2011 International Nuclear Atlantic Conference - INAC 2011
Minas Gerais,MG, Brazil, October 24 to 28, 2011
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
***ISBN:* 978-85-99141-04-5**

OPTIMIZATION OF LABELING PROCEDURE OF ^{188}Re-DMSA(V)

Danielle M. Dantas[1], Tânia P. Brambilla[1], Nicoli F. Reis, 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
danielle_2705@hotmail.com
taniabrambilla@yahoo.com.br
jaosso@ipen.br

ABSTRACT

Radionuclide therapy (RNT) is emerging as an important tool of nuclear medicine. Apart from the well established ^{131}I, several other promising radionuclides have been identified, among them ^{188}Re, ^{90}Y and ^{177}Lu. ^{188}Re has received a lot of attention in the past decade, due to its favourable nuclear characteristics [$t_{1/2}$ 16.9 h, $E_{\beta\ max}$ 2.12 MeV and E_{γ} 155 keV (15%) suitable for imaging, including the fact that it is carrier-free and can be obtained cost-effectively through the generator ^{188}W-^{188}Re. Biodistribution studies of ^{188}Re-DMSA(V) have shown that its general pharmacokinetic properties are similar to that of ^{99m}Tc-DMSA(V), so this agent could be used for targeted radiotherapy of medullary thyroid carcinoma, bone metastases, soft tissue and others tumors. The aim of this work is to evaluate two labeling procedures for the preparation of ^{188}Re-DMSA(V). The first method was prepared using a commercial kit of DMSA(III) for labeling with ^{99m}Tc at high temperature (100^{o}C). The second method was prepared in a vial containing 2.5 mg of DMSA, 1.00 mg of $SnCl_2.2H_2O$ and 10 mg of sodium oxalate, 10 mg of ciclodextrin, in a total volume of 2.0 mL. The pH was adjusted to 3 with 37% HCl. After labeling the solution was stirred and incubated for 30 min at room temperature. The radiochemical purity was determined using TLC-SG developed with two different solvent systems: Acetone and glycine. Preliminary results for both methods of labeling ^{188}Re-DMSA(V) showed that the labeling yield was >95%. Further experiments are also necessary to optimize the labeling methodology of ^{188}Re-DMSA(V).

1. INTRODUCTION

Radionuclide therapy (RNT) is emerging as an important tool of nuclear medicine. Apart from the well established ^{131}I, several other promising radionuclides have been identified, among them ^{188}Re, ^{90}Y and ^{177}Lu are considered to be the most promising radionuclides for *in vivo* therapy (Sarkar et al.,2009). ^{188}Re has received a lot of attention in the past decade, due to its favourable nuclear characteristics: $t_{1/2}$ 16.9 h, $E_{\beta\ max}$ 2.12 MeV and E_{γ} 155 keV (15%) suitable for imaging, including the fact that it is carrier-free and can be obtained cost-effectively through the generator ^{188}W-^{188}Re (Brambilla, 2009). The radionuclide ^{188}Re is a β^- particle emitter with excellent physical properties for therapeutic use in nuclear medicine. In addition, it emits gamma rays (γ) that can be used to monitor the effectiveness of therapy by means of scintigraphic images and perform a dosimetric study (Marczewki, 2006). ^{188}Re has been administered along with therapeutic molecules, peptides and monoclonal antibodies. Biphosphonates labeled with^{188}Re are used in the treatment of bone metastasis and pain in the palliative treatment of metastases, giving excellent selectivity. (Knapp, 1998) Thyroid carcinomas (Magalhães et al., 2003), prostate and soft tissues treatments are also a proposed for radiopharmaceuticals prepared with^{188}Re $^{\cdot}$(Knapp,1998). Biodistribution studies of ^{188}Re-DMSA(V) have shown that its general pharmacokinetic properties are similar to that of ^{99m}Tc-DMSA(V), so this agent could be used for targeted radiotherapy of medullary thyroid

carcinoma, bone metastases, soft tissue and others tumors. The aim of this work is to evaluate two labeling procedures for the preparation of ^{188}Re-DMSA(V) by two different methods.

2. MATERIALS AND METHODS

2.1. Preparation of ^{188}Re-DMSA(V)

2.1.1. Method I

Initially ^{188}Re-DMSA(V) was prepared using a commercial kit of DMSA (III) for labeling with ^{99m}Tc (IPEN-CNEN/SP). The kit contained 1.0 mg de DMSA, 0.44 mg de $SnCl_2.2H_2O$, 0.70 mg of ascorbic acid and 50 mg de inositol. The labeling was done with 1 mL of $^{188}ReO_4^-$ (~185MBq) and the reaction time was 30 minutes at high temperature (100 ^oC). The variables studied were: reaction temperature (100 °C and room temperature), reaction time (20 and 30 minutes) and volume of $^{188}ReO_4^-$ (1.0 and 2.0 mL).

2.1.2. Method II

This method is based on the formulation of ^{188}Re-DMSA (V) described by Bolzati et. al., 2000, which contained 0.2 mg of $SnCl_2.2H_2O$, 2.5 mg of DMSA, 10 mg of γ-ciclodextrina and 13mg of potassium oxalate. The pH of the solution is kept around 5 and labeled with 0.25 mL of $^{188}ReO_4$, with reaction time of only 15 minutes without heating. The variables studied were:

- $SnCl_2.2H_2O$ mass (0.2, 0.6, 1.0, 1.5 and 2.0 mg);
- Mass of DMSA (2.5 and 5.0 mg);
- Mass of sodium oxalate (10, 20, 30 mg);
- Mass of cyclodextrin (5, 10, 20 mg);
- Labeling time (0, 15, 30 min);
- pH (3.5, 4.0 and 5.0);
- Volume of ^{188}Re (1ml and 2 ml);
- Labeling Stability: 1, 2, 4 and 24 hours at room temperature.

2.1.3. Radiochemical quality control

The radiochemical purity was evaluated by thin layer chromatography 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. Acetone was used in order to separate ReO_4^- (R_f 1) from ^{188}Re-DMSA(V) and ReO_2 (R_f 0) and 5% glycine was used in order to separate ReO_2 (R_f 0) from ^{188}Re-DMSA(V) and ReO_4^- (R_f 1).

The distribution of radioactivity on the TLC-SG strips was determined using a calibrated Germanium hyperpure detector model GX1518 (HPGe) coupled to a multichannel analyzer system (Canberra Inc., USA).

3. RESULTS AND DISCUSSION

3.1. Preparation of ^{188}Re-DMSA(V)

3.1.1. Method I

Figures 1, 2 and 3 show the results of the effect of the variation of reaction temperature and time and volume on the labeling efficiency of ^{188}Re-DMSA (V) prepared with a commercial kit of ^{99m}Tc-DMSA (III). The best results of labeling yield (> 98%) were achieved when it was used 30 minutes of reaction time with heating at 100 °C and 1 mL of $^{188}ReO_4^-$.

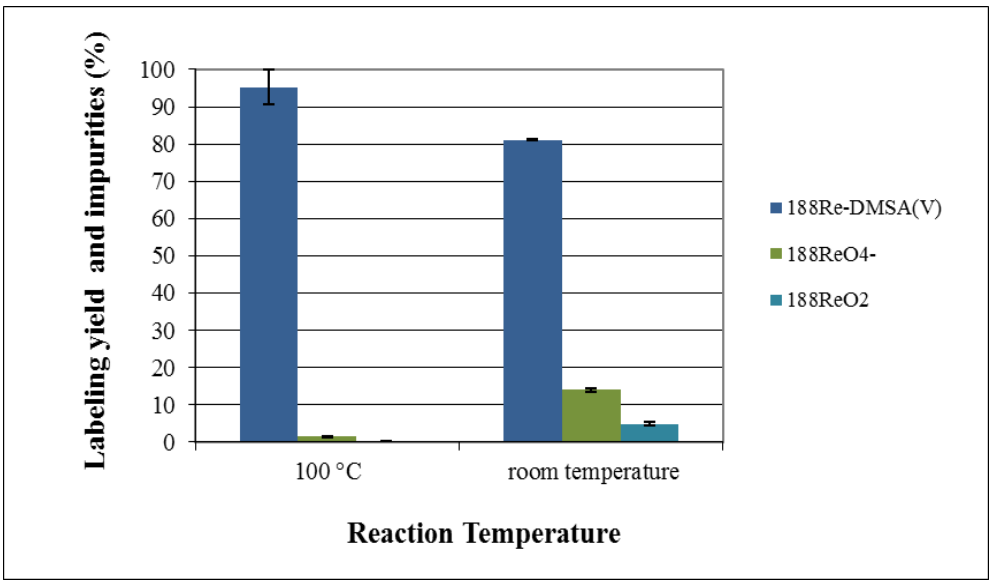

Figure 1: Labeling yield with the variation of reaction temperature.

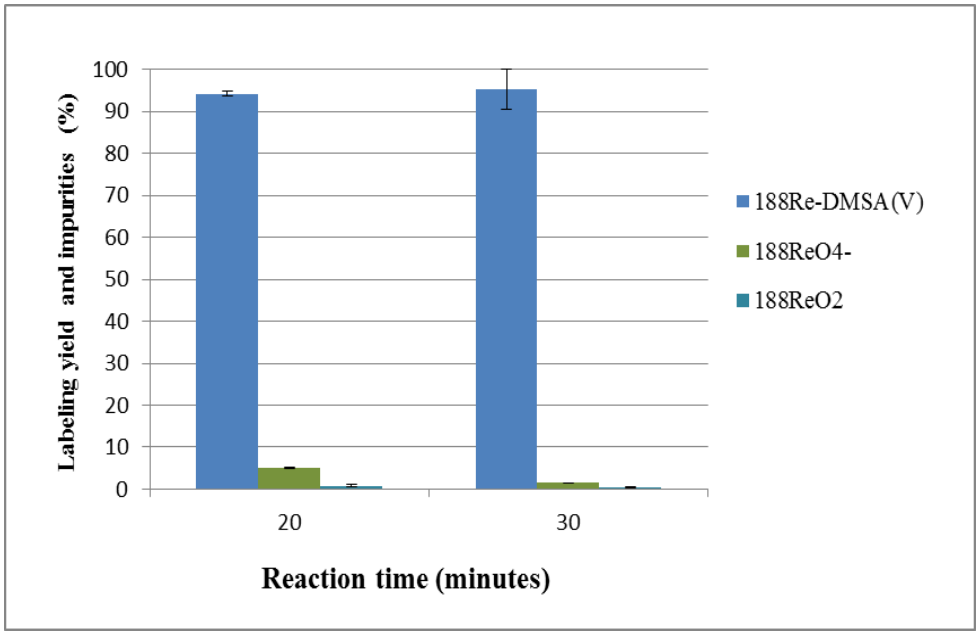

Figure 2: Labeling yield with the variation of reaction time.

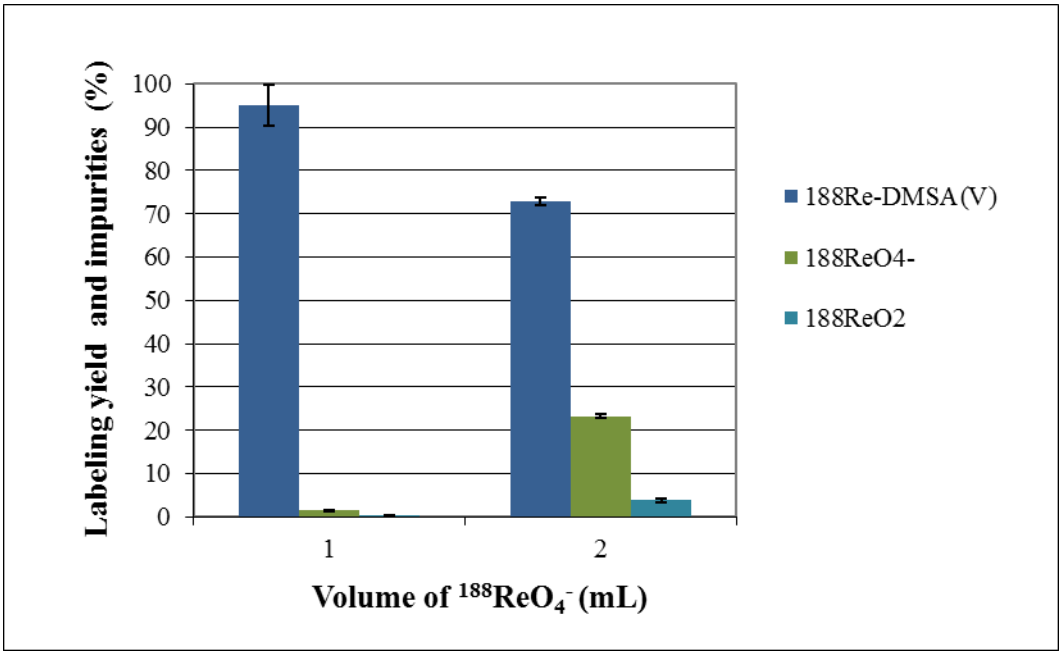

Figure 3: Labeling yield with the variation of volume of $^{188}ReO_4^-$.

3.1.2. Method II

Figure 4 shows the results of the variation of the mass of oxalate using the conditions described in method II. The best results were achieved with 10mg of oxalate.

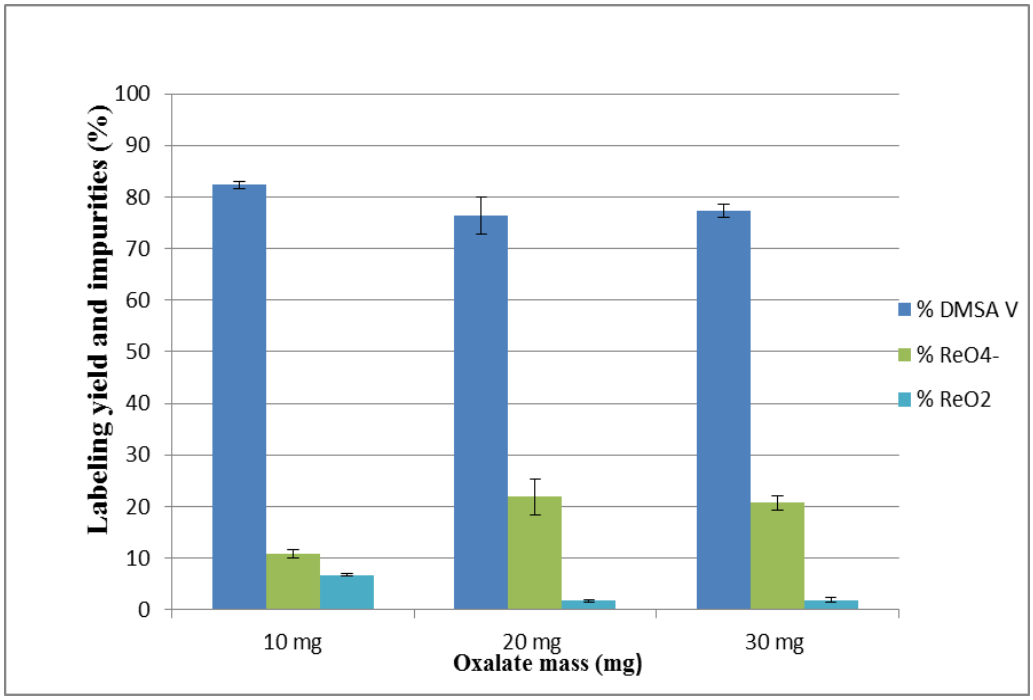

Figure 4: Labeling yield with the variation of sodium oxalate mass.

The results of the variation of the mass of the reducing agent $SnCl_2.2H_2O$ are shown in Figure 5. The minimum mass that gave good yield was 1.0 mg.

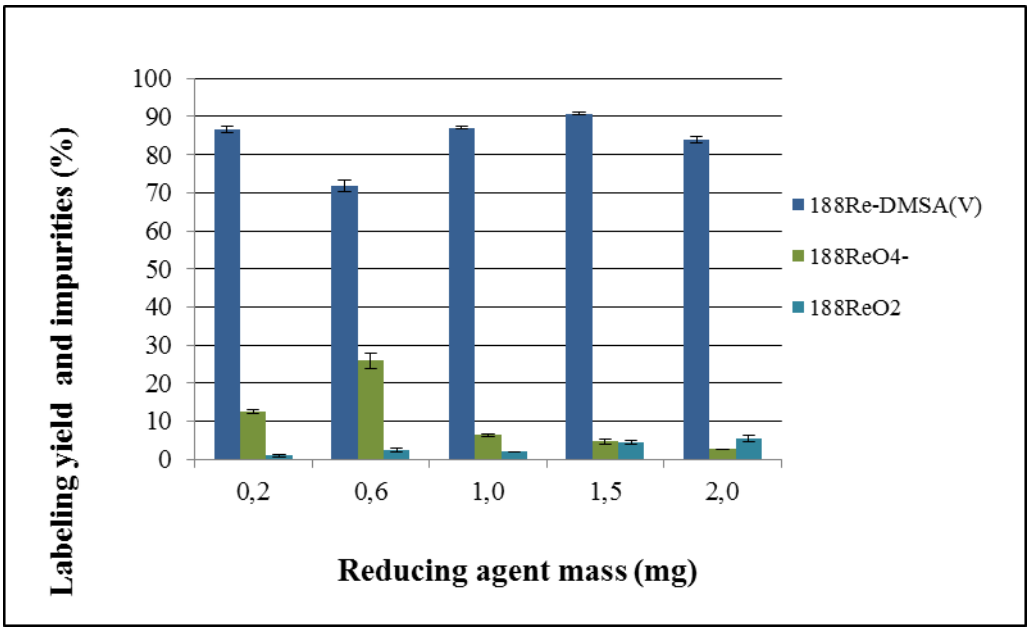

Figure 5: Labeling yield with the variation of reducing agent mass.

Regarding the reaction time, the best results were achieved with 30 minutes of reaction, as seen in Figure 6.

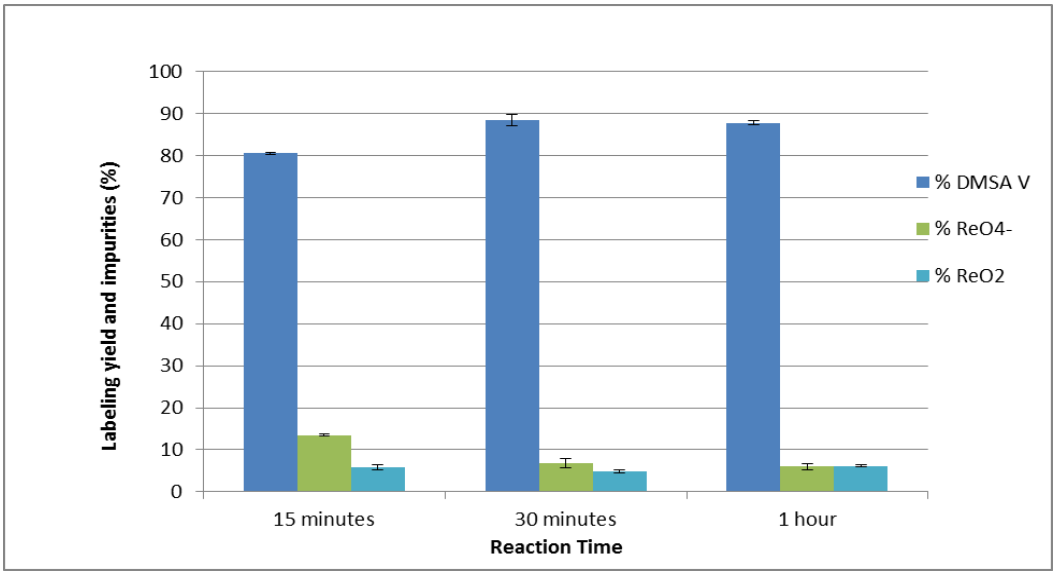

Figure 6: Labeling yield with the variation of labeling time.

Figure 7 shows that the results of the variation of the mass of DMSA. The mass of 2.5 mg of DMSA gave the best yield.

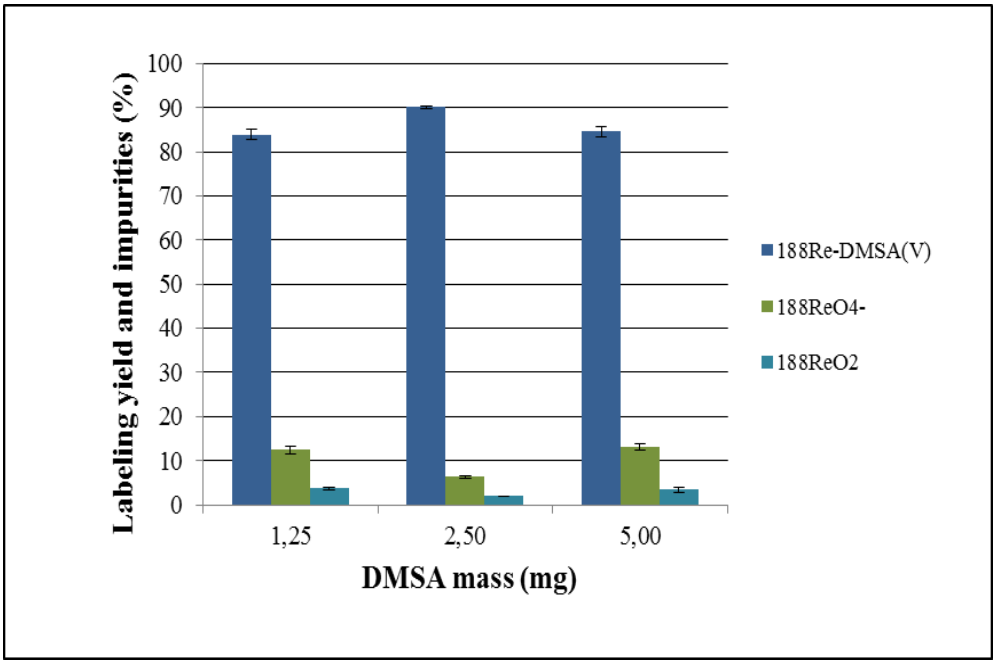

Figure 7: Labeling yield with the variation of DMSA mass.

Adding 10 mg of γ-cyclodextrin, another reagent that facilitates the reduction of the ^{188}Re (VII) to ^{188}Re (V), an increase in the labeling yield was obtained as seen in figure 8.

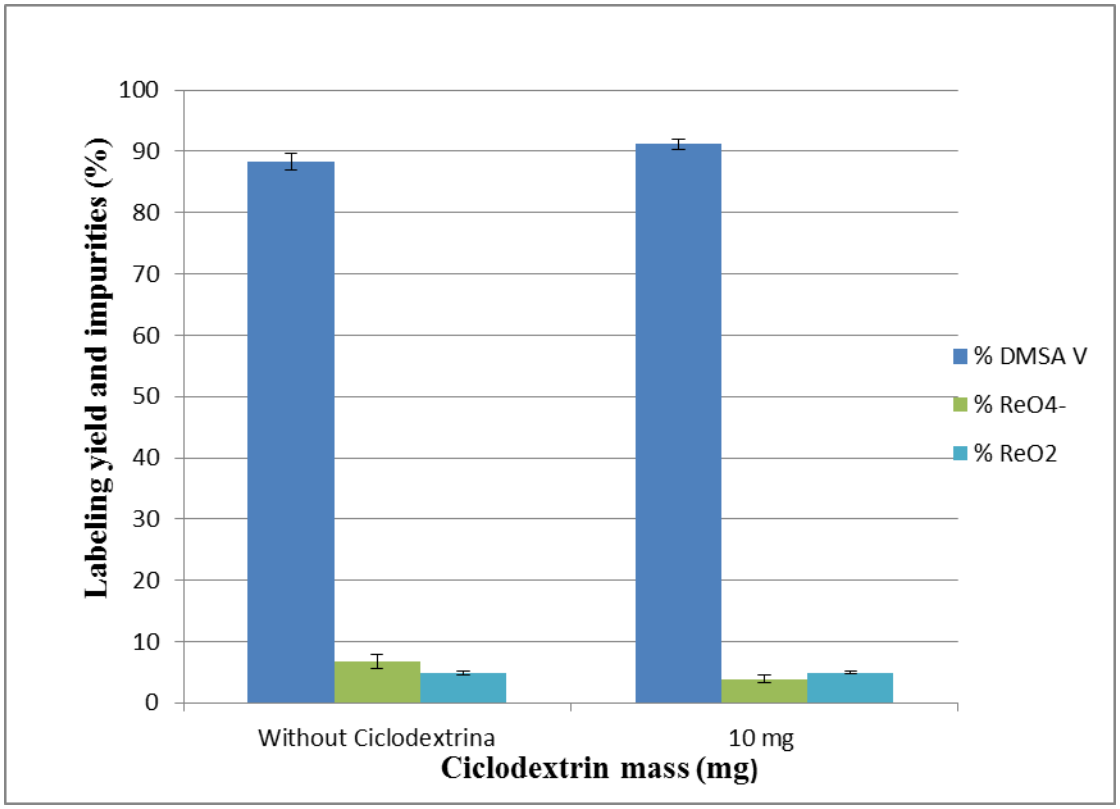

Figure 8: Labeling yield with the variation of γ-ciclodextrin mass.

The pH of the reaction was varied between 3.5 and 5, showing an increase in the labeling yield as the pH decreased (Figure 9).

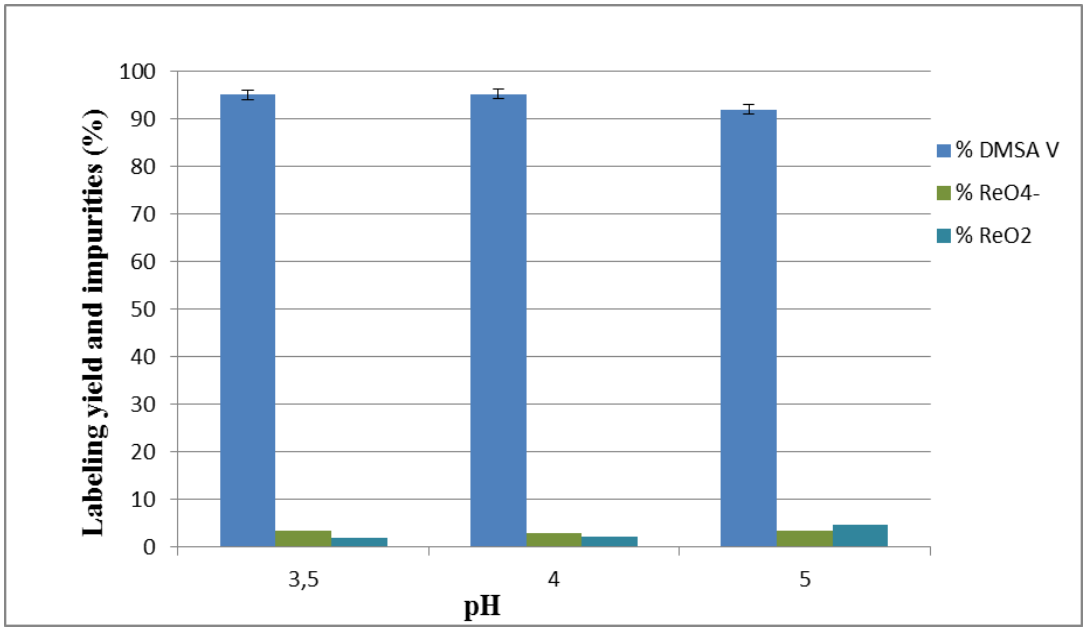

Figure 9: Labeling yield with the variation of pH.

The change in the volume from 1 mL to 2 mL of ^{188}Re showed a slight increase in the labeling yield (Figure 10).

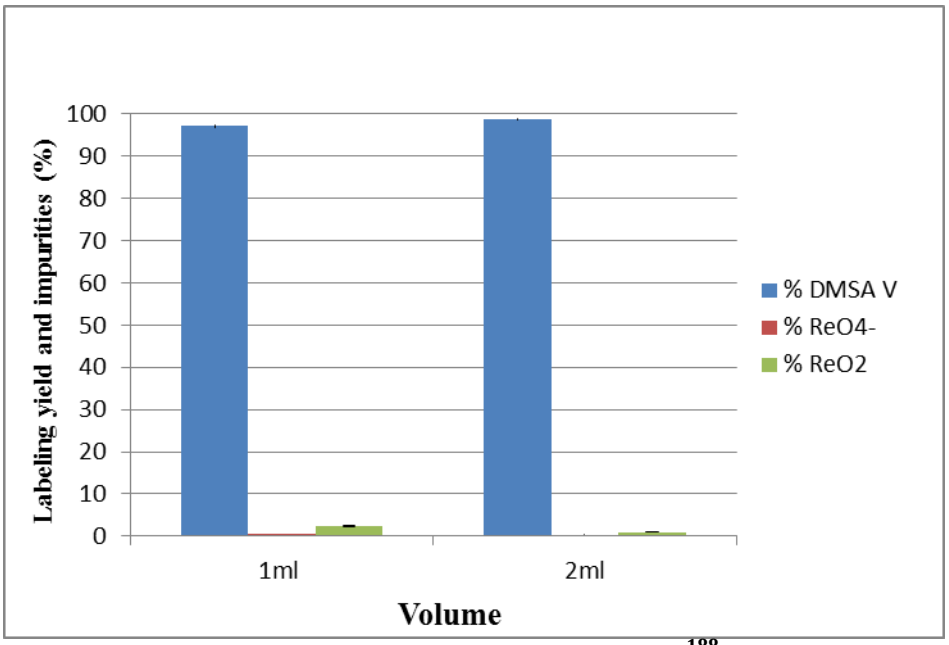

Figure 10: Labeling yield with the variation of ^{188}Re volume.

Stability studies revealed that the labeling reaction is stable up to 6 hours in a room temperature (25 °C) as can be seen in (Figure 11).

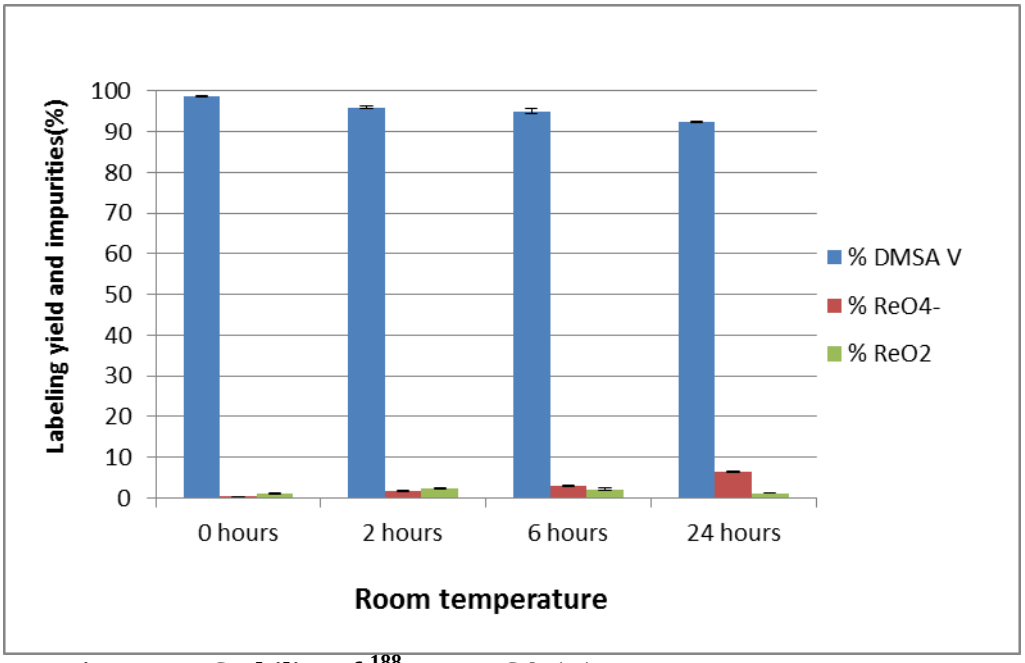

Figure 11: Stability of ^{188}Re-DMSA (V)

The best formulation was:
- $SnCl_2.2H_2O$ mass - 1.0mg;
- Mass of DMSA - 2.5mg
- Mass of sodium oxalate - 10mg;
- Mass of cyclodextrin - 10mg;
- Labeling time - 30 min;
- pH – 3.5;
- Volume of ^{188}Re – 2ml;

The formulation has stability labeling of up to 6 hours.

The advantage of this method is that it does not require high temperatures to achieve good labeling yields due to the use of oxalate. This compound complexes with Re in a more appropriate geometry and kinetics promoting a more efficient reduction of $^{188}ReO_4^-$ when compared with the method I (Bozalti et al., 2000).

4. CONCLUSIONS

The results for both methods of labeling ^{188}Re-DMSA(V) showed that the labeling yield was >95%. The second method has an advantage over the first method because it does not require heating and has stability labeling of up to 6 hours.

The great contribution of this work was the optimization of two labeling methods. The results could eneble the application in medicine nuclear of the radiopharmacetical ^{188}Re-DMSA(V).

ACKNOWLEDGMENTS

The authors wish to thank CNEN and IPEN for grating fellowships for this work.

REFERENCES

1. SARKAR, S.K.; VENKATESH, M.; RAMAMOORTHY, N.; *Evaluation of two methods for concentrating perrhenate (^{188}Re) eluates obtained from ^{188}W-^{188}Re gerador*. Applied Radiation and Isotopes. Vol. 67, p. 234-239, (2009).

2. BRAMBILLA, T.P. *Desenvolvimento de métodos para marcação de DMSA pentavalente com ^{99m}Tc e ^{188}Re.* (2009). Dissertação (Mestrado) - Instituto de Pesquisas Energéticas e Nucleares, São Paulo.

3. MARCZEWSKI, B. *Estudos de marcação do etidronato com ^{188}Re proveniente de diferentes geradores de ^{188}W / ^{188}Re.* (2006). Tese (Doutorado) – Instituto de Pesquisas Energéticas e Nucleares, São Paulo.

4. KNAPP, Jr., F.F.R. *A Generator –Derived Radioisotope for Cancer Therapy*. Cancer Biotherapy & Radiopharmaceuticals. Vol. 13, nº 5, pp. 337-349, (1998).

5. MAGALHÃES, P.K.R.; CASTRO, M.; ELIAS, L. L.K.; MACIEL, L. M.Z. *Carcinoma Medular de Tireóide: da Definição às Bases Moleculares*. Arq. Bras. Endocrinol. Metab. Vol. 47, nº 5, pp. 515-529. Outubro, (2003).

6. BOLZATI, C.; BOSCHI, A.; UCCELLI, L.; DUATTI, A.; FRANCESCHINI, R.; PIFFANELLI, A. *An Alternative Approach to the Preparation of ^{188}Re Radiopharmaceuticals from Generator-Proced [^{188}ReO$_4$]$^-$: Efficient Synthesis of ^{188}Re(V)-meso-2,3-Dimercaptosuccinic Acid*. Nuclear Medicine and Biology, vol. 27, pp. 309-314, (2000).