Fully Automated Synthesis of Nitrogen-13-NH$_3$ by SHIs HM-18 Cyclotron and Dedicated Module for Routine Clinical Studies: Our Institutional Experiences

Abstract

Aims: The production of nitrogen-13 ($^{13}$N)-NH$_3$ by ethanol method using automated synthesizer and accessing the production yield, quality control for clinical application. Context: $^{13}$N, together with $^{99}$Tc, $^{18}$F, $^{11}$O, and $^{123}$I, is one of the positron emitters that can be produced on the multi-gigabecquerel scale in biomedical cyclotrons. ($^{13}$N)-ammonia is frequently used for cardiac PET studies. It is widely applied for the evaluation of myocardial perfusion in the clinical assessment of cardiac disorders. Simple, fast, and reliable preparation methods have contributed to the routine application of this tracer. Although only two methods are available, a challenge remains to adopt a more efficient and consistent approach to its production. For clinical application, routine production of this tracer is mandatory in compliance with regulatory guidelines. Being at hospital radiopharmacy it is our responsibility to support the clinical service with uninterrupted production and supply of ($^{13}$N)-NH$_3$.

Materials and Methods: The chemicals were used commercially available from Sigma Aldrich, India, Ltd., and Fisher Scientific, India, Ltd. (Mumbai, India), Sep-Pak CM cartridges (Waters India Pvt., Ltd.), Radio-thin layer chromatography was carried out using aluminum sheets precoated with silica gel 60 F254 (E. Merck, India). Results: The protocol developed with MPS-100 synthesizer yield ($^{13}$N)-NH$_3$ 95–97% (EOB) with a synthesis time of around 7 min. Conclusions: With the installation of HM-18 cyclotron at our hospital, center is capable to produce ($^{13}$N)-NH$_3$ of good yield and purity through the ethanol method, for myocardial perfusion studies. Our protocol is simple, reproducible, and robust.

Keywords: Nitrogen-13-NH$_3$, automation, quality control, synthesizer

Introduction

Cardiovascular disease remains the leading cause of death in developed countries as well as in most of developing countries.[1] Myocardial perfusion imaging, a noninvasive measure of blood flow in the heart, is commonly used to determine areas of reversible ischemia, characterize infarcted tissue, and assess left ventricular function.[2] At present, single-photon emission computed tomography imaging with the radioactive transition metal technetium-99 m ($^{99m}$Tc; $t_{1/2} = 6.2$ h) incorporated into monocationic complexes (e.g. $^{99m}$Tc-sestamibi, $^{99m}$Tc-tetrofosmin) is widely used.[2,3] Following intravenous injection, these $^{99m}$Tc complexes distribute into heart tissue in proportion to blood flow and remain trapped for times sufficient to image perfusion territories.

$^{13}$N was one of the earliest positron emitters to be produced; it was discovered in 1934 by Joliot and Curie.[4] The ($^{13}$N)-NH$_3$ has been widely used for myocardial perfusion scans.[5–8] The centers having biomedical cyclotron and suitable infrastructure to produce the ($^{13}$N)-NH$_3$ use these PET tracer-based studies. It could be used in the assessment of the ischemic area and severity level of coronary artery disease and also provides absolute quantification imaging and valuable information for prognosis and strategy selection for precision treatment. Thus, the clinical demand and significance of ($^{13}$N)-NH$_3$ are continuously increasing over time. Synthesis of ($^{13}$N)-NH$_3$ is well documented in the literature but with two routes of production: (1) DeVardas’s method[9] and (2) Ethanol method.[10–13] In DeVardas’s method, the produced ($^{13}$N)-NOX through $^{16}$O (p, a) $^{13}$N reaction subsequently reduced with...
DeVarda’s alloy to yield the $^{13}$N-NH$_3$ wherein ethanol method, 5 mM of EtOH (aq) as an additive in O-16 water target is used to produce the $^{13}$N-NH$_3$ directly during irradiation. The ethanol method is currently widely used in most centers around the globe. It has the advantage of low input cost, ease of handling, and higher production yield. The addition of a small amount of ethanol works as a scavenger that is capable to oxidize hydroxyl radical during irradiation whereas, in DeVarda’s method, the alloy acts as a reducing agent to reduce the oxides of nitrogen to ammonium ions, high input cost, handling or preparation of DeVarda’s alloys, needs special care and precaution, although this having good yield.

The production of $^{13}$N-NH$_3$ by alpha-particle irradiation of boron nitride followed by heating with sodium hydroxide was first proposed by Joliot and Curie.[4] Hunter et al.[14] also used a similar synthetic strategy for the production of $^{13}$N, they produced it by irradiation of an AlC$_4$ solid-state target with 8-12 MeV deuterons followed by target treatment with KOH aqueous solution, and $^{13}$N-NH$_3$ was distilled and trapped in an acidic solution as $^{13}$N-NH$_2$OH. The Welch and Lifton,[15] using 7 MeV deuteron energy, studied the formation of $^{13}$N-labeled species in different inorganic carbides but AlC$_4$ yielded a maximum percentage of $^{13}$N-NH$_3$ (75%-90%) depending on integrated current in the target. During irradiation of aluminium carbide with deuterons, $^{28}$Al is also produced (2.4 min half-life, high energy gamma, and beta radiation); to prevent the formation of this by-product, an alternative method based on the irradiation of continuously flowing methane with 8 MeV deuterons was proposed.[16] Several other production protocols are using different organic phases such as acetic acid, but only two (DeVarda’s alloy method and Ethanol methods) are mostly used for the production of $^{13}$N-NH$_3$ in clinical studies by different groups as summarized in below Table 1.

Hence, every radiopharmacy has its challenges to achieve as respective hospital demands. At hospital pharmacy, the daily challenges for production of radiopharmaceuticals are (i) need for rapid and reliable manufacturing, (ii) adhere by radiation safety protocols due to production of multi-curies, (iii) follow current good manufacturing practice (cGMP) as recommended by regulatory bodies (the Food and Drug Administration [FDA] 21CFR212, EU),[17,18] and (iv) cost-effective to be bear by patients. All this applies to the production of $^{13}$N-NH$_3$ too. Hence, keeping the above points, the production of $^{13}$N-NH$_3$ is exclusively shifted to automated protocols, which compiles the clinical use of it as a drug following 21 CFR 212 (FDA) or local compliances. Various commercial dedicated automated radio-synthesizers are available such as Tracsis, GE TracerLab MX, Bioscan, Inc., IBA Synthera, and Sumitomo’s MPS-100 which are designed to produce ($^{13}$N)-NH$_3$ as per GMP standards and it complies the quality controls tests. These synthesizers are mostly cassette-based modules, run by software template that is sterile, manufactured according to cGMP, and are compatible with validated clinical procedures and used for producing a large quantity of ($^{13}$N)-NH$_3$. In addition, the transition to automated systems ensures safety to radiochemists while adhering to the as low as reasonably achievable principle during producing an excessively larger quantity of production.

All the commercially available synthesis modules work on the trap and release method, of which in the target $^{13}$N-NH$_3$ was first trapped over anion exchange cartridge and finally eluted with the 0.9% sterile saline to product via through 0.22 mm sterile filter.

We, at our center, have established routine production of $^{13}$N-NH$_3$ as required by our hospital. For this purpose, the production and quality assurance of $^{13}$N-NH$_3$ by using the ethanol method was through dedicated multipurpose automated synthesizers termed as MPS-100. In this paper, we document our experience in synthesizing the $^{13}$N-NH$_3$ by automated and QC tests of the final product.

### Materials and Methods

The chemicals were used without any further purification and were commercially available from Sigma Aldrich India Ltd and Fisher Scientific, India, Ltd. (Mumbai, India). USP-grade 0.9% NaCl, and sterile water for injection were purchased from B. Braun India Pvt. Ltd., Sep-Pak CM cartridges (Waters India Pvt. Ltd., WAT20550) were used. Radio-thin layer chromatography (TLC) was carried out using aluminum sheets precoated with silica gel 60 F254 (E. Merck, India) (ratio of propionic acid: acetone: water: 2:2:1 as mobile phase).

### Discussion

#### Production of $^{13}$N-radionuclide through $^{16}$O (p, α) $^{13}$N nuclear reaction

The production of ($^{13}$N)-Nitrogen radionuclide was achieved by nuclear reaction of $^{16}$O (p, α) $^{13}$N at Sumitomo Heavy Industries HM-18 Cyclotron having liquid target capacity. The cyclotron is equipped with Niobium as chamber material with a target volume of 2.5 ml and a

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**Table 1: Summary of the $^{15}$N-NH$_3$ irradiation of 10 mM EtOH Water**

| Load volume (ml) | Beam (mA) | Irradiation time (minutes) | Yield EOS (mCi) |
|------------------|-----------|---------------------------|-----------------|
| 2.5              | 30        | 10                        | 269             |
| 2.5              | 30        | 10                        | 264             |
| 2.5              | 30        | 10                        | 287             |
| 2.5              | 30        | 10                        | 275             |
| 2.5              | 30        | 08                        | 226             |
| 2.5              | 30        | 08                        | 242             |
| 2.5              | 30        | 08                        | 233             |
| 2.5              | 30        | 12                        | 300             |
| 2.5              | 30        | 15                        | 400             |
target holding volume of 1.7 ml. The production yield of the \( ^{13}\text{N}}\text{NH}_3 \) is in the range of 8.0-15 GBq at 30 \( \mu \text{A} \) with of bombardment time of 8–15 min [Scheme 1].

**Automated synthesis of \( ^{13}\text{N}\text{NH}_3 \) using MPS-100 synthesis module**

The synthesis of \( ^{13}\text{N}\text{NH}_3 \) was performed in this dedicated synthesizer. The system was configured with a synthesis software template and hardware named ammonia tray. This tray consists of the two-syringe unit and four magnetic valves [as depicted in Figure 1] for performing the different commands for the production of \( ^{13}\text{N}\text{NH}_3 \).

Our priority was to establish the synthesis of \( ^{13}\text{N}\text{NH}_3 \) and then to access its quality for clinical application. Performed the optimization runs using the chemistry protocol as programmed with synthesis module [Figure 2].

The \( ^{13}\text{N}\text{NH}_3 \) solution is a clear, colorless, and isotonic solution. It is subjected to pass the quality tests before being used for clinical studies. After successfully performing ten hot runs and analyzing the QC data, the \( ^{13}\text{N}\text{NH}_3 \) PET Tracer is used for clinical studies.

Summarizing the synthesis of \( ^{13}\text{N}\text{NH}_3 \) through the ethanol method [Table 1], we first bombarded the 10 mm ethanolic water with a proton at 30 \( \mu \text{A} \) beam current designated energy and time (around 10 min for each run), after that the target water was transferred to the synthesis module from the cyclotron target through delivery line, the target water pass through the precondition ion exchange cartridge (Waters Accell Plus CM, 10 ml water and then 10 ml 0.9% saline) which traps \( ^{13}\text{N}\text{ammonium} \) ion and pass through the excess water to waste vial. The cartridge was washed with the water twice to remove any impurities and finally the \( ^{13}\text{N}\text{NH}_3 \) was eluted out with 5 ml of 0.9% saline to the product vial through the sterile syringe filter into the product vial and this product is delivered for clinical studies.

Each batch of the production was subjected to various quality control tests as summarized in Table 2 for five runs of \( ^{13}\text{N}\text{NH}_3 \), such as appearance, pH of the final solution, radionuclide purity, radiochemical purity, half-life, sterility, bacterial endotoxin test were performed before releasing for clinical studies. Since the beginning of the production till date, the average yield is in the range of 10 GBq at the end of the synthesis with 10 min of bombardment time.

**Results**

**Quality control test**

Summarized the different quality tests performed for each batch of \( ^{13}\text{N}\text{NH}_3 \) produced [Table 2]. All the parameters of the different quality tests comply with the standard set by the various regulatory agencies for clinical usage. The

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**Table 2: Quality Control Test for \( ^{13}\text{N}\text{NH}_3 \) runs**

| Test performed       | Hot Run 1 | Hot Run 2 | Hot Run 3 | Hot Run 4 | Hot Run 5 |
|----------------------|-----------|-----------|-----------|-----------|-----------|
| Particulate Test     | Clear, colorless | Clear, colorless | Clear, colorless | Clear, colorless | Clear, colorless |
| Radionuclide Purity  | 99.99     | 99.96     | 99.98     | 99.99     | 99.97     |
| Filter Integrity (> 50 psig) | 55       | 51        | 54        | 55        | 56        |
| pH (5-7)             | 7         | 6         | 7         | 6         | 6         |
| RTLC (Rf)            | 0.61      | 0.59      | 0.6       | 0.6       | 0.58      |
| Radio Chemical purity (>95%) | 97.8     | 98.3      | 98.7      | 97.9      | 98.2      |
| Half life (10 min)   | 10        | 10        | 10        | 10        | 10        |
| Measured Endotoxin (<175 EU/mL) | Pass    | Pass      | Pass      | Pass      | Pass      |
| Sterility            | No Growth | No Growth | No Growth | No Growth | No Growth |

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**Figure 1**: Pictorial presentation of the synthesizer MPS-100 ammonia tray

**Figure 2**: Software template screen of the ammonia tray of MPS-100
synthesizers have produced the final product in more than 95% radiochemical purity. The program template designed for MPS-100 complies with the test parameters that are set for the QC data to product required yield and purity of the final product.

With this production setup, uninterrupted service to the patients is offered. The installation of the cyclotron facility as well radiochemistry facility in the year 2015 and the number of scans, as well as the number of successful hot runs self-explain the robustness and reliability of the machine.

**Conclusions**

The ethanol method of production of ($^{13}$N)-NH$_3$ is well developed in our center, and our experience concluded that it has numerous benefits such as lower cost, low exposure to the worker, shorter synthesis time, and ease of operation. With our MPS-100 automated synthesizer, we have produced the clinical usable ($^{13}$N)-NH$_3$. Hence, our protocol is simple, reproducible, and robust to work over it.

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**Conflicts of interest**

There are no conflicts of interest.

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