

Synthesis, Radiochemical Purity Control and Stability of Scandium-46-1,4,7-triazacyclononane-1,4,7-triacetic acid (⁴⁶Sc-NOTA)

Duyeh Setiawan^{1,*}, Iwan Hastiawan², and Asri Nurul Bashiroh²

¹Center for Applied Nuclear Science and Technology, National Nuclear Energy Agency, Jl. Tamansari 71, Bandung 40132, Indonesia

²Department of Chemistry, Faculty Mathematics and Natural Sciences, Padjadjaran University, Jl. Raya Bandung-Sumedang km 21, Jatinangor-Sumedang 45363, Indonesia

Received April 26, 2017; Accepted July 31, 2017

ABSTRACT

The purposes of this research are to synthesize of scandium-46-1,4,7-triazacyclononane-1,4,7-triacetic acid (⁴⁶Sc-NOTA) labeled compound based on formation of stoichiometric complex bonding between radioisotope ⁴⁶Sc and 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) ligand, then to determine radiochemical purity and stability of ⁴⁶Sc-NOTA. In this research, two variables, namely mole ratio of radioisotope to and ligand and pH were investigated. The results showed that optimum condition was at the mole ratio of radioisotope to ligand (⁴⁶Sc : NOTA) of 1:2 and pH 5. The radiochemical purity and stability assay of ⁴⁶Sc-NOTA were evaluated with paper chromatography and the result showed that ⁴⁶Sc-NOTA gave radiochemical purity of 99.09 ± 0.2% and was stable for 9 days.

Keywords: radioisotope ⁴⁶Sc; NOTA; synthesis; stability; radiotherapy

ABSTRAK

Tujuan dari penelitian ini adalah sintesis senyawa bertanda skandium-46-1,4,7-triazacyclononane-1,4,7-triacetic acid (⁴⁶Sc-NOTA) yang didasari oleh pembentukan ikatan kompleks antara radioisotop ⁴⁶Sc dan ligan 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) secara stoikiometri, uji kemurnian radiokimia dan stabilitas dari ⁴⁶Sc-NOTA. Dalam penelitian ini dipelajari dua variabel, yaitu perbandingan mol ⁴⁶Sc : NOTA dan pH. Hasil penelitian didapatkan kondisi optimum pada perbandingan mol ⁴⁶Sc : NOTA 1:2 dan pH 5. Kemurnian radiokimia dan uji kestabilan ⁴⁶Sc-NOTA diuji dengan kromatografi kertas dan hasil menunjukkan bahwa kemurnian radiokimia sebesar 99,09 ± 0,2 % dan masih stabil selama 9 hari.

Kata Kunci: radioisotop ⁴⁶Sc; NOTA; sintesis; stabilitas; radioterapi

INTRODUCTION

In recent years, research on the compound complexes containing macrocyclic ligand with functional groups on its cyclic bonding has attracted the attention of researchers especially for the radiopharmaceutical and biomedical purpose [1].

One of ligand often used information of radioactive complex compounds is macrocyclic ligand its complex stable compared to non-cyclic ligands. The stability complex compounds to be considered for success in radiotherapy and diagnostic imaging application [2-8].

Radionuclide scandium-47, ⁴⁷Sc (T_{1/2} = 3.35 days) which it serves as β-emitter (0.600 MeV 31.5%) and gamma energy (159 keV 68.5%) widely used in medical studies as radionuclide therapy and diagnosis. Due to

relatively short of the half life (⁴⁷Sc), the study of chemical nature and its stability of scandium are mostly common using ⁴⁶Sc instead of ⁴⁷Sc even both had similar chemical behavior, but only had different half-life (T_{1/2} = 83 days) [9-13].

In present study, scandium-46 is easily produced because of its abundance in nature and can be used as an analog of scandium-47. Meanwhile, in the process of production of scandium-47 through the nuclear reaction of ⁴⁷Ti (n,p) ⁴⁷Sc required fraction of fast neutrons with high energy and the results have not been promising due to difficult manufacturing techniques [14]. In this study, for complexing the radionuclide scandium complex a macrocyclic ligand, namely, 1,4,7-triazacyclononane-1,4,7-triacetate acid (NOTA) has been chosen.

* Corresponding author. Tel : +62-22-2503997
Email address : d_setiawan@batan.go.id

EXPERIMENTAL SECTION

Materials

Chemicals used in this study were 1,4,7-triazacyclononane-1,4,7-triacetate acid (NOTA) ligand [10], radioisotopes $^{46}\text{ScCl}_3$ [9], hydrochloric acid, ammonium acetate 10%, ammonia 25%, DTPA (diethylene triamine penta acetate), methanol, all obtained from E. Merck.

Instrumentation

The equipment of glass tools used in this study was typically used in laboratory. The instruments used were the magnetic stirrer, dose calibrator, analytical balance, pH indicators, Multichannel Analyzer with HPGe detector (High Purity Germanium), single-channel Analyzer with NaI detector and vortex, electrophoresis.

Procedure

Synthesis of $^{46}\text{Sc-NOTA}$

Determination of optimum condition with mol variation. NOTA ligand solution added to radionuclides $^{46}\text{ScCl}_3$ solution with variation of mole ratio (2:1, 1:1, 1:2, 1:3). The solutions were incubated for 24 h, after that the radiochemical analysis was carried out by ascending paper chromatography developed with ammonia: distilled water (1:25). The solution then spotted onto chromatography paper and counted with using Single Channel Analyzer-NaI(TL).

pH effect assay. NOTA ligand was labeled with $^{46}\text{ScCl}_3$ at pH 5 and pH 13 and then incubated for 24 h. Labeled compound was spotted onto chromatography paper that developed in mobile phase consisting of ammonia: distilled water (1:25). The result of distribution activities were counted using Single Channel Analyzer-NaI(TL).

Optimum condition assay. The labeled compound, $^{46}\text{Sc-NOTA}$ was spotted onto three kind of chromatography papers, namely, 3 MM, ET 31 then each chromatography papers developed in different mobile phases, ammonia : distilled water (1:25), 10 mM DTPA pH 5 and ammonium acetate 10% : methanol (1:1) respectively. The results were counted using Single Channel Analyzer-NaI(TL).

Electrophoresis of $^{46}\text{Sc-NOTA}$. The labeled compound $^{46}\text{Sc-NOTA}$ was spotted onto electrophoresis paper, submerged in HCl 0.01 M for 1 h in 350 voltage DC.

Stability assay. The labeled compound $^{46}\text{Sc-NOTA}$ was spotted onto 3 MM chromatography paper and developed in mobile phase consisting of ammonia: distilled water (1:25). The result of distribution activities were counted using Single Channel Analyzer-NaI(TL). Stability towards storage was examined by performing labeling test at day 1 until day 9.

RESULT AND DISCUSSION

Determination of Optimum Condition with Mol Variation

$^{46}\text{Sc-NOTA}$ compound has R_f value of range 0.8–0.9 [10] and radioisotope ^{46}Sc has R_f value of 0 [9,11], these values show the difference of distribution velocity and interaction in solvent.

The moles ratio of ^{46}Sc and NOTA ligand, very significantly influenced radiochemical purity of $^{46}\text{Sc-NOTA}$. The mole ratio of ^{46}Sc and NOTA 2:1 caused low radiochemical purity (34.08%). This result showed that with the less number of NOTA, ^{46}Sc has not fully reacted with NOTA and therefore free Sc are still remain (Fig. 1a). For a ratio of 1:1 (Fig. 1b), the radiochemical purity of 64.40% was obtained. This indicates that the ratio of 1:1 does not qualified as radiopharmaceutical preparation. With the ratios 1:2 (Fig. 1c) and 1:3 (Fig. 1d), radiochemical purities obtained were of 99.09% and 99.72%, respectively. There indicated that the mole ratio has been achieved stoichiometrically, so that all the reactants were reacted completely. Therefore formed Sc-NOTA complexes were in high purity because there is no excessive ^{46}Sc . The high radiochemical purity of Sc-NOTA (> 95%), the product has fulfilled the requirements of radiochemical purity for the preparation of radiopharmaceutical (> 95%) [15].

pH Effect Assay

Fig. 2 shows the chromatogram at pH 13 with 1.05% radiochemical purity peaks formed at $R_f = 0$ which showed that free Sc was still formed (Fig. 2b). While at pH 5 with a radiochemical purity of 99.09% peak formed is at $R_f = 0.9$ (Fig. 2a). This result showed that pH value to complex the radioisotope compound may be applied for radiotherapy at range of pH 5-7 that matches with pH of human blood.

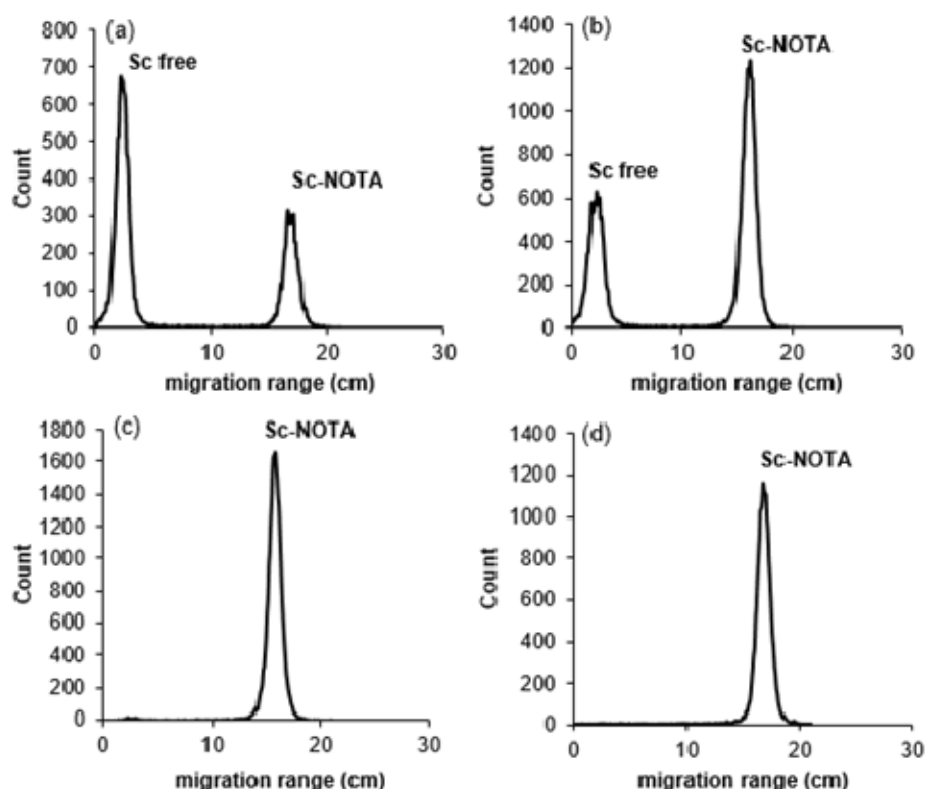


Fig 1. Chromatogram of ^{46}Sc -NOTA variation moles ratio of ^{46}Sc with NOTA ligand in mobile phase ammonia : distilled water (1:25) using 3 MM chromatography paper; (a) Sc: NOTA 2:1, (b) Sc: NOTA 1:1, (c) Sc: NOTA 1:2, (d) Sc: NOTA 1:3

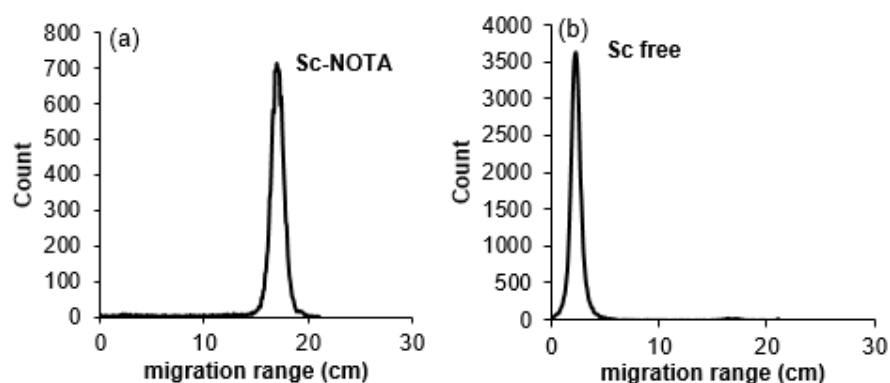


Fig 2. ^{46}Sc -NOTA's chromatogram in mobile phase ammonia : distilled water (1:25) using 3 MM chromatography paper; (a) Sc: NOTA at pH 5, (b) Sc: NOTA at pH 13

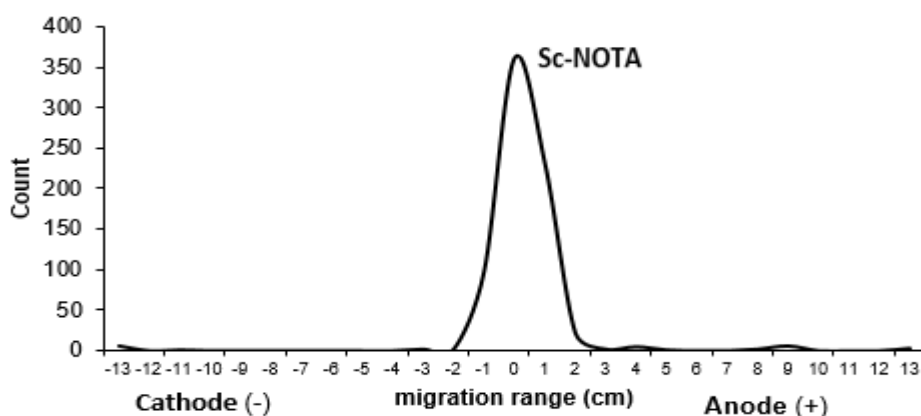
Determining the Optimum Conditions of Chromatography Paper

Variation of solvent was conducted to determine which more effective solvent for use is in the present study of the chromatographic processing that can separate the complex from its impurity with a good resolution. The effectiveness was determined from the result of chromatography paper that demonstrated the good resolution of the separation peak of the complex

and its impurity. The selection of paper has chosen by the power of capillarity for chromatography its acts as the passage of the mobile phase. The most effective paper chromatography for this study was 3 MM chromatography paper. Although the time of solvent rise in 3 M chromatography paper is relatively long but the radiochemical purity is better than the 31 E paper. The results of paper chromatographic variations are summarized in Table 1.

Table 1. The result of variation of chromatography system

No	System		Rf		Elution time (in minute)	Result
	Stationary Phase	Mobile Phase	$^{46}\text{ScCl}_3$	$^{46}\text{Sc-NOTA}$		
1	3 MM	Ammonia : distilled water (1:25)	0	0.9-1	60	Useable
2	3 MM	DTPA 10 mM pH 5	0.9-1	1	65	Useable
3	3 MM	Ammonium acetate (10%) : Methanol (1:1)	0-0.2 (tailing)	0.5-0.7	75	Unusable
4	Paper 31 ET	Ammonia : distilled water (1:25)	0	1	30	Useable
5	Paper 31 ET	DTPA 10 mM pH 5	1	1	25	Useable
6	Paper 31 ET	Ammonium acetate (10%) : Methanol (1:1)	0 (tailing)	0.7-0.9 (tailing)	60	Unusable

**Fig 3.** $^{46}\text{Sc-NOTA}$ electrogram in hydrochloric acid 0.01 M

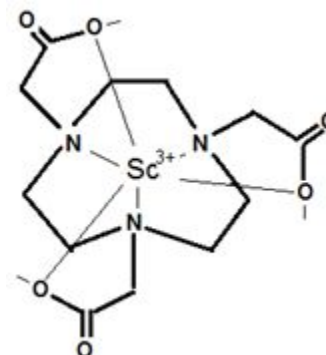
In ammonia : distilled water (1:25) with 3 MM and paper 31 ET chromatography paper, the chromatograms do not produce tailings, $R_f = 0$ (^{46}Sc), $R_f = 0.9-1$ ($^{46}\text{Sc-NOTA}$) and the elution time take 60 min and 30 min.

Electrophoresis of $^{46}\text{Sc-NOTA}$

The determination of the charge of $^{46}\text{Sc-NOTA}$ was performed using electrophoresis on 3 MM paper with an electrolyte solution, hydrochloric acid 0.01 M at 350 voltage DC for 1 h.

The results of electrophoresis (Fig. 3) showed that NOTA was a three dentate ligand and had a charge of 3- (NOTA^{3-}). NOTA Sc^{3+} binds to metals through coordination bonding of three amine group with the carboxylic group to form uncharged $^{46}\text{Sc-NOTA}$. The complex structures can be seen in Fig. 4.

For the complexation of Sc^{3+} , macrocyclic ligand was chosen because it can form complexes with solid metal cations M^{3+} , which are stable thermodynamically and kinetically. Structural factors such as stiffness, cavity size and the nature and the number of donor atoms in the macrocyclic chelating agent has dual function and it has an important role in chemical and biological behavior of the complex form [2].

**Fig 4.** Structure of $^{46}\text{Sc-1,4,7-triazacyclononane-1,4,7-triacetic acid [NOTA]$ [15]

Stability Assay

The stability assay of $^{46}\text{Sc-NOTA}$ was conducted using 3 MM paper chromatography that were developed in mobile phase of ammonia: distilled water (1:25). The complex compounds of $^{46}\text{Sc-NOTA}$ was quite stable with a radiochemical purity (RCP) 95% stands for 9 days as shown in Fig. 5.

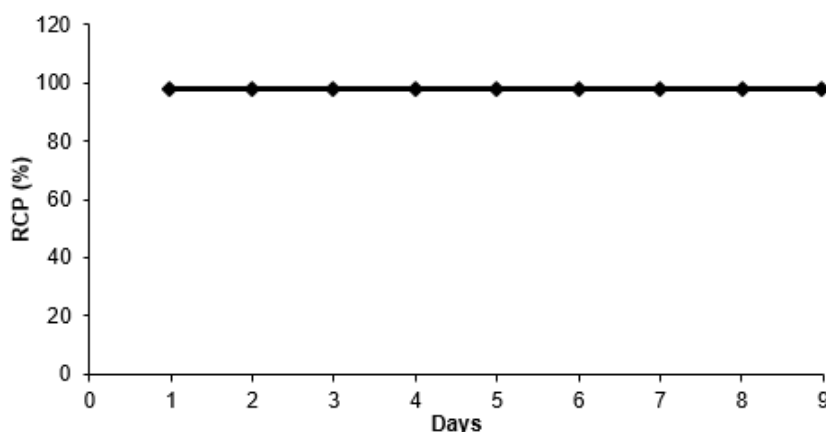


Fig 5. The results of stability assay of ⁴⁶Sc-NOTA for 9 days in graph

CONCLUSION

The complex compound of scandium-NOTA synthesized from NOTA ligand and scandium-46 with the optimum mole ratio of 1:2 and pH 5 gave radiochemical purity of $99.09 \pm 0.2\%$. The complex showed stable for 9 days with no significant reduction of purity ($> 95\%$).

ACKNOWLEDGEMENT

The authors are thankful to colleagues in Labeled Compound and Radiometric of BATAN and also Padjadjaran University for their contributions in this research.

REFERENCES

- [1] Martins, P.A., da Silva, J.L., Ramos, M.P.S., de Oliveira, I.M., Felgueiras, C.F., Herrerias, R., Júnior, C.L.Z., Mengatti, J., Fukumori, N.T.O., and Matsuda, M.M.N., 2011, Radiochemical stability of radiopharmaceutical preparations, *International Nuclear Atlantic Conference - INAC 2011*, Belo Horizonte, MG, Brazil, October 24-28, 2011.
- [2] Majkowska-Pilip A., and Bilewicz, A., 2011, Macrocyclic complexes of scandium radionuclides as precursors for diagnostic and therapeutic radiopharmaceuticals, *J. Inorg. Biochem.*, 105 (2), 313–320.
- [3] Notni, J., Hermann, P., Havlíčková, J., Kotek, J., Kubíček, V., Plutnar, J., Loktionova, N., Riss, P.J., Rösch, F., and Lukes, I., 2010, A triazacyclononane-based bifunctional phosphinate ligand for the preparation of multimeric ⁶⁸Ga tracers for positron emission tomography, *Chem. Eur. J.*, 16 (24), 7174–7185.
- [4] Varasteh, Z., Velikyan, I., Lindeberg, G., Sörensen, J., Larhed, M., Sandström, M., Selvaraju, R.K., Malmberg, J., Tolmachev, V., and Orlova, A., 2013, Synthesis and characterization of a high affinity NOTA-conjugated bombesin antagonist for GRPR-targeted tumor imaging, *Bioconjugate Chem.*, 24 (7), 1144–1153.
- [5] Anissa, I.T., Setiawan, D., and Hastiawan, I., 2014, *Synthesis of complex compounds (scandium-47)-1,4,7-triazacyclononane-1,4,7-triacetate acid (NOTA) as precursor to radiotherapy and diagnostic imaging*, Essay, Undergraduate Studies Program Faculty of Chemistry, Padjadjaran University, Bandung - Indonesia.
- [6] Pniok, M., Kubíček, V., Havlíčková, J., Kotek, J., Sabatie-Gogová, A., Plutnar, J., Huclier-Markai, S., and Hermann, P., 2014, Thermodynamic and kinetic study of scandium(III) complexes of DTPA and DOTA: a step toward scandium radiopharmaceuticals, *Chem. Eur. J.*, 20 (26), 7944–7955.
- [7] Setiawan, D., and Hastiawan, I., 2014, Sintesis dan karakterisasi 1,4,7-triazasiklononana-1,4,7-asam triasetat (NOTA) sebagai ligan dalam senyawa kompleks sediaan radioterapi, *JCNA*, 2 (1), 66–71.
- [8] Holub, G.J., Meckel, M., Kubíček, V., Rösch, F., and Hermann, P., 2015, Gallium(III) complexes of NOTA-bis (phosphonate) conjugates as PET radiotracers for bone imaging, *Contrast Media Mol. Imaging*, 10 (2), 122–134.
- [9] Setiawan, D., and Anggraeni, R., 2015, Preparation and characterization of radioisotopes ⁴⁶ScCl₃ results irradiation natural scandium oxide materials target, *Proceedings of the National Seminar on Nuclear Science and Technology*, PSTNT-BATAN, 207–213.
- [10] Idzni, F., Setiawan, D., and Hastiawan, I., 2015, *Synthesis and characterization of ligand 1,4,7-triazacyclononane-N,N',N''-triacetate acid (NOTA)*

- with chloroacetic acid precursors, Essay, Undergraduate Studies Program Faculty of Chemistry, Padjadjaran University, Bandung - Indonesia.
- [11] Setiawan, D., and Mulyati, T.S., 2015, Pembuatan dan analisis fisiko-kimia radioisotop skandium-47 (^{47}Sc) dari bahan sasaran titanium oksida alam, *JSTNI*, 16 (1), 63–70.
- [12] Setiadi, Y., Setiawan, D., Daruwati, I., Hastiawan, I., and Bashiroh, A.N., 2015, Synthesis and characterization of a Scandium-46 complexes with polyammino carboxylate based bifunctional agent as precursor in bleomycin labeling, *Proceedings of the National Seminar on Nuclear Energy and Technology*, Denpasar, 591–598.
- [13] Moghaddam-Banaem, L., Jalilian, A.R., Pourjavid, M., Bahrami-Samani, A., Mazidi, M., Ghannadi-and Maragheh, M., 2012, Preparation and quality control of scandium-46 bleomycin as a possible therapeutic agent, *Iran. J. Nucl. Med.*, 20 (1), 19–24.
- [14] Mamtimin, M., Harmon, F., and Starovoitova, V.N., 2015, Sc-47 production from titanium targets using electron linacs, *Appl. Radiat. Isot.*, 102, 1–42.
- [15] Maskur, Setiyowati, S., Ramli, M, Cecep, R.T., Subur, M., and Misyetti, 2011, Preparation, characterization, and stability test radiopharmaceuticals ^{177}Lu -CTMP for palliative pain bone metastasis, *Proceedings of Scientific Meeting Radioisotopes, Radiopharmaceuticals and Cyclotron*, Radioisotopes and Radiopharmaceuticals Center-Batan Serpong.