Preparation and optimization of SBA-16-Al nanomaterials labeled Technetium-99m for radiation imaging applications

Witri Nuräeni 1, Edy Giri Rachman Putra, Isti Daruwati 1 and Maria Christina Prihatiningshih 2

1 Center of Applied Nuclear Science and Technology, National Nuclear Energy Agency (BATAN), Jl.Tamansari 71 Bandung 40132
2 Polytechnic Institute of Nuclear Technology, National Nuclear Energy Agency (BATAN), Jl. Babarsari PO Box 6101 YKBP Telp(0274)484085, Fax.(0274)489715 Yogyakarta, Indonesia 55281

Email: witri@batan.go.id

Abstract. In today's of the conventional cancer diagnosis, there are still many problems encountered, such as the ineffectiveness of drug loading, instability, and biocompatibility issues. One alternative method for early detection of cancer is using nuclear techniques using radioactive compounds such as Technetium-99m. In the present research work, a silica mesoporous nanomaterial SBA-16-Al will be introduced as a carrier of imaging agent of radioactive compounds that play a role in radiation imaging. The Al-SBA-16 nanomaterials labeled with 99mTc (99mTc-SBA-16-Al) radioisotope exhibited very interesting nuclear properties for applications in imaging radionuclide diagnoses. Preparation by treatment of milling for 30 hours and ultrasonication bath for 60 minutes produced particle sizes of 510-3900 nm with a median of 1587 nm. Based on the results of this study, the optimum radiochemical purity was obtained at 85.66 ± 0.72% with pH 9 of solutions, the ratio of the amount of SnCl2 and SBA-16-Al to 75 µg compared to 1000 µg (1: 13.3), and incubation time during 20 minutes, with the radioactivity of technetium-99m ranging from 0.21 to 1.23 mCi.

The results of the electrophoresis test showed that 99mTc-SBA-16-Al is a neutrally charged compound.

1. Introduction

Each year, more than 14 million people are diagnosed with cancer, the majority of whom live in low- and middle-income countries (LMICs). In 2015, 8.8 million died from cancer, representing one in six deaths globally. The number of deaths due to cancer in LMICs exceeds those due to HIV/AIDS, tuberculosis, and malaria combined. Approximately two-thirds of global cancer deaths are in less developed countries, where case fatality rates are higher due to late-stage presentation and less accessible treatment. The consequences of delays in diagnosis and treatment cancer are that the likelihood of death and disability from cancer increases significantly as cancer progresses. It is, therefore, critical to identify barriers to timely diagnosis and treatment and to implement programs that provide access to care for all [1].

The best method for cancer prevention and control is early diagnosis and treatment. Nanotechnology has developed with wide applications for targeted molecular imaging, molecular diagnosis, and targeted therapy of cancer. It plays an important role in realizing the goal of detecting
transforming cell populations early by in vivo imaging. This allows the combination with the right agent to be chosen so that it can target the agent for early cancerous lesions without side effects on healthy tissue and monitoring the effects of treatment in real-time [2].

Recently, the combination of nanotechnology with drug delivery in the field of cancer therapy has been a research hotspot. Though organic nanocarriers such as nanocapsules, liposomes, polymeric micelles, and nanoparticles can easily encapsulate anticancer drugs, their physicochemical instability and unexpected drug leakage have severely impeded their application. In contrast, inorganic silicate (SiO\textsubscript{2}) carriers have several merits, such as excellent biochemical and physicochemical stability, biocompatibility, and degradability. Among the recent breakthroughs that bring exciting new possibilities to this area are Mesopore silica nanoparticles (MSNs). They have been generally suggested as effective carriers for anticancer drugs due to their excellent drug administration and endocytic behavior [3].

Several studies have been carried out to characterize and evaluate MSNs that have been labeled technetium-99m radionuclides. The results of the study of Tamba et al., revealed that MSNs characterized by radionuclides have improved properties, namely reducing toxicity with low side effects, increased biodistribution, labeling and stability of high-labeled compounds so that they have the potential to be applied in nuclear medicine as radiotracers [4]. Previous studies conducted by Hakiki N.W et al., that have successfully labeled M41S-NH\textsubscript{2} with technetium-99m using the direct method and producing radiochemical purity of 98.45 ± 0.30% [5].

SBA-16 (Santa Barbara Amorphous-16) is an interesting type of MSNs because its mesoporous has a three-dimensional cubic arrangement making it very promising for biomedical applications [6]. In this study, SBA-16 was modified to SBA-16-Al. To make smaller the particle size of SBA-16-Al, preparations were made using milling and ultrasonication treatments. Furthermore, the labeling optimization was carried out on the pH, ratio of the number of reducing agents and SBA-16-Al, and the incubation time. The optimization of labeling is carried out in order to obtain high radiochemical purity. This research is expected to be a method in the SBA-16-Al marking procedure with technetium-99m for cancer diagnosis applications. In addition, it can be a reference for labeling SBA-16-Al with Rhenium-186 radionuclide for radiotherapy applications because it has chemical properties similar to technetium-99m.

2. Materials and methods

2.1. Preparation of SBA-16-Al

2.1.1. Optimization of Ultrasonication. A total of 5 mg of SBA-16-Al was dispersed with 5 mL aqua dest. Then the sonication was carried out using a bransonic ultrasonic bath, an ultrasonic processor with a diameter probe of 2 mm, and a diameter probe of 3 mm. Each ultrasonication is done with time variations. Then the size and distribution of the particles are determined using Particle Size Analyzer (PSA) Light Dynamic Scattering LB-550.

2.1.2. Optimization of Milling. SBA-16-Al was put into a vial containing a ball mill, then grinding at a speed of 2500 rpm with short time variations (1, 2, and 3 hours) and a long time (16, 24, and 30 hours). After that, 5 mg SBA-16-Al dispersion with 0.9% NaCl then ultrasonic bath for 60 minutes. The size and distribution of particles is determined using PSA.

2.2. Labeling SBA-16-Al with a direct method

Technetium-99m radionuclide taken from \textsuperscript{99}Mo/\textsuperscript{99m}Tc generator was eluted using 0.9% NaCl to produce Na\textsuperscript{99m}TcO\textsubscript{4}\. By using the SnCl\textsubscript{2} reducing agent, the oxidation state of \textsuperscript{99m}Tc(VII) will be reduced so that it can bind to SBA-16-Al. From this reaction, it is expected that \textsuperscript{99m}Tc-SBA-16-Al can be formed. In addition to the labeled compounds obtained, it can also produce \textsuperscript{99m}TcO\textsubscript{4} free and \textsuperscript{99m}TcO\textsubscript{2} reduced (\textsuperscript{99m}TcO\textsubscript{2}). By optimizing the pH, the ratio of the number of reducing agent and SBA-
16-Al, and the incubation time, it is expected to obtain a practical labeling method with high radiochemical purity.

2.3. Determination of labeling efficiency

The determination of labeling efficiency was done simultaneously with the determination of the radiochemical purity of 99mTc-kanamycin using ascending paper chromatography method using Whatman 31 ET paper (10 × 1 cm) as the stationary phase and 0.9% NaCl as the mobile phase to separate the impurities 99mTcO₄⁻ free at Rf = 1.0. Meanwhile, to separate the impurity of 99mTcO₂, ITLC-SA (10 × 1 cm) was used as the stationary phase and ethanol-water-ammonia (2:5:1) as a mobile phase where Rf of 99mTcO₂ = 0.0. The chromatograms were dried in an oven at 80 oC for five minutes and then measured using TLC-Scanner.

2.4. Labeling optimization

2.4.1 Optimization of pH. Into three vials, each containing 1 mL SBA-16-Al (1 mg/mL), 100 µL of SnCl₂ solution (0.1 mg/mL) was added. The mixture was shaken until homogeneous. The pH of the solutions in those vials was adjusted to 5, 7, and 9, respectively, by adding 0.1 N NaOH, and then to the mixture was added a solution of Na⁹⁹mTcO₄ with the activity of 0.2-1.2 mCi. The mixtures were shaken until homogeneous and incubated at room temperature for 60 minutes. The optimum pH was determined from the labeling efficiency of ⁹⁹mTc-SBA-16-Al using a paper chromatographic method.

2.4.2 Optimization of the ratio of reducing agents and SBA-16-Al. Into four vials, each containing 1 mL SBA-16-Al (1 mg/mL), solutions of SnCl₂ of varying amounts (50, 75, 100, and 125 µL) were added. So that the ratio of the number of SnCl₂ and SBA-16-Al from each vial is 1:8; 1:10; 1:13; and 1:20. The mixture was shaken until homogeneous. The pH of the mixture was adjusted to 9 by adding 0.1 N NaOH, and then to the mixture was added a solution of Na⁹⁹mTcO₄ with the activity of 0.2-1.2 mCi. The mixtures were shaken until homogeneous and incubated at room temperature for 60 minutes. The optimum of the ratio of reducing agents and SBA-16-Al was determined from the labeling efficiency of ⁹⁹mTc-SBA-16-Al using a paper chromatographic method.

2.4.3 Optimization of incubation time. Labeling was done by adding 75 µL of SnCl₂ solution (1 mg/mL) into a vial containing 1 mg of SBA-16-Al that was a dispersion in 1 mL of 0.9% NaCl and stirring until homogeneous. The pH was adjusted to 9 by addition of 0.1 N NaOH, and then 0.2-1.2 mCi of Na⁹⁹mTcO₄ solution was added. The mixture was stirred until homogeneous and incubated at room temperature for varying periods of 5, 10, 20, 30, and 60 minutes. The labeling efficiency was determined using a paper chromatographic method.

2.5 Determination of electrical charge

The determination of the electric charge is done by the paper electrophoresis method. 1 x 37 cm Whatman 1 paper is marked with a pencil from -16 to +16. The labeled compound is dripped at zero points. The zero points of the paper are positioned in the middle of the electrophoresis chamber. The edge of the negative side of the paper is dipped in a phosphate buffer solution connected to the cathode and the positive side of the paper at the anode. The electrophoresis chamber is closed, then an electric current is supplied with a voltage difference of 300 volts for 1-2 hours. After finishing, the paper is removed and dried and then counted every 1 cm of paper using a Single Channel Analyzer (SCA).

3. Result and discussion

The SBA-16-Al material used in this study had a surface area of 1412.1543 m²/gram, a pore volume of 1.180046 cc/gram, and a pore diameter of 33.4254 Å. This study used a combination of ball-mill and sonication methods for the preparation of SBA-16-Al material. Before weighing, the SBA-16-Al powder was ground using a ball mill for 10 minutes at a speed of 2500 rpm. After that, mixing is done
with the solvent and stirred using a vortex mixer for one minute, then ultrasonication is performed. The vial containing the mixture/suspension is immersed in an ultrasonic pool filled with water. Ultrasonic waves (frequency range of 20 kHz-10 MHz) can cause the molecules contained in the solution to oscillate to their average position. The solution will experience stretching and density. When the energy provided by this ultrasonic wave is large enough, the strain of the wave can break bonds between molecules of solution [7].

Before the particle size analysis is carried out, the refractometer index is first measured. The refractive index is the ratio (ratio) between the speed of light in a vacuum against the speed of light in a material. The propagation of light in a vacuum has a velocity \(c\), then after entering a certain medium, the velocity will change to \(v\) with \(v \ll c\) [8].

Figure 1. shows a particle size chart for SBA-16-Al with bath ultrasonication time variations. The results showed that the ultrasound treatment of bath for one hour obtained a decrease in particle size higher than the others. Before ultrasonication, the mixture is still in the form of coarse dispersions containing solids which are insoluble and not evenly distributed in the liquid phase. With ultrasonication, the solid material can split into small particles and be evenly distributed. However, at the time of ultrasonication that is too long, small particles that have formed can occur aggregation and slowly settle.

![Figure 1. SBA-16-Al Particle Size in Variation of Time Ultrasonication Bath](image)

The SBA-16-Al treatment was then performed using an ultrasonic probe. In contrast to the ultrasonic bath, this ultrasonic treatment is carried out using a probe that is dipped directly into the suspension. In this study, two probe sizes were used, namely 2 mm in diameter and 3 mm in diameter. Based on the results of this study, the process of breaking down molecules into smaller particles using an ultrasonic probe can provide faster results. Either using a 2 mm or 3 mm probe, the 5-minute treatment gives a particle size that is almost the same as the ultrasonic bath treatment for one hour. However, there was an increase of particle size in the 7.5th minute using a 2 mm probe and in the 10th minute using a 3 mm probe.
The next optimization is done by varying the time of grinding using a ball mill, then ultrasonication for 15-60 minutes. With the addition of grinding time, there was a sharp decrease in particle size until the 3rd hour. However, at the time of milling for 4 hours, the aggregation occurred, so that particle size analysis was not possible.

Milling time variations are also carried out with a long time, which is for 16, 24, 30, and 36 hours. Particle measurement results show that the minimum size that can be obtained is by grinding treatment for 30 hours. The obtained particle size is in the range of 590-3900 nm, with a median of 1587.6 nm. Milling for 36 hours of aggregation occurred, causing the particle size not detected in the PSA device.

Based on this research, the grinding process with a ball-mill gives a sharp drop in particle size compared to just ultrasonic. In addition, with the ball-mill treatment, an increase in the value of the refractive index from 1.334 to 1.335. This shows that the dispersion of the material with the ball-mill treatment results in a higher density so that the speed of light in the medium becomes slower. This shows that there is a breakthrough in more particles with a high surface area.
For the optimization of the labeling SBA-16-Al with technetium-99m, the milling treatment method is used 24 hours and 30 hours with 15-60 minutes ultrasonication bath. The long-time milling method gives a fairly good homogeneity of particles and is more stable during storage.

The first parameter that is carried out optimization is the degree of acidity (pH). A radiopharmaceutical must have ionic strength suitable for human administration. The pH of a radiopharmaceutical can vary from two to nine. In this study, the pH optimization was carried out with variations of pH 5, 7, and 9. Based on the results of the study, the pH 9 obtained higher radiochemical purity compared to pH 5 and 7. Figure 6 shows that pH 5 contains 99mTcO2 impurities that are high, but do not contain 99mTcO4- free impurities. This shows that at pH 5 all 99mTcO4- reacts to another form of radiochemistry. However, 85.50% changed to 99mTcO2 and only 14.5% formed to 99mTc-SBA-16-Al. Whereas at pH 7 it has 99mTcO2 impurities which are higher than at pH 9.
IOP Conf. Series: Journal of Physics: Conf. Series 1436 (2020) 012121

Table 1. pH optimization of teknetium-99m labeled SBA-16-Al

| pH | Impurity (%) | 99mTcO\textsubscript{4}\textsuperscript{-} pure | 99mTcO\textsubscript{2} (%) | Radiochemical purity (%) | Radioactivity (mCi) |
|----|--------------|---------------------------------|-----------------|--------------------------|-------------------|
| 5  | 0,00 ± 0,00  | 85,50 ± 3,52                    |                 | 14,50 ± 4,06             |                   |
| 7  | 12,21 ± 4,71 | 29,15 ± 0,66                    |                 | 58,64 ± 5,73             | 0,95-1,23         |
| 9  | 12,64 ± 5,64 | 13,49 ± 5,33                    |                 | 73,87 ± 0,10             |                   |

Furthermore, optimization of the amount of SnCl\textsubscript{2} and SBA-16-Al is performed. Variations are made at the ratio of 1:20, 1: 13,3, 1:10, and 1: 8. From Figure 7, it can be seen that the smaller the number of SBA-16-Al, the higher 99mTcO\textsubscript{2} impurities. This shows that 99mTcO\textsubscript{4} cannot react optimally at a smaller amount of SBA-16-Al so that more 99mTcO\textsubscript{4} changes to 99mTcO\textsubscript{2}. Whereas at the ratio of 1:20 where the highest number of SBA-16-Al has 99mTcO\textsubscript{4} impurities, which is higher than the ratio of 1: 13.3. This can be caused by the ability of SnCl2 to reduce 99mTcO\textsubscript{4} reduced due to the large number of SBA-16-Al. Therefore, based on this study, the optimum ratio of SnCl\textsubscript{2} and SBA-16-Al is 1: 13.3 with radiochemical purity 81.11 ± 2.32%.

Table 2. Optimization of the ratio of SnCl\textsubscript{2} and SBA-16-Al amount

| Rasio Jumlah SnCl\textsubscript{2} dan SBA-16-Al | Pengotor (%) | KRK (%) | Radioaktivitas (mCi) |
|-------------------------------------------------|-------------|---------|---------------------|
| 1:20                                            | 11,78 ± 2,59 | 8,14 ± 1,24 | 80,50 ± 2,94 |
| 1:13,3                                          | 6,07 ± 0,43  | 13,4 ± 1,76 | 81,11 ± 2,32 |
| 1:10                                           | 8,68 ± 1,77  | 18,17 ± 0,58 | 73,40 ± 2,40 |
| 1:8                                            | 12,23 ± 2,02 | 20,98 ± 1,62 | 68,66 ± 2,76 |

Figure 6. Grafik of pH optimization of teknetium-99m labeled SBA-16-Al
After obtaining the optimum pH and the ratio of SnCl$_2$ and SBA-16-Al amount, optimization is then performed for incubation time. Incubation time is the time needed for a compound to react in a room. This optimization is carried out at room temperature with variations of 5, 10, 20, 30, and 60 minutes. Based on the results of this study, the incubation time of 20 minutes produced the highest radiochemical purity, which was 85.66 ± 0.72%. At the fifth minute, $^{99m}$TcO$_4^-$ impurities are still high. This can be caused by the lack of time needed for SnCl$_2$ to reduce $^{99m}$TcO$_4^-$ optimally. In this incubation time optimization, the radioactivity used ranges from 0.21 to 0.46 mCi which is smaller than the previous optimization. Small radioactivity with the same volume can cause loss of the reducing power of SnCl$_2$ due to radiolysis [9]. Radiation can break down water solvents to produce free radicals, which can oxidize other molecules such as SnCl$_2$.

| Time incubation (minute) | Impurity (%) | Radiochemical purity (%) | Radioactivity (mCi) |
|--------------------------|--------------|--------------------------|---------------------|
|                          | $^{99m}$TcO$_4^-$ | $^{99m}$TcO$_2$   |                    |
| 5                        | 17.86 ± 2.95  | 10.52 ± 0.15            | 71.62 ± 2.80       |
| 10                       | 7.52 ± 2.07   | 7.63 ± 1.74             | 84.84 ± 1.33       |
| 20                       | 7.75 ± 1.44   | 6.59 ± 1.74             | 85.66 ± 0.72       |
| 30                       | 11.06 ± 2.60  | 9.01 ± 2.19             | 79.94 ± 2.23       |
| 60                       | 14.47 ± 2.11  | 8.53 ± 3.58             | 77.00 ± 1.51       |

Figure 7. Optimization of the ratio of SnCl$_2$ and SBA-16-Al amount
The electrical charge test was carried out using the paper electrophoresis method. Electrophoresis is a method of separation based on the movement of molecules in an electric field. Based on this research, the $^{99m}$Tc-SBA-16-Al has a neutral charge, which is characterized by not moving of the $^{99m}$Tc-SBA-16-Al compound both to the cathode and the anode. The electric charge of a labeled compound can be taken into consideration to determine the target organ to be addressed. Neutral electric charges can be used to target brain organs, cation-shaped compounds (positively charged) can be used to target cardiac organs, and anionic compounds can be used to target kidney organs. Based on the electric charge, the $^{99m}$Tc-SBA-16-Al labeled compound is ideal for brain imaging because of its neutral charge. Besaid being neutral, another ideal trait for brain organ target is small and lipophilic [10]. With its small particle size, SBA-16-Al silica mesoporous can be a potential carrier material for technetium-99m for cancer imaging, especially neutral charged organs such as the brain.

Figure 8. Optimization of time incubation of teknetium-99m labeled SBA-16-Al

Figure 9. Grafik of $^{99m}$Tc-SBA-16-Al Paper Electrophoresis
4. Conclusion

Based on the research, the smallest SBA-16-Al particle size was obtained in milling treatment for 30 hours and pond ultrasonication for 15-60 minutes with a range of 509-3904 nm and a median of 1587 nm. The optimization results of SBA-16-Al labeling with technetium-99m were obtained at pH 9, the ratio of SnCl2 and SBA-16-Al amount was 75 compared to 1000 (1: 13.33), and the incubation time for 20 minutes at room temperature with radiochemical purity 85.66 ± 0.72%. Paper electrophoresis tests showed ~Tc-SBA-16-Al with a neutral charge.

References
[1] World Health Organization. (2017). Guide to cancer early diagnosis. Geneva: World Health Organization
[2] Vartika Rai and Pranita Roy. (2014). Molecular diagnosis of cancer. BioEvolution, ISBN 978-8(January 2014), 12–19.
[3] Zhou, Y., Quan, G., Wu, Q., Zhang, X., Niu, B., Wu, B., Wu, C. (2018). Mesoporous silica nanoparticles for drug and gene delivery. Acta Pharmaceutica Sinica B, 8(2), 165–177.
[4] Tamba B.I.A. Dondas,M. Leon,A.N. Neagu,G. Dodi,C. Stefanescu,A. Tijani, Silica nanoparticles: Preparation, characterization and in vitro/in vivo biodistribution studies, European Journal of Pharmaceutical Sciences Volume 71, 25 April 2015, Pages 46-55.
[5] Hakiki N.W., dkk. (2013). Penandaan M41S-NH2 dengan Radionuklida Teknesium-99m: Perbandingan Metode Langsung dengan Metode Tidak Langsung dalam Aplikasi Radiosinovektomi, The 2nd International Conference of the Indonesian Chemical Society 2013 October, 22-23th 2013.
[6] Andrade, G. F., Soares, D. C. F., Dos Santos, R. G., & Sousa, E. M. B. (2013). Mesoporous silica SBA-16 nanoparticles: Synthesis, physicochemical characterization, release profile, and in vitro cytocompatibility studies, Microporous and Mesoporous Materials, 168, 102–110, https://doi.org/10.1016/j.micromeso.2012.09.034
[7] Roobab U AR, Madni GM, Bekhit AED. 2018. The impact of nonthermal technologies on the microbiological quality of juices: A review. Comprehensive Reviews in Food Science and Food Safety:1–21.
[8] Matthew D. Schwartz. (2014). Quantum Field Theory and the Standard Model, chapter 14 : Polarization. Massachusetts: Harvard University.
[9] Saha, Gopal B. (2010). Fundamental of Nuclear Pharmacy. Sixth Edition. New York : Springer.
[10] Oekar, Nanny Kartini. (2016). Prinsip Pembuatan Senyawa Bertanda. Pelatihan Petugas dan Supervisor Proses Radioisotop dan Senyawa Bertanda. Bandung, 1-15 Februari 2016. Pusat Pendidikan dan Pelatihan-BATAN.