PRODUCTION OF ^{13}N-LABELLED MOLECULAR NITROGEN FOR PULMONARY FUNCTION STUDIES

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SUMMARY

A method for the production of ^{13}NN either as a gas or as an injectable solution is described. The method is based on the $^{16}O(p,\alpha)^{13}N$ reaction and water as target material. Following irradiation, the $^{13}NO_2^-$ and $^{13}NO_3^-$ are reduced to $^{13}NH_3$, followed by NaOBr oxidation to ^{13}NN. With a beam current of 1 μA and 20 min irradiation time the injectable solution contained 148 MBq (4 mCi) per ml.

Key words : Nitrogen-13, injectable solution, pulmonary function

INTRODUCTION

Nitrogen-13 ($t_{\frac{1}{2}}$ = 9.96 min, β^+) labelled molecular nitrogen, either in the gaseous form for regional ventilation measurements or as an injectable solution for perfusion studies, has been used extensively (1,2,3,4). Compared with ^{133}Xe, this radiopharmaceutical offers several distinct advantages for pulmonary investigations; first, the lower solubility of ^{13}NN in tissue, and second, the positron decay which permits tomographic measurements by coincidence imaging devices. The short half life of ^{13}N necessitates production of the radioactivity directly in the vicinity of the nuclear medicine department. Continuous flow as well as batch processes have been described (1,5,6,7). The $^{12}C(d,n)^{13}N$ reaction has been used most commonly, but the $^{13}C(p,n)^{13}N$ reaction has also

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been used. Either CO_2, graphite or activated charcoal have been used as target material. Production systems, including target design and performance have been described in detail (5). A drawback of the reported methods is that only injectable solutions with relatively low radioactivity per ml can be obtained. We report here a batch method for the production of ^{13}NN either as a gas or as an injectable solution. The method is based on the $^{16}O(p,\alpha)^{13}N$ reaction. The main advantages of this method are the high absolute yield of ^{13}N and the very high radioactivity per ml of nitrogen gas or per ml of injectable ^{13}NN solution. Moreover the convenient transportation of the radioactivity as $^{13}NH_4^+$ from the cyclotron to the clinical facility and the simplicity of generating ^{13}NN, immediately before application, increase the present and potential use of these radiopharmaceuticals.

MATERIALS AND METHODS

All reagents used were pharmaceutical grade and were dissolved in sterilized water. With the exception of the ascorbic acid solution and the NaOBr solution, all the solutions were sterilized by heating for 20 minutes in saturated steam at 120^oC.

The preparation of ^{13}N-labelled molecular nitrogen, either in gaseous form or as an injectable solution for lung perfusion measurements is based on the oxidation of $^{13}NH_3$ by sodium hypobromite (7).

$$2\ ^{13}NH_3 + 3\ NaOBr \longrightarrow\ ^{13}N_2 + 3\ NaBr + 3\ H_2O$$

The ^{13}N-ammonia was prepared by irradiation of water with 20 MeV protons, followed by the reduction of $^{13}NO_3^-$ and $^{13}NO_2^-$ formed by consequence of the $^{16}O(p,\alpha)^{13}N$ reaction (8). The irradiations were carried out with the 280 cm AVF cyclotron of the University of Groningen. The reduction was accomplished with Devarda's alloy in alkaline solution and the $^{13}NH_3$ was collected by steam distillation (9).

The NaOBr solution was prepared by adding 3 ml Br_2 to an ice-cooled, sterilized solution of 8 g NaOH in 100 ml of water. This solution was used for

no longer than one month and was stored in the cold ($4^{o}C$) in the dark.

A 0.5 M phosphate buffer (pH 7.4) was prepared by dissolving 13.84 g of $Na_2HPO_4.2H_2O$ and 3.47 g of $NaH_2PO_4.2H_2O$ in water, adding 1 N NaOH or 1 N H_3PO_4 for pH adjustment, and bringing to a volume of 200 ml. This solution was put into 1 ml ampoules, that were sealed and sterilized.

An ascorbic acid solution was prepared by dissolving 20 g of ascorbic acid in 100 ml water. After aseptic membrane filtration the solution was put into sterile 1 ml ampoules, that were sealed and stored in the cold and in the dark.

For the preparation of an injectable ^{13}NN solution, the steam distillation was carried out in sterilized equipment. NaOBr solution (0.2 ml) was added to the first 2 ml of the distillate in a 3 ml volume sterilized vial. After 10 minutes 0.1 ml ascorbic acid solution was added, followed by 0.2 ml phosphate buffer; this resulted in a clear and colourless solution. The preparation was ready for injection 15 to 20 minutes after the end of bombardment. With a beam current of 1 μA and 20 minutes irradiation time the final preparation contained 370 MBq (10 mCi) in 2.5 ml.

When the labelled nitrogen gas was applied for lung ventilation studies, about 5 ml of the $^{13}NH_3$ steam distillate was collected in a vial containing 1 ml of a 1 M NH_4Cl solution. To generate the ^{13}NN, NaOBr solution was added (about 5 ml) to the stirred NH_4Cl solution until the yellow colour of the NaOBr remained. The radioactive N_2 which evolved was swept directly into a "bag in box" spirometer system or collected in a syringe.

DISCUSSION

When producing ^{13}NN gas for lung ventilation studies the volume of the steam distillate containing $^{13}NH_3$ was not critical. After mixing the distillate with the NaOBr solution more than 95% of the ^{13}N-radioactivity could be harvested. However, when preparing an injectable ^{13}NN solution, the fraction of the distillate with the highest amount of radioactivity was used. When the distillation was carried out as described previously (9) 49% of the $^{13}NH_3$ activity was in the first 2.0 ml. However, the 0.1 ml ethanol which was added

to the distillation mixture would also be in the distillate initially collected. This ethanol interferes with the sodium hypobromite reaction, forming acetaldehyde and acetate, and partially disturbs the oxidation of the ammonia. In a distillation without ethanol, only 24% of the $^{13}NH_3$ was in the first 2.0 ml. This can be explained by the large hold up in the bridge between reaction mixture and cooler (fig. 1). Heating the bridge to 105°C with heating bandage increased the $^{13}NH_3$ yield in the first 2.0 ml to 57%.

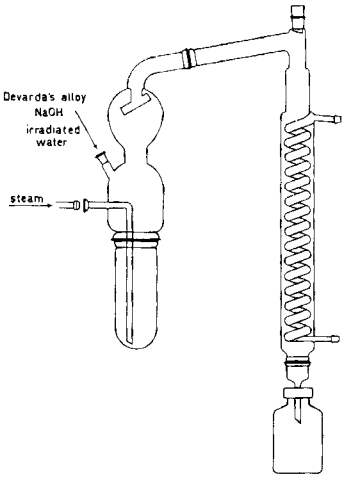

fig. 1. Steam distillation apparatus for the preparation of $^{13}NH_3$.

To study the oxidation of $^{13}NH_3$ into $^{13}N_2$, 0.5 ml 4N HCl (rather than the phosphate buffer) was added to the final product. The ^{13}NN was swept out by bubbling air through this solution. As a mean value from 10 experiments, 3.6% unconverted $^{13}NH_3$ was found after a 10 minute oxidation period. The $^{13}N_2$ was identified by radiogaschromatography.

Because NaOBr is not acceptable in a solution to be injected, removal of the excess NaOBr was tried by passing the solution through an ion-retardation resin. In view of the irreproducable results, possible contamination with pyrogens, and more difficulty in handling, the suggestion of Krizek (10) to eliminate the excess OBr^- with ascorbic acid was followed:

$$OBr^- + \text{ascorbic acid} \longrightarrow Br^- + \text{dehydroascorbic acid}$$

Under our conditions more than 99.5% of the hypobromite was destroyed, as determined with spectrophotometry. The dehydroascorbic acid that is formed can also react with hypobromite to form oxalic acid and threonic acid a normal urinary organic acid (11-15). When all the ascorbic acid in the injectable solution is converted into oxalic acid the maximum concentration would be 4 mg/ml. Conversion of all the ascorbic acid into threonic acid would result in a threonic acid concentration of less than 6 mg/ml.

After adding the ascorbic acid, the pH of the preparation is slightly acidic ($\sim$ pH 4). Since rapid intravenous injection could cause irritation, the radiopharmaceutical had to be buffered. Due to the similarity to the blood buffer system, a carbonate/bicarbonate buffer is best suited. Our choice, however, was a phosphate buffer, because in acidic medium the carbonate/bicarbonate buffer produces CO_2, and the CO_2 drives the ^{13}NN out of the solution.

The stability of the NaOBr solution is optimal at pH 11.4. A stability study of the sodium hypobromite solution was performed. The solution was analysed spectrophotometrically using the absorbance maximum at 330 nm and diluting 0.5 ml of the NaOBr solution to 100 ml with water. The shelf life was taken to be the time required for 10% decomposition. When stored in the light and at room temperature the tenability was two days. When the solution was stored in the cold and the dark the shelf life was one month.

Preferentially a solution with the same osmotic value as blood (290 mOsm) is used for injection. The mean osmotic value of ten of our preparations was 535 mOsm, which is acceptable when only small volumes are injected. The osmolarity could be reduced by dilution if necessary.

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