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Research Article

Assessment of Stability and Economic Viability of Reconstituted HMPAO Formulations for Brain Imaging Applications

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Abstract

Background: The unusual *in vitro* instability of ^{99m}Tc-HMPAO is considered a major disadvantage as the labeled product must be injected within 30 minutes of reconstitution of HMPAO vial. In the present studies it was our interest to check whether HMPAO once reconstituted could be radiolabeled later with sufficient efficiency when stored at appropriate storage conditions.

Methodology: Percentage labeling efficiency of ^{99m}Tc-HMPAO, following storage of reconstituted HMPAO at different temperatures (-20°C to + 20°C) for different storage time (day zero to day 21 of storage), was investigated.

Results: The percentage of primary ^{99m}Tc-HMPAO on day 1 of storage of fractionated HMPAO at -20°C was greater than 80% which is considered suitable for brain uptake studies. The percentage decreased to 46 and 49 following storage at 4°C and 20°C respectively. Further, percentage of primary ^{99m}Tc-HMPAO was found to be maximum on the day of preparation i.e. day zero of storage and decreased steadily with time till Day 21. Similar pattern was observed on subjecting the fractionated HMPAO to different storage temperatures.

Conclusion: These results demonstrated that a vial of HMPAO after reconstitution can be fractionated and stored at -20°C for next 24 hours with no loss of radiolabeling efficiency and considerable cost savings. Storage longer than 24 hours at any temperature, does not improve the radiolabeling efficiency of ^{99m}Tc-HMPAO.

Keywords: HMPAO; Reconstitution; Stannous chloride; *In vitro* stability

Introduction

Over the years several new radiopharmaceuticals, labeled with different radioisotopes (^{75}Se, ^{123}I, ^{201}Tl) have been tested for routine imaging of regional Cerebral Blood Flow (rCBF), using rotating head Single Photon Emission Computed Tomography (SPECT) [1-5]. However, due to reasons such as poor physical characteristics, high production cost and limited availability, none of these agents found wide application on routine cerebral blood flow imaging. Thus considerable work was initiated on the development of technecium (^{99m}Tc) agents as ^{99m}Tc has certain advantages over other radionuclides such as easy availability, low generation cost and reduced radiation exposure to patients [6].

In order to cross the Blood Brain Barrier (BBB), the technetium complex should be lipophilic in nature, small in size and should have a net zero charge. The main biologic requirement for such a radiopharmaceutical besides the penetration of BBB is to distribute in the brain, proportional to the blood flow. Once in the brain, the tracer should have a fixed regional distribution for time, sufficient to permit image acquisition, which for a rotating gamma camera, is typically 20-30 min.

During the last few years a series of ligands based upon Propylene Amine Oxime (PnAO) were developed and evaluated as possible technetium labeled brain imaging agents. The research finally led to the development of d, l- HMPAO (Hexamethylene Propylene Amine Oxime), which has been selected as the preferred ligand for ^{99m}Tc, in cerebral perfusion imaging [7].

However, the unusual in-vitro instability of the complex is considered a major disadvantage [8-15] and thus, formulation of instant freeze dried kits for the preparation of good quality ^{99m}Tc-HMPAO complex, requires several precautions during manufacturing process. After reconstitution, the labeled HMPAO complex must be injected within 30 minutes. The chemical structures of HMPAO includes the lipophilic d,l form and the hydrophilic meso form and both structures can be reduced to 5 positive charges using tin and labeling with ^{99m}Tc to produce Tc-^{99m} HMPAO. Under normal situations, the d, l form of HMPAO is spontaneously converted to the meso form, and it then loses its lipophilic property to cross the BBB [8,9]. Therefore, some studies were conducted in attempts to improve or prolong the stability of the d, l form of Tc-^{99m} HMPAO.

Many attempts have been made to improve the utilization of the ^{99m}Tc-HMPAO [10-18]. One approach was addition of stabilizers like cobalt chloride, methylene blue and gentisic acid while radiolabeling, in order to improve the stability of the labeled complex, and the other approach was fractionating and freezing HMPAO vial contents for

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subsequent use. The latter approach is concerned with employing an effective way of utilizing HMPAO rather than prolonging its stability after radiolabeling. This could be done by fractionating the vial contents after reconstitution so that the stored aliquots can be used as and when required. In this regard, the stability of the stored aliquots needs further investigation with regard to storage temperatures as a function of time, since previous reports that demonstrate increase in the stability of labeled HMPAO following storage of reconstituted, fractionated HMPAO at low temperatures [19-21], show varied results and therefore it is difficult to conclude the time for which reconstituted HMPAO would be suitable for use. In the present study, it was our interest to see whether HMPAO once reconstituted can be used again, if it is stored at an appropriate temperature and for suitable time duration without affecting its radiolabeling efficiency.

Materials and Methods

Chemicals

Sodium Pertechnetate ($^{99m}TcO_4^-$) was procured from Post Graduate Institute of Medical Education and Research (PGIMER) Chandigarh. Hexamethylene Propylene Amine Oxime (d, l-HMPAO) (Exametazine) was purchased commercially from CERETECTM GE Healthcare, UK. Stannous Chloride was purchased from Sigma. Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) strips were purchased from MERCK, Sodium Chloride (NaCl), was purchased from SRL.

Fractionation of reconstituted HMPAO

Fractionation of HMPAO cold kit was performed inside a laminar flow. A vial of HMPAO was reconstituted with 1.3ml of N_2 purged normal saline. The content of the vial was then fractionated into aliquots in aseptic vials with each containing 50µl of reconstituted HMPAO and then purged with N_2 before being sealed with rubber caps and stored in the freezer. The fractionated vials were kept at different storage temperatures (-20°C, +4°C and +20°C), with 8 vials at each temperature condition. One vial from each lot was labeled with ^{99m}Tc and was tested for quality control on DAY 1, DAY 7, DAY 14, and DAY 21 and the day of reconstitution of the stock vial was considered as Day 0.

Preparation of ^{99}mTc HMPAO

Sodium Pertechnetate ($^{99m}TcO_4^-$) was eluted with N_2 purged saline within 24 hours of receiving the $^{99Mo/99m}Tc$ generator (ISORAD Israel) and eluted ^{99m}Tc was used within 1hr of elution.^{99}Mo content in eluate was checked and found to be within limit (0.015µCi/mCi). Vials stored at different temperatures were brought to room temperature. Top of the vial was wiped with 70% isopropyl alcohol and allowed to dry and then 60µCi (2.22 MBq) of ^{99m}Tc was added to the vial. The contents in the shielded vial were mixed by inverting the vial. ^{99m}Tc-HMPAO was used within 30 minutes.

Radiochemical purity

Radiochemical purity of ^{99m}Tc-HMPAO was determined by carrying out chromatographic studies. Briefly, a single spot from the preparation was applied on the Whatman no.1 paper and ITLC-SG strips of appropriate width and length (0.5 x10cm). Strips were then placed in tubes containing 0.9% Saline, Acetonitrile (ACN): Distilled water(1:1) and Butan-2-one, as running solvents to measure the amount of free $^{99m}TcO_4^-$, reduced/hydrolyzed-^{99m}Tc (^{99m}Tc-RH) fraction, lipophilic technetium Exametazine complex [^{99m}Tc-HMPAO] and secondary technetium Exametazine complex [^{99m}Tc-SHMPAO] in the preparation. After the solvent touched the earmarked line on the chromatographic paper, the strips were air dried, cut into 0.5cm long sections and then counted for activity using well-type gamma-sensitive probe (Nucleonix, India). Interpretation of chromatogram systems was done according to the literature reported procedure [22,23]. The percentage of impurities was calculated using the formula:

^{99m}Tc-HMPAO (lipophilic) = 100 – (A + B)

Where,

A = ^{99m}Tc-SHMPAO + ^{99m}Tc-RH (i.e. system 1 origin);

B = free $^{99m}TcO_4^-$ (i.e. system 2, solvent front);

C = ^{99m}Tc-RH (i.e. system 3, origin).

Interpretation of chromatograms

System 1 - ITLC: butan-2-one

^{99m}Tc-SHMPAO and ^{99m}Tc-RH remain at the origin; Rf= 0

Lipophilic ^{99m}Tc-HMPAO and free $^{99m}TcO_4^-$ migrate to solvent front; Rf = 0.8-1.0.

System 2 - ITLC: 0.9% sodium chloride

Lipophilic ^{99m}Tc-HMPAO, ^{99m}Tc-SHMPAO and ^{99m}Tc-RH remain at the origin; Rf= 0

Free $^{99m}TcO_4^-$ migrates to solvent front; Rf =0.8-1.0.

System 3-Whatman paper 1: ACN: distilled water (1:1)

Lipophilic ^{99m}Tc-HMPAO, ^{99m}Tc-SHMPAO migrate to solvent front; Rf =0.8-1.0.

^{99m}Tc-RH remain at the origin; Rf= 0

Results and Discussion

A significant variation in radiochemical purity of ^{99m}Tc-HMPAO was observed following storage of reconstituted HMPAO formulations at different temperatures and storage time. The results show that there is an overall decrease in percent radiolabeling efficiency of HMPAO formulation when stored for different time durations and at varied temperature conditions. However, the rate is slower at lower temperatures as compared to higher ones.

As shown in (Table 1,2 and 3), percentage of primary ^{99m}Tc-HMPAO was found to be highest on the day of reconstitution (Day 0) and decreased steadily with time till the third week. Similar pattern was observed on subjecting the fractionated HMPAO to different storage temperatures. The percentage of primary ^{99m}Tc-HMPAO on DAY 1 of storage of fractionated HMPAO at -20°C (Table 1) was greater than 80% which is considered suitable for brain uptake studies while the percentage was as low as 46 and 49 percent, following storage for the same time period at 4°C and 20°C respectively (Table 2 and 3). The ^{99m}Tc-RH component was less than 10% and free $^{99m}TcO_4^-$ was less than 6% when HMPAO formulations stored at -20°C for 24 hours were radiolabeled with ^{99m}Tc.

Baker [16] in his study of stannous pyrophosphate augmentation

Table 1: Time dependant variation in % labeling efficiency of ^{99m}Tc-HMPAO following storage of fractionated HMPAO at -20°C.

DAYS	% RHT + % ^{99m}Tc-SHMPAO	% Free ^{99m}Tc	% ^{99m}Tc-RH	% LABELING
DAY 0	3.9±0.8	6.4±2.2	2.8±0.3	89.7±3.3
DAY 1	7.4±1.6	9.6±2.3	5.8±1.3	83.0±2.6
DAY 7	7.4±3.4	22.5±3.5	11.8±3.2	60.0±4.5
DAY 14	10.4±2.4	57.0±3.4	9.2±2.4	32.6±5.7
DAY 21	4.3±1.2	67.2±5.5	3.9±3.2	28.6±6.7

Results are expressed as mean± standard deviation
Reduced hydrolysed Technecium (RHT), Technecium labeled Secondary HMPAO (^{99m}Tc- **S**HMPAO**)**

Table 2: Time dependant variation in % labeling efficiency of ^{99m}Tc -HMPAO following storage of fractionated HMPAO at +4°C.

DAYS	% RHT + % ^{99m}Tc-SHMPAO	% Free ^{99m}Tc	% ^{99m}Tc-RH	% LABELING
DAY 0	3.9±1.2	6.4±1.3	2.8±1.1	89.7±3.2
DAY 1	6±1.1	47.8±3.3	5.2±1.4	46.2±3.2
DAY 7	7.2±2.3	64.2±3.5	6.7±2.2	28.6±3.6
DAY 14	1.4± 0.8	76.7±5.5	1.2±0.3	21.8±4.2
DAY 21	2.1±1.1	79±4.6	1.4±0.6	18.9±5.2

Results are expressed as mean± standard deviation
Reduced hydrolysed Technecium (RHT), Technecium labeled Secondary HMPAO (^{99m}Tc- **S**HMPAO**)**

of fractionated cold kits at -20°C came up with the results that those vials passed the quality control till 1 week, giving a radiochemical purity of > 90% irrespective of the length of storage time. However, the results in the present study shows that the contents of the vial were usable (radiochemical purity of > 70%) only up to 24 hours following fractionation and storage at -20°C.

Hawkins et al. [17] in his study of fractionation of HMPAO cold kits at low temperature storage at -66°C showed that the vials were stable with radiochemical purity value > 55% till 24th day, whereas results in the present study show that >55% values of quality control was seen in vials stored at -20°C till 1 week, and vials stored at 4°C and 20°C showed <55% after 24 hours.

The results in the present study clearly indicate that if the reconstituted HMPAO fractions are stored at -20°C, then the chemical stability of HMPAO can easily be maintained up to 24 hours post reconstitution, which would be an economically viable proposition for laboratories with limited financial resource.

Conclusion

The study highlights that reconstituted HMPAO fractions when stored at -20°C for up to 24 hours can be successfully radiolabeled with a radiochemical purity of >80%.

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Table 3: Time dependant variation in % labeling efficiency of ^{99m}Tc-HMPAO following storage of fractionated HMPAO at +20°C.

DAYS	% RHT + % ^{99m}Tc-SHMPAO	% Free ^{99m}Tc	% ^{99m}Tc-RH	% LABELING
DAY 0	3.9±1.2	6.4±1.3	2.8±1.1	89.7±3.2
DAY 1	7.7±2.2	42.6±3.1	5.9±1.4	49.7±3.3
DAY 7	9.2±2.2	60.6±4.3	7.8±1.5	30.2±2.4
DAY 14	6.1±2.3	62.4±1.2	4.9±2.4	31.4±3.6
DAY 21	18.7±3.3	71.6±4.5	6.8±1.3	9.7±2.1

Results are expressed as mean± standard deviation
Reduced Hydrolysed Technecium (RHT), Technecium labeled Secondary HMPAO (^{99m}Tc- **S**HMPAO**)**

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