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PRELIMINARY STUDIES FOR PREPARATION OF DMSA-V KIT FOR LABELLING WITH ^{99m}Tc

Tânia P. Brambilla[1], João A. Osso Jr.[1]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
taniabrambilla@yahoo.com.br
jaosso@ipen.br

ABSTRACT

Technetium-99m is the most useful radionuclide in diagnostic imaging procedures in Nuclear Medicine, more than 80 percent of radiopharmaceuticals are ^{99m}Tc-labeled compounds. The reason for such a position of ^{99m}Tc in clinical use is its favorable physical characteristics (half life of 6.02 h, gamma emission of low energy, 140 keV, and absence of particulated emission). ^{99m}Tc-DMSA (V) is used for the detection of medullary thyroid cancer and other soft tissue tumors. Other studies carried out by this compound include head and neck tumors, liver and skeletal metastases from breast carcinoma. The aim of this work is the development of a lyophilized DMSA-V kit for the one step labeling with ^{99m}Tc, and to compare it with the two steps labeling method performed by pH adjustment of a DMSA (III) kit. The radiochemical purity was evaluated by thin layer chromatography on sílica gel (TLC-SG), developing with n-butanol/acetic acid/ H_2O (3:2:3) solvent, ^{99m}Tc-DMSA (III) stayed at the origin (Rf= 0), and ^{99m}Tc-DMSA (V) (Rf = 0.7) migrated more slowly toward the solvent front than $^{99m}TcO4^-$ (Rf = 1). When developing with water, $^{99m}TcO_2$ stayed at the origin and the other species migrated to the solvent front. The results so far showed good labeling yields of ^{99m}Tc-DMSA (V).

1. INTRODUCTION

Technetium-99m is the most useful radionuclide in diagnostic imaging procedures in Nuclear Medicine, more than 80 percent of radiopharmaceuticals are ^{99m}Tc-labeled compounds. The reason for such a position of ^{99m}Tc in clinical use is its favorable physical characteristics of decay, like short half life of 6.02 h, gamma emission of low energy (140 keV) and absence of particulated emission. It can be easily obtained from a ^{99}Mo/^{99m}Tc generator system, that allows the use of this radiopharmaceutical in different locals, far from the production center. This radionuclide is formed by the β^- decay of ^{99}Mo, adsorbed onto an alumina column of the generator, and collected in the form of sodium pertechnetate ($Na^{99m}TcO_4$) by elution with saline solution (0.9%). Another advantage of the ^{99m}Tc is that it can efficiently label many kits by one step method [1,2]. One of the many kits labeled with ^{99m}Tc is the DMSA (dimercaptosuccinic acid), in two different forms: ^{99m}Tc-DMSA (III) and ^{99m}Tc-DMSA (V). The ^{99m}Tc-DMSA (III) is prepared in acidic medium (pH 2 - 3) whereas ^{99m}Tc-DMSA (V) is prepared in basic medium (pH 8.0 - 8.5) and so their biological behavior is different [2,3]. ^{99m}Tc-DMSA (III) is widely used for renal cortical imaging and ^{99m}Tc-DMSA (V) has been reported to accumulate in medullary carcinoma of the thyroid (MCT) and some other soft tissue tumors. Other studies carried out by this agent include head and neck tumors, brain, liver and skeletal metastases from breast carcinoma [4]. Recently it has been studied for imaging of small cell and non small cell lung cancer, imaging patients with bone metastases, hepatocellular carcinoma and bone tumors [5].

^{99m}Tc-DMSA (V) can be prepared by two methods, one of them, here called two steps method, uses a commercial kit of ^{99m}Tc-DMSA (III) and a certain amount of $NaHCO_3$ to elevate the pH to 8.0 – 8.5. The other method, here called one step method, is a lyophilized formulation of the DMSA(V), where the product will be ready to be labeled with ^{99m}Tc. The Brazilian Nuclear Medicine community has great interest in this radiopharmaceutical and the final result of this project is to make possible its routine production with the adequate quality requirements. Adams et al [6] and Hirano et al [7] have used a commercial DMSA(III) kit to prepare the ^{99m}Tc-DMSA (V) by adding 7% $NaHCO_3$ to the kit and then $Na^{99m}TcO_4$ in 0.9% NaCl. The obtained labeling yields were › 90% and 83%, respectively. Mushtaq et al [4] prepared ^{99m}Tc-DMSA (V) by the two methods and the labeling yield for both was › 95%.

The aim of this work is the development of a lyophilized DMSA (V) kit for the one step labeling with ^{99m}Tc. In order to achieve it this study started with the definition of the radiochemical quality control methodology, evaluation of the labeling yields of the ^{99m}Tc-DMSA (V) prepared using a commercial DMSA (III) and then the preliminary results from the direct formulation of DMSA (V) and comparison of the results.

2. MATERIALS AND METHODS

2.1. Preparation and labeling of DMSA (V)

2.1.1. Two steps method

^{99m}Tc-DMSA (V) was prepared using a commercial DMSA (III) kit from IPEN-CNEN/SP. The kit contained 1.0 mg of DMSA, 0.44 mg of $SnCl_2.2H_2O$, 0.70 mg of ascorbic acid and 50 mg of inositol. The kit was dissolved with 0.05 mL of 7% $NaHCO_3$ that also elevated the pH to 8.5 and then 630 MBq of $Na^{99m}TcO_4$ in 1 mL of 0.9% NaCl was added. $^{99m}TcO_4^-$ was eluted from a ^{99}Mo-^{99m}Tc generator from IPEN-CNEN/SP. The solution was stirred and let to react for different lengths of time (0, 0.5, 1, 2 and 4 hours) at room temperature. Samples were then taken to perform the radiochemical quality control.

2.1.2. One step method

^{99m}Tc-DMSA (V) was prepared in a liquid kit that contained 1.8 mg of DMSA and 1.12 mg of $SnCl_2.2H_2O$ in a total volume of 1 mL. DMSA from Merck was dissolved in 7% $NaHCO_3$ and $SnCl_2.2H_2O$ was dissolved in 0.1 N HCl and left for 20 min under a N_2 gas stream. The pH of the liquid kit was adjusted to about 8.5 with NaOH and then 740 MBq of $Na^{99m}TcO_4$ in 1 mL of 0,9% NaCl was added to the liquid kit. $^{99m}TcO_4^-$ was eluted from a ^{99}Mo-^{99m}Tc generator from IPEN-CNEN/SP. The solution was stirred and incubated for 30 minutes at room temperature. Samples were then taken to perform the radiochemical quality control.

2.2. Radiochemical quality control

The radiochemical purity was evaluated by thin layer chromatography (TLC) on silica gel (TLC-SG) to determine the labeling efficiency and impurity formation. TLC-SG strips (1.5 x 12 cm) were developed in two different solvent systems. A solvent system containing: n-butanol/acetic acid/ H_2O (3:2:3 by volume) was used in order to separate ^{99m}Tc-DMSA (V) from $^{99m}TcO_4^-$ and ^{99m}Tc-DMSA (III). The second system used water as solvent in order to determine $^{99m}TcO_2$, that stayed at the origin and the other species migrated with the solvent front.

The R_f of the different species was confirmed experimentally, in particular the R_f for TcO_2 in the first solvent, that was not stated in the original publication [7]. High amounts of TcO_2 were prepared by adding a high mass of Sn to freshly eluted $^{99m}TcO_4^-$. The R_f value of DMSA (III) was also confirmed by labeling the commercial kit according to manufacture's instructions.

After the TLC run, the strips were cut in 1 cm pieces and the radioactivity determined using a calibrated hyperpure Germanium detector model GX1518 (HPGe) coupled to a multichannel analyzer system (Canberra Inc., USA).

3. RESULTS AND DISCUSSION

3.1 Radiochemical quality control

Table 1 shows the R_f values for the different species that can be found in the labeling of DMSA (V). It is clear that all the species can be evaluated using the two solvent systems

Table 1. R_f of different species of ^{99m}Tc

TLC-SG	R_f of different species			
Solvent	^{99m}Tc-DMSA V	^{99m}Tc-DMSA III	$^{99m}TcO_4^-$	$^{99m}TcO_2$
n-butanol/ acetic acid/ H_2O (3:2:3)	0.7	0-0.1	1	0-0.1
H_2O	1	1	1	0

Figure 1 shows the chromatogram of $^{99m}TcO_2$ when the solvent n-butanol/ acetic acid/ H_2O was employed. The R_f of $^{99m}TcO_2$ is well determined to be 0-0.1, while a small peak of $^{99m}TcO_4^-$ can be seen at R_f 0.9 and also a small peak at R_f 0.6-0.7 probably due to a different reduced specie of ^{99m}Tc.

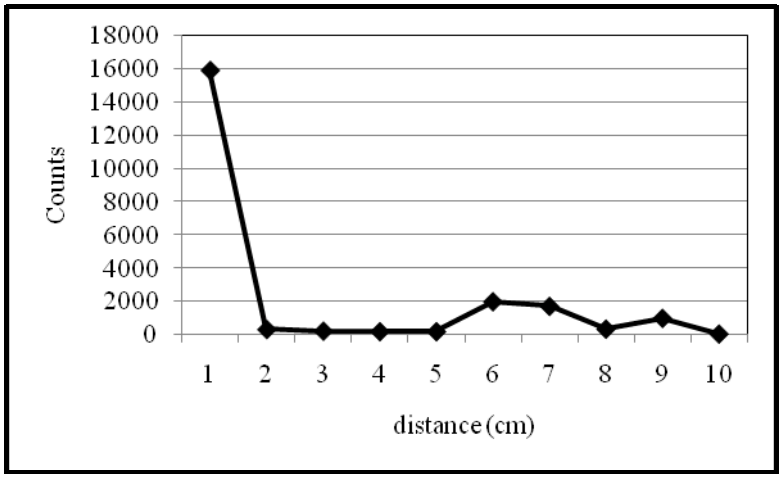

Figure 1. Radiochromatogram of $^{99m}TcO_2$ for the solvent n-butanol/ acetic acid/ H_2O

3.2. Preparation and labeling of DMSA (V)

3.2.1. Two steps method

The results of the labeling of DMSA (V) using the two steps method at different reaction times can be seen in figure 2.

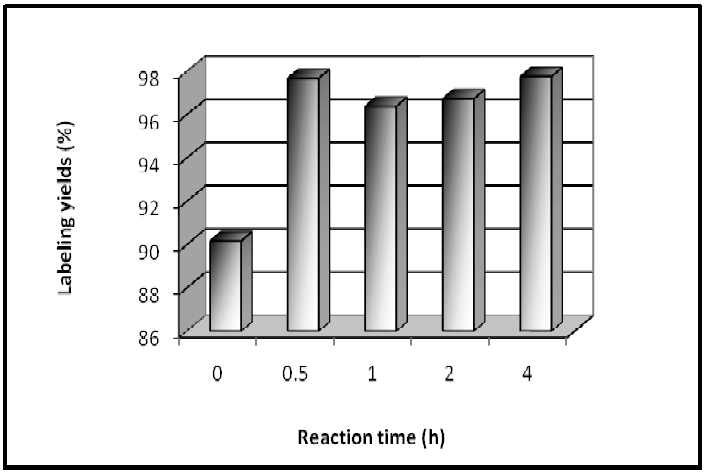

Figure 2. Labeling yield of DMSA (V) with the variation of reaction time using the two steps method

All the labeling yields were high (>95%), with the exception of the result of the instantaneous labeling of DMSA (V). For production purposes the reaction time of choice was 30 min, because of the high yield and less loss of ^{99m}Tc due to its decay. These results also represented the stability of the labeled compound at room temperature, that had a high radiochemical percentage even 3.5 hours after the labeling.

3.2.2. One step method

The preliminary experiments with the liquid kit showed a low labeling yield, less than 65%. A typical result can be seen in Table 2.

Table 2. Typical results of the labelling of DMSA (V) with the one step method

^{99m}Tc Species	(%)
DMSA (V)	65.06
DMSA (III)	24.82
TcO_4^-	1.05
TcO_2	9.07

According to Table 2, the highest impurity was DMSA (III) that suggests that the pH is not appropriate for the labeling in these conditions, although this pH gave excellent results for the two steps labeling method. One must also remember that the commercial DMSA (III) kit had other components that were not present in the one step labeling method.

4. CONCLUSIONS

The very first results obtained with the one step method of labeling of DMSA (V) with ^{99m}Tc were presented and several parameters must be optimized in order to get a good labeling yield and later start with the lyophilization studies. Very good results were shown with the two steps method and a throughout study of the radiochemical quality control technique was also performed in order to certify the results obtained.

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