{18}F-FDG has been found to be 100% by using TLC method (Fig. 6).
- The radionuclidic purity was greater than 99.9% (determined by HPGe) (Fig. 7).
- The presence of Kryptofix in the final product was found to be less than 22 µg/ml.
- The residual solvent ethanol and ACN in ^{18}F-FDG were within the specified value (GC method) (Fig. 8).
- HPLC study is required to know any radiochemical impurities like $^{18}F^-$, ^{18}F-FDM and ^{18}F-ClDG are present or not (Fig. 9 and Fig. 10).
- The Bacterial endotoxin in ^{18}F-FDG was found <10 EU/ml determined by PTS method.
- Each batch was evaluated for sterility test and each batch passes the sterility test.
- PET-CT scan of ^{18}F-FDG was carried out in North City Centre, Kolkata (Fig. 11).
- Results are shown in Table 1.

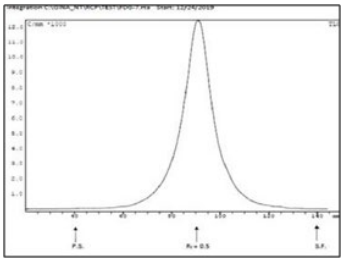

FIG. 6. TLC spectra of ^{18}F-FDG

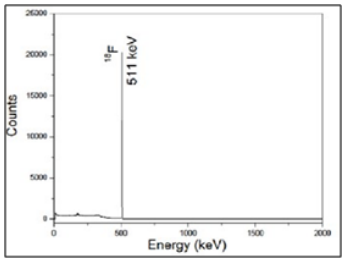

FIG. 7. HPGe spectra of ^{18}F

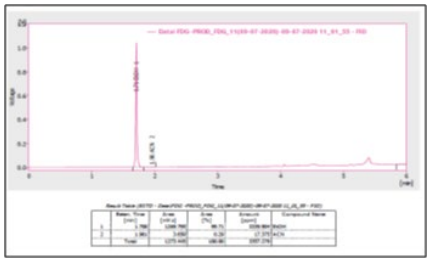

FIG. 8. GC spectra of ^{18}F-FDG

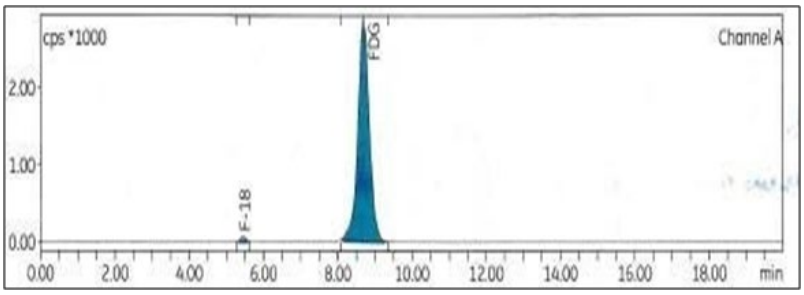

FIG. 9. HPLC spectra of ^{18}F-FDG

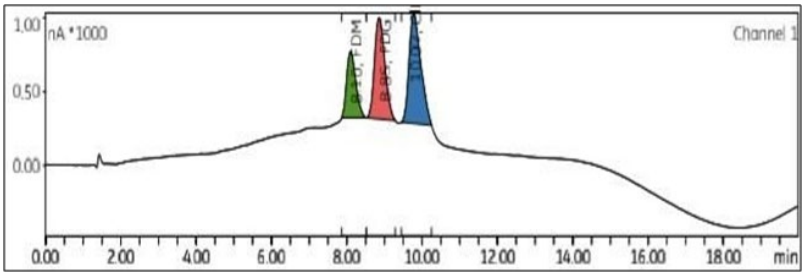

FIG. 10. HPLC spectra of cold samples of FDM, FDG & ClDG

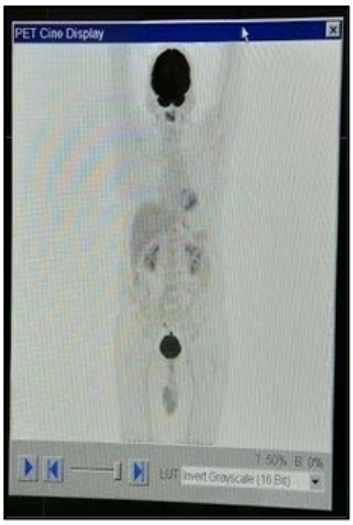

FIG. 11. PET-CT scan of 18F-FDG carried out in North City Centre, Kolkata

TABLE 1. PHYSICOCHEMICAL AND BIOLOGICAL QUALITY CONTROL TESTS OF ^{18}F-FDG

Batch no.	Appearance	pH	Half life (min)	RC Purity (%)	RN Purity (%)	Kryptofix (<22 µg/ml)	Acetonitrile (ppm)	Ethanol (ppm)	BET test (<10 EU/ml)	Sterility test
1	Clear solution	6.5	109.1	100	99.9	Passed	17.88	1705.96	Passed	Passed
2	Clear solution	6.0	110.76	100	99.9	Passed	14.04	1756.94	Passed	Passed
3	Clear solution	6.0	109.9	100	99.9	Passed	18.21	1641.40	Passed	Passed
4	Clear solution	6.5	108.9	100	99.9	Passed	<5	1344.48	Passed	Passed
5	Clear solution	6.0	109.5	100	99.9	Passed	14.60	1481.30	Passed	Passed
6	Clear solution	6.5	109.8	100	99.9	Passed	15.59	1506.85	Passed	Passed

2.2. Radioactive Thallium-201 in the form of ^{201}TlCl suitable for diagnostic uses in patients

2.2.1. Irradiation of the target

^{201}Tl has been produced on medium energy cyclotron, Cyclone-30, via the ^{203}Tl(p,3n)^{201}Pb → ^{201}Tl nuclear reaction. The starting material is a solid target in the form of a target plate electrodeposited with enriched Thallium-203 (Fig. 12 and Fig. 13), which leads to significantly higher yields. Further the ^{201}Tl must be separated from the bulk parent ^{203}Tl and ^{201}Pb isotope and purified to remove any unwanted metal contaminants. The end product has been supplied as a ready-to-use sterile, pyrogen free, isotonic aqueous solution of radioactive Thallium-201 (^{201}Tl) in the form of thallous chloride solution for intravenous administration. Irradiations of the

electrodeposited (~ 74 - 75 µm) ^{203}Tl targets were carried out with the 28MeV proton beam energy and 50µA beam current for up to 6-8h (n=6) at 6° angle. During the irradiation the target assembly (Fig. 14 and Fig. 15) was water cooled with a flow rate of 9 liter/min. Beam current/charge deposited on the target was monitored with a current integrator.

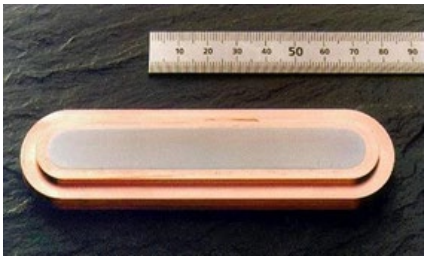

FIG. 12. ^{203}Tl Target

FIG.13. Electrodeposition vessel

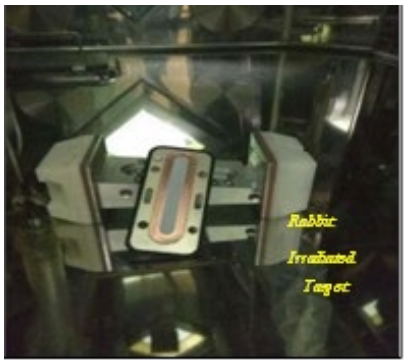

FIG. 14. Irradiated target system received in the receiving Hotcell.

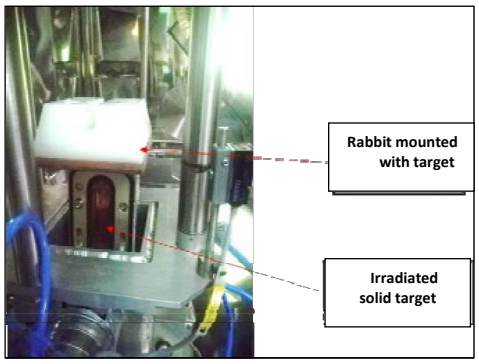

FIG. 15. Irradiated enriched Tl-203 Target with Rabbit System in Receiving Hotcell.

2.2.2. Dissolution of the irradiated thallium target and separation of ^{201}Tl from ^{201}Pb

The original script for the Tl-201 chemistry-1 and chemistry-2 was supplied for production of [Tl-201]TlCl and thus required modifications while working on our system (Fig. 16 and Fig. 17).

Chemistry-I: The irradiated target was dissolved in 25 ml of 0.7 N HNO_3 (containing 100 mg $Pb(NO_3)_2$). 201Pb was precipitated as $^{201}PbSO_4$ by using 10 ml of 3.6 N H_2SO_4. The first dissolution of $^{201}PbSO_4$ was carried out with 10 ml of 0.1 M Na_2EDTA (pH ~ 9.0), while second dissolution was carried out with 10 ml of 0.1 M Na_2EDTA (pH ~ 5.4). $^{203}Tl^{3+}$ was reduced to $^{203}Tl^{+}$ by bubbling SO_2 gas. Ion Exchange Chromatography using Dowex 50W-X8 resin (100-200 mesh, H^+ form) was employed to remove co-precipitated $^{203}Tl^{+}$. Cation exchange chromatography was employed to adsorb $^{203}Tl^{+}$ in the column while the ^{201}PbEDTA complex was collected in column eluate. $^{201}PbEDTA^{2-}$ complex was stored for 32 h for decay of $^{201}Pb^{2+}$ either to $^{201}Tl^{3+}$ or $^{201}Tl^{+}$.

Chemistry-II: Post 32 h decay, $^{201}Pb^{2+}$ (in the form of ^{201}PbEDTA mother solution) was converted to either $^{201}Tl^{3+}$ or $^{201}Tl^{+}$. The reduction of $^{201}Tl^{3+}$ to $^{201}Tl^{+}$ was carried out by bubbling SO_2 gas through the mother PbEDTA solution until a pH~3 is attained. Post reduction, the pH of reduced $^{201}Tl^{+}$ in mother PbEDTA solution was adjusted to ~ 5.4 by using 1 N NaOH. The mother PbEDTA solution containing $^{201}Tl^{+}$ was passed through Dowex 50W-X8 resin (100-200 mesh, H^+ form) chromatographic column. $^{201}Tl^{+}$ was adsorbed in the column while PbEDTA was collected as eluate in waste flask. The adsorbed $^{201}Tl^{+}$ was eluted from cation exchange chromatographic column using 15 ml of 6 N HCl. Further $^{201}Tl^{+}$ was oxidized to $^{201}Tl^{3+}$ using ozone. Solvent extraction of $^{201}Tl^{3+}$ from aqueous phase (HCl) to organic phase (DIPE) was carried out utilizing 20 ml DIPE (DIPE saturated with 6 N HCl). Reduction of $^{201}Tl^{3+}$ to $^{201}Tl^{+}$ was carried out by SO_2 gas in aqueous phase (0.005N HCl). Post reduction, $^{201}Tl^{+}$ was back extracted into 20 ml of 0.005N HCl. After successful removal of DIPE from aqueous phase, finally 201TlCl ($^{201}Tl^{+}$ form, in 0.005N HCl) was collected. pH of ^{201}TlCl was adjusted to 6 - 7 using 1N NaOH and was diluted with 0.9% NaCl. ^{201}TlCl (in 0.9% NaCl) was filtered with sterile pyrogen free 0.20 µm PES membrane syringe filter. ^{201}TlCl ($^{201}Tl^{+}$ form) solution obtained was assayed for radioactive concentration and suitable activity was dispensed into sterile pyrogen free glass vials for supply.

The entire operation was carried out in aseptic environment using ultrapure grade chemicals and sterile and pyrogen-free glassware to ensure the purity (radionuclide, radiochemical and chemical), sterility and apyrogenicity of the product. The physicochemical quality control and BET assay was completed prior to supply of the product. Sterility was initiated within the same day of production. Following the revised configurations and the designed steps, the script was accordingly modified. The whole process of chemical separation and purification has been carried out in GMP certified hot cell and semi-automated radiochemistry module under aseptic environment for efficient, rapid and easy handling.

FIG. 16. Thallium Chemistry hotcell with Tl-chemistry module

FIG. 17. Thallium Chemistry External Panel: computer-based controller system

2.2.3. Quality Control Results

- The radiochemical purity of ^{201}TlCl was 100% (PC method) (Fig. 18).
- The metal content of ^{201}TlCl (Fe, Cu and Tl) were within the specified values.
- The Bacterial Endotoxin in ^{201}TlCl was < 6 EU/ml (PTS method).
- The residual solvent DIPE in 201TlCl was within the specified value (GC method) (Fig. 19).
- The radionuclidic purity of 201Tl was > 99% (determined by HPGe) (Fig. 20).
- Each batch were evaluated for sterility test and each batch passed the sterility test.
- PECT-CT scan of ^{201}TlCl was carried out in NH Rabindranath Tagore, International Institute of Cardiac Sciences, Kolkata (Fig. 21).
- Results are shown in Table 2.

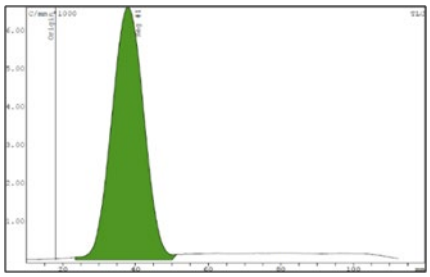

FIG. 18. PC spectra of ^{201}TlCl

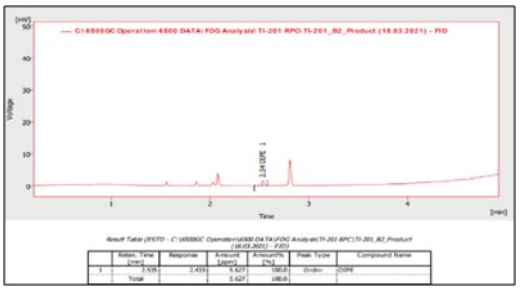

FIG. 19. GC spectra of ^{201}TlCl

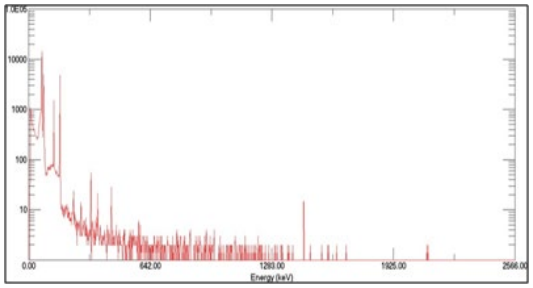

FIG. 20. HPGe spectra of ^{201}TlCl

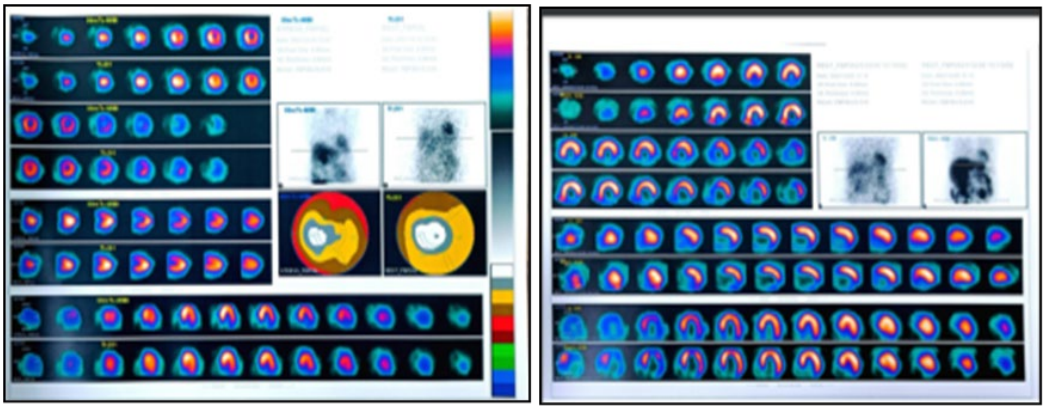

FIG. 21. Two cardiac studies (Rest-Stress on 18/12/2021 for ischemia evaluation and rest only for viability assessment on 20/12/2021) using GE Discovery 670DR SPECT-CT scanner in patients for suspected coronary artery disease evaluation: A comparison between ^{201}TlCl vs ^{99m}Tc-MIBI performed on same patients.

TABLE 2. PHYSICOCHEMICAL AND BIOLOGICAL QUALITY CONTROL TESTS OF ^{201}TlCl

Batch no.	Appearance	pH	Half life (hrs)	RC Purity (%)	RN Purity (%)	Fe (Fe^{2+}) µg/ml)	Cu (Cu^{2+}) µg/ml)	Tl (Tl^{+}) µg/ml)	BET test (<6 EU/ml)	Sterility test
1	Clear solution	5.0	73.68	100	99.79	< 3	BDL	< 2	Passed	Passed
2	Clear solution	6.0	73.20	100	99.98	< 3	BDL	< 2	Passed	Passed
3	Clear solution	5.0	73.08	100	99.88	< 3	BDL	< 2	Passed	Passed
4	Clear solution	6.5	73.57	100	99.85	< 3	BDL	< 2	Passed	Passed
5	Clear solution	5.0	73.08	100	99.80	< 3	BDL	< 2	Passed	Passed
6	Clear solution	5.5	73.18	100	99.82	< 3	BDL	< 2	Passed	Passed

2.3. Indigenous module for $^{68}GaCl_3$ radiochemical and ^{68}Ga-PSMA-11 radiopharmaceutical synthesis

2.3.1. Irradiation of the target

Irradiations of the electrodeposited (~ 94.5 – 95.5 µm) ^{68}Zn targets (Fig. 22) were carried out with the 15MeV proton beam of up to 60µA for 25 minutes (n=6) at 6° angle. During the irradiation the target assembly was water cooled with a flow rate of 9 liter/min. Beam current/charge deposited on the target was monitored with a current integrator.

2.3.2. Dissolution of the irradiated gallium target and separation of ^{68}Ga from ^{68}Zn

The original script for the Ga-67 chemistry was supplied by VUB for production of Ga-67 radiochemical and thus required modifications while working on our system. Beam energy was accordingly adjusted to obtain Ga-68 in curie quantity.

FIG. 22. Electroplated Zinc-68 targets

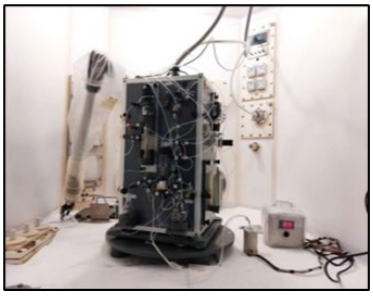

FIG. 23. Module for $^{68}GaCl_3$ and ^{68}Ga-PSMA-11 synthesis

2.3.3. Production of $^{68}GaCl_3$ radiochemical

Irradiated target was placed in the dissolution unit of automated radiochemistry module (Fig. 23) using master-slave manipulator. It was then dissolved using 20 ml of 10 N HCl (containing 100 µL H_2O_2). The enriched ^{68}Zn and carrier free Ga rapidly dissolved in this medium. Upon complete dissolution of the target material, about ~ 10 mg of Cu from Cu backings was co-dissolved. Dowex 50W-X8 resin (100-200 mesh, H^+ form) was packed in column (dimension of column: 1.33 cm2 internal cross section area x 6 cm height). The column was preconditioned with 40 ml of 9 N HCl at a flow-rate of 2.0 ml / min. Separation of ^{68}Ga from Cu and ^{68}Zn was carried out by cation exchange chromatography using preconditioned Dowex 50W-X8 resin column (100 - 200 mesh, H^+ form). Stripping solution was applied to the chromatographic column at a flow-rate of 1.7 ml / min. The ^{68}Ga is adsorbed quantitatively, while the Cu and ^{68}Zn pass into the storage flask (68Zn recovery storage flask). Interstitial Zn and Cu was removed from the column with 25 ml of 9 N HCl. ^{68}Ga was eluted with 20 ml of 3.75 N HCl from the column and the eluate is collected in extractor present inside radiochemistry module. Concentration of HCl was adjusted prior to extraction of ^{68}Ga in DIPE for optimum extraction. 7 N HCl is the optimum concentration of HCl for ^{68}Ga extraction from HCl into DIPE. In extractor (containing ^{68}Ga eluate), 20 ml of 10 N HCl was added so as the concentration of HCl increases from 3.75 N to 7 N. Solvent extraction of ^{68}Ga from HCl to DIPE takes place by introducing 15 ml DIPE (DIPE saturated with 7N HCl) to extractor. Both the layers {aqueous (HCl) and organic(DIPE)} were mixed by bubbling N_2 gas through the aqueous layer. Post separation of both the phases, the HCl layer was transferred to waste-flask inside the radiochemistry module.

2.3.4. Preparation of $^{68}GaCl_3$ radiochemical

Back extraction was performed with DIPE in extractor after addition of 10 - 20 ml of 0.005N HCl. Finally, 0.005 N HCl layers was collected in the $^{68}GaCl_3$ flask inside radiochemistry module, whereas DIPE phase was transferred to the waste flask inside the radiochemistry module. Traces of DIPE was removed from ^{68}GaCl3 solution present in the flask and homogenization of the content was carried out by bubbling N_2 through the solution for 5 minutes at 90°C.

2.3.5. Synthesis of ^{68}Ga-PSMA-11 radiopharmaceutical

Radiolabelling was performed by adding buffer + peptide (PSMA-11, 100 µg) mixture (3 ml) to the reaction vial and heating for 10 mins at 95°C. 3 ml water for injection was added to the reaction vial. The mixture was passed through C-18 column and the waste was collected in the waste vial. 3 ml water for injection was again added to wash the C-18 column; collected in the waste vial. ^{68}Ga-PSMA-11 was eluted from the C-18 column using 3 ml 50% (v/v) EtOH and collected in the product vial containing 5 ml 0.9% saline. Column was washed with 2 ml water for injection and collected in the product vial.

2.3.6. Dispensing of ^{68}Ga-PSMA-11 radiopharmaceutical

The resultant ^{68}Ga-PSMA-11 radiopharmaceutical solution was filtered using sterile pyrogen free 0.20 µm PES membrane syringe filter. Small aliquots (0.5 ml) of clinical grade ^{68}Ga-PSMA-11 solution was dispensed into sterile, pyrogen-free glass vials using the automatic dispensing system as per customer requirement. The glass vials were sealed with 25 KGy γ irradiated, sterile, pyrogen-free bromobutyl rubber closures and crimped with aluminum caps (pre swabbed with 70% ethanol). The sealed glass vials were transferred to a cylindrical lead container (LP-30), surrounded by thermocol and placed inside an outer container made up of HDPE (TPPL-1) and sealed before being dispatched to hospitals.

The entire operation was carried out in an aseptic environment using ultrapure grade chemicals and sterile and pyrogen-free glassware to ensure the purity (radionuclide, radiochemical and chemical), sterility and apyrogenicity of the product. Physico-chemical and biological quality control of [^{68}Ga]Ga-PSMA-11 were optimized and carried out and they are in accordance with USP monograph, International Pharmacopeia and Indian Pharmacopeia. The clinical results from PET-CT Cardiac studies performed at Netaji Subhas Chandra Bose Cancer Hospital, AMRI Hospitals (Dhakuria), Command Hospital (Eastern Command, Alipore Road), Kolkata add support to the use of our ^{68}Ga-PSMA-11 as a pharmaceutical grade diagnostic radiopharmaceutical.

2.3.7. *Quality Control Results*

- The radiochemical purity of $^{68}GaCl_3$ was ≥ 99.9% (TLC & HPLC method) (Fig. 24 and Fig. 25).
- The residual solvent DIPE in $^{68}GaCl_3$ was within the specified value (GC method) (Fig. 26).
- The radionuclidic purity of ^{68}Ga was > 98% (determined by HPGe) (Fig. 27).
- The radiochemical purity of ^{68}Ga-PSMA-11 was ≥ 95% (TLC, PC & HPLC method) (Fig. 28, Fig. 29 and Fig. 30).
- The metal content of $^{68}GaCl_3$ (Fe, Cu and Zn) were within the specified values.
- The Bacterial endotoxin in $^{68}GaCl_3$ and ^{68}Ga-PSMA-11 was < 5 EU/mL (PTS method).
- Each batch were evaluated for sterility test and each batch passed the sterility test.
- A typical PET-CT scan of ^{68}Ga-PSMA-11 of a patient diagnosed with prostate carcinoma is given below (Fig. 31).
- Results are shown in Table 3.

TABLE 3. PHYSICOCHEMICAL AND BIOLOGICAL QUALITY CONTROL TESTS OF $^{68}GaCl_3$

Batch no.	**Appearance**	**pH**	**Half life (min)**	**RC Purity (%)**	**RN Purity ^{68}Ga (^{67}Ga) (%)**	**Fe µg/ml**	**Cu µg/ml**	**Zn µg/ml**	**BET test (<3 EU/ml)**	**Sterility test**
1	Clear solution	< 2	69	100	99.95 (0.05)	< 3	BDL	BDL	Passed	Passed
2	Clear solution	< 2	69	100	99.89 (0.11)	< 3	BDL	BDL	Passed	Passed
3	Clear solution	< 2	69	100	99.85 (0.15)	< 3	BDL	BDL	Passed	Passed
4	Clear solution	< 2	69	100	99.97 (0.03)	< 3	BDL	BDL	Passed	Passed
5	Clear solution	< 2	69	100	99.81 (0.19)	< 3	BDL	BDL	Passed	Passed
6	Clear solution	< 2	69	100	99.83 (0.17)	< 3	BDL	BDL	Passed	Passed

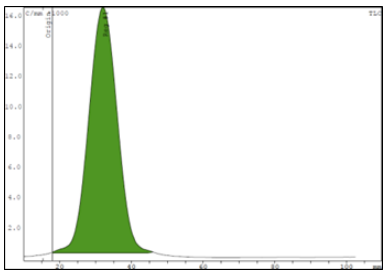

FIG. 24. TLC spectra of $^{68}GaCl_3$

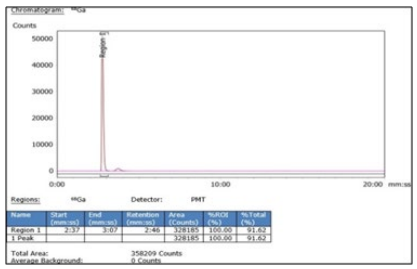

FIG.25. HPLC spectra of $^{68}GaCl_3$

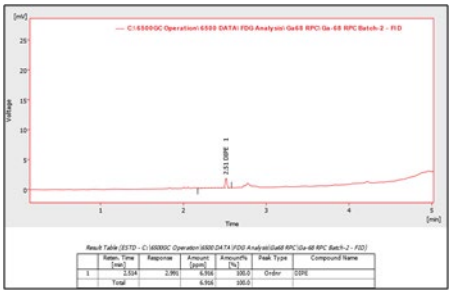

FIG. 26. GC spectra of $^{68}GaCl_3$

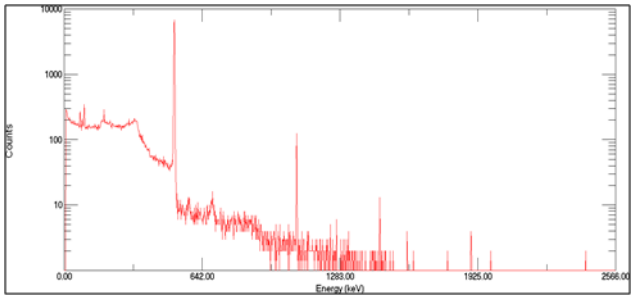

FIG. 27. HPGe spectra of $^{68}GaCl_3$

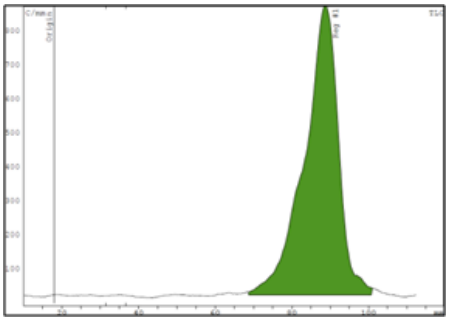

FIG. 28. TLC spectra of [^{68}Ga]Ga-PSMA-11

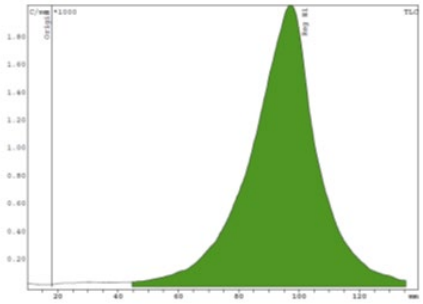

FIG. 29. PC spectra of [68Ga]Ga-PSMA-11

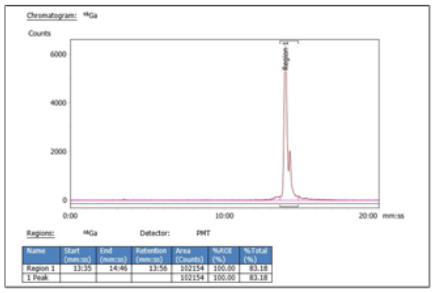

FIG. 30. HPLC spectra of [68Ga]Ga-PSMA-11

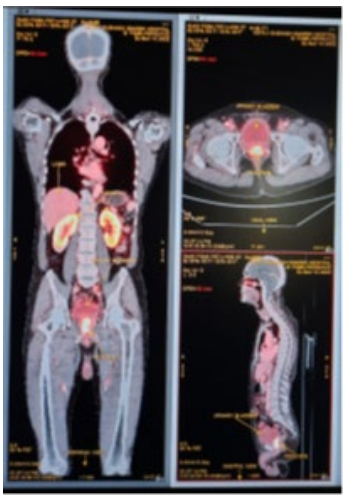

FIG. 31. PET-CT image of [68Ga]Ga-PSMA-11

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REFERENCES

[1] MEYER, G.J., MATZKE, K.H., HAMACHER, K., FUCHTNER, F., STEINBACH, J., NOTOHAMIPRODJO, G., ZIJLSTRA, S., The stability of 2-[F]fluoro-deoxy-D-glucose towards epimerisation under alkaline conditions. Applied Radiation and Isotopes, Volume 51, Issue 1 (July 1999) 37-41.

[2] RIJIN, CJSV., HERSCHEID, JCM., VISSER. GWM., HOEKSTRA. A., On the stereoselectivity of the reaction of [18F]acetylhypofluoride with glucals. International Journal of Applied Radiation and Isotope. (1984) 36:111– 115.

[3] LEBOWITZ, E., GREENE, M. W., FAIRCHILD, R., BRADLEY-MOORE, P. R., ATKINS, H. L., ANSARI, A. N., RICHARDS, P., BELGRAVE, E., Thallium-201 for medical use. I. Journal of Nuclear Medicine. (1975) 16(2),151-155.

[4] SCHWAIGER, M., MELIN, J., Cardiological applications of nuclear medicine. THE LANCET (1999) 354, 661-666.

[5] MIKOLAJCZAK, R., GARNUSZEK, P., Radiopharmaceuticals in cardiology. Nuclear Medicine Review. 15(1), (2012) 39–45.

[6] QAIM, S. M., WEINREICH. R., OLLIG. H., 1979. Production of ^{201}T1 and ^{203}Pb via proton induced nuclear reactions on natural thallium. International Journal of Applied Radiation and Isotope. (1979) 30(2), 85-95.

[7] USP Monographs: Thallous Chloride Tl-201 Injection. 2008. USP29-NF24. Page 2108.

[8] Cyclotron Produced Radionuclides: Physical Characteristics and Production Methods. IAEA-TRS-468. IAEA, Vienna, (2009).

[9] VELIKYAN, I., ^{68}Ga-based radiopharmaceuticals: production and application relationship. Molecules 20 (2015) 12913–12943.

[10] [10] RAHBAR, K., et al, German multicenter study investigating ^{177}Lu-PSMA-617 radioligand therapy in advanced prostate cancer patients. Journal of Nuclear Medicine 58 (2017) 85–90

[11] AMOR-COARASA, A., SCHOENDORF, M., MECKEL, M., VALLABHAJOSULA, S., BABICH, J.W., Comprehensive quality control of the ITG ^{68}Ge/^{68}Ga generator and synthesis of ^{68}Ga-DOTATOC and ^{68}Ga-PSMA-HBED-CC for clinical imaging. J. Nucl. Med. 57, (2012) 1402–1405.

[12] BANERJEE, S.R., POMPER, M.G., Clinical applications of gallium-68. Applied Radiation and Isotopes 76, (2013) 2–13.

[13] OBERG, K., Gallium-68 somatostatin receptor PET/CT: is it time to replace ^{111}Indium DTPA octreotide for patients with neuroendocrine tumors? Endocrine 42, (2012) 3–4.

[14] SCHWENCK, J., REMPP, H., REISCHL, G., KRUCK, S., STENZL, A., NIKOLAOU, K., PFANNENBERG, C., LA FOUGERE, C., Comparison of ^{68}Ga-labelled PSMA-11 and ^{11}C-choline in the detection of prostate cancer metastases by PET/CT. Eur. J. Nucl. Med. Mol. Imaging 44, (2017) 92–101.

[15] TIROSH, A., KEBEBEW, E., The utility of ^{68}Ga-DOTATATE positron-emission tomography/computed tomography in the diagnosis, management, follow-up and prognosis of neuroendocrine tumors. Future Oncol. 14, (2018) 111–122.

[16] ALVES, F., ALVES, V.H., NEVES, A.C.B., DO CARMO, S.J.C., NACTERGAL, B., HELLAS, V., KRAL, E., Gonçalves-Gameiro, C., Abrunhosa, A.J., 2017. Cyclotron production of Ga-68 for human use from liquid targets: from theory to practice. AIP Conf. Proc. 1845, 020001 https://doi.org/10.1063/1.4983532

[17] BLASER, J.P., BOEHM, F., MARMIER, P., PEASLEE, D.C., Fonctions d'excitation de la reaction (p, n). (I). Helv. Phys. Acta 24, (1951) 3–38

[18] HOWE, H.A., (p, n) cross sections of copper and zinc. Physical. Review. (1958) 109, 6–8.

[19] SZELECSENYI, F., BOOTHE, T.E., TAKACS, S., TARKANYI, F., TAVANO, E., 1998. Evaluated cross section and thick target yield data bases of Zn + p processes for practical applications. Applied Radiation and Isotope 49, (1998) 1005–1032.

[20] International Atomic Energy Agency. Gallium-68 Cyclotron Production. IAEA-TECDOC-1863. IAEA, Vienna, (2019).

[21] SADEGHI, M., KAKAVAND, T., RAJABIFAR, S., MOKHTARI, L., RAHIMI-NEZHAD, A., Cyclotron production of ^{68}Ga via proton-induced reaction on ^{68}Zn target, Nukleonika 54 (2009) 25.

[22] BOIS, F., NOIROT, C., DIETEMANN, S., MAINTA, I. C., ZILLI, T., GARIBOTTO, V., WALTER, M. A., [^{68}Ga]Ga-PSMA-11 in prostate cancer : a comprehensive review. Am J Nucl Med Mol Imaging 10(6), (2020) 349-374.