Volume 18, No 2 | International Journal of Radiation Research, April 2020

Optimized production, quality control and biological assessment of ^{68}Ga-bleomycin as a possible PET imaging agent

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► ***Original article***

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Revised: July 2019
Accepted: August 2019

Int. J. Radiat. Res., April 2020; 18(2): 235-241

DOI: 10.18869/acadpub.ijrr.18.2.235

[Downloaded from mail.ijrr.com on 2026-10-12]

[DOI: 10.18869/acadpub.ijrr.18.2.235]

ABSTRACT

Background: Recently, several diagnostic radiopharmaceuticals including bleomycin (BLM) derivatives have been developed. Due to the suitable physical and chemical characteristics of ^{68}Ga as a radionuclide for PET imaging, in this study, optimized production, quality control and preclinical evaluation of ^{68}Ga-BLM as a new PET imaging agent is reported for the first time. ***Materials and Methods:*** Labeling of BLM with ^{68}Ga was performed using ^{68}Ge/^{68}Ga generator. Experiments were carried out by changing BLM concentration, temperature and pH of the reaction to determine the optimum parameters while the radiochemical purity was checked by radio thin layer chromatography at different times post labeling. Stability of the radiolabeled complex was studied at room temperature and in human serum at 37 °C. Biodistribution of the complex in BALB/c mice was assessed after intravenous injection and by counting the activity of each organ. Also, images were acquired up to 120 min by dual-head SPECT system. ***Results:*** The purity of this complex >96% (ITLC). At the optimized conditions for preparation of ^{68}Ga-BLM (pH= 3.5-4, temperature = 90 ºC, reaction time = 15 minutes and ligand concentration of 1 mg/ml) , the special activity of the labeled BLM reached to around 17.5 GBq/mmol**.** Biodistribution study showed significant accumulation of radioactivity in lung and bladder that was different pharmacokinetic compared to free ^{68}Ga cation. ***Conclusion:*** Results show that ^{68}Ga-BLM can be prepared in high radiochemical purity and high special activity only in 15 minutes and totally can be considered as a high potential agent for PET imaging.

Keywords: *Bleomycin, Gallium-68, radiolabeled compound, PET.*

INTRODUCTION

Early detection of cancers can greatly increase the chance of successful treatment and radiolabeled tracers can play an important role for primitive diagnosis of tumors. Positron emission tomography (PET) has rapidly become an indispensable tool for clinicians in the locating and staging of tumors, as well as measuring their response to interventional therapies. PET experienced rapid development and has become an accepted approach for clinical routine diagnostics. Therefore, the expansion of new radiopharmaceuticals for development of clinical PET is very essential [(1)].

Excellent physical specifications of ^{68}Ga such as relatively short half-life of 67.71 min, positron emission of 89 % and low abundance of 1077

keV photon emission (3.22 %) as well as it's availability in the form of ^{68}Ge/^{68}Ga generator at a reasonable cost make this radioisotope an attractive choice for developing new radiopharmaceuticals specially for the countries with limited or no cyclotron facilities [2,3]. Gallium-68 has high tumor affinity and it is being used in nuclear medicine for detection and imaging of malignant and inflammatory sites in the body for a short time. The number of publications related to basic and clinical researches of ^{68}Ga-radiopharmaceutical has drastically promoted during recent years [1,4-7].

Bleomycins (BLMs) are family of glycopeptide antibiotics that have potent antitumor activity against a range of lymphomas, head and neck cancers and germ-cell tumors and therefore, are widely used for chemotherapy of cancers. The mechanisms of BLM induced cytotoxicity have been studied [8,9] and when in presence of Fe^{+2} ion and oxygen, it transforms into its active form and binds to DNA molecules. Several structure variations of BLMs have been identified from fermentation broths, primarily differing at the C-terminus of the glycopeptide. Structurally and biosynthetically related to the BLMs are the phleomycins (PLMs), such as PLM 12 or PLM D1 [10,11], and tallysomycins (TLMs), such as TLM S2B and TLM S10B [12,13] (figure 1).

During the past three decades, complexes of BLM with radioactive elements as a radiopharmaceutical and their body distribution and resultant biological effects, were intensively studied. Several radiolabeled BLM derivatives contain indium-111 [14], cobalt-57 [15], technetium-99m [16], radioferric salts [17], rhodium-105 [13] and radiolanthanides such as lutetium-177, samarium-153 and holmium-166 [16-18] have been developed for imaging and/or therapy of neoplastic tissues.

However, the positron-emitting gallium (III) radioisotopes, $^{66}Ga^{3+}$ and $^{68}Ga^{3+}$, have been proposed for applications in PET imaging, according to the best of our knowledge, preparation, quality control and biological of ^{68}Ga-BLM complex have not been reported until now. Therefore, in this research study, due to the suitable physical characteristics of ^{68}Ga and desirable tumor seeking properties of BLM, optimized conditions for preparation of ^{68}Ga-BLM complex were investigated. Different affecting parameters on the labeling yield such as pH, temperature, and time and ligand concentration were studied. The quality control of the complex was done by radio thin layer chromatography (RTLC) method and the stability and biodistribution of the radiolabeled compound were checked in normal animal model. Also, the imaging of the animal was acquired by a dual-head SPECT system after injection of the radiolabeled compound to the animals.

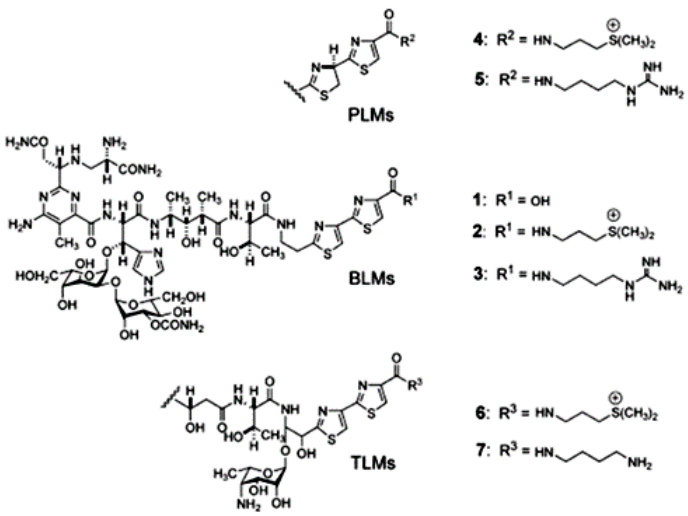

Figure 1. Structures of bleomycinic acid, bleomycin A2, bleomycin B2, phleomycin 12, phleomycin D1, and tallysomycin S2B, and tallysomycin S10B.

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MATERIALS AND METHODS

^{68}Ge/^{68}Ga generator with the nominal activity of 50 mCi was provided from Pars Isotope Co. (Tehran, Iran) and its chemical purity was check by an ICP-OES spectrometer (Varian Co., model Turbo-AX-150-Liberty). BLM sulfate was purchased from Merck (Darmstadt, Germany). HCL, buffer acetate, ammonium acetate, methanol and other chemical reagents were obtained from Sigma Aldrich (UK). A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer was used for radionuclidic purity assessment. Also, radiochemical purity analysis was done by a bioscan AR-2000 radio TLC scanner instrument (Bioscan, Europe Ltd CO., France) using Whatman No. 2 paper (Whatman, Maidstone, UK). Student T-test was used to compare the obtained data and the final values were expressed as the mean ± standard deviation (Mean ± SD). Statistical significance was defined as $P<0.05$.

Preparation and quality control of $^{68}GaCl_3$

The QC/QA protocols for used in this study have been reported elsewhere [21, 4]. To prepare $^{68}GaCl_3$ solution, ^{68}Ge/^{68}Ga generator was eluted by 2 mL HCl (0.6 M) and gathered in separate fractions. The first 0.5 mL of the eluate was discarded and the next 1.5 ml was used for labeling purposes after the activity measurement. The radionuclidic purity of the product was investigated by gamma spectrometry for 1000 s. The sample was counted 48 h later for breakthrough measurement of ^{68}Ge in the sample. Radiochemical purity of $^{68}GaCl_3$ solution was checked in 10 % ammonium acetate/methanol mixture on silica gel sheets and in 10 mM DTPA solution (pH = 4) on Whatman No. 2 paper.

Radiolabeling of BLM with $^{68}GaCl_3$

BLM powder was dissolved in pure water to prepare the stock solution with concentration of 1 mg/mL. $^{68}GaCl_3$ solution was added to the 10-mL conical vial and evaporated by slight heating under nitrogen gas flow. Different volumes of BLM solution (0.5–2 mmol) were added to the vials. To determine the optimum parameters for reaction, temperature and pH of the reaction changed from 25–100 °C and 1–5, respectively. While, the maximum reaction time considered being 1 h, the radiochemical purity of the complex was checked at different times post labeling. Radiochemical purity tests were carried out by RTLC with various mobile phase mixtures including 10% Ammoniumacetate:Methanol (1:1); H_2O:Methanol: Acetic acid (4:4:2).

Stability tests of ^{68}Ga-BLM

The stability of ^{68}Ga-BLM was performed at room temperature up to 120 min post preparation by checking the radiochemical purity of the complex at the specified intervals. Also, the stability of the complex in human serum was done by adding a 37 MBq of ^{68}Ga-BLM to 500 µL of freshly prepared human serum following by incubation of the mixture at 37 °C for 48 h. Finally, aliquots were analyzed by ITLC method at various intervals after preparation.

Biological evaluation of ^{68}Ga-BLM in BALB/c mice

The protocol of the study has been approved by the institutional review board (Nuclear Science and Technology Research Institute (NSTRI)- License No.18, date 2013). The female BALB/c mice of age 28±4 days weighting 18.5-22.7 g was prepared from Pasteur institute (Tehran, Iran) and kept at routine day/night light program under common rodent diet pellets. 100 µL of $^{68}GaCl_3$ and ^{68}Ga-BLM (~ 37 MBq) solutions was intravenously injected into the mice through their tail veins. The mice sacrificed at the selected intervals (15, 30, 45 and 60 min) after injection (n=4). The tissues including liver, lung, spleen, muscle, skin, heart, kidney, colon, colon content, and bladder were rinsed with normal saline and weighed and their activities were determined using the HPGe detector. Finally, the activity concentration of each organ (A) was computed according to equation (1) [22].

$$A = \frac{N}{\varepsilon \gamma t_s m k_1 k_2 k_3 k_4 k_5} \quad (1)$$

where N is the corrected net peak area of the

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corresponding photopeak; ε is the efficiency at photopeak energy, γ is the emission probability of the gamma line corresponding to the peak energy, ts is the live time of the sample spectrum collection in seconds, m is the mass (kg) of the measured sample, k1 , k2, k3, k4 and k5 are the correction factors for the nuclide decay from the time the sample was collected to the start of the measurement, the nuclide decay during counting period, self-attenuation in the measured sample, pulses loss due to random summing and the coincidence, respectively.

Imaging studies

100 µL of ^{68}Ga-BLM solution (37 MBq) was injected intravenously to normal mice through their tail veins for imaging studies. PET images were taken at 30 and 120 min post administration by a dual-head SPECT system. The distances of mice to high-energy septa were 12 cm. The useful field of view (UFOV) was 540 mm×400 mm.

Statistical analysis

All values were expressed as mean ± standard deviation and the data were compared using Student's T-test. Once a t value was calculated, the p-value was determined using the table of values from student's t distribution. Statistical significance was defined as $P < 0.05$.

RESULTS

Preparation and quality control of $^{68}GaCl_3$

The radionuclidic purity assessment of $^{68}GaCl_3$ solution was done by gamma spectrometry method demonstrated the presence of 511 and 1077 keV, all originating from ^{68}Ga. Also, the radiochemical purity of the sample used in this study was checked by ITLC method resulted in the radiochemical purity of more than 99.8%.

Radiolabeling of BLM with $^{68}GaCl_3$

To achieve optimized conditions for radiolabeling of BLM with ^{68}Ga, various experiments were done and the parameters including pH of reaction (1-5), temperature (25, 50, 75, 90 and 100°C), ligand concentration (0.5, 1, 1.5 and 2 mg), and reaction time (up to 1 h) were changed. While, 10% ammoniumacetate:methanol (1:1) mixture was considered as a better mobile phase for control of the radiochemical purity, the optimization process was checked by this mobile phase. RTLC tests of the produced complex showed two distinct radio peaks at R_fs of 0.10 and 0.80 as indicated in figure 2.

Increasing the temperature of complex mixture during reaction time to 90ºC increased the yield, which remained constant for temperatures up to 90-100 ºC (figure 3). The best pH for labeling was found 3.5-4 at the temperature of 90 ºC as indicated in figure 4. At the optimum reaction temperature and pH, the yield reached a maximum within 15 minutes which remained approximately constant for longer reaction times (figure 5).

Finally, the optimized conditions for radiolabeling of higher than 96 % were pH of 3.5 -4, temperature of 90 ºC and the reaction time of 15 minutes that results in the special activity of around 17.5 GBq/mmol for ^{68}Ga-BLM.

Stability tests of ^{68}Ga-BLM

The stability tests of the radiolabeled complex including stability at room temperature and stability in human serum at 37 °C were studied by ITLC method. The results showed that the radiochemical purity of ^{68}Ga-BLM was greater than 96 % after 120 min of preparation both at room temperature and in human serum at 37 °C.

Biological evaluation of $^{68}GaCl_3$ and ^{68}Ga-BLM in BALB/c mice

Biodistribution of $^{68}GaCl_3$ and ^{68}Ga-BLM was studied in BALB/c mice at 15, 30, 45 and 60 min post injection (figures 6 and 7).

Imaging studies

PET images were taken from normal BALB/c mice at 30 and 120 min post injection of ^{68}Ga-BLM solution. As shown in Fig. 8, the radiolabeled complex is mainly washed out from the urinary tract and the most accumulation occurred in bladder and the major remained activity is accumulated in lung and excretory organs.

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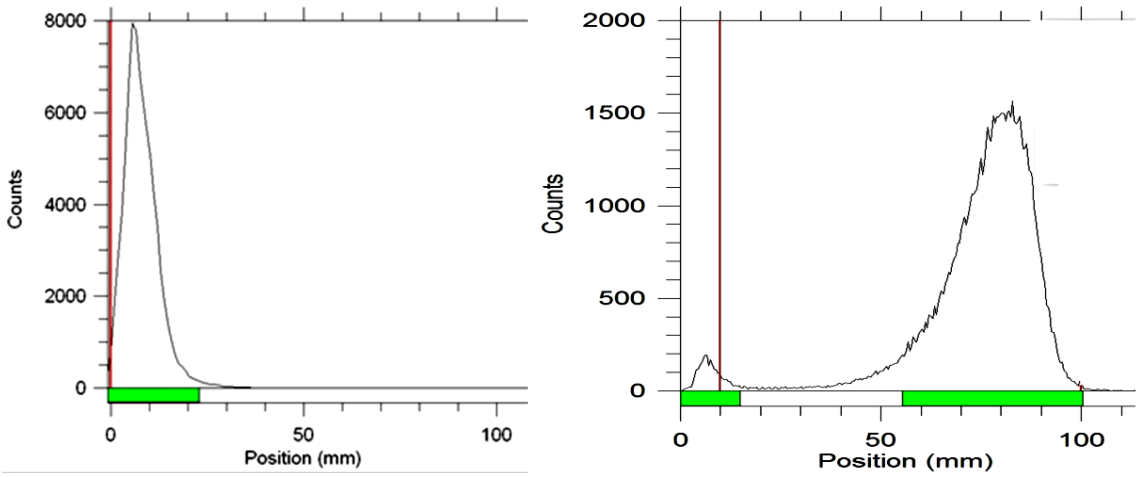

Figure 2. RTLC chromatogram of $^{68}Ga^{3+}$ (left), ^{68}Ga-BLM (right) in 10 % ammonium acetate/methanol mixture on silica gel sheets at optimized condition.

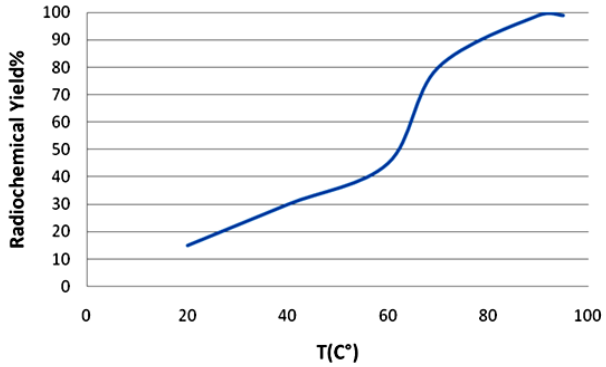

Figure 3. Effect of temperature on radiochemical yield of ^{68}Ga-BLM.

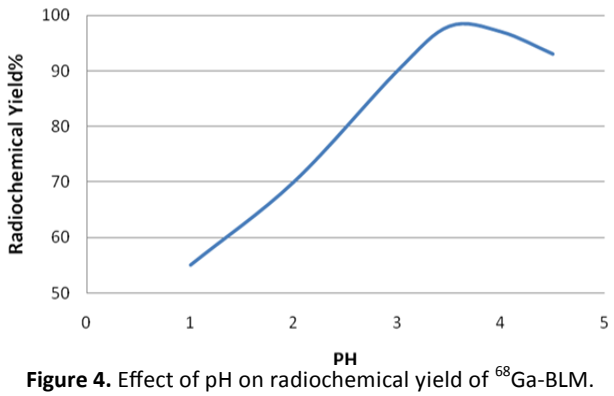

Figure 4. Effect of pH on radiochemical yield of ^{68}Ga-BLM.

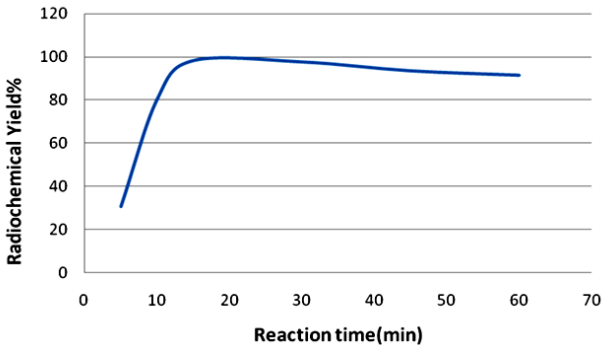

Figure 5. Effect of reaction time on radiochemical yield of ^{68}Ga-BLM.

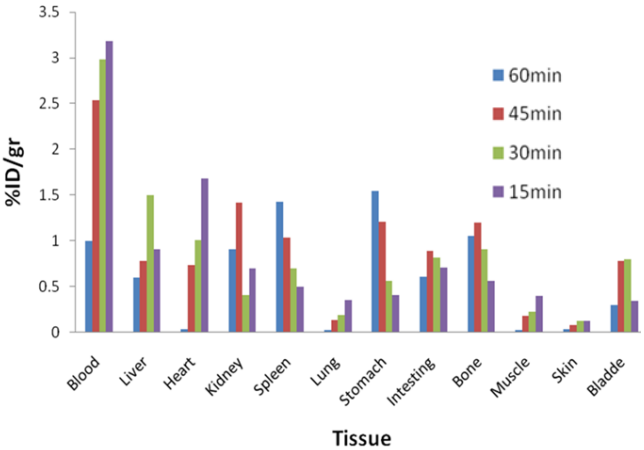

Figure 6. Biodistribution of ^{68}GaCl3 in Balb/c mice up to 60 min after injection.

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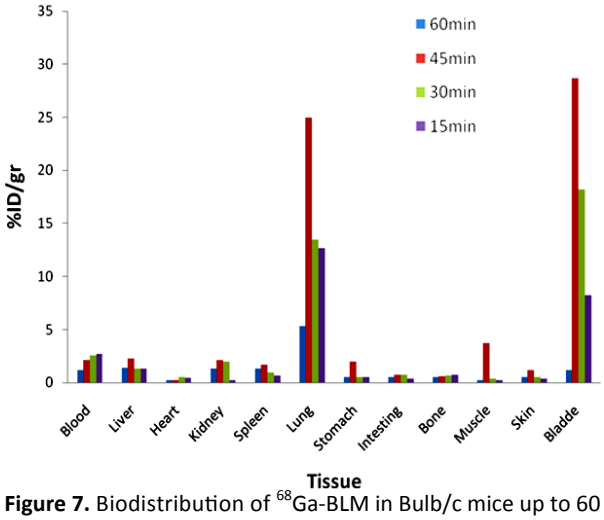

Figure 7. Biodistribution of ^{68}Ga-BLM in Bulb/c mice up to 60 min after injection.

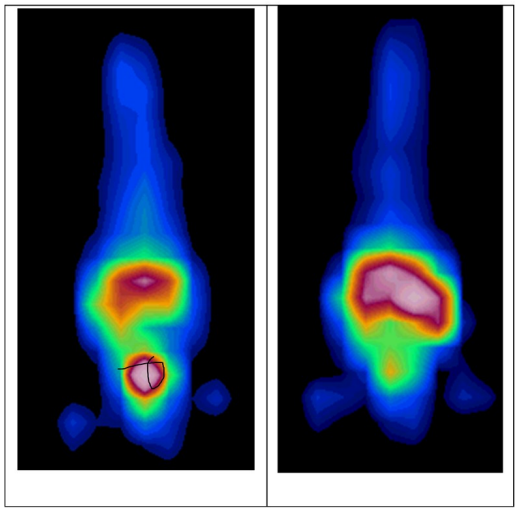

Figure 8. Images of ^{68}Ga-BLM 30 and 120 min post Injection.

DISCUSSION

In this research study, due the special properties of ^{68}Ga and BLM, the ^{68}Ga-BLM complex was prepared as a new PET imaging agent. The results of ^{68}Ge/^{68}Ga generator elution, the breakthrough of the generator and its possible chemical ionic impurities and the quality control of the eluted $^{68}GaCl_3$ are very similar to the other literature [4, 21].

The optimized conditions for radiolabeling of this radiolabeled complex were pH = 3.5-4; temperature = 90 ºC; and reaction time = 15 min. The radiochemical purity of the complex was >96 % and the special activity of the final solution was > 17.4 GBq/mmol. The optimum reaction time for the complex found in this study agrees with Thakur method for labeling of ^{111}In-BLM complex [14]. Compared to the ^{177}Lu-BLM [18] and ^{153}Sm-BLM [19], the optimized pH for labeling of BLM with ^{177}Lu and ^{153}Sm (5.5 –7) has changed in labeling with ^{68}Ga (3.5-4). Also, the labeling of BLM with ^{68}Ga need to higher temperature (90 °C), however, the labeling time is shorter (15 min).

The biodistribution of ^{68}Ga-BLM in normal BALB/c mice showed different pattern compared with free ^{68}Ga cation. The water solubility of the cation cause its rapid removal from the blood circulation and the cation mainly extracted via urinary tract. The remained activity of $^{68}GaCl_3$ in the body was accumulated in the stomach, spleen, liver and bone.

The biodistribution results of the radiolabeled compound clearly showed the main portion of the remained activity in the body was accumulated in lung and bladder. Also, the main extraction route of the compound is via the kidneys due the water solubility of the ^{68}Ga-BLM complex.

Since high resolution images for mice required an animal PET device and we did not have access to this instrument, the imaging was done by a human dual-head SPECT device, and therefore, the resulting images do not have suitable resolution, which is one of the limitations of this study. Other limitations include the lack of access to tumoral mice to investigate the biodistribution of radiolabeled compound in tumoral model. Given the anti-cancer properties of BLM on the one hand and the physical properties of gallium-68 on the other hand, the study of this radiolabeled compound in tumor model is suggested.

CONCLUSION

In this research study, ^{68}Ga-BLM was prepared in high radiochemical purity (>96 %) with high special activity (> 17.4 GBq/mmol) at a short time (15 min) using a portable ^{68}Ge/^{68}Ga generator. The biodistribution study of the radiolabeled complex showed completely

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different pattern compared to the free gallium cation and lung and bladder were the accumulation sites for the remained activity. Generally, it seems ^{68}Ga-BLM can be considered as a high potential candidate for PET imaging; however, more biological studies in tumoral mammals are still needed.

ACKNOWLEDGMENTS

The authors gratefully acknowledge support from the Jaber Ibn Hayyan Laboratory Foundation of Nuclear Science and Technology Research Institute (NSTRI).

***Conflicts of interest*: Declared none.**

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