Journal of Applied Pharmaceutical Science Vol. 6 (11), pp. 126-134, November, 2016
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2016.601120
ISSN 2231-3354

The stability of ^{99m}Tc-MIBI (Sestamibi) complex samples which prepared under ultrasound irradiation technique versus boiling water bath method

Alireza Doroudi[1*], Farkondeh Saraji[1], Mostafa Erfani[2], Seyyed Mostafa Saadati[3], Ali Kisast[3], Faramarz Ahmadi[3], Behrooz Etesami[3]

[1]School of Pharmacy , Ahvaz Jundishapur University of Medical Sciences , Ahvaz ,Iran.
[2]Radiation Application Research School , Nuclear Science and Technology Research Institute (NSTRI) , Tehran , Iran
[3]Nuclear Medicine Department, Golestan General Hospital Ahvaz Jundishapur University of Medical Sciences , Ahvaz ,Iran.

ARTICLE INFO

Article history:
Received on: 19/07/2016
Revised on: 14/08/2016
Accepted on: 06/09/2016
Available online: 29/11/2016

Key words:
Green chemistry, MIBI, Sestamibi, ^{99m}Tc-MIBI, Ultrasound irradiation.

Abbreviations
Bq: Becquerel , Ci: Curie, Mo: Molybdenium , TcO_4^-: Pertechnetate , Tc: Technetium.

ABSTRACT

Objectives: The main propose of this investigation is to evaluate the value of ultrasound irradiation technique versus boiling water bath in the reconstitution cold MIBI (Sestamibi) kits by technetium 99m.
Material and Methods: Twenty Sestamibi cold kits from various batches have been chosen. The vials were randomly divided into two equal groups. The first group was reconstituted by boiling water bath as standard method and the other group reconstituted by new developed technique. The stability of radio-complex samples were examined by thin layer chromatography (ITLC) and Radio high pressure liquid chromatography (Radio-HPLC) up to 24 hours post reconstitution. The partition coefficient and protein bonding of radiotracer samples were analyzed.
Results: The significant differences have not been observed in the yields and stability of radiotracer samples which were prepared by either two methods in ITLC and Radio-HPLC investigations. The partition coefficient and protein bonding assays demonstrated that radiotracer samples which were prepared by boiling water bath were a little more lipophilic than the radio-complex samples which were reconstituted by new developed technique.
Conclusion: Green chemistry is convenient and efficient modality to reconstitute the cold Sestamibi kit by technetium 99m. It can be established as an alternative method to prepared ^{99m}Tc-Sestamibi in the clinical practice.

INTRODUCTION

The assessment of a subject with chest pain is still challenging dilemma in emergency departments. Electro-cardiogram finding and clinical evaluations at the time of presentation have a low sensitivity and specificity (18-65%), (69%) respectively (Weissman *et al.*, 1996). The potential risk of inappropriate discharge could lead to unnecessary high number of admissions to coronary care unit in the hospitals. Therefore, preferentially diagnosis of coronary disease could be refrained unnecessary admission at the hospitals and prevented to loss the golden time in order to avoid any delay in acute therapy for myocardial infarction. Radioisotope scintigraphy imaging can be considered as noninvasive diagnostic modalities in this regard. For this reason, different radiotracers have been successfully suggested in Single Photon Emission Computerized Tomography (SPECT) like ^{201}Tl and ^{99m}Tc- 2-methoxy isobutyl (^{99m}Tc-MIBI) and Positron Emission Tomography (PET) like ^{82}Rb and ^{18}F-DG for myocardial perfusion imaging (Gould, 1991; Berman *et al.*, 1994; Merhige *et al.*, 2007; Salerno and Beller, 2009; Jain and He, 2015). PET scan provides to obtain images with higher resolution than SPECT scintigraphy imaging.

* *Corresponding Author*
Dr Alireza Doroudi, E-mail address: Doroudi-a@ajums.ac.ir
Postal Code: 6135733184, Tel:986133738378 , Fax: 986133738381

But SPECT scintigraphy imaging is cheaper and more available than PET scan imaging especially for undeveloped countries. Thallium 201 is supplied as thallous chloride in isotonic solution. It is generated in a cyclotron and must be transferred to the nuclear medicine departments. ^{201}Tl is a potassium analog and the most primitive radioisotope for this purpose. It has been taken up by viable myocyte cells in proportion to blood flow, but unfortunately it has suffered from the following disadvantages. It produces gamma rays 170 keV (~ 10 %), 135 keV (~3%). The energies of photons are 69-80 keV which are abundance and used for scintigraphy imaging resulted from electron capture decay. These low energies are not suitable for imaging analysis. The physical half-life is long approximately 72 h. Therefore, ionizing irradiation dosimetry is unfavorable because of the high total body absorbed dose (Einstein *et al.,* 2007; Kusmierek and Plachcinska, 2012). In addition to the above mentioned factors, ^{201}Tl radioisotope is the product of cyclotron and relative high expensive and is not available as a generator.^{99m}Tc-MIBI (called Sestamibi) radiotracer is a promising radiotracer for myocardial perfusion imaging (Manka-Waluch *et al.,* 2006; Fard-Esfahani *et al.,* 2010; Fukushima *et al.,* 2010) . The popularity of ^{99m}Tc-MIBI is related to label by technetium-99m. ^{99m}Tc is widely used in diagnostic procedures in clinical practice. Its popularity is mainly to the matter that the radioisotope can be readily produced by ^{99}Mo/^{99m}TC generators. It has the ideal γ-ray energy (140keV) which is suitable for gamma camera detection. It is necessary to prepare the ^{99m}Tc-MIBI radiotracer rapidly to provide the myocardial perfusion imaging. According to the labeling protocol that is provided by manufacturer, the reconstitution of ^{99m}Tc-MIBI by technetium -99m radioisotope requires 10-15 min to bring the water bath to a boiling state. The mixture solution in the shielded lead vial must be heated on boiling water bath for 10 min and removed the shield vial from the water bath, placed in the lead shield and allowed cooling for 15 min. The labeling process of MIBI by ^{99m}Tc is time-consuming by boiling water bath as standard or conventional method. It is highly desirable to reduce the time of labeling process particularly in emergency situations in clinical practice. The ^{99m}Tc-MIBI has also been recommended to assess the tumor size and the treatment efficiency of tumors of breast (Vecchio and Salvatore, 2004), parathyroid (Spanu *et al.,* 2004) and lung (Dirlik *et al.,* 2002).Ultrasound irradiation technique is routinely used in the different branches of chemistry. The reactions are carried out in order to obtain higher yields, shorter time or milder conditions under ultrasound irradiation modality (Khalaj *et al.,* 2006; Khalaj *et al.,* 2009 ; Pizzuti *et al.,* 2010). In our previous study, 37 MBq (1mCi) ^{99m}Tc-MIBI radiopharmaceutical samples with appropriate yields could be reconstituted under ultrasound irradiation technique (Doroudi *et al.,* 2013).Thereafter, the bio-distribution of ^{99m}Tc-MIBI complex samples were studied in the rat. Then the activity was scaled up to the amounts that could be used for myocardial perfusion imaging. Finally the new developed technique has been suggested for preparation of ^{99m}Tc-MIBI samples in clinical practice (Doroudi *et al.,* 2015) .We continued our achievement to the other radiopharmaceutical kit which the reconstitution is time-consuming in nuclear medicine departments (Doroudi *et al.,* 2015). The ^{99m}Tc-UBI complex samples could be prepared with the sufficient yields under ultrasound irradiation technique. This study was launched to validate the new developed technique as a reliable alternative method for preparation ^{99m}Tc-MIBI complex samples in nuclear medicine departments. Therefore, the stability and pharmacokinetic parameters such as partition coefficient and protein bonding of ^{99m}Tc-MIBI complex samples were investigated which the radio-complex samples were reconstituted by boiling water bath or ultrasound irradiation methods.

MATERIAL AND METHODS

All chemical materials have been purchased from Merck and Fluka. The chemicals and solvents were the highest purity and analytical grade and used without further purification. The freeze-dried MIBI kits and ^{99}Mo/^{99m}Tc generators have been provided by Radioisotope Division of Atomic Energy Organization of Iran (AEOI). Technetium 99m as sodium pertechnetate was obtained from an in-house ^{99}Mo/^{99m}Tc generator using 0.9 % saline.

Labeling of MIBI by ^{99m}Tc radioisotope

The commercial freeze-dried Sestamibi kit (AEOI) contains 2-methoxy isobutyl isonitrile as a performed copper (I) complex, Cu $(MIBI)_4^-BF_4^-$ [Tetrakis (2-methoxy isobutyl isonitrile) Cu (I) tetrafluroroborate]. Reconstitution of sestamibi kit with pertechnetate 99m sodium (Na $^{99m}TcO_4$) is performed according to the manufacturer's instructions. On the basis of instructions, the volume 1-3 ml of freshly eluted Na $^{99m}TcO_4$ 925-5550 MBq (25-150 mCi) could be added to the sestamibi vial aseptically. A total number of twenty commercial cold kits were chosen from different batches. The vials were randomly divided into two equal groups. The vials were reconstituted by boiling water bath as a standard method in one group. The vials in other group were reconstituted by ultrasound irradiation as a newly developed technique in the optimum conditions that were determined and reported to the literature (Doroudi *et al.,* 2013; Doroudi *et al.,* 2015). Consequently, technetium Na $^{99m}TcO_4$was obtained from an in-house ^{99}Mo/^{99m}Tc generator using 0.9 % saline. The vials were put in the lead shield. The shielded vials were shaked for 30 seconds .The vials (n=10) were heated on a boiling water bath for 10 min , according to the instructions provided by manufacturer (AEOI) or the vials (n=10) were sonicated in the thermo stated bath (Elma, P= 95 w made in Germany) for 1 min at 65ºC .

Quality control

Radiochemical impurities and labeling efficiency were investigated by Instant Thin Layer Chromatography (ITLC) and Radio High Pressure Liquid Chromatography (Radio-HPLC).ITLC analysis was performed by using Whatman No.3 filter paper chromatography as the stationary phase. Normal saline solution and methanol as two different

solvent systems were used as mobile phase. The strips in 2 cm width and 10 cm length were used. The samples containing ^{99m}Tc-MIBI (2 µl) were prepared by either two above mentioned methods applied 1 cm from the bottom of ITLC strips. The strips were allowed to dry at room temperature and then placed in air-tight containers. The radio-complex and reduced technetium- 99m ($^{99m}TcO_2$) remained at the spotting point and free $^{99m}TcO_4$ traveled to solvent front, when normal saline solution was used as mobile phase. The $^{99m}TcO_2$ remained at the spotting point, when methanol was used as another mobile phase. But the radio-complex and free $^{99m}TcO_4$ moved to the solvent front. After migration of mobile phase to 1 cm from the top in all studies, the strips were removed from the air-tight containers and allowed to dry at room temperature. The strips were cut to ⅓ lower and ⅔ upper pieces.

Each piece was counted for 2 minutes under a single head camera equipped with low energy all-propose collimator using an energy peak centered a140keV with NaI (Tl) detector. The Radio-HPLC analysis was performed with analytical reverse-phase on a JASCO 880-PU intelligent pump HPLC system (Tokyo, Japan) equipped with a multiwavelength detector and a flow-through Raytest-Gabi g-detector CC 250/4.6 Nucleosil 120-5 C-18 column from Teknokroma was used for HPLC. For radionuclide analysis of ^{99m}Tc-MIBI complex by HPLC, a volume of 10µl of the test solution was injected into the C-18 reverse-phase column and trifluoroacetic acid 0.1%/water (solvent A) and acetonitrile (solvent B) were used as a mobile in following gradient : 0 min A 95% (B 5%), 5 min A 95% (B 5%) , 25 min A 0% (B 100%) and 30 min A 0% (B 100%) , flow= 1 ml/min Figure1 and 2.

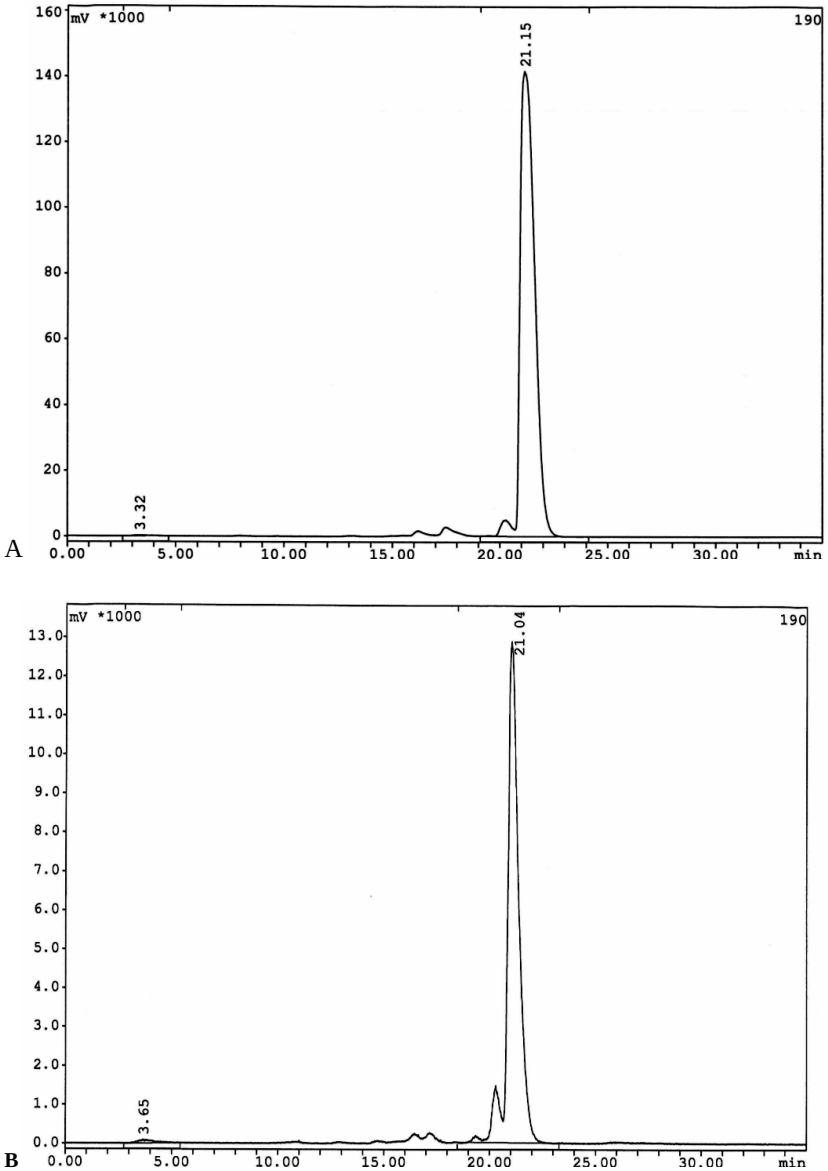

Fig 1. The radiochemical stability of ^{99m}Tc-MIBI (Sestamibi) in saline after 0.5 h reconstitution.
The Radio-chromatogram profiles of ^{99m}Tc-MIBI complex samples were prepared by a: water boiling bath b: ultrasound irradiation methods.

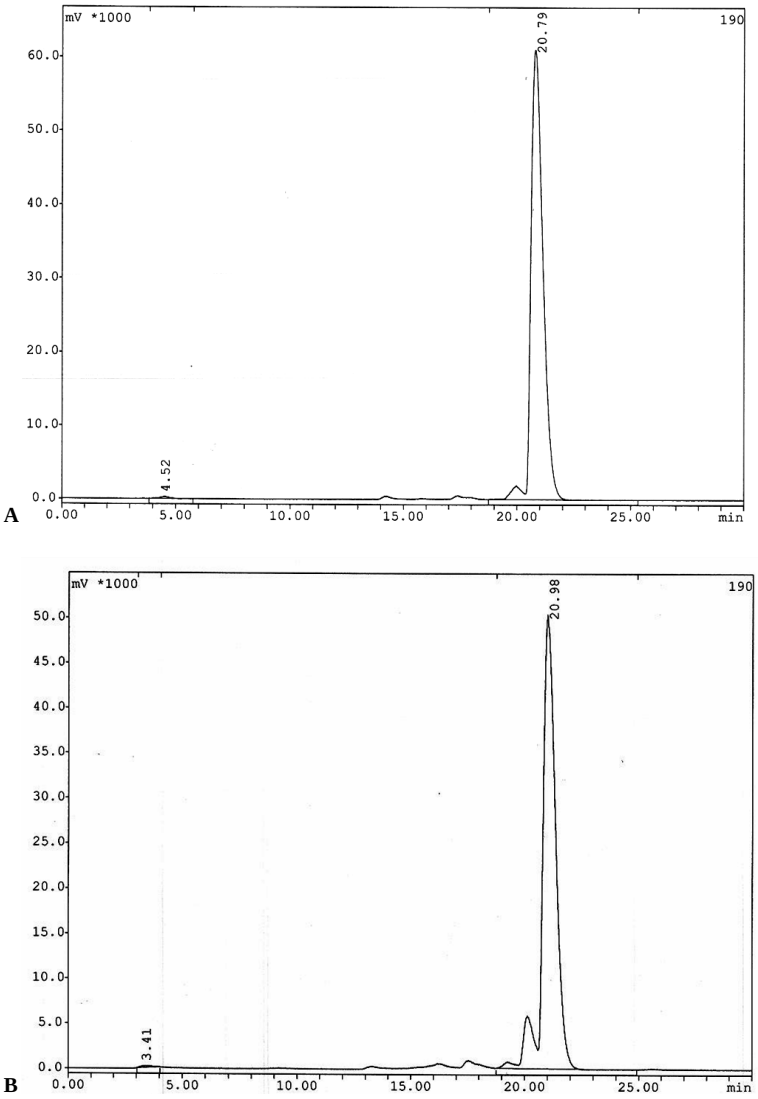

Fig. 2: The radiochemical stability of ^{99m}Tc-MIBI (Sestamibi) in saline after 24 h reconstitution.
The Radio-chromatogram profiles of 99mTc-MIBI complex samples were reconstituted by a: water boiling bath b: ultrasound irradiation methods.

Table 1: The yield of ^{99m}Tc-MIBI (Sestamibi) complex and radiochemical impurities in saline solution have been obtained by Instant Thin Layer Chromatography (ITLC).

Time / Yield %	0.5 h	1 h	2 h	4 h	6 h	24 h
$^{99m}TcO_2$	3.23± 1.24	2.3±1.26	3.47± 1.21	3.41± 2.31	4.12± 2.28	18.79± 1.87
	3.15±1.28	3.76± 1.45	3.7± 1.86	4. 5± 2.11	4.30±2.43	17.48± 2.65
$^{99m}TcO_4$	1.32± 0.85	2.16±0.83	2.73± 1.10	3.59± 1.72	3.82± 1.65	10.47± 2.34
	2.88±1.78	3.14± 1.61	3.14± 1.61	4.5± 1.88	4.78± 2.02	10.55± 1.77
^{99m}Tc-MIBI	95.45± 2.19	95.54±2.41	93.9±2.15	93.00± 2.1	92.06± 2.19	70.74± 1.42
	93.97±1.35	93.10±1.12	93.16± 1.71	91.25± 1.15	90.92±1.42	71.97± 2.08

The 1480 MBq (40 mCi) Na $^{99m}TcO_4$ freshly eluted solution was added to Sestamibi kit. The mean yields of $^{99m}TcO_2$, $^{99m}TcO_4$ and ^{99m}Tc-MIBI have been obtained by ITLC 0.5, 1, 2,4,6 and 24 h post the reconstitution sestamibi kits . The top of each row belongs to the radiotracer samples (n= 10) which were prepared by boiling water bath method. The bottom of each row belongs to the radiotracer samples (n= 10) which were prepared by ultrasound irradiation technique.

Physical stability analysis

The stability analysis of radio-complex samples was performed. Therefore, the yield of radiotracer and purity of ^{99m}Tc-MIBI complex samples and radiochemical impurities were assessed by ITLC and Radio-HPLC at different intervals up to 24 h post the reconstitution kits. Aliquots were taken out at 0.5,1,2,4,6 and 24 h at room temperature and analyzed by ITLC and Radio-HPLC.

The data have been obtained from this investigation are shown in table 1 and 2.

Table 2: The yield of ^{99m}Tc-MIBI (Sestamibi) complex and radiochemical impurity ($^{99m}TcO_4$) in saline solution have been obtained by Radio-HPLC analysis.

Yield % \ Time	0.5 h	1 h	2 h	4 h	6 h	24 h
$^{99m}TcO_4$	0.45± 0.12	0.96± 0.11	0.93± 0.21	0.32± 0.16	0.55± 0.21	2.30± 0.92
	0.91± 0.06	0.9± 0.14	0.72± 0.08	0.26± 0.1	1.64± 0.65	3.32± 1.21
^{99m}Tc-MIBI	99.55± 0.18	99.04± 0.16	99.07± 0.28	99.68±0.19	99.45± 0.44	97.70± 0.66
	99.09± 0.24	99.1± 0.2	99.28± 0.18	99.74± 0.25	98.36± 0.70	96.68± 1.08

The mean yields of $^{99m}TcO_4$ and ^{99m}Tc-MIBI have been measured by Radio-HPLC analysis. The top of each row is related to the radio-complex samples (n= 10) which were reconstituted by boiling water bath method. The bottom of each row is related to the radio-complex samples (n= 10) which were reconstituted by ultrasound irradiation technique.

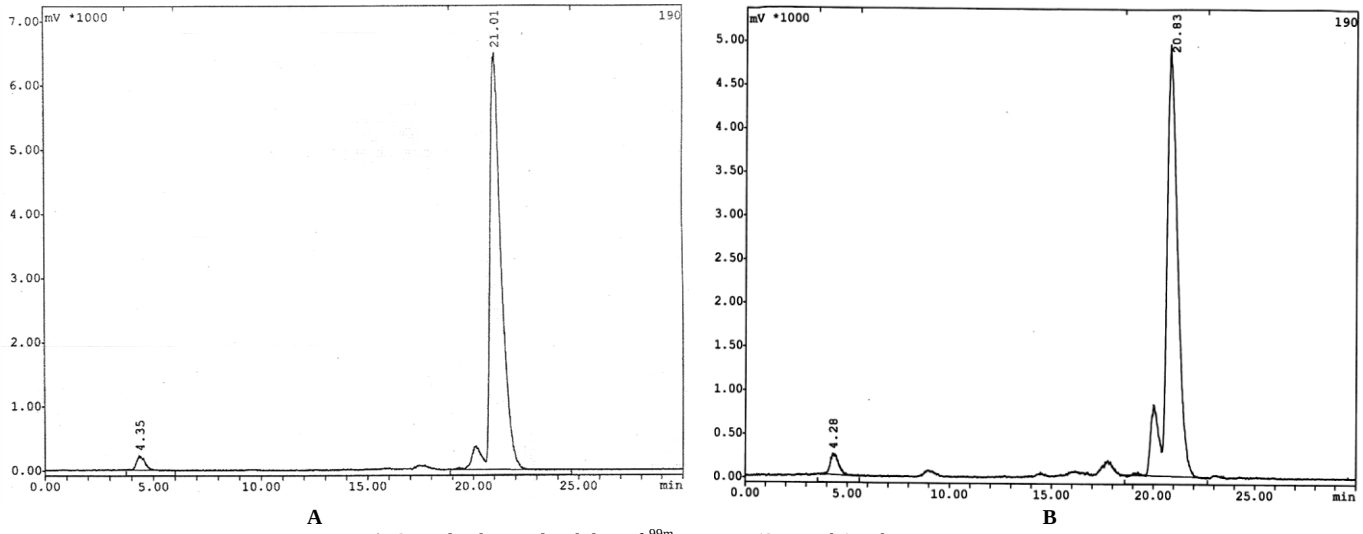

Fig 3: Radiochemical stability of ^{99m}Tc-MIBI (Sestamibi) in human serum.
Radio-HPLC chromatograms have been obtained from the supernatant solution of ^{99m}Tc-MIBI (Sestamibi) complex samples in human serum. The radio-complex samples were reconstituted due to a: boiling water bath b: ultrasound irradiation techniques.

Partition coefficient

Octanol and water solvents were chosen in order to determine and calculate the partition coefficient of radio-complex samples which were prepared by boiling water bath or ultrasound irradiation techniques two hours after the preparation radio-conjugate samples. The amount of 50µl of test solution was mixed with 2 ml octanol and 2 ml water in a tube. The tube was vigorously vortexed over a period of 5 min and centrifuged at 500×g for 5 min. Three aliquots of 50 µl were sampled from each layer and counted in the gamma counter. The mean activities from the octanol and water layers were calculated for each radiotracer test sample. The octanol to water partition coefficient (P O/W) was measured by dividing the counts of the octanol phase by that of the aqueous phase. The value of log partition coefficient (log P O/W) was considered for lipophilic characteristic of the radiotracer complex sample.

Protein bonding

Protein bonding investigation was performed 2 h after the preparation of ^{99m}Tc-Sestamibi radiopharmaceutical kits. Protein bonding analysis of the ^{99m}Tc-MIBI complex samples were undertaken as follow: 100 µl of each sample test was added to 1 ml freshly human serum albumin in micro tube. The mixtures were shaked by shaker 15 min and incubated at 37ºC in incubator for 12 h. The samples were removed from incubator. Each sample test was treated by 1 ml ethanol and centrifuged for 15 min at 500×g in order to precipitate serum albumin. Sediment and supernatant solution were separated and counted by gamma counter .The activity of each section was measured and quantified. The protein bonding of radiotracer complex sample or radio-metal transferred to serum albumin was calculated by dividing the activity of precipitated protein to the total activity of sediment and supernatant multiple 100.In the other word ,Protein bonding radiotracer % = (activity of sediment / activity sediment+ activity supernatant)× 100. The supernatant for each sample tube was also assayed with Radio-HPLC to determine serum stability for radiotracer complex sample Fig 3.

Statistics

The calculations of means and standard deviations were made on Microsoft Excel. The data were shown as the mean ± SD.

RESULTS

Limitations such as reduction fuel in Tehran Research Nuclear Reactor due to imposed sanctions against peaceful nuclear program of Iran by foreign countries, as well as our nuclear medicine department in Golestan hospital is only general section to satisfy the needs of people who are living in the southwest of Iran. The activities higher than 1480 MBq (40 mCi) $Na^{99m}TcO_4$ were unavailable in this research work, for this reason all Sestamibi kits were reconstituted by1480 MBq (40 mCi) $Na^{99m}TcO_4$. Free $^{99m}TcO_4$ and $^{99m}TcO_2$ are two main impurities produced during the labeling of Sestamibi kit with^{99m}TC. The $^{99m}TcO_2$, $^{99m}TcO_4$ and radiotracer complex sample can be readily identified and quantified by ITLC analysis. But the $^{99m}TcO_2$ impurity cannot be detected and determined by Radio-HPLC study. ^{99m} Tc-Sestamibi and $^{99m}TcO_4$ are identified by Radio-HPLC analysis. Only the proportion of $^{99m}TcO_4$ and radio-complex are detected and measured due to Radio-HPLC assay. On the basis of data have been obtained from ITLC study, the yields of radiotracer samples were prepared by boiling water bath were 95.45±2.19, 95.54±2.41, 93.9± 2.15 , 93.00 ±2.10, 92.06± 2.19 and 70.74±1.42% after 0.5 ,1, 2, 4, 6 and 24 h post the reconstitution Sestamibi kits (n= 10). The ILTC analysis indicated that radiotracer samples were completely stable until 6 h post the reconstitution kits. The percent of $^{99m}TcO_2$ impurity has been considerably increased after 6 h and reached from 4.12± 2.28 to maximum 18.79± 1.87 % after 24 h post the reconstitution kits. It is confirmed this matter, the yield of desirable radiotracer complex samples can be influenced by the gamma ray emission from technetium 99m radioisotope. The bonding between Sestamibi ligand and technetium 99m is not strong like the covalent or ionic bonding. The radioisotope can be released from radiotracer sample and reduced and hydrolyzed by stannous chloride that is present in the aqueous environment to produce $^{99m}TcO_2$ radiochemical impurity. The data have been obtained by ITLC analysis demonstrated that the percent of yields of radiotracer complex samples were 93.97±1.35, 93.10±1.12, 93.16±1.71,91.25±1.15, 90.92± 1.42and 71.97± 2.08 % after 0.5,1,2,4,6 and 24 h post the reconstitution which the samples were reconstituted by new developed modality. The radio-complex samples have been observed to be stable maximum 6 h post the reconstitution kits. The yields of labeling by ultrasound irradiation technique at different times were very similar to the yields of radio-complex samples which were prepared by the conventional method. The free $^{99m}TcO_4$ and ^{99m}Tc-Sestamibi can be readily detected and quantified by Radio-HPLC analysis. Therefore, the yields of radiotracer complex samples were 99.55± 0.18, 99.04± 0.16, 99.07± 0.28, 99.68±0.19, 99.45± 0.44 and 97.70± 0.66 % after 0.5,1, 2, 4, 6 and 24 h post the reconstitution kits which were prepared by standard method. The yields were 99.09± 0.24, 99.1± 0.2, 99.28± 0.18, 99.74± 0.25, 98.36± 0.70 and 96.68± 1.08% for the above mentioned time intervals for radio-complex samples which were reconstituted by the new developed modality. The Radio-HPLC analysis indicated that the radio-complex samples were reconstituted by either two methods was stable up to 24 h post the reconstitution kits Fig 2. The decomposition of the radiotracer samples has not been observed in this period, suggesting its high stability in normal saline at room temperature. As it can be seen in Fig1,2 and 3, the retention times of free $^{99m}TcO_4$ and ^{99m}Tc-sestamibi were approximately 4 min and 21 min when the radiotracer complex samples were reconstituted by boiling water bath or conventional method. The retention times of ^{99m}Tc-Sestambi complex samples were identical which were prepared by either two methods. Radio-HPLC analysis indicated that the reactions were leaded to a single radio-complex and its retention times were found to be approximately 21min. The outcome of our approach demonstrated that the successful reconstitution of ^{99m}Tc-Sestamibi by new developed technique. The partition coefficient and protein bonding were 0.711± 0.14 and 74.40±1.87 % respectively for the radio-complex samples (n= 10) which were prepared by boiling water bath method. These parameters were 0.67 ± 0.07 and 68.39±2.28% for radio-conjugate samples (n= 10) which were reconstituted due to ultrasound irradiation technique. It revealed this matter that the radiotracer samples were reconstituted by the conventional method was a little more lipophile than the radio-complex samples were prepared by the new developed technique. It could be related to this fact that the high temperature and long period of heating is used in the boiling water bath method versus the ultrasound irradiation technique. Therefore, the different isomer of radio-complexes could be formed and influenced the partition coefficient and protein bonding parameters. The stability of radiotracer samples was checked in human serum at 37 °C. As it is stated in Fig 3, the radio-complex samples showed good stability in human serum.

DISCUSSION

Technetium 99m radioisotope in the $^{99m}TcO_4$ form is present in the elution solution which is usually obtained from in-house ^{99} MO/^{99m}Tc generators. Technetium is in the highest valance state. This form of technetium 99m cannot react with any ligands in order to produce the desirable radio-complex. The reducing agent such as stannous chloride is widely used in the formulation of radiopharmaceutical kits. The pivotal role of stannous chloride is the reduction of the highest oxidation state of technetium 99m to the lower valence state which can be able to react with unshared electrons of ligands. Therefore, the structure of ligand must have electron donor groups like oxygen, nitrogen, phosphorus or sulfur in order to form the bonding between the unshared electrons of ligand and the empty orbitals of technetium 99m.It is obvious the nature of this kind of bonding is not strong as covalent or ionic bonding. The labeling process of lipophilic cationic ^{99m}Tc (I)-hexakis (2-methoxy isobutyl isonitrile) tetrafluoroborate is occurred by reaction tetrakis (2-methoxy isobutyl isonitrile) copper (I) tetrafluoroborate adduct with freshly eluted solution of $^{99m}TcO_4$. The exact structure of ^{99m}Tc-Sestamibi radio-complex has not been completely elucidated. It is assumed that hexakis (alkyl) technetium (I) is monovalent cation with a

central technetium (I) core surrounded by six identical lipophilic ligands coordinated through the isonitrile carbon in an octahedral geometry (Piwnica-Worms *et al.,* 1993). group acts as an electron donor group in the ligand structure. The unshared electrons of isonitrile group are not readily available for the bond formation. Therefore, the labeling process of Sestamibi kits with technetium 99m is facilitated by heating at the elevated temperature. The boiling water bath method is routinely used as heating source in nuclear medicine departments. But the conventional technique is time-consuming for the reconstitution Sestamibi kits especially in the emergency situation. The microwave heating method has been recommended as an alternative modality for the preparation Sestamibi radiotracer. This technique has been introduced not only for synthesis of Sestamibi (Lima *et al.,* 2005) but also for labeling of Sestamibi by technetium 99m radioisotope (Gagnon *et al.,* 1991; Hung *et al.,* 1991). When microwave irradiation was used as heating source, the required time for the preparation radiotracer complex samples was reduced to 10 seconds. The new developed technique was suffered from the following precautions when the vials were placed inside the microwave oven apparatus. The potential risk of sparking for the presence of metal cap of the vial must be considered.

The geometry of vial samples is important factor. Any residual gas left in the head space of the vial could cause an ejection of the rubber stopper due to the excess steam pressure built up the vial. Microwave devices with digital control panel are more suitable for setting short heating time, since they can be accurately set at the required heating period. The loss or variation of microwave output and frequency related to extended use of the microwave oven must be evaluated on a long-term usage. Any technical error in setting the instrument heating time below or beyond the predetermined time may lead in the ^{99m}Tc-Sestamibi solution being rendered inappropriate for clinical use. In spite of rapid reconstituted of ^{99m}Tc-Sestamibi complex samples by microwave oven method, for the above mentioned factors the new developed technique is not commonly used practically. Sono-chemistry is a branch of chemical research dealing with the application of ultrasound waves. Ultrasound irradiation occurs at a frequency, higher than the audible threshold of human hearing, and is typically associated with the frequency range of 20 KHz to 500 MHz.

Low intensity, high frequency ultrasound irradiation does not alter the state of the medium through which it travels and is commonly used for non-destructive assessment and medical diagnosis. However, high intensity, low frequency ultrasound irradiation can alter the state of the medium and is the type of ultrasound irradiation is typically used for sono-chemistry applications. Ultrasound irradiation has demonstrated to be a very useful laboratory tool in enhancing the reaction rates in a different of reacting systems (Moon *et al.,* 1979; Kristol *et al.,* 1981). This modality has successfully improved the yield of the reactions, changed the reaction pathway or initiated the reaction. In addition to the above mentioned factors, ultrasound irradiation can decrease the number of synthesis steps and provide the use of lower purity reagents and solvents or enhance the activity of existing catalysts. For this reason the application of ultrasound irradiation technique appears to be a promising alternative for high value chemical and pharmaceutical products (Berlan and Mason, 1992; Brenmer, 1994; Luche , 1996; Mitragotri *et al.,* 1996; Dhumal *et al.,* 2008 ; Park *et al.,* 2013; Jagtap *et al.,* 2015). Ultrasound irradiation sounds have two direct and indirect effects. The direct effects of ultrasound waves have low energies in order to alter electronic, vibrational and rotational molecular states. The indirect effect of ultrasound waves are caused by cavitation bubbles which are generated during the rarefaction or negative pressure. During the negative pressure cycle, the liquid is pulled apart at sites containing some gaseous impurities, forming void. Pressure wave cycle exceeds the attractive forces of the molecules, if the power of generating ultrasound waves is appropriate. Cavitation bubbles can be formed and bubbles grown over a few cycles. Bubbles rapidly suffer expansion and finally bubbles are collapsed.The collapse of bubbles caused by cavitation produces intense local heating and high pressure, with very short lifetimes. Therefore, cavitation phenomenon generates enough energy to alter vibrational and rotational molecular states (Gong and Hart, 1998; Tuziuti *et al.,* 2006). The cavitational activity is directly proportional to the number density of particles present in the medium (Madanshetty and Apfel, 1991).

The ambient conditions of reaction system can greatly influence the intensity of cavitation, which directly affects the reaction rate or yield. These conditions include the reaction temperature, hydrostatic pressure, irradiation frequency, acoustic power and ultrasound intensity. The appropriate conditions for the preparation of ^{99m}Tc-Sestamibi complex samples due to ultrasound irradiation technique have been examined in our previous studies. Our investigations indicated that two main factors are crucial role in the reconstitution Sestamibi kits by ultrasound irradiation technique. These factors are the temperature reaction and the time period of reaction. The locally induced heating by ultrasound irradiation could not produce radiotracer samples with suitable yields. The heating required at 65ºC in order to produce the radio-complex samples with sufficient yields. An overall decreased the yields of radio-complex samples have been observed when the temperature reaction was used above 65ºC. Another important factor is reaction time.

The bonding between the ligand and technetium 99m radioisotope is not strong as covalent or ionic bonding. Therefore, the reaction time for more than 1 min at 65 ºC could influence the nature of bonding or disintegrate the structure of radio-complex samples (Doroudi *et al.,* 2013). The following advantages can be considered for the reconstitution of Sestamibi kits by ^{99m}Tc due to ultrasound irradiation technique versus boiling water bath method. The reaction time has been significantly decreased by using new developed technique. It is highly favourable in the emergency situations in clinical practice. It is permitted a fast and reliable method to make ^{99m}Tc-Sestamibi samples, if ultrasound facility is available in any nuclear medicine departments. The potential risk of absorbed ionization irradiation dose can be reduced to the staff

members who are working in nuclear medicine departments. The labeling of Sestamibi kits can be readily carried out by ultrasound instrument. There is no potential risk of sparking for the presence of metal cap and shielded lead container. The geometry of samples in the instrument is not important factor. The radiotracer samples can be obtained with the sufficient yields. Energy consumption can be saved by ultrasound irradiation technique versus boiling water bath method. Different ultrasound irradiation devices with varieties power are available in markets. It is necessary to set out this new developed technique in order to find out the optimum conditions from the aspect of temperature and reaction time on the basis of power of instrument. Then the reconstitution of Sestamibi kits with ^{99m}Tc can be easily carried out according to the suitable and ideal conditions that would be determined.

CONCLUSION

The outcome of our investigations demonstrated that the application of ultrasound irradiation technique is an interesting alternative approach for the reconstitution ^{99m}Tc-MIBI complex samples. The reaction time for the preparation radio-complex can be reduced significantly in comparison to boiling water bath method. This major achievement may be decreased potential risk to the patient in order to prevent any delay in emergency situations such as acute therapy particularly for myocardial infarction patients in clinical practice. Green chemistry can be recommended for the preparation of the radiopharmaceutical kits that the reconstitution of kits is time-consuming in nuclear medicine departments.

ACKNOWLEDGMENTS

This approach is part of Pharm-D thesis of Farkondeh Saraji. The authors gratefully acknowledge Head and staff of Radioisotope Division of Atomic Energy Organization Iran for their assistance and good Co-operation.

Financial support and sponsorship: This work has been carried out with financial support from Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Conflict of Interests: Authors have no relevant financial interests related to the material in this manuscript. They also have no conflict of interests to declare.

REFERENCES

Berlan J, Mason TJ. Sonochemistry from research laboratories to industrial plants. Ultrasonics 1992;30:203-212

Berman DS, Kiat HS, Van Train KF, Germano G, Maddahi J, Friedman JD. Myocardial perfusion imaging with technetium-99m-Sestamibi: comparative analysis of available imaging protocol. J Nucl Med 1994;35:681-688

Brenmer DH. Recent advances in organic synthesis utilizing ultrasound. Ultrson Sonochem 1994;1:119-124

Dhumal RS, Biradar SV, Yamamura S, Paradkar A, York P. Preparation of amorphous cefuroxime axetil nanoparticles by sonoprecipitation for enhancement of bioavailability. Eur J Pharm Biopharm 2008;70:109–115

Dirlik A, Burak Z, Goksel T, Erinc R, Karakus H, Ozcan Z, Veral A, Ozcan M . The role of 99mTc- Sestamibi in predicting clinical response to chemotherapy in lung cancer. Ann Nucl Med 2002;16:103-108

Doroudi A, Erfani M, Norouzi B, Sadaati SM, Kiasat A, Ahmadi F, Etesami B, Bagersad MH. Clinical application of ultrasound for preparation of ^{99m}Tc-Sestamibi complex. Ann Nucl Med 2015;29:295-301

Doroudi A, Erfani M, Zaheri M, Saadati SM, Ahmadi F, Kiast A, Etesami B, Meghdadi H, Rezaee S. Preparation of ^{99m}Tc-UBI$_{29-41}$ complex samples under ultrasound irradiation technique. Inter J Curr Res 2015;7:21795-21801

Doroudi A, Saadati SM, Hassanpour H, Ahmadi F, Erfani M, Rezaee S, Khodayar MJ, Samaee H, Etesami B. Preparation of ^{99m}Tc-MIBI under ultrasound irradiation. J Radioanal Nucl Chem 2013;298:1185-1190

Einstein AJ, Moser KW, Thompson RC, Cerqueira MD, Henzlova MJ. Radiation dose to patients from cardiac diagnostic imaging. Circulation 2007;116:1290-1305

Fard-Esfahani A, Fallahi B, Mohaghegh A, Assadi M, Beiki D, Eftekhari M, Saghari M, Takavar A. The value of myocardial perfusion imaging with ^{99m}Tc-MIBI for the prediction of perfusion improvement after percutaneous transluminal coronary angioplasty. Iran J Nucl Med 2010;18:7-13

Fukushima K, Mamosa M, Kondo C, Higuchi T, Kuasakabe K, Hagiwara N. Myocardial ^{99m}Tc-Sestamibi extraction and washout in hypertensive heart failure using an isolated rat heart.

Nucl Med Biol 2010;37:1005-1012

Gagnon A, Taillerfer R, Bavaria G, Leveille J. Fast labeling of technetium-99m –Sestamibi with microwave oven heating. J Nucl Med Technol 1991;19:90-93

Gong C, Hart DP. Ultrasound induced cavitation and sonochemical yields. J Acoust Soc Am 1998;104:2675-2682

Gould KL. PET perfusion imaging and nuclear cardiology. J Nucl Med 1991;32:579-606

Jain D, He ZX. Direct myocardial ischemia imaging with exercise ^{18}FDG. J Nucl Cardiol 2015;22: 111-114

Hung JC, Wilson ME, Brown ML, Gibbons RJ. Rapid preparation and quality control method for technetium-99m-2-methoxy isobutyl isonitrile (technetium-99m-Sestamibi). J Nucl Med 1991; 32: 2162-2168.

Jagtap VA, Vidyasagar G, Dvivedi SC. Solubility enhancement of rosiglitazone by using melt

sonocrystallization techniques. J Ultrasound 2014;17:27-32

Khalaj A, Doroudi A, Ostad SN, Khoshayand MR, Babai M, Adibpour N. Synthesis ,aerobic,

cytotoxicity and radiosensitizing activity of 2,4-dinitophenylamine tethered 5- fluorouracil and hydroxyurea. Bioorg Med Chem Lett 2006;16:6034-6038

Khalaj A, Doroudi A, Adibpour N, Araghi M. N-alkylation and N-acylation of 2,4-dinitrophenylamine by ultrasound irradiation. Asian J Chem 2009;21:997-1001

Kristol DS, Klotz H, Parker RC. Effect of ultrasound on the alkaline hydrolysis of nitrophenyl esters.Tetrahedron Lett 1981; 22: 907-908

Kusmierek J, Plachcinska A. Patient exposure to ionizing radiation due to nuclear medicine cardiac procedures. Neu Med Rev Cent East Eur 2012; 15: 71-74

Lima MJC, Marques FLN, Okamoto MRY, Garcez AT, Sapienza MT, Buchpigqel CA. Preparation and evaluation of modified composition for lyophilized kits of $[Cu(MIBI)_4]BF_4$ for [99mTc]technetium labeling . Braz Arch Biol Technol 2005;48:1-8

Luche JL. Synthetically useful sonochemical reactions in solution. Ultrson Sonochem 1996;3:215-221

Madanshetty SL, Apfel RE. Acoustic microcavitation: enhancement and application. J Acoust Soc Am. 1991; 90: 1508-1514

Manka-Waluch A, Palmedo H, Reinhardt MJ, Joe A, Manka C, Guhlke S, Biersack HJ, Bucerius J. Myocardial uptake characteristics of three 99mTc-labeled tracers for myocardial perfusion imaging one hour after rest injection. Ann Nucl Med 2006;20:663-670

Merhige ME, Breen WJ, Shelton V, Houstons T, Arcy BJD, Perna AF. Impact of myocardial perfusion imaging with PET and 82 Rb on downstream invasive procedure utilization, costs and outcomes in coronary disease management. J Nucl Med 2007;48:1069-1076

Mitragotri S, Blankschein D, Langer R. Transdermal drug delivery using low-frequency sonophoresis . Pharm Res 1996;13:411-420

Moon S, Duschin L, Cooney JV. Application of ultrasound to organic reaction: ultrasound catalysis on hydrolysis of carboxylic acid esters. Tetrahedron Lett 1979;20:3917-3920

Park D, Park H , Seo J, Lee S. Sonophoresis in transdermal drug delivery system. Ultrasounics 2013;54:56-65

Piwnica-Worms D, Chiu M, Budding M, Kronauge J, Kramer R, Croop J. Functional imaging of multidrug-resistant p-glycoprotein with an organotechnetium complex. Cancer Res 1993;53:977-984

Pizzuti L, Martins PLG, Ribeiro BA, Quina FH, Flores AFC, Venzke D, Pereira CMP. Efficient sonochemical synthesis of novel 3,5-diaryl-dihydro-1 H-pyrazole-1- carboximidamides. Ultrson Sonochem 2010;17:34-37

Salerno M, Beller GA. Noninvasive assessment of myocardial perfusion. Circ Cardiovasc Imaging 2009;2:412-424

Spanu A, Falchi A, Manca A, Marongiu P, Cossu A, Pisu N, Chessa F, Nuvoli S, Madeddu G. The usefulness of neck pinhole SPECT as a complementary tool to planar scintigraphy in primary and secondary hyperparathyroidism . J Nucl Med . 2004 ;45:40-48

Tuziuti T, Yasui K, Kozuka T, Towata A, Ioda Y. Enhancement of sonochemical reaction rate by addition of micrometer-sized air bubbles. J Phy Chem A 2006;110:10720-10724

Vecchio SD, Salvatore M. 99mTc-MIBI in the evaluation of breast cancer biology. Eur J Nucl Med Mol Imaging 2004;31:88-96

Weissman IA, Dickinson CZ, Dworkin HJ, ONeill WW, Juni JE. Cost effectiveness of myocardial perfusion imaging with SPECT in the emergency department evaluation of patients with unexplained chest pain. Radiology 1996; 199:353-357

How to cite this article:

Doroudi A, Saraji F, Erfani M, Saadati SM, Kisast A, Ahmadi F, Etesami B. The stability of ^{99m}Tc-MIBI (Sestamibi) complex samples which prepared under ultrasound irradiation technique versus boiling water bath method. J App Pharm Sci, 2016; 6 (11): 126-134.