• *Technical note*

Preparation and quality control of ^{153}Sm-[*tris*(1,10 -phenanthroline) samarium (III)] complex

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Background: The ^{153}Sm-[tris(1,10-phenanthroline) Samarium(III)]complex (^{153}Sm-PL$_3$) was prepared in view of development of targeting therapeutic compounds for malignancies, and interesting *in-vitro* anti-tumor activities of lanthanide phenanthroline complexes,. ***Materials and Methods:*** Sm-153 chloride was obtained by thermal neutron flux (4×10^{13} n.cm^{-2}.s^{-1}) of enriched $^{152}Sm_2O_3$ sample, dissolved in acidic media. The labeling was performed in ethanol in 24h, controlled by ITLC (1.0mM DTPA, pH.5, as mobile phase). The partition coefficient for the labeled compound was also determined. ***Results:*** A radiochemical yield of more than 95% was obtained. Radiochemical purity of 96% was obtained using ITLC with specific activity of about 27.75 GBq/mg. The radio-labeled complex was stable in aqueous solution at least 24 hours and no significant amount of free ^{153}Sm was released from the complex. The partition coefficient for the labeled compound was determined (log P. 3.4). The complex was stable in final formulation for 66h. The biological evaluation of the compound is under investigation. ***Conclusion:*** The radiolabeled compound used in this study was a very inexpensive and useful agent for the use as a therapeutic compound. **Iran. J. Radiat. Res., 2012; 10(1): 59-62**

Keywords: *Samarium-153, 1, 10-phenanthroline, radiolabeling, quality control.*

INTRODUCTION

[Tris(1,10-phenanthroline)lanthanum (III)] (La-PL$_3$) has been prepared previously and the rigid planar 1,10-phenanthroline (PL) molecule demonstrated distinct effects on *in vitro* cultured cells. The complex also has shown to stop DNA synthesis in CCRF-CEM and Ehrlich ascites cells leading to a cell cycle arrest in G0/G1 (1, 2) based on the metal chelating ability of 1,10-phen (3, 4), several metal ions including copper, ruthenium and cobalt has shown to enhance the anticancer activity of PL (5).

On the other hand, several complexes of vanadium with PL derivatives has shown to demonstrate apoptotic effect *in vivo* and *in vitro* (6-8). Recently, La-PL$_3$ demonstrated anticancer activity via potent induction of cell cycle arrest and/or apoptosis with promising in vivo anticancer activity against a human colon cancer xenograft, suggesting, La-PL$_3$ as a new anticancer metal-drug (9).

Radioisotopes with medium-energy beta emissions and half-life of a few days are attractive candidates for systemic delivery of targeted irradiation (10), such as ^{153}Sm ($T_{1/2}$ = 46.7 h), also having medium-energy gamma photon (103 keV) which is suitable for imaging.

In this research, ^{153}Sm-PL$_3$ (figure 1) complex was prepared and the effects of various production conditions were investigated on its labeling yield.

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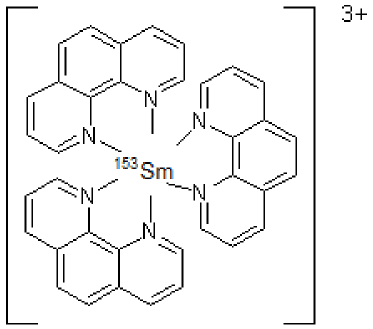

Figure 1. Possible chemical formula for ^{153}Sm-PL$_3$.

MATERIALS AND METHODS

Production of ^{153}Sm was performed at Tehran Research Reactor (TRR) using ^{152}Sm (n, g) ^{153}Sm reaction with ^{152}Sm in purity of 98.7% (ISOTEC Inc.) 1,10-phenanthroline was purchased from Aldrich Co., Germany, without further purification. Chromatography paper (Whatman No. 2) was obtained from Whatman (Maidstone, UK). Radiochromatography was performed using a bioscan AR-2000 radio TLC scanner instrument (Bioscan, Paris, France). A high purity germanium (HPGe) detector, coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany), were used for counting distributed activity in rat organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Calculations were based on the 103 keV peak for ^{153}Sm. All values were expressed as mean ± standard deviation (Mean± SD), and the data were compared using Student's T-test. Statistical significance was defined as $P<0.05$.

Synthesis of ^{153}Sm-PL$_3$ complex

The ^{153}Sm was produced by neutron irradiation of 100 µg of enriched $^{152}Sm_2O_3$ according to reported procedures [(11)] at a thermal neutron flux of 4×10^{13} n.cm^{-2}.s^{-1} for 5 days. Specific activity of the ^{153}Sm was 27.75 GBq/mg. The irradiated target was dissolved in 200 µl of 1.0 mol/L HCl, to prepare $^{153}SmCl_3$ and diluted to the appropriate volume with ultra pure water, to produce a stock solution. The mixture was filtered through a 0.22 µm biological filter and sent for use in the radiolableing step. Radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy and HPGe spectroscopy to detect various interfering beta and gamma emitting radionuclides. The radiochemical purity was also checked by Whatman No.2 chromatography paper, and developed in a mixture of 1.0 mmol/L DTPA solution as mobile phase.

The acidic solution (0.2 ml) of $^{153}SmCl_3$ (111 MBq, 3 mCi) was transferred to a 5 ml-borosilicate vial and heated to dryness using a flow of N_2 gas at 50-60°C. Two hundred microlitres of 1,10-phenanthroline monohydrate (PL) in absolute ethanol (5 mg/ml) was added to the activity-containing vial and the mixture was diluted by the addition of normal saline (300 µl) followed by vortexing at 60°C for 30-60 min. The active solution was checked for radiochemical purity by ITLC. The final solution was then passed through a 0.22 mm filter and pH was adjusted to 5.5-7. From the final product, 5 µl was applied to a Whatman No.2 strip and followed by developing in 1mM DTPA (pH.5). Radioactivity was determined by a RTLC scanner.

Stability of ^{153}Sm-PL$_3$ in final formulation

Stability tests were based on previous studies performed for radiolabeled metal complexes [(12)]. A sample of ^{153}Sm-PL$_3$ (100 MBq) in aqueous solution was kept at room temperature for 6 hours while checked by RTLC. Micro-samples (5 µl) taken from the shaken mixture were transferred the TLC papers and the ratio of ^{153}Sm cation to ^{153}Sm -PL$_3$ were checked (eluent: 1mM DTPA).

Determination of partition coefficient

Partition coefficient (log P) of ^{153}Sm-PL$_3$ complex was calculated followed by the determination of P (P= the ration of specific activities of the organic and aqueous phases) [(13)]. A mixture of 1 ml of 2-octanol and 1 ml of radiolabeled samarium complex at 37°C was vortexes for 2 hours and left for 30 minutes in room temperature. Then the octanol and aqueous phases were sampled (5 µl) and counted in HPGe detector for 1000 seconds.

RESULTS

The radionuclide was prepared in a research reactor according to regular methods with a range of specific activity 600 -750 mCi/mg for radiolabeling use, after counting the samples on an HPGe detector for 5 hours, very slight amount of impurities were recorded and shown to be Eu radionuclides as shown in table 1 (figure 2).

Table 1. The radionuclidic impurities and their percentages in the final Sm-153 samples produced form enriched Sm-152 (n=5).

Radionuclides	Impurity (%)
Eu-154	< 2.27e-4
Eu-155	< 1.02e-4
Eu-156	< 4.90e-4

The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated for obtaining the desired pH and volume followed by sterile filtering.

To determine stability of complex in aqueous solution, a sample of ^{153}Sm-phenantroline in aqueous solution was kept at room temperature for 6 hours while checked by RTLC. Micro-samples (5 µl) taken from the shaken mixture were transferred to the TLC papers, and the ratio of free radio Samarium to ^{153}Sm-Phenantroline were checked (eluent: 1mM DTPA) (figure 3).

As expected, the lipophilicity of the compound is rather high. The measured water/octanol partition coefficient, P, for the ^{153}Sm-complex found to be 3.4 at pH,7.

DISCUSSION

The radiochemical purity of the ^{153}Sm solution was checked in 1mM DTPA, and free Sm^{3+} cation is complexed to more

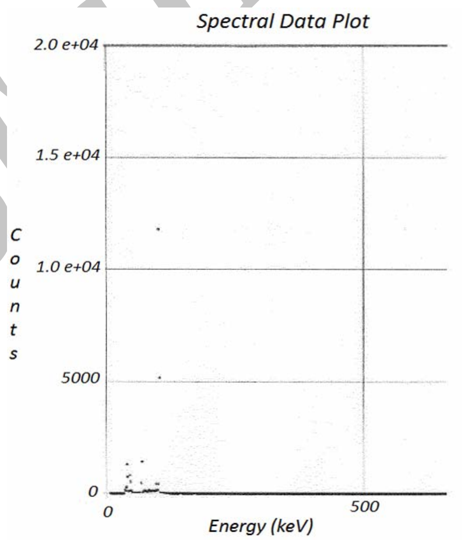

Figure 2. gamma spectrum for Sm-153 prepared by neutron irradiation of Sm-152 sample using an HPGe detector.

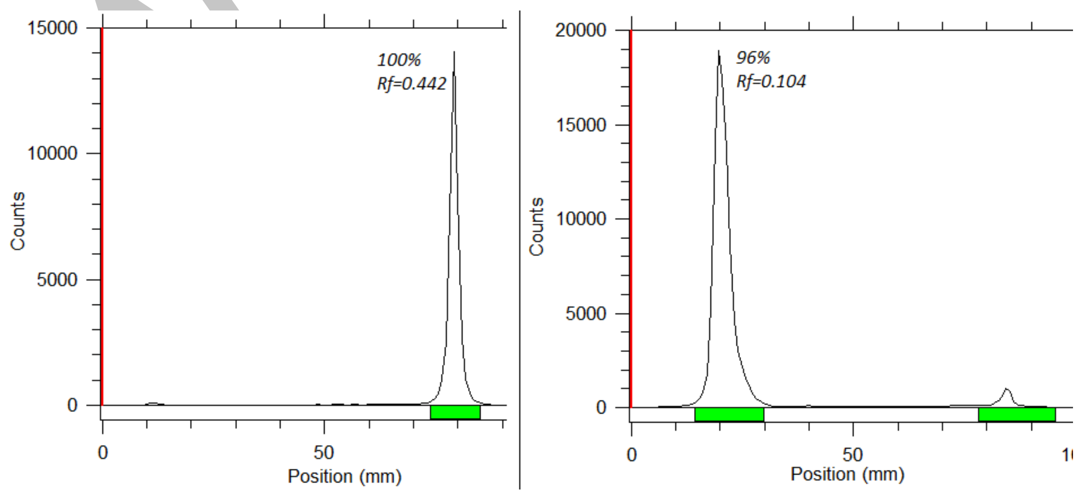

Figure 3. RTLC chromatograms of ^{153}Sm-SmCl$_3$ solution (left) and final ^{153}Sm-PL$_3$ solution (right) using 1mMDTPA solution.

lipophilic SmDTPA form, and migrated to higher R_f, while small radioactive fraction remained at the origin, which could be related to other Lu ionic species, not forming SmDTPA complex, such as $SmCl_4^-$, etc. and/or colloids. While in case of ^{153}Sm-PL_3, the complex slowly migrated to Rf. 0.1, which was easily distinguishable from the free cation. The complexation ability of the eluting DTPA should not exceed the stability of the complex in the media since the complex can be destroyed in high molar ratios of DTPA, this phenomenon had been already reported and applied in the determination of various radionuclidic samples (14).

The free ^{153}Sm/ ^{153}Sm-phenantroline ratio in the labeled sample remained unchanged 4:96. The complex had significant stability in the aqueous media which made it possible to formulate the compound for upcoming biological studies in experimental animals. Stability of such complexes has also been reported while the stability in presence of human serum was also enough (>97% in 10 hours), and appropriate for future works. The lanthanide-phenanthroline complexes had logK of higher than 15 (6-8).

Regarding the high lipophilicity ratio, the high lipophilicity of the complex would be helpful since the complex could easily penetrate through blood-brain barrier (BBB) as well as other biological membranes and has allowed rapid uptake though cells, as well as lipophilic nucleus membrane (4, 5).

CONCLUSION

The method used in this research for the production of ^{153}Sm-PL_3 was quite simple and cost effective. The radiochemical purity was 96% and the labeling and quality control took 24 hours. Trace amounts of $^{153}SmCl_3$ (~4%) were detected by ITLC which showed that radiochemical purity of the ^{153}Sm-PL_3 was higher than 96%. (specific activity, 4.20 TBq/mmol). The radio-labeled complex was stable in aqueous solution at least 6 hours and no significant amount of free ^{153}Sm was released from the complex. Our experiments on this compound have shown satisfactory quality, and stability for future therapeutic studies.

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