

## Preparation and Evaluation of Modified Composition for Lyophilized Kits of $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ for $[\text{}^{99\text{m}}\text{Tc}]$ Technetium Labeling

Marisa de Jesus de C. Lima<sup>1</sup>, Fabio Luiz Navarro Marques<sup>1\*</sup>, Miriam Roseli Yoshie Okamoto<sup>1</sup>, Alexandre Tales Garcez<sup>1</sup>, Marcelo Tatit Sapienza<sup>1</sup> and Carlos Alberto. Buchpiguel<sup>1</sup>

<sup>1</sup>Centro de Medicina Nuclear; Departamento de Radiologia; Faculdade de Medicina; Universidade de São Paulo; Trav. R. Dr. Ovídio Pires de Campos s/n; fabio.marques@hcnet.usp.br; 054030-010; São Paulo - SP - Brasil

### ABSTRACT

The  $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$  complex was synthesized and different formulations for lyophilized kits that could be cost-effectively used with different routines in nuclear medicine laboratories were investigated. In one preparation the kit components were kept similar to the Cardiolite®, except that the  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  concentration was increased to 0.150 mg. In a second formulation, component concentrations were reduced to 1/5 of the original value and the  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  concentration was adjusted to 0.04 mg. These products were labeled with maximum activities of 55.5 GBq and 8.14 GBq, respectively, and have shown an average radiochemical purity of 95 %. Biodistribution of the products was assessed by dissection in mice and in rabbits, and did not show any statistical difference when compared to Cardiolite®. In the synthesis of  $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$  a new procedure was introduced for the synthesis of *N*-(2-methyl-propenyl)-formamide, with the use of microwave radiation as heat source. This modification reduced the reaction time to 25 seconds, while maintaining a yield of 68%.

**Key words:**  $[\text{}^{99\text{m}}\text{Tc}]$ technetium,  $[\text{}^{99\text{m}}\text{Tc}](\text{MIBI})_6^+$ , radiopharmaceutical, biodistribution

### INTRODUCTION

Nuclear Medicine had a great development in the 1980s, when new classes of compounds, such as the tetraamines, aminethiols and isonitriles, were synthesized and evaluated as possible new  $[\text{}^{99\text{m}}\text{Tc}]$ technetium radiopharmaceuticals (Jurrisson et al., 1993). In the isonitrile class prepared by Jones (Jones et al., 1984), 1-isociane-2-methoxy-2-methyl-propane, currently named MIBI, exhibited excellent characteristics for use in myocardial perfusion studies (Piwnica-Worms et al., 1989), with a quality superior to that of

(<sup>201</sup>Tl) thallium (Taillefer et al., 1997). Also, it was indicated for use in breast cancer detection and parathyroid scanning (Bagni et al., 2003; Geatti et al., 1994). Because of these important applications and the high cost of the commercial product Cardiolite®, several nuclear medicine laboratories have introduced routines to make the product cost-effective, such as labeling the product with higher activities than that established by the manufacturer or using a cold kit fractionation technique, allowing labeling of fractions in different times (Lazarus et al., 1998; Millar, 1999). However, because of the low concentration of stannous ion

\* Author for correspondence

( $\text{Sn}^{2+}$ ) in the formulation (max 0.075 mg), the use of these procedures can lead to a failure in the labeling process, resulting in a product with low radiochemical purity (Fleming et al., 1992; Decristoforo et al., 1996; Kumar, 1997). An alternative for those procedures is the production of an in-house kit, with kit components being adjusted to the clinical demand according to the number of studies performed per day. A limitation in this case is that MIBI, the ligand to [ $^{99\text{m}}$ Tc]technetium, is a non-commercial product and needs to be synthesized (Van Wyk et al., 1991; Muradian et al., 1992; Ferro Flores et al., 1994; Lee et al., 1996; Deicas et al., 1997), a task that cannot be easily accomplished in all localities. In this paper a modified synthesis of MIBI is presented, introducing the use of microwave radiation in one step of the synthesis. Two formulations will also be presented, one preparation being named *FULL*, to be labeled with high activities, and another named *FRACTION*, to be labeled with low activities. This procedure furnishes a cost-effective product to be used in the routine of nuclear medicine laboratories of various sizes.

## MATERIALS AND METHODS

Domestic microwave (Panasonic-model NN 5556B) was used as a heat source in the synthesis step.  $^1\text{H-NMR}$  spectra were recorded (Bruker AC-200 system) using TMS as internal reference and the chemical shifts were registered in  $\delta$  (ppm). Lyophilizations was performed in a Lyophilizator Supermodoly (Edwards-BOC). Radioactive samples were measured in a dose calibrator (Victoreen - mod 34-056) and in a well counter (Gamma Nuclear). Animal imaging was acquired in a planar gamma-camera (Siemens - mod LEM) using a low energy all purpose (LEAP) collimator. The [ $^{99\text{m}}$ Tc] $\text{O}_4^-$  was obtained from  $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$  generator (IPEN/CNEN-Brazil). All the chemical products were used under analytical grade and without purification, except  $\text{POCl}_3$  that was distilled. Animal experiments were performed in compliance with regulations of this Institution and approved by the Ethical Committee. Statistical analyses were performed using the Student *t* test with 5 % level of significance.

### Synthesis of [ $\text{Cu}(\text{MIBI})_4$ ] $\text{BF}_4$

The synthesis of 1-isociane-2-methoxy-2-methylpropane (compound 3), as a cooper complex (compound 4), was performed using a previously published synthetic route (Van Wyk et al., 1991) and adaptations published later (Muradian et al., 1992; Deicas et al., 1997), as shown in fig 1. A modification was introduced in the preparation of [2-methyl-propenyl]-formamide (Step 1) as described below.

In a round bottomed pyrex glass flask, fitted with a dry ice condenser, 2-methyl-propenylamine hydrochloride (5.35 g – 50 mmols) and formamide (3.38 g – 75 mmols) were mixed and irradiated at the center of a domestic microwave oven at 240 W for 25 seconds. The flask was cooled in a water bath, 10 mL of  $\text{CHCl}_3$  was added, the mixture was filtered, and the solid was washed with 5 mL of  $\text{CHCl}_3$ . The filtrate was concentrated in a rotary evaporator, the crude product distilled at reduced pressure (0.9 mmHg) and the product collected at 72-6°C, yielding 3.36 g (68.0%).  $^1\text{H-NMR}$  (200 MHz,  $\text{CDCl}_3/\delta$  pm) 1.72 (s, 3H,  $\text{CH}_3$ ); 3.77 (d, 2H,  $J_{\text{NH}}$  6.05 Hz,  $\text{CH}_2$ ); 4.85 (d, 2H,  $\text{HC}=\text{C}$ ); 6.94 (br s, 1H, NH); 8.19 (s, 1H, CHO).

### Kit preparation

L-Cysteine hydrochloride monohydrate, [ $\text{Cu}(\text{MIBI})_4$ ] $\text{BF}_4$ ,  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , sodium citrate and mannitol were dissolved in water previously flushed with nitrogen, and the pH was adjusted to 6.0 using NaOH (0.1N) to obtain two types of solution with the final concentration (per mL) presented below. ***FULL* content:** 1.0 mg of [ $\text{Cu}(\text{MIBI})_4$ ] $\text{BF}_4$ , 0.15 mg of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , 1.0 mg of L-cysteine hydrochloride monohydrate, 2.6 mg of sodium citrate and 20.0 mg of mannitol. ***FRACTION* content:** 0.2 mg of [ $\text{Cu}(\text{MIBI})_4$ ] $\text{BF}_4$ , 0.04 mg of  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.2 mg of L-cysteine monohydrate hydrochloride, 0.41 mg of sodium citrate and 4.0 mg of mannitol. The solutions were sterilized by membrane filtration (0.22  $\mu\text{m}$ ) and lyophilized for 24 hours.

### Radiochemical labeling

The lyophilized products were reconstituted using fresh [ $^{99\text{m}}$ Tc] $\text{O}_4^-$  solution from generators eluted previously at 24, 48 or 72 h, using activities below 55.5 GBq, for *FULL* content, and below 8.14 GBq for *FRACTION* content. The vials were heated on a boiling water bath during 10 minutes. Cardiolite® was labeled according to the

manufacturer's instructions, except for the activities that reached 18.5 GBq.

#### Labeling efficiency assessment

Labeling efficiencies (LE) of  $[[^{99m}\text{Tc}](\text{MIBI})_6]^+$  preparations were determined by ascendant chromatography or by solvent extraction. In the former procedure, a drop of labeled product was applied to the TLC- $\text{Al}_2\text{O}_3$  Baker-Flex chromatographic strip (7.5x2.0 cm) and the product was developed using ethanol as mobile phase. The strips were dried, cut in three equal parts and the radioactivity was measured in a well counter. The  $[[^{99m}\text{Tc}](\text{MIBI})_6]^+$  was found at  $R_f = 0.3-0.6$ . In the latter procedure, a drop of labeled product was added to a test tube containing 3 mL  $\text{CHCl}_3$  and 3 mL NaCl 0.9 % solution. The mixture was shaken and 1 mL of each phase was removed and transferred to two new tubes and radioactivity was measured in a dose calibrator (the radiopharmaceutical must be in the  $\text{CHCl}_3$  phase).

#### Animal biodistribution

Quantitative biodistribution studies were performed in Swiss mice (male, seven weeks old, about 45 g). Unanesthetized animals were injected with 0.37 MBq of  $[[^{99m}\text{Tc}](\text{MIBI})_6]^+$  via the tail vein. At 5, 30 and 60 minutes post-injection, the animals were anesthetized with ether and sacrificed by decapitation. The organs of interest (heart, liver, lungs, kidneys and blood) were removed and weighed. The radioactivity was assayed in a well counter and expressed as mean injected dose/gram  $\pm$  SD ( $n = 6$  for each time point).

Also, dynamic imaging studies were performed in male, three-year-old, New Zealand white rabbits (about 3.5 kg). Immobilized animals were injected with 18.5-37.0 MBq/kg of  $[[^{99m}\text{Tc}](\text{MIBI})_6]^+$  in the marginal ear vein and 56 serials images (30 sec/frame - matrix 256x256) were immediately collected in a planar gamma-camera. A ROI was drawn over the heart, lung and liver and time-activity curves were obtained with background subtraction.

## RESULTS

#### Ligand synthesis

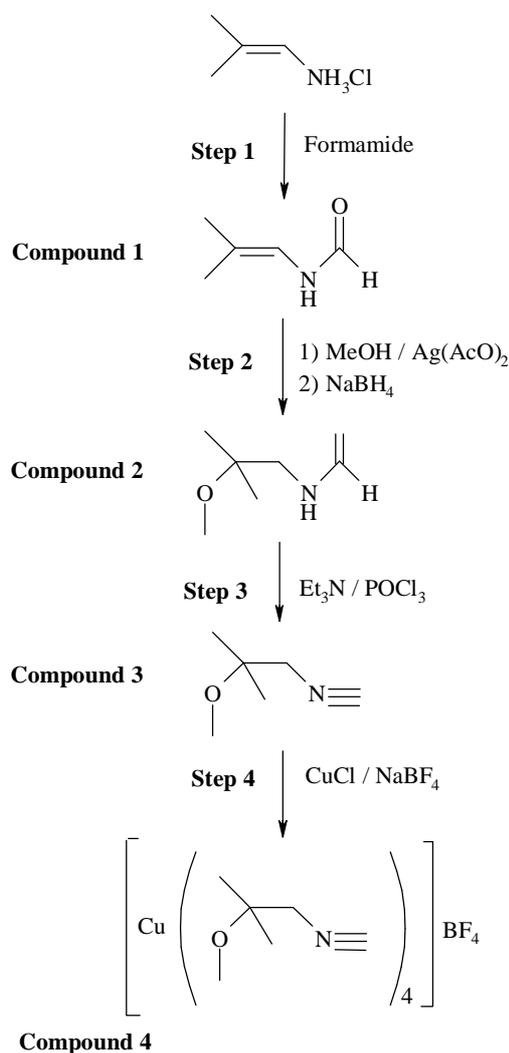
The complex  $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$  was synthesized using a route established (Van Wyk et al, 1991) with previously described modifications (Muradian et al., 1992; Deicas et al., 1997) in an overall yield of 9.38%. A new approach introduced in the reaction between N-(2-methylpropenyl)-amine hydrochloride and formamide permitted the attainment of a product in 68% yield, with a maximum reaction time of 25 seconds. Spectral data and physical chemical characteristics of all compounds were in agreement with previously published results.

#### Kit preparation, labeling and quality control.

The change in kit composition was focused on  $\text{Sn}^{2+}$  augmentation. Compared to the Cardiolite® kit, the *FULL* content kit had twice the maximum concentration of  $\text{Sn}^{2+}$  the reducing agent and the *FRACTION* content kit had the equivalent of 1.6 times more. These modifications permitted labeling with activities that ranged from 3.7 GBq to 55.5 GBq for *FULL* content and 1.11 GBq to 8.14 GBq for *FRACTION* content, giving a labeling efficiency yield, in 40 samples each, of  $96.66 \pm 1.48\%$  and  $96.45 \pm 1.67\%$ , respectively. The maximum activities were achieved using fresh eluate from 24 h previously eluted generator. The maximum activities for other elution profile are shown in table 1. Chromatographic and extraction methods for determination of labeling efficiency were compared in 40 samples, obtaining values of  $95.46 \pm 1.38\%$  and  $96.72 \pm 1.71\%$ , respectively.

#### Animal biodistribution

Quantitative biodistribution studies performed in mice showed no statistical difference, at the  $p < 0.05$  significance level, for the concentration of the three products in the organs of interest (Table 2). Compounds showed fast clearance from the lung and blood, considered background compartments, and concentration in the heart, liver and kidneys, these last two being organs of excretion. The ratio of heart/liver activities (around 1.09% ID/g organ) and heart/lung activities (around 11.07% ID/g organ) at 30 minutes after product administration showed a product with good characteristics for image purposes.



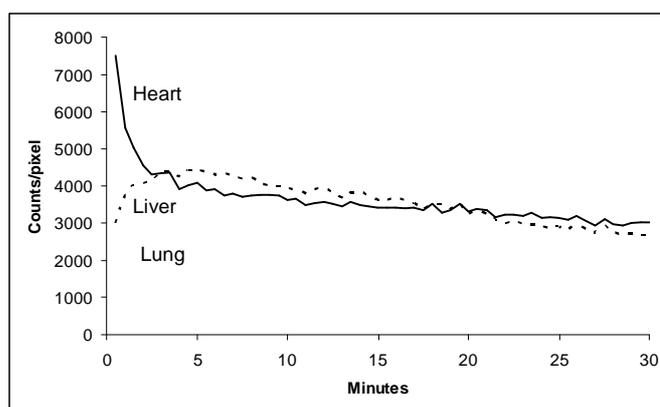
**Figure 1** – Synthesis scheme for [Cu(MIBI)<sub>4</sub>]BF<sub>4</sub> complex.

**Table 1** – Labeling efficiency (LE) of the [<sup>99m</sup>Tc](MIBI)<sub>6</sub><sup>+</sup> complex, expressed as mean ± SD, for six samples for each time and product, as a function of the product characteristic, interval between elution of the generators and maximum activities used.

Product	Previous generator elution (h)	Activity (GBq)	LE (%)
<i>FULL</i>	24	55.5	97.50 ± 0.78
	48	44.4	95.95 ± 1.13
	72	33.3	97.30 ± 1.40
<i>FRACTION</i>	24	8.14	97.02 ± 1.53
	48	6.29	96.29 ± 2.24
	72	4.81	94.65 ± 1.65

A dynamic gamma-camera imaging study, performed in rabbits, permitted a direct comparison of pharmacokinetics for different products. The time-activity curve (Fig. 2) from the rabbits image showed a fast accumulation of the compounds in heart, liver and kidneys, followed by a moderate flushing from the hepatobiliary system and kidneys and a fast transit of the

material through the lungs. After the dynamic study, a static acquisition evaluated the relationship between heart and the background organs, liver and lungs, as expressed in Table 3. The heart/liver ratio of around 1.1 was identical for rabbits and mice. The same was not true for the heart/lung ratio, whose value in mice was around 11.6 and, in rabbits, was 4.1.



**Figure 2** - Time-activity curve for fasted and non-anesthetized rabbit during 30 minutes after i.v. administration of the  $[[^{99m}\text{Tc}](\text{MIBI})_6]^+$  obtained from the *FULL* content kit. Regions of interest are normalized to counts/pixel.

**Table 2** - Biodistribution of  $[[^{99m}\text{Tc}]$ technetium complexes with Cardiolite®, *FULL* and *FRACTION* content kits, expressed as % injected dose/g of organs  $\pm$  SD for six mice, and the heart/liver and heart/lung ratios at 5, 30 and 60 minutes after injection.

	5 min			30 min			60 min		
	Cardiolite	<i>FULL</i>	<i>FRACTION</i>	Cardiolite	<i>FULL</i>	<i>FRACTION</i>	Cardiolite	<i>FULL</i>	<i>FRACTION</i>
Heart	4.38 $\pm$ 0.37	4.40 $\pm$ 0.39	4.52 $\pm$ 0.35	4.08 $\pm$ 0.20	4.11 $\pm$ 0.34	4.13 $\pm$ 0.30	4.07 $\pm$ 0.38	4.05 $\pm$ 0.28	4.08 $\pm$ 0.33
Liver	4.10 $\pm$ 0.28	4.29 $\pm$ 0.47	4.42 $\pm$ 0.42	3.71 $\pm$ 0.26	3.80 $\pm$ 0.29	3.74 $\pm$ 0.41	3.62 $\pm$ 0.33	3.50 $\pm$ 0.40	3.35 $\pm$ 0.30
Lung	1.05 $\pm$ 0.16	0.97 $\pm$ 0.09	1.10 $\pm$ 0.11	0.36 $\pm$ 0.09	0.37 $\pm$ 0.11	0.39 $\pm$ 0.07	0.28 $\pm$ 0.06	0.27 $\pm$ 0.04	0.27 $\pm$ 0.03
Kidneys	12.39 $\pm$ 1.16	13.22 $\pm$ 1.30	14.01 $\pm$ 0.78	11.44 $\pm$ 2.01	11.58 $\pm$ 1.19	12.17 $\pm$ 1.73	11.27 $\pm$ 1.20	12.33 $\pm$ 1.50	11.73 $\pm$ 0.68
Blood	0.15 $\pm$ 0.03	0.13 $\pm$ 0.02	0.20 $\pm$ 0.03	0.06 $\pm$ 0.02	0.04 $\pm$ 0.03	0.09 $\pm$ 0.05	0.09 $\pm$ 0.03	0.04 $\pm$ 0.02	0.06 $\pm$ 0.02
Heart/liver	1.07 $\pm$ 0.08	1.03 $\pm$ 0.09	1.04 $\pm$ 0.17	1.10 $\pm$ 0.06	1.09 $\pm$ 0.15	1.12 $\pm$ 0.16	1.14 $\pm$ 0.17	1.17 $\pm$ 0.17	1.23 $\pm$ 0.16
Heart/lung	4.22 $\pm$ 0.55	4.53 $\pm$ 0.30	4.18 $\pm$ 0.64	11.79 $\pm$ 2.69	11.62 $\pm$ 2.75	11.07 $\pm$ 2.41	15.05 $\pm$ 2.65	15.15 $\pm$ 2.80	15.22 $\pm$ 1.85

**Table 3** - Heart/liver and heart/lungs ratios expressed as mean  $\pm$  SD of the counts/pixel from the image of three rabbits obtained at 30 minutes after injection of  $[[^{99m}\text{Tc}](\text{MIBI})_6]^+$ .

Product	Heart/liver ratio	Heart/lung ratio
Cardiolite®	1.22 $\pm$ 0.25	4,01 $\pm$ 0,19
<i>FULL</i>	1.39 $\pm$ 0.20	4,11 $\pm$ 0,22
<i>FRACTION</i>	1.24 $\pm$ 0.35	4,09 $\pm$ 0,20

## DISCUSSION

Although MIBI synthesis is not an original problem, since it has been performed by several routes, our contribution in this work was to introduce the microwave radiation as a heat source, in the reaction between N-(2-methyl-propenyl)-amine hydrochloride and formamide in a system without solvent. This modification reduced the reaction time to 25 seconds, with a yield of 68%, a value similar to those obtained in previous works, where the reaction between N-(2-methyl-propenyl)-amine hydrochloride and formamide was performed in refluxing toluene for three hours (Van Wyk et al., 1991) and in a non-solvent system, heating the sample for 30 minutes in an oil bath (Muradian et al., 1992). Although the results have shown a great advantage in the use of microwave radiation over other heating techniques or reaction conditions, care must be taken when microwave radiation is used in organic synthesis, because several variables need to be considered, for instance, the power of the equipment, the position of the sample inside of the microwave oven, the sample geometry, among others (Stone-Elander and Elander, 1991). Thus, to scale up the synthesis from that established in this work, new reactions conditions need to be found. Other reaction steps were performed according to the previously published procedure (Muradian et al., 1992), except for the copper complex  $[\text{Cu}(\text{MIBI})_4]^+$  that, instead of using  $\text{Cl}^-$  as the counter-ion, was obtained as the  $\text{BF}_4^-$  salt (Deicas et al., 1997). This modification furnished a product with better characteristics and stability.

A critical issue with the Cardiolite<sup>®</sup> lyophilized kit is the low  $\text{Sn}^{2+}$  content (maximum 75  $\mu\text{g}$  and minimum 25  $\mu\text{g}$ ) that could permit the labeling of the product using activities below 5.55 GBq, as established by the Cardiolite<sup>®</sup> manufacturer, although it is known that activities such as 18.5 GBq could be used with relative security (Hung et al., 1996). However, care needs to be taken since, depending on the generator elution intervals, age of eluted solution and activity used, the  $\text{Sn}^{2+}$  content might not be sufficient to completely reduce the  $[\text{}^{99\text{m}}\text{Tc}]\text{O}_4^-$  (Marques et al., 2001), and the  $\text{Sn}^{2+}$  content may be diminished even more during the cold fractionation technique. Thus, the addition of extra  $\text{Sn}^{2+}$  in an “in-house” prepared kit,

permitted labeling of the products using activities as high as 55.5 GBq with fresh eluate from a generator eluted after 24 hours, and this maximum activity decreased to 33.3 GBq when the generator was eluted after 72 hours. This activity is related to the increase in  $[\text{}^{99}\text{Tc}]$  technetium isomer content, which does not contribute with gamma radioactivity, but is chemically equal to  $[\text{}^{99\text{m}}\text{Tc}]$ technetium and reacts with kit components. Although the content of the *FRACTION* product had been reduced five-fold, an additional quantity of  $\text{Sn}^{2+}$  was necessary to permit the use of an activity equivalent to that used in the *FULL* product (Table 3). The final concentration of  $\text{Sn}^{2+}$  in the *FRACTION* product (40  $\mu\text{g}$ ) was similar that presented in the literature for other fractioned preparation procedures (Chowdhury and Hung, 1995). Labeling efficiency evaluated by chromatography and by the extraction procedure confirmed that the latter furnished results similar to the chromatographic method, indication that both could be used in nuclear medicine laboratories. However, when using the extraction procedure, extra care needs to be taken, due the personal and place contamination risk.

Modification of the kit content and use of large amounts of  $[\text{}^{99}\text{Tc} + \text{}^{99\text{m}}\text{Tc}]$ technetium could give a product with different biological characteristics (Ikeda et al, 1977). Thus, the  $[[\text{}^{99\text{m}}\text{Tc}](\text{MIBI})_6]^+$  complex was assessed by two distinct techniques and animal models, since it is known that biodistribution of the  $[\text{}^{99\text{m}}\text{Tc}]$ technetium isonitrile complex can change in different animal species (Kronauge et al., 1992; Barbarics et al., 1994). The assessment of biodistribution in mice permitted absolute quantification of the concentration of the  $[[\text{}^{99\text{m}}\text{Tc}](\text{MIBI})_6]^+$  in the target organs demonstrating that the products obtained from modified formulations behaved similarly to that obtained from Cardiolite<sup>®</sup>. This result was in agreement with results obtained by Barbarics (Barbarics et al, 1994), but with characteristics different from that obtained by Bouquillon (Bouquillon et al., 1995). Imaging assessment permitted comparison of the pharmacokinetic behaviour of the radiopharmaceuticals and quality of diagnostic with these compounds. The heart/liver ratio observed in this work was similar that established in the literature (Barbarics et al., 1994), around 0.9-1.4, but the

heart/lung ratio was different. Barbarics et al. (1994) observed a ratio of 1.6 to 1.8, a ratio of approximately 4 was observed in this study. This difference could be related to a lack of background correction in Barbaric's work.

## CONCLUSION

The introduction of microwave radiation in MIBI synthesis was an interesting approach since the reaction time in that step could be decreased drastically. Changes in kit composition introduced in this work did not alter the biological behaviour, when compared with Cardiolite<sup>®</sup>, but presented the advantage of being labeled with high activities or of being used as fractionated a preparation.

## ACKNOWLEDGEMENTS

The authors would like to thank Profa. Dra. Liliana Marzorati for laboratorial support and to FUNDAP for the fellow to Marisa J.C. Lima.

## RESUMO

Neste trabalho são apresentados dados relativos a síntese do complexo  $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$  e à utilização do mesmo para produção de kit liofilizado para obtenção do radiofármaco  $[[^{99\text{m}}\text{Tc}](\text{MIBI})_6]^+$ . No processo de síntese foi utilizado radiação de microondas como fonte de aquecimento em uma etapa do processo, permitindo que a reação fosse executada em 25 segundos, obtendo rendimento de 68 %. Na preparação dos kits liofilizados, um deles denominado *FULL*, nos quais a quantidade dos componentes foi mantida igual ao produto comercial Cardiolite<sup>®</sup>, e outro denominado *FRACTION*, nos quais as quantidades foram reduzidas a 1/5 do total, foram adicionadas quantidades extras de  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ . Deste modo, tornou-se possível marcar os kits liofilizados com atividades máximas de 55,5 GBq e 8,14 GBq, respectivamente. A biodistribuição dos produtos marcados, em camundongos e coelhos, não mostrou diferença estatística ( $p > 0,05$ ), para o teste *t* de Student, quando comparada ao

produto obtido pela reconstituição do Cardiolite<sup>®</sup>.

## REFERENCES

- Bagni, B.; Franceschetto, A.; Casolo, A.; De Santis, M.; Bagni, I.; Pansini, F. and Di Leo, C. (2003), Scintimammography with  $^{99\text{m}}\text{Tc}$ -MIBI and magnetic resonance imaging in the evaluation of breast cancer. *Eur. J. Nucl. Med. Mol. Imag.*, **30**, 1383-1388.
- Barbarics, E.; Kronauge, J. F.; Costello, C. E.; Janoki, G. A.; Holman, B. L.; Davison, A. and Jones, A. G. (1994), In vivo metabolism of the technetium isonitrile complex  $[\text{Tc}(\text{2-ethoxy-2-methyl-1-isocyanopropane})_6]^+$ . *Nucl. Med. Biol.*, **21**, 583-591.
- Bouquillon, S.; Coulais, Y.; Dartiguenave, M.; Tafani, J. A. M. and Guiraud, R. (1995), Synthesis, characterization and biodistribution of a new technetium-99m complex with trimethylsilylmethylisonitrile. Comparison with  $^{99\text{m}}\text{Tc}$ -TBI and  $^{99\text{m}}\text{Tc}$ -MIBI. *Nucl. Med. Biol.*, **22**, 585-588.
- Chowdhury, S. and Hung, J. C. (1995), Reconstitution and fractionation of Cardiolite<sup>®</sup> into multi-dose cold kits for cost saving. *J. Nucl. Med.*, **36**, 80.
- Decristoforo, C. and Riccabona, G. (1996), Problems with fractionated cold kits. *J. Nucl. Med.*, **37**, 1912-1913.
- Deicas, B.; Gambino, D.; Kremer, C.; Kremer, E.; Mombro, A.; Suescun, L.; Mariezcurrena, R.; Gonzalez, O.; Rey, A.; Mallo, L. and Leon, A. (1997), Synthesis, characterization and spectroscopic properties of  $[\text{Cu}(\text{alkylisocyanide})_4]\text{BF}_4$  complexes. X-ray crystal structures of  $[\text{Cu}(\text{MIBI})_4]\text{BF}_4$ . *Polyhedron*, **16**, 2397-2403.
- Ferro-Flores, G.; Ramirez-Garcia, L.; Lezama, J.; Garcia-Garcia, P. and Tendilla, J. I. (1994), An improved procedure for the synthesis of 2-methoxyisobutylisonitrile - an efficient complexing agent for Tc-99m. *J. Radioanal. Nucl. Chem. Letters*, **188**, 409-415.
- Fleming, W. K.; Jay, M. and Ryo, U. Y. (1992), Reconstitution and fractionation of radiopharmaceuticals kits. *J. Nucl. Med.*, **33**, 1915.
- Geatti, O.; Shapiro, B.; Orsolon, P. G.; Proto, G.; Guerra, U. P.; Antonucci, F. and Gasparini, D. (1994), Localization of parathyroid enlargement - experience with  $^{99\text{m}}\text{Tc}$ -methoxyisobutylisonitrile and  $^{201}\text{Tl}$  scintigraphy, ultrasonography and computed-tomography. *Eur. J. Nucl. Med.*, **21**, 17-22.

- Hung, J. C.; Herold, T. J. and Gibbons, R. J. (1996), Optimal conditions of  $^{99m}\text{Tc}$  eluate for the radiolabeling of  $^{99m}\text{Tc}$ -sestamibi. *Nucl. Med. Biol.*, **23**, 599-603.
- Ikeda, I.; Inoue, O. and Kurata, K. (1977), Preparation of various Tc-99m dimercaptosuccinate complex and their evaluation as radiotracers. *J. Nucl. Med.*, **18**, 1222-1229.
- Jones, A. G.; Abrams, M. J.; Davison, A.; Brodack, J. W.; Toothaker, A. K.; Adelstein, S. J. and Kassiss, A. I. (1984), Biological studies of a new class of technetium complex: the hexakis(alkylisonitrile) technetium(I) cations. *J. Nucl. Med. Biol.*, **11**, 225-34.
- Jurrisson, S.; Berning, D.; Jia, W. and Ma, D. (1993), Coordination compounds in Nuclear Medicine. *Chem. Rev.*, **93**, 1137-1156.
- Kronauge, J. F.; Noska, M. A.; Davison, A.; Holman, B. L. and Jones, A. G. (1992), Interspecies variation in biodistribution of technetium(2-carbomethoxy-2-isocyanopropane) $_6^+$ . *J. Nucl. Med.*, **33**, 1357-1365.
- Kumar, V. (1997), Fractionated cold-kits: Address the critical issues to obviate problems. *J. Nucl. Med.*, **38**, 1664.
- Lazarus, C.; Palmer, A. M. and Hesslewood, S. R. (1998), Radiopharmaceutical kit modification. *Nucl. Med. Comm.*, **19**, 613-614.
- Lee, T. W.; Su, C. S. and Ting, G. (1996), Synthesis, reactivity and  $^{99m}\text{Tc}$  labelling of 2-alkoxyisobutylisonitrile. *Appl. Radiat. Isot.*, **47**, 207-210.
- Marques, F. L. N.; Okamoto, M. R. Y. and Buchpiguel, C. A. (2001), Technetium-99m generators and radiopharmaceuticals and quality control tests. *Radiol. Bras.*, **34**, 233-239.
- Millar, A. M. (1999), Radiopharmaceutical kit modification. *Nucl. Med. Comm.*, **20**, 105-106.
- Muradian, J.; Marques, F. L. N.; Rockmann, T. M. B. and Dias Neto, A. L. (1992), Synthesis of the 2-methoxyisobutylisonitrila (MIBI) for medical use. *Radiol. Bras.*, **25**, 171-172.
- Piwnica-Worms, D.; Kronauge, J. F.; Holman, B. L.; Davison, A. and Jones, A. G. (1989), Comparative myocardial uptake characteristics of hexakis(alkylisonitrile) technetium(I) complex. Effect of lipophilicity. *Invest. Radiol.*, **24**, 25-29.
- Stone-Elander, S. and Elander, N. (1991), Microwave cavities - some parameters affecting their use in radiolabeling. *Appl. Radiat. Isot.*, **42**, 885-887.
- Taillefer, R.; DePuey, E. G.; Udelson, J. E.; Beller, G. A.; Latour, Y. and Reeves, F. (1997), Comparative diagnostic accuracy of Tl-201 and Tc-99m sestamibi SPECT imaging (perfusion and ECG-gated SPECT) in detecting coronary artery disease in women. *J. Am. Coll. Cardiol.*, **29**, 69-77.
- Van Wyk, A. J.; Van Aswegen, A.; Knoesen, O.; Fourie, P. J.; Koekemoer, J.; Herbst, C. P.; Otto, A. C. and Lotter, M. G. (1991), Synthesis and  $^{99m}\text{Tc}$  labelling of MMI (MIBI) and its ethyl analogue EMI. *Appl. Radiat. Isot.*, **42**, 687-689.

Received: July 02, 2005;

Revised: July 14, 2005;

Accepted: August 01, 2005.