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Preparation of DOTA-TATE and DOTA-NOC freeze-dried kits for formulation of patient doses of ^{177}Lu-labeled agents and their comparison for peptide receptor radionuclide therapy application

Tapas Das · Mohini Bhadwal · Sharmila Banerjee · H. D. Sarma · Ajit Shinto · K. K. Kamaleshwaran

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Abstract The objective of the present work is to prepare freeze-dried DOTA-TATE and DOTA-NOC kits for the easy and convenient preparation of patient doses of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC, respectively at the hospital radiopharmacy and to compare the radio-peptides with respect to their radiochemical and biological behaviors. Freeze-dried kits of DOTA-TATE and DOTA-NOC, comprising a lyophilized mixture of 200 μg of DOTA-peptide, 80 mg of gentisic acid and 13.9 mg of ammonium acetate were prepared. Therapeutic doses of ^{177}Lu-labeled peptides (up to 200 mCi, 7.4 GBq) were prepared using these kits and ^{177}Lu, produced in-house, with >99 % radiochemical purity and high stability following an easy and convenient protocol. Comparative pharmacokinetic behavior of the radio-peptides was studied by carrying out biodistribution studies in normal Wistar rats which revealed higher retention of activity in several major organs and slower renal clearance for ^{177}Lu-DOTA-NOC compared to that of ^{177}Lu-DOTA-TATE. Preliminary pharmacokinetic studies, carried out in limited number of patients suffering from cancers of neuroendocrine origins, showed lower accumulation of activity in vital organs and faster renal clearance of ^{177}Lu-DOTA-TATE compared to that of ^{177}Lu-DOTA-NOC.

Keywords Freeze-dried DOTA-TATE kit · Freeze-dried DOTA-NOC kit · ^{177}Lu-DOTA-TATE · ^{177}Lu-DOTA-NOC · PRRNT · Neuroendocrine cancers

T. Das (✉) · M. Bhadwal · S. Banerjee
Isotope Applications and Radiopharmaceuticals Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India
e-mail: tdas@barc.gov.in

M. Bhadwal
e-mail: mohini@barc.gov.in

S. Banerjee
e-mail: sharmila@barc.gov.in

H. D. Sarma
Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India
e-mail: hdsarma@barc.gov.in

A. Shinto · K. K. Kamaleshwaran
Department of Nuclear Medicine & PET, Kovai Medical Centre and Hospital, Coimbatore 641 014, Tamil Nadu, India
e-mail: ajitshinto@gmail.com

K. K. Kamaleshwaran
e-mail: dr.kamaleshwar@gmail.com

Introduction

Peptide receptor radionuclide therapy (PRRNT) employing radiolabeled somatostatin analogue peptides has become an established procedure for the treatment of patients suffering from inoperable neuroendocrine cancers over-expressing somatostatin receptors (SSTR) in the last decade [1–6]. The use of several radiolabeled peptides such as, ^{90}Y-DOTA-TATE (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid coupled Tyr^3-octreotate), ^{90}Y-DOTA-TOC (DOTA coupled Tyr^3-octreotide), ^{177}Lu-DOTA-TATE, ^{177}Lu-DOTA-TOC and ^{177}Lu-DOTA-NOC (DOTA coupled Nal^3-octreotide) have been documented for this purpose [1–11]. Amongst the ^{177}Lu-labeled peptides, ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-TOC have been used more frequently for treating the neuroendocrine cancer patients while only limited use of ^{177}Lu-DOTA-NOC have been reported [12]. In India, ^{177}Lu-DOTA-TATE is regularly used in several nuclear medicine centers for providing radiotherapeutic treatment to the patients suffering from various types of inoperable neuroendocrine cancers [13,

14]. However, recent non-availability of DOTA-TATE and DOTA-TOC due to intellectual property right issues has created inconvenience to the patients who are undergoing radiotherapeutic regimen as well as for the new patients who are scheduled to undergo PRRNT using ^{177}Lu-DOTA-TATE. This necessitated studying other DOTA coupled somatostatin analog peptides for labeling with ^{177}Lu. The availability of DOTA-NOC coupled with the fact that it has the highest affinity towards SSTR-3 and SSTR-5 and high affinity towards SSTR-2 among all the somatostatin analogs considered [12, 15, 16], has prompted us to radiolabel it with ^{177}Lu and also to carry out a comparative study between ^{177}Lu-DOTA-NOC and ^{177}Lu-DOTA-TATE in biological systems.

The success of PRRNT, along with several other factors, primarily depends on the availability of the radiolabeled peptide with adequately high specific activity, so that required therapeutic efficacy can be achieved without saturating the limited number of receptors available on the target lesions [17–19]. This in turn directly depends on the specific activity of the radionuclide at the time of preparation of the agent. Therefore, ^{177}Lu-DOTA-TATE, the agent which is most commonly used in India for treating neuroendocrine cancer patients, is prepared in the hospital radiopharmacy just prior to its administration in patients [13, 14, 20]. This ensures preparation of the agent with maximum possible therapeutic efficacy depending on the specific activity of ^{177}Lu available at the time of preparation. However, successful preparation of the agent following this methodology is largely dependent on the availability of the trained personnel at the respective nuclear medicine centers, as this procedure requires stringent adjustment of certain parameters prior to incubation [20]. A small deviation from the standard procedure may lead to failure of the batch and consequently loss of expensive peptide and radionuclide. Moreover, this may adversely affect the treatment schedule of the patients. However, all these problems can be circumvented if the freeze-dried kits, which will enable the preparation of the radiolabeled peptide in an easy and single-step, could be developed. The use of kits is also expected to reduce the exposure of the personnel working in the hospital radiopharmacy as well as number of batch failures.

Working in this direction, we have developed freeze-dried DOTA-NOC kits which can be utilized to prepare therapeutic doses of ^{177}Lu-DOTA-NOC using ^{177}Lu having a certain minimum specific activity at the time of preparation of the agent. A similar attempt was also made to prepare freeze-dried DOTA-TATE kits, suitable for the preparation of patient doses of ^{177}Lu-DOTA-TATE, in order to make a comparison between these two agents. It is worthwhile to mention that the use of freeze-dried DOTA-TATE kits for the formulation of ^{177}Lu-DOTA-TATE have already been documented in the literature [21, 22]. However, none of these articles describe the detailed methodologies of formulation of such kits and merely mention their use for the formulation of patient dose of ^{177}Lu-DOTA-TATE. Moreover, no information is available regarding the rationale behind taking certain amount of DOTA-TATE, shelf-life of the kits and most importantly, about the maximum dose that can be prepared using a single kit vial. Apart from this, none of these kits have been utilized for the preparation of usually administrated therapeutic dose of 5.55–7.4 GBq (150–200 mCi) ^{177}Lu-DOTA-TATE.

In the present paper, we describe the detailed methodologies of preparation of freeze-dried DOTA-TATE and DOTA-NOC kits, whose one kit vial is sufficient for the preparation of up to 7.4 GBq (200 mCi) patient dose of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC, respectively. Herein we also report a comparative study of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC, prepared using the respective kits, in terms of their radiochemical and biological behaviors in animal models. Our experience of using these kits to treat limited number of neuroendocrine cancer patients is also documented in the present paper.

Materials and methods

DOTA-TATE acetate and DOTA-NOC acetate were obtained from ABX Advanced Biochemical Compounds (Germany). Gentisic acid (2,5-dihydroxybenzoic acid) and ammonium acetate were procured from Aldrich Chemical Company (USA). Lutetium oxide (82 % enriched in ^{176}Lu, spectroscopic grade, >99.999 % chemically pure) was obtained from Centre for Molecular Research (Russia). High purity supra-pure water and supra-pure HCl were obtained from Merck (Germany). All other chemicals and solvents used were of analytical reagent (AR) grade and supplied by reputed local chemical manufacturers. Radionuclidic purity of ^{177}Lu was ascertained by high resolution gamma ray spectrometry using a HPGe detector (EGG Ortec/Canberra detector, USA) coupled to a 4K multichannel analyzer (MCA) system after radiochemical processing. All other radioactivity measurements were carried out by using well-type NaI(Tl) scintillation counter (Electronic Corporation of India Limited, India), unless mentioned otherwise, by keeping the baseline and window at 150 and 100 keV, respectively; thereby utilizing the 208 keV gamma photon of ^{177}Lu. Lyophilization was done by using the Labocene Coolsafe^{TM} 55-4 freeze-drier (Denmark). Paper chromatography (PC) strips were purchased from Whatman (UK). The high performance liquid chromatography (HPLC) system (PU 1580) was obtained from Jasco (Japan). The elution profile was monitored by detecting the associated radioactivity signal using a well-type NaI(Tl) detector (Jasco, Japan) coupled with the HPLC system. All the solvents used for HPLC were degassed and filtered prior to use and were of HPLC grade.

Animal experimentations were carried out in normal Wistar rats which were bred and reared in the laboratory animal facility of our Institute under standard management practice. Radioactive counting associated with the animal studies were carried out using a flat-type NaI(Tl) scintillation counter (Electronics Corporation of India Limited, India) fixing the baseline at 150 keV and keeping a window of 100 keV. All the animal experiments were carried out in strict compliance with the relevant national laws relating to the conduct of animal experimentation.

Clinical studies with ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC, prepared using the corresponding freeze-dried kits, were carried out by administering the preparation to the patients suffering from inoperable or metastasized cancers of neuroendocrine origin. The patients showed somatostatin receptor positive disease in somatostatin receptor scintigraphy (SRS) carried out with ^{99m}Tc-HYNIC-TOC (hydroxynicotinamide coupled Tyr^3-octreotide), prepared using in-house HYNIC-TOC kit, one month prior to the therapy. All the patients exhibited significantly higher uptake of activity in the tumors and metastatic lesions compared to that in liver in SRS study. Aminoven 10 % intra-venous infusion, an injectible solution of the mixed amino acids, was obtained from Fresenius Kabi (Austria). Post-therapy whole-body scans and SPECT-CT (SPECT: single photon emission computed tomography, CT: computed tomography) acquisitions of the abdomen area were performed using the dual-head gamma camera (Symbia T-200) procured from Siemens (Germany). The necessary ethical clearances for administration of the agents in human patients were obtained from the competent authority and written consents were collected from the patients prior to the administration of the agents.

Experimental

Formulation of freeze-dried DOTA-TATE and DOTA-NOC kits

Freeze-dried DOTA-TATE and DOTA-NOC kits (ten numbers in each batch) were prepared following the protocol mentioned below. A solution of gentisic acid in buffer was prepared by dissolving 800 mg of gentisic acid in 18 mL of 0.1 M ammonium acetate buffer (pH 5) by gentle warming under aseptic conditions. A solution of the peptide, prepared by dissolving 2 mg of the DOTA-TATE or DOTA-NOC in 2 mL of high purity supra-pure water, was added with the buffer solution containing gentisic acid. The resultant solution was thoroughly mixed and its pH was adjusted to $\sim$5. The solution was subsequently passed through Millipore® (0.22 μ) filter paper and aliquoted into ten sterile glass vials, each vial containing 2 mL of the solution. All these preparative steps were carried out under aseptic conditions. The vials were incubated for a period of 24 h at −4 °C followed by another 24 h at −40 °C. Finally, the vials were freeze-dried in a lyophilizer for $\sim$8 h, whereby the kits were obtained. The kits were stored at −4 °C temperature.

Production and radiochemical processing of $^{177}LuCl_3$

Lu-177, which is regularly produced in our laboratory for the commercial deployment to various nuclear medicine centers [23], was used for the preparation of patient doses using the kits developed in-house. Typically, 200 μg of isotopically enriched Lu_2O_3 target (82 % in ^{176}Lu) was irradiated at a thermal neutron flux of $\sim 9 \times 10^{13}$ n cm^{-2} s^{-1} for a period of 21 days in our Institute's reactor. The irradiated target was dissolved in 0.01 M supra-pure HCl by gentle warming. The resulting solution was evaporated to near dryness and reconstituted with supra-pure water. The evaporation and volume reconstitution steps were repeated two to three times in order to obtain $^{177}LuCl_3$ in the pH range of 3–4. The radioactive solution was allowed to attain room temperature and subsequently passed through the Millipore® (0.22 μ) filter paper in order to obtain $^{177}LuCl_3$ in the sterile condition. $^{177}LuCl_3$, thus obtained, was directly used for the preparation of therapeutic doses of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC.

The total ^{177}Lu radioactivity produced and its radionuclidic purity were determined following the procedure mentioned in the literature [24].

Preparation of therapeutic dose of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC using the freeze-dried kits

Therapeutic dose of ^{177}Lu-DOTA-TATE or ^{177}Lu-DOTA-NOC was prepared by adding the required volume of $^{177}LuCl_3$ [200–400 μL, up to 200 mCi (7.4 GBq)] with the kit material dissolved in water for injection and subsequently incubating the reaction mixture at 85–90 °C for a period of 45 min. Dissolution of the kit material was carried out using water such that the total volume of the preparation, after the addition of $^{177}LuCl_3$, becomes 2 mL. When the radiochemical preparation attained room temperature, an aliquot was withdrawn and used to ascertain the radiochemical purity of the preparation using the quality control procedures mentioned below.

Quality control studies

The radiochemical purity of the complex was determined by PC and radio-HPLC. In PC, a small drop of the reaction mixture was spotted at 1.5 cm from one end of the chromatography paper strip (10 × 1 cm). The strip was developed using 50 % aqueous acetonitrile (1:1, v/v) as the

solvent, dried, cut into 1 cm segments and the activity associated with each segment was recorded using NaI(Tl) detector.

HPLC was carried out using a dual pump HPLC unit with a C-18 reversed phase HiQ-Sil (5 μM, 25 cm × 0.46 cm) column. Water (A) and acetonitrile (B) mixtures with 0.1 % trifluoroacetic acid were used as the mobile phase and the following gradient elution technique was adopted for the separation (0–4 min 95 % A, 4–15 min 95 % A to 5 % A, 15–20 min 5 % A, 20–25 min 5 % A to 95 % A, 25–30 min 95 % A). Flow rate was maintained at 1 mL/min.

Radiochemical studies

In order to optimize the amount of DOTA coupled peptides required to be used in the kits, so that the radio-peptides could be prepared with adequately high radiochemical purity; attempt was made to prepare kits with different metal: DOTA-peptide molar ratios. While calculating the metal: DOTA-peptide molar ratios, it was considered that the kits will be used only when ^{177}Lu will have a specific activity of ≥20 mCi/μg (740 MBq/μg). Freeze-dried kits, thus prepared with different amount DOTA coupled peptide were labeled with ^{177}Lu and the corresponding radiochemical purity of the preparation was determined by the quality control methods described above. All the radiolabeling experiments were carried out by incubating the ingredients at the reported optimized conditions i.e. at 85–90 °C for a period of 45 min to 1 h [17, 19, 20]. The final pH of the reconstituted kit vial was automatically adjusted to the desired value i.e. ~5, prior to incubation.

Stability of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC prepared using the freeze-dried kits

The stability of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC, prepared using the corresponding kits, was checked by storing the preparations at room temperature and determining the radiochemical purities of the preparations at different time intervals following the quality control procedures mentioned above.

Biodistribution studies

Biological behaviors of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC, prepared using the corresponding freeze-dried kits, were studied by carrying out biodistribution studies in normal Wistar rats each weighing 225–250 g. The radiochemical preparations were diluted with normal saline prior to administration in animals and each animal was injected with ~3.7 MBq (100 μL, 100 μCi) of the preparation through the tail vein. For each time point, five animals were used. The animals were sacrificed by overdose of CO_2 at 3 h, 1 d, 2 d and 7 d post-administration. Subsequent to sacrifice, the organs were excised, washed with saline, dried, weighed in a weighing balance and radioactivity associated with each organ was measured using a flat-type NaI(Tl) counter. Blood was collected immediately after sacrifice through cardiac puncture and counted in the same counter for determining the associated blood activity. The percentage of injected activity (%IA) accumulated in various organs/tissue was calculated from the above data. Total activity accumulated in the blood, muscle and bone was determined by considering the blood, muscle and bone weight to be 7, 40 and 10 % of the total body weight, respectively [25, 26]. The activity excreted was indirectly determined from the difference between total injected activity (IA) and %IA accounted for all the organs.

Clinical studies

Therapeutic doses of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC (up to 200 mCi, 7.4 GBq) were prepared following the protocol described above and administered to the patients, suffering from cancers of neuroendocrine origin. About 1,000 mL of mixed amino acid solution (aminoven 10 %, composition: isoleucine 5 g, leucine 7.4 g, lysine acetate 9.31 g, methionine 4.3 g, phenylalanine 5.1 g; threonine 4.4 g, tryptophane 2 g; valine 6.2 g, arginine 12 g, histidine 3 g, alanine 14 g, glycine 11 g, proline 11.2 g, serine 6.5 g, tyrosine 0.4 g, taurine 1 g) was infused to each patient 4 h prior to administration of radiopeptides and continued for another 24 h after administration to reduce the uptake in the kidneys. Post-therapy whole-body scans (1024 × 256 matrix size, scan speed 15 cm/min) and SPECT-CT acquisition of the abdomen (128 × 128 matrix size, 20 s/projection, 16 projections) were performed using a dual-head gamma camera with high-energy-general-purpose collimators using the energy window centered on 113 and 208 keV photo-peaks of ^{177}Lu with a window width of ±20 %.

Results and discussion

Formulation of freeze-dried DOTA-TATE and DOTA-NOC kits

Radiochemical studies carried out by labeling the kits having different molar ratios of DOTA coupled peptides and Lu showed that these two ingredients should be taken in at least 2:1 molar ratio in order to obtain ^{177}Lu labeled peptides with >98 % radiochemical purity. This indicates that minimum 163 μg of DOTA-TATE or 165 μg of

DOTA-NOC has to be used in the kit in order to obtain ^{177}Lu labeled peptides with adequately high radiochemical purity when labeled with ^{177}Lu having a minimum specific activity of 20 mCi/μg (740 MBq/μg). Therefore, each kit was formulated using 200 μg of DOTA-coupled peptide. Each kit vial actually comprises a lyophilized mixture of either 200 μg of DOTA-TATE or DOTA-NOC along with 80 mg of gentisic acid and 13.9 mg of ammonium acetate. The kit vials were stored at −4 °C immediately after lyophilization. The vials were allowed to attain room temperature before the preparation of therapeutic doses of ^{177}Lu-DOTA-TATE or ^{177}Lu-DOTA-NOC for human administration.

Production of ^{177}Lu

The kits were evaluated using the ^{177}Lu obtained from different batches having specific activities in the range of 24.3 mCi/μg (899.1 MBq/μg) to 32.1 mCi/μg (1,187.7 MBq/μg). Lu-177m was found to be the only other radionuclide present in the processed ^{177}Lu. After three weeks of irradiation at a thermal neutron flux of $\sim 9 \times 10^{13}$ n cm^{-2} s^{-1}, it was found that only 0.15 μCi (5.55 kBq) of ^{177m}Lu was present in per mCi (37 MBq) of ^{177}Lu at the end-of-bombardment (EOB) [24]. This indicates that ^{177}Lu was produced with >99.98 % radionuclidic purity at EOB. The radioactive concentration of the processed ^{177}Lu was maintained between 500 mCi/mL (18.5 GBq/mL) and 1 Ci/mL (37 GBq/mL) while the pH was between 3 and 4.

Quality control studies

In PC, carried out using 50 % aqueous acetonitrile (1:1, v/v) as the eluting solvent, it was observed that the activity corresponding to ^{177}Lu-DOTA-TATE or ^{177}Lu-DOTA-NOC moved towards the solvent front ($R_f = 0.7 - 1$), while uncomplexed ^{177}Lu remained at the point of spotting ($R_f = 0 - 0.1$) under identical conditions. However, there exists a small difference between the R_f values exhibited by these two agents. While ^{177}Lu-DOTA-NOC moved almost up to the solvent front ($R_f = 0.8 - 1$), ^{177}Lu-DOTA-TATE exhibited R_f value in the range of 0.7 − 0.9. The typical PC patterns of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC are shown in Fig. 1a, b, respectively. Similar difference was also observed in the retention time of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC in radio-HPLC studies. It was observed that ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC exhibit retention times of ∼18 and ∼20 min, respectively, while uncomplexed ^{177}Lu was eluted from the column at ∼4 min. Radiochemical purity of ^{177}Lu-labeled peptides was determined by employing the above two techniques and was found to be >99 % when radiolabeling was done by using ^{177}Lu having specific activity ≥20 mCi/μg (740 MBq/μg). Typical HPLC profiles of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC, prepared using the cold kits, are shown in Fig. 2a, b, respectively.

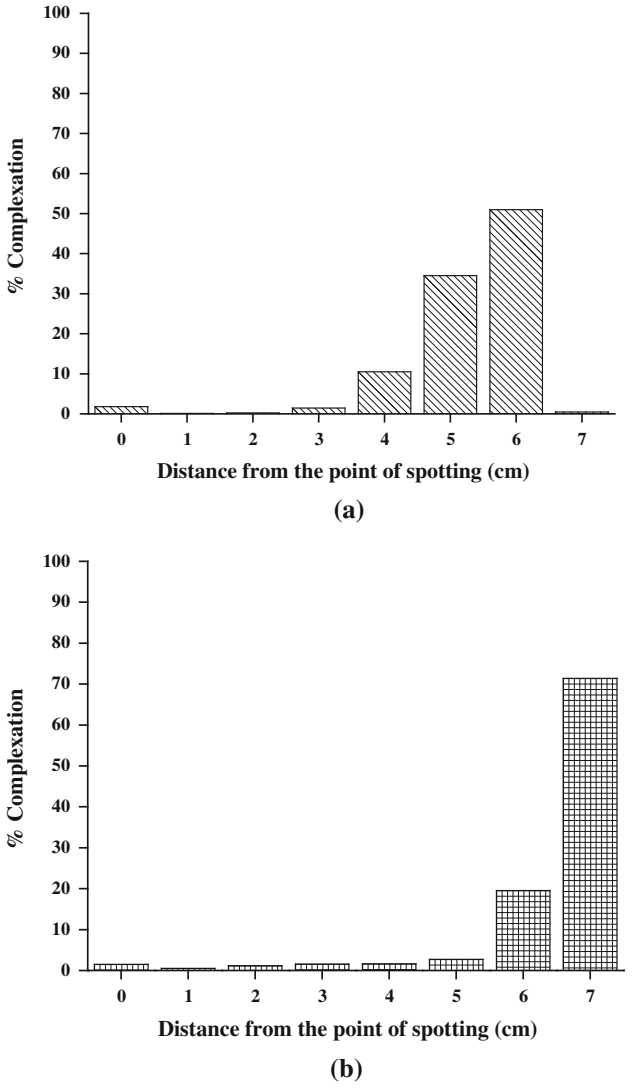

Fig. 1 PC profile of **a** ^{177}Lu-DOTA-TATE and **b** ^{177}Lu-DOTA-NOC, prepared using the corresponding freeze-dried kits

Stability of ^{177}Lu-DOTA-TATE and ^{177}Lu-DOTA-NOC prepared using the freeze-dried kits

The stability of ^{177}Lu-labeled peptides, prepared using the corresponding freeze-dried kits, was studied by storing the preparations at room temperature and determining the radiochemical purity of the preparations at different time intervals using the standard quality control techniques mentioned above. It was observed that both the complexes maintained their radiochemical purity of >99 % till 3 days post-preparation, up to which the study was continued.

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