Fast Labeling of Technetium-99m-Sestamibi with Microwave Oven Heating

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Technetium-99m-methoxy isobutyl isonitrile (^{99m}Tc-MIBI) imaging can be useful to assess myocardial salvage following thrombolysis. In such acute conditions, MIBI must be readily available. The standard labeling method requires a 10-min boiling period in a boiling water bath (BWB). The purpose of this study was to compare the BWB technique to a microwave oven heating (MOH) method. Two series of 10 MIBI vials (8 cc)—one for BWB and one for MOH—were evaluated after removal of 7-8 cc of head space volume and the addition of 100 mCi (3 cc) of [^{99m}Tc]-pertechnetate. The two heating processes were evaluated as follows: (1) BWB: vials were put in boiling water for 10 min, (2) MOH: vials were put in a 0.4-cu ft lead-shielded microwave oven for 13 sec. A styrofoam cap protected the aluminum seal from sparking. Labeling efficiency was evaluated by radiochromatography with Baker flex aluminum oxide plates/absolute ethanol system at 1 min, 10 min, 6 hr and 24 hr after reconstitution. The labeling efficiency (percentage of primary complex) was determined and the results showed no significant differences between the two methods. MOH considerably reduces the heating period (13 sec) and provides a good labeling efficiency for in vivo imaging.

Myocardial perfusion imaging with technetium-99m-(^{99m}Tc)sestamibi (Cardiolite, DuPont Merck Pharmaceuticals, N. Billerica, MA) has proven to be a valuable tool in the assessment of coronary artery disease (*1–5*). It offers interesting physical and biologic properties that are optimal for imaging purposes. Unlike thallium-201 (^{201}Tl),^{99m}Tc-sestamibi remains bound to cytosolic proteins and thus does not show a clinically relevant redistribution over time. Since it does not redistribute, timing after injection is less critical than with ^{201}Tl. This unique property can be useful in assessing myocardial perfusion in acute conditions such as spontaneous chest pain or in evaluating the results of different therapies such as thrombolysis after acute myocardial infarction (*6–12*).

Even though ^{99m}Tc-sestamibi is readily available as a "kit," the standard labeling procedure requires 30–40 min of preparation before it is ready for injection. This preparation requires a 10-min boiling step in order to attain a labeling efficiency exceeding 90%. This relatively long preparation period is not ideal for emergency situations, as it would possibly delay initiation of therapy if the product had not been previously prepared. This might be the case for the evaluation of patients who arrive at the emergency ward at night and need to be injected with ^{99m}Tc-sestamibi as soon as possible prior to thrombolysis.

In order to accelerate the labeling process, a microwave oven heating technique has been developed which reduces the preparation time to a minimum. Injection of ^{99m}Tc-sestamibi within 5 min of kit reconstitution is feasible with this fast labeling procedure. This reduction in preparation time also provides a reduction in radiation exposure to the technical staff. The purpose of the study was to compare the labeling efficiency of ^{99m}Tc-sestamibi using the standard boiling water bath procedure and a microwave oven technique.

MATERIALS AND METHODS

In order to evaluate the heating process, temperature measurements as a function of time were obtained using an IT-660 electro-thermal temperature probe (Electro Medics, Inc., Denver, CO) for both the boiling water bath and microwave oven techniques. Temperature measurements were made on two vials in each of the methods tested. Temperature/time curves were then generated (Fig. 1).

The boiling water bath method served as a standard by which the microwave oven method was compared. The optimum heating time for the microwave oven method was extrapolated from the curves and set at 13 sec.

Labeling efficiency was evaluated by the manufacturer-recommended radiochromatography procedure in 20 vials. Ten vials were prepared by the standard boiling water bath method and the labeling efficiency was compared to 10 vials prepared using the 13-sec microwave method. The labeling stability over time was also evaluated by successive radiochromatographies at 6 hr and 24 hr after the ^{99m}Tc-sestamibi preparation.

Each 8-cc capacity vial of sestamibi contains in lyophilized form: 1.0 mg of $[Cu(MIBI_4)]BF_4$, 0.075 mg of stannous chloride dihydrate, 20.0 mg of mannitol, 2.6 mg of sodium citrate dihydrate, and 1.0 mg L-cisteine hydrochloride monohydrate.

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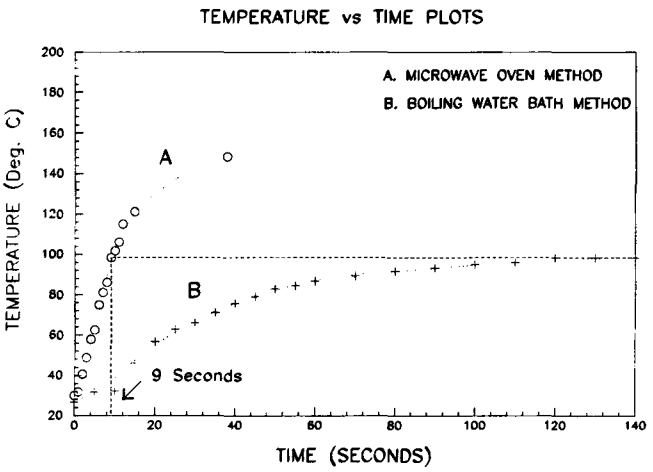

FIG. 1. Temperature measurements within the sestamibi vial as a function of time, comparison of (A) microwave oven method and (B) boiling water bath method. The 9-sec mark on the x-axis represents the extrapolated time with the microwave oven method corresponding to the maximum temperature found in the vial with the standard boiling bath method.

Vials were stored at room temperature and protected from the light before reconstitution.

Boiling Water Bath Method

After disinfection of the top of the ^{99m}Tc-sestamibi vial closure surface with an alcohol swab, the vial was placed in a suitable radiation shield. Using a sterile syringe, 3 ml of solution containing 100 mCi (3.7 GBq) of nonpyrogenic [^{99m}Tc]pertechnetate was obtained aseptically. The ^{99m}Tc solution was then aseptically added to the vial in the lead shield. Without withdrawing the needle, an equal volume of head space was removed to maintain atmospheric pressure within the vial.

The contents of the vial were then swirled for 15 sec. The vial was placed with the lead shield upright in a boiling water bath (Corning hot plate PC35 with water tank, Corning, NY) for 10 min. Timing of the 10 min-period began as soon as the water in the tank started to boil. After removal of the vial from the water bath, radiochemical purity was assessed by thin-layer chromatography (TLC).

Microwave Oven Method

After disinfection of the top of the ^{99m}Tc-sestamibi vial closure surface with an alcohol swab, the vial was placed in a suitable radiation shield. Using a sterile syringe, 3 ml of solution containing 100 mCi (3.7 GBq) of nonpyrogenic [^{99m}Tc]sodium pertechnetate was obtained aseptically. The ^{99m}Tc solution was then aseptically added to the vial in the lead shield. Without withdrawing the needle, an excess of 8 ml of head space was removed to prevent any buildup of positive pressure within the vial while heating. The contents of the vial were then swirled for 15 sec. A styrofoam cap (2 × 2 in) was then placed tightly over the vial top to prevent electrical sparking during heating procedure (Fig. 2).

The vial was taken out of the lead shield and placed in the lead-shielded microwave oven in the middle of the "cooking

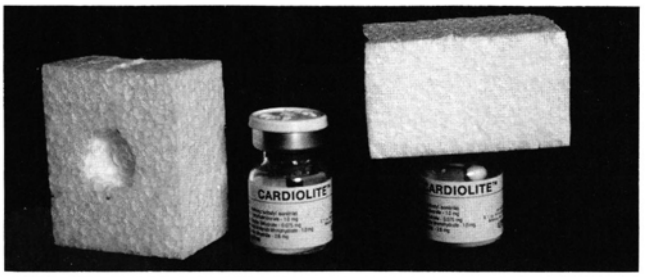

FIG. 2. A styrofoam cover is tightly placed over the sestamibi vial top to prevent electrical sparking.

surface" for exactly 13 sec at the highest intensity level. After the vial had been removed from the microwave oven, it was then placed in the radiation shield and radiochemical purity was assessed by TLC.

A domestic microwave oven was used for the tests. The heating of the samples was done at maximum intensity in all cases. A list of the technical specifications of the microwave are presented in Table 1.

Radiochemical Purity Assessment

Radiochemical purity was assessed by TLC and was performed on all vials. This procedure evaluates the percentage of labeled primary complex (^{99m}Tc-sestamibi) present in solution.

After preparation of the ^{99m}Tc-sestamibi, and without waiting for the product to cool down, TLC was then performed using the following procedure. In a 250-ml "beaker" tank, 10 ml of ethanol (100%) was added in order to get a depth of 3–4 mm of solvent in the bottom of the tank. The tank was covered to allow equilibration (saturation of ethanol gas in the tank) for 10 min. Using a 1-ml syringe with a 26-gauge needle, one drop of ethanol was applied onto the alumimum oxide (Baker flex) TLC plates to form a spot 1 cm from the bottom. Without allowing the ethanol spot to dry, a small volume of ^{99m}Tc-sestamibi was withdrawn from the vial and one drop was applied on top of the ethanol spot. The plate was then dried before it was developed for its entire length. The strip was taken out of the tank and cut at the midpoint of the migration. Each piece was measured in a dose calibrator. Calculation of the labeling efficiency in percent was performed as follows:

$$\frac{\text{net } \mu\text{Ci top portion (solvent front)} \times 100}{\text{net } \mu\text{Ci top portion (solvent front)} + \text{net } \mu\text{Ci bottom portion (origin)}}.$$

TABLE 1. Microwave Oven Specifications

Power input	120V, 60Hz, 7.5A, AC only 3-prong grounded plug
Power output	450 Watts
Microwave frequency	2,450 MHz
Outer dimensions	16.9 in (W) × 9.6 in (H) × 10.8 in (D)
Cavity dimensions	10.8 in (W) × 5.9 in (H) × 10.8 in (D)
Cavity volume	0.4 ft^3

The microwave oven was shielded with ¼-in. thick lead panel on three sides and the door side was protected with a lead glass shield. Wipe tests were performed before and after heating to evaluate contamination level within the microwave oven.

RESULTS

The product was prepared using the standard 10-min BWB method and demonstrated labeling efficiencies as shown in Table 2. No particulates or discoloration of the product were noted after visual examination of the test vial. No vials showed any cracks or signs of damage from the heating process. The average preparation time was 23.4 ± 3.0 min. This relatively long preparation time is mainly attributable to two of the preparation steps: (1) bringing the tap water to a boiling state; and (2) requirement of a 10-min boiling period.

The product prepared using the 13-sec MOH method demonstrated labeling efficiencies as shown in Table 2. No particulates or discoloration of the product were noted after visual examination of the test vial heated for 13 sec.

Wipe test results indicated no traces of contamination inside the microwave oven after the heating procedure.

DISCUSSION

Taillefer et al. (*19*) demonstrated that a 3-min period in a BWB was sufficient in order to obtain a 99% labeling efficiency with ^{99m}Tc-sestamibi. With that information, heating time was extrapolated from the temperature/time curve. After 3 min of boiling process in a BWB, the temperature reached 99°C. The temperature of 99°C extrapolated on the MOH method curve corresponds to a 9.0-sec heating period. To optimize the method, four seconds of heating process was added arbitrarily to the extrapolated heating time.

The results show no significant differences between the labeling efficiency of the ^{99m}Tc-sestamibi prepared with the BWB method versus the MOH method. The two methods showed an average of more than 95% labeling efficiency which is considered acceptable by the manufacturer standards for in vivo imaging. The radiopharmaceutical produced from either method was stable over a 24-hr period.

We have compared the relative complexity of both techniques and have found that the MOH method is simpler. The extra step to isolate the vial with styrofoam adds only a few seconds to the procedure. The withdrawal of 8 cc of head space (gas) from the vial is no more complicated or time-consuming for the MOH method than it is when withdrawing 3 cc for the BWB method. In addition, the MOH method circumvents the necessity of bringing the water bath to a boiling state, a significant time-consuming step in the heating process.

The TLC as described previously requires 10-15 min for migration, the slowest step in the preparation process. However new and faster TLC methods have been published (*19*) and may reduce preparation time even more.

Some attention had to be paid to the fact that the temperature within the vial follows laws of chemical equilibrium. The temperature in a boiling bath is more homogeneous and is not a matter of concern. However, the temperature within the vial could be much higher than the predicted solution boiling point when heated in the microwave oven. Due to the constant volume of the vial and the rising pressure in the vial, the temperature could exceed 138°C. Even though our results showed no damage to the vial or breakdown of the product after 20 sec of microwave heating, it is conceivable that accidents could occur with extreme overheating of the product. *One important step is to remove an excess of 8 ml of head space from the vial before microwave heating in order to avoid excessive pressure buildup in the vial.* More than 20 sec microwave heating might result in a discoloration of the material, rendering the solution unsuitable for injection. Radiation safety results showed no radioactive contamination inside the microwave oven after heating.

The styrofoam cover is an absolute necessity. Without it, sparking will occur and will damage the microwave oven. One solution to this problem would be for the manufacturer to provide vials with a plastic ring retainer for the rubber stopper.

The BWB method requires some special attention. The lead shield that surrounds the vial in the water bath serves to isolate the vial while boiling. Water inside the shield needs to be in contact with the water bath for an effective heating to occur, otherwise insufficient heating will result.

The test only considered a 3-ml volume within the vial. This was a logistic choice since the desirable concentration for injection is 25-35 mCi/ml. Any heating procedure with a lesser or greater volume has to be tested, since different volumes will show different temperature variation responses in the microwave oven. Loss of output power related to an extensive use of the microwave oven has not been evaluated over a long period of time. We did not consider this important, since only short heating periods are involved.

Refrigeration of the agent while the chromatography is performed is recommended since at room temperature the product would not have time to decrease its temperature to a level which is suitable for injection. Digital microwave ovens are preferable since they are more adaptable for short heating periods. Any microwave oven with different power output

TABLE 2. Technetium-99m-Sestamibi Labeling Efficiency Percentage of Primary Complex After Preparation

	1 min	10 min	6 hr	24 hr
Boiling bath	99.7% ± 0.3%	99.7% ± 0.3%	99.7% ± 0.3%	99.8% ± 0.3%
Microwave oven	96.4% ± 0.4%	98.2% ± 0.5%	98.6% ± 0.4%	98.5% ± 0.5%

and/or different cavity volume cannot be used to prepare ^{99m}Tc-sestamibi with the method described earlier. These different specifications would significantly change the heating process within the vial and in certain cases would probably make the ^{99m}Tc-sestamibi unsuitable for injection. The purpose of the study was not aimed at finding a reproducible MOH method. Thus the reproduction of the method with a commercially available microwave oven is not recommended without testing.

Presently the MOH method described in this study cannot be used on a routine basis in any laboratories since government regulations require strict compliance with product monograph for radiopharmaceuticals preparation.

CONCLUSION

The results of this study have demonstrated that the MOH method provides a reproducible heating technique to label ^{99m}Tc-sestamibi. With a labeling efficiency over 96.4% after 13 sec of heating, this procedure simplified and shortened the work involved in preparing this new radiopharmaceutical. Twenty minutes of heating process with the standard method (that includes 10 min to bring water to a boiling state) is reduced to 13 sec with the microwave. Even compared with a 3-min BWB method (*15*), it reduces heating time from 13 min to 13 sec. It is reliable and well suited for emergency situations when the agent needs to be available almost immediately. This new method will eliminate the need to prepare in advance many vials per day in case of emergency. Combined with a fast chromatographic method, the MOH method makes ^{99m}Tc-sestamibi available within 5 min after initiation of the preparation process.

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