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Aspects of yield and specific activity of (n,γ) produced ^{177}Lu used in targeted radionuclide therapy

K. V. Vimalnath · Priyalata Shetty · Sharad P. Lohar · V. C. Adya · S. K. Thulasidas · Sudipta Chakraborty · Ashutosh Dash

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Abstract ^{177}Lu-labeled receptor avid peptides and monoclonal antibodies have been effectively used in targeted tumor therapy, owing to the ideally suited decay properties and favourable production logistics of ^{177}Lu [$T_{½}$ = 6.65 days; $E_{\beta(max)}$ = 497 keV (78.6 %); E_{γ} = 208 keV (11.0 %)]. The specific activity of ^{177}Lu produced by the (n,γ) route is one of the important criteria, which determines the efficacy of ^{177}Lu-labeled receptor-avid biomolecules. The present article highlights that the specific activity of (n,γ) produced ^{177}Lu cannot be calculated by simply dividing the produced activity by the mass of the target irradiated, unlike other (n,γ) produced medical radioisotopes and there is a significant enhancement of specific activity due to the burn up of the Lu target during irradiation, which is an added advantage towards the utilization of ^{177}Lu in receptor specific therapeutic radiopharmaceuticals.

Keywords ^{177}Lu · Targeted tumor therapy · Specific activity · Target burn-up

Introduction

Owing to its suitable nuclear decay properties, favourable production logistics and straightforward coordination chemistry, ^{177}Lu [$T_{½}$ = 6.65 days; $E_{\beta(max)}$ = 497 keV (78.6 %), 385 keV (9.1 %), 176 keV (12.2 %); E_{γ} = 208 keV (11.0 %), 113 keV (6.4 %)] has emerged as one of the pivotal radionuclides in therapeutic nuclear medicine during the last decade [1–3]. Presently, there is a great deal of interest in the use of ^{177}Lu for in vivo targeted tumor therapy using regulatory peptides (Peptide Receptor Radionuclide Therapy, PRRT) [4–6] and monoclonal antibodies (Radioimmunotherapy, RIT) [7, 8]. Among the wide variety of ^{177}Lu-labeled receptor-avid peptides studied for PRRT, ^{177}Lu-labeled DOTA^{0}-Tyr^{3}-Octreotate (DOTA-TATE) has been extensively investigated and found to be very effective in PRRT [5, 9, 10]. Scrutiny of literature reveals that in order to achieve desired therapeutic efficacy, the formulation of clinical dose of ^{177}Lu-DOTA-TATE requires ^{177}Lu to be available with a specific activity of at least 740 GBq/mg (20 Ci/mg) [4, 11, 12]. This is also the prerequisite when other peptide derivatives or monoclonal antibodies are used as targeting vectors. Two different strategies, namely, direct thermal neutron activation of enriched (in ^{176}Lu) lutetium target and thermal neutron activation of enriched (in ^{176}Yb) yitterbium target leading to the formation of ^{177}Lu from the β^{-} decay of the short-lived activation product ^{177}Yb ($T_{½}$ = 1.9 h) could be utilized to produce ^{177}Lu having specific activity more than 740 GBq/mg [1–3, 13, 14]. Eventhough the indirect method of production using enriched ^{176}Yb provides no-carrier-added (NCA) ^{177}Lu (theoretical specific activity 40.33 TBq/mg, 1090 Ci/mg), the implicit need of a complex radiochemical separation procedure to obtain ^{177}Lu of requisite purity and poor yield become the major impediments in this method [2, 14]. Direct (n,γ) route can be utilized for large-scale production of ^{177}Lu with specific activity adequate for targeted tumor therapy in nuclear reactors having thermal neutron flux of $\sim 1.0 \times 10^{14}$ n/cm^2 s or higher using enriched target of 80 % or more in ^{176}Lu [15]. This is possible due to the

K. V. Vimalnath · P. Shetty · S. P. Lohar · S. Chakraborty (✉) · A. Dash
Isotope Application and Radiopharmaceuticals Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India
e-mail: sudipta63@rediffmail.com; sudipta@barc.gov.in

V. C. Adya · S. K. Thulasidas
Radiochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

following two reasons, (i) ^{176}Lu has very high thermal neutron capture cross section (σ = 2090 b, I_0 = 1087 b) for formation of ^{177}Lu and (ii) neutron capture cross section of ^{176}Lu does not follow 1/v law and there is a stong resonance very close to the thermal region [16]. This is the least intricate route to access ^{177}Lu in the desired chemical form, apart from being inexpensive.

The yield of ^{177}Lu 'A' (in Bq) produced via ^{176}Lu(n,γ)^{177}Lu at the end of irradiation (EOI) is calculated using the formula

$$A = \frac{N_o \sigma_1 \varphi k \lambda}{\lambda + \varphi(\sigma_2 - \sigma_1 k)} \left[e^{-\sigma_1 k \varphi t} - e^{-(\lambda + \sigma_2 \varphi)t}\right] \quad (1)$$

where, N_0 = number of ^{176}Lu atoms used as target (at t = 0), λ = decay constant of ^{177}Lu (in s^{-1}), σ_1 = cross section of nuclear reaction ^{176}Lu(n,γ)^{177}Lu (2090 b), σ_2 = cross section of nuclear reaction ^{177}Lu (n,γ)^{178}Lu (1000 b), ϕ = thermal neutron flux of the reactor (in /cm^2/s), t = time of irradiation and 'k' is k-factor (correction of the activation rate as a function of the thermal neutron flux temperature due to non-1/v behavior), the value of 'k' is reported to be between 1.5 and 2.5 [17].

In general, the specific activity (S) of a radioisotope produced via (n,γ) route is calculated from its total activity (A) using the equation

$$S = \frac{A}{m_o} \quad (2)$$

where, m_o is the initial mass of the target irradiated. However, in case of (n,γ) produced ^{177}Lu, decay of the radioisotope during the irradiation of ^{176}Lu target in the reactor leads to the formation of $^{177/178}$Hf to a significant extent, thereby reducing the mass of lutetium. As a result, the actual specific activity of ^{177}Lu is significantly different, since the actual mass of lutetium present in the system post irradiation is different from the initial mass of the irradiated target. Using the burn-up correction, the actual specific activity S (Bq/mol) of ^{177}Lu can be expressed as

$$S = \frac{A}{n + n_o\left(e^{-\sigma_1 k \varphi t} + \frac{\sigma_1 \varphi k}{(\lambda + \varphi(\sigma_2 - \sigma_1 k))}\left[e^{-\sigma_1 k \varphi t} - e^{-(\lambda + \sigma_2 \varphi)t}\right]\right)} \quad (3)$$

where, n_0 is the number of moles of the target isotope ^{176}Lu at the start of irradiation and n is the number of moles of other lutetium isotopes which do not lead to the formation of ^{177}Lu by (n,γ) process.

The present article gives an account of the interesting aspects of yield and specific activity of ^{177}Lu produced by thermal neutron irradiation of enriched ^{176}Lu (82 % in ^{176}Lu) at a flux of 1.2×10^{14} n/cm^2/s in the Dhruva research reactor, India. Theoretically calculated values are compared with experimental data. The enhancement of specific activity of ^{177}Lu due to the loss of target during irradiation and its advantages in the use of ^{177}Lu for targeted tumor therapy are also highlighted.

Experimental

Materials and equipments

Lutetium oxide (99.99 % chemically pure, 82 % enriched in ^{176}Lu) was procured from Trace Science International, Canada. Suprapure HCl and de-ionized water (resistivity higher than 18.2 MΩ cm) were procured from E-merck, Germany. Whatman 3 MM chromatography paper (UK) was used for paper chromatography studies. Radioactivity assay and radionuclidic purity of ^{177}Lu activity produced subsequent to radiochemical processing was carried out by high resolution gamma ray spectrometry using an HPGe detector (EGG Ortec/Canberra detector) coupled to a 4K multichannel analyzer (MCA) system. Energy and efficiency calibration of the detector was carried out using ^{152}Eu reference source obtained from Amersham Inc., USA. All other radioactivity measurements were carried out by using a well type NaI(Tl) scintillation counter (Mucha, Raytest, Germany) utilizing the 208 keV gamma photon of ^{177}Lu. Assay of the concentration of lutetium in the $^{177}LuCl_3$ solution after radiochemical processing of irradiated target was carried out by Inductively Coupled Plasma (ICP)-Atomic Emission Spectrometry (AES) technique using a Jobin–Yvon Ultima high resolution ICP-AES (Horiba, France) equipped with 35 analytical channels for analyses of 35 elements having a detection limit 0.05 μg/mL for Lu.

Production of ^{177}Lu

10 mg Lu_2O_3 (82 % in ^{176}Lu) was accurately weighed and dissolved in 0.1 M HNO_3 solution by gentle heating. The solution was evaporated to near-dryness and reconstituted in deionized water to prepare a stock solution of lutetium target with a concentration of ~2 mg/mL. The exact lutetium concentration in the stock solution was determined by ICP-AES technique. A known aliquot of this solution (typically 10 μL) was dispensed in a quartz ampoule and carefully evaporated to dryness under IR lamp to obtain a thin film of the target. The ampoule was subsequently flame sealed and encapsulated in irradiation container [22 mm (ϕ) × 44 mm (l)] made of 1S aluminum. Targets thereby prepared were irradiated in Dhruva research reactor at BARC for varying durations

(7, 14, 21 and 28 days) at a thermal neutron flux of 1.2×10^{14} n/cm^2/s.

Subsequent to irradiation, radiochemical processing was performed in lead shielded processing plants with remote handling provisions. The targets were retrieved from the quartz ampoules and dissolved in 20 mL of 0.01 M suprapure HCl by heating under reflux for a period of 15 min in a round bottom flask. The resultant solution was evaporated to near dryness, cooled and reconstituted in 5 mL of deionized water and ^{177}Lu radioactivity content was ascertained. Batch yield, radionuclide purity, radiochemical purity and specific activity were determined for all the batches.

Assay of yield and radionuclidic purity

Assay of the total activity of ^{177}Lu produced was carried out by gamma ray spectrometry using a HPGe detector coupled to a 4K MCA system. Energy as well as efficiency calibration of the detector were carried out using a ^{152}Eu reference source prior to the recording of gamma ray spectra. Appropriately diluted aliquots of the processed $^{177}LuCl_3$ solution were measured for 1 h. The activity of ^{177}Lu in the aliquot was ascertained utilising the 208 keV photopeak of ^{177}Lu.

Radionuclidic purity was also determined using the same technique. The trace level of ^{177m}Lu co-produced with ^{177}Lu was determined by recording the gamma ray spectra of the sample aliquot, initially having high radioactive concentration of ^{177}Lu, after complete decay of ^{177}Lu activity (8–10 $T_{1/2}$ of ^{177}Lu, i.e. for a period of 50–70 days). The characteristics gamma peaks of ^{177m}Lu are 128, 153, 228, 378, 414 and 418 keV.

Assay of specific activity

Actual specific activity of ^{177}Lu at EOI was detrmined by dividing the batch yield with the amount of lutetium present in the target after irradiation. Amount of lutetium present in the $^{177}LuCl_3$ solution after radiochemical processing was determined by ICP-AES technique. Multi point standardization was carried out by feeding standard solutions of Lu (0.05–1,000 μg/mL concentration range) prior to the measurement of the test samples. Concentration of Lu in the test solutions of $^{177}LuCl_3$ were subsequently measured with ±5 % accuracy using the standard curve. The total amount lutetium in the batch at EOI was determined from this after incorporating the required correction to account for the loss of mass of lutetium due to decay of ^{177}Lu when the analysis was done. The actual specific activities of ^{177}Lu produced at EOI in different batches were calculated from this data.

Results and discussions

Table 1 shows the practically obtained yield (per mg of irradiated target) and specific activity values of ^{177}Lu at EOI when irradiation of enriched (82 % in^{176}Lu) Lu target was carried out for 7, 14, 21 and 28 days at a thermal neutron flux of 1.2×10^{14} n/cm^2/s. It is observed that the maximum yield of ^{177}Lu is achieved when the irradiation is carried out for 14 days duration. On the other hand, the specific activity passes through maxima at 21 days for longer irradiation durations. This phenomenon can be explained in the following way. As reported by Zhernosekov et. al. [18], the actual specific activity of ^{177}Lu will be different from the value obtained by dividing the production yield of ^{177}Lu by the mass of the target irradiated, since the actual mass of lutetium present in the system post irradiation is different from the initial mass of the target irradiated. Using the burn-up correction, the actual specific activity of ^{177}Lu can be expressed by Equation [3]. Fig. 1 compares the variation of the burn-up corrected specific activity of ^{177}Lu calculated using Equation 3 with that calculated without taking target burn-up into consideration (Equation 1 and 2). In all theoretical calculations the value of k is taken to be 2.5. It is evident from the figure that, the period of irradiation at which the maximum yield of ^{177}Lu is achieved does not provide the highest available specific activity. The practically obtained values of production yield of ^{177}Lu per mg of irradiated Lu target and specific activity of ^{177}Lu (Table 1) are also given in Fig. 1 alongside the theoretical values. It is evident that while the yields of ^{177}Lu per mg of irradiated Lu target for the different irradiation periods are in good agreement with specific activity values calculated (using $k = 2.5$) without burn-up correction, the actual or available specific activity values determined using the Lu contents measured by ICP-AES are very close to the specific activity values calculated taking into account the burn-up correction. Both the theoretically calculated and practically measured data show that

Table 1 Measured values of yield and specific activity of ^{177}Lu at EOI when enriched (82 % in ^{176}Lu) Lu target was irradiated at a thermal neutron flux of 1.2×10^{14} n/cm^2/s

Duration of irradiation (days)	Yield of ^{177}Lu (GBq) per mg of target irradiated	Specific activity of ^{177}Lu[a] (GBq/mg Lu)
7	693 ± 14	774 ± 31
14	791 ± 11	919 ± 27
21	701 ± 17	1,094 ± 23
28	546 ± 29	1,037 ± 23

mean ± SD values are reported for three independent measurements

[a] Lu content determined using ICP-AES technique

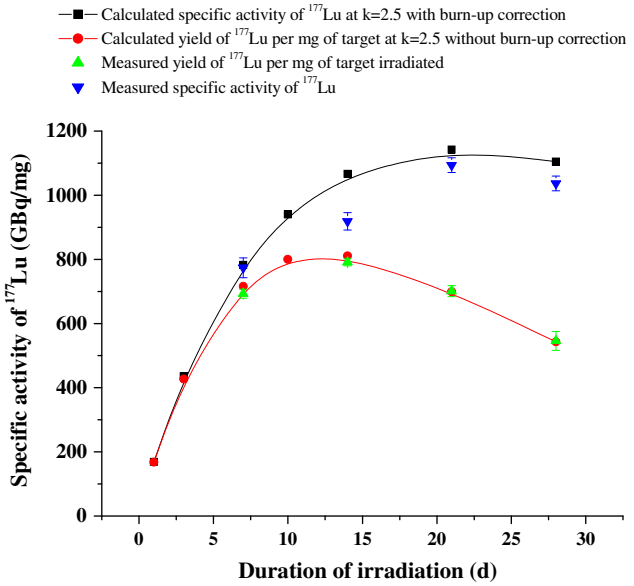

Fig. 1 Variation of calculated and measured specific activity of ^{177}Lu with respect to the duration of irradiation with and without burn up correction

the available specific activity (burn-up corrected) of ^{177}Lu passes through a maxima at ~21 days of irradiation for the enriched Lu target (82 % ^{176}Lu), when irradiated at thermal neutron flux of 1.2×10^{14} n/cm^2/s. This duration is significantly higher than the theoretically calculated 't_{max}' of ^{177}Lu yield, which is ~14 days at the same irradiation condition. Irradiation longer than the 't_{max}' leads to some loss of activity but also to an increased ^{177}Lu/^{176}Lu ratio, and hence increased specific activity due to burn up of ^{176}Lu. The significant enhancement of specific activity due to the burn up of Lu target during irradiation is an added advantage for ^{177}Lu towards its utilization in receptor specific therapeutic radiopharmaceuticals.

The radionuclidic purity of ^{177}Lu as determined by gamma ray spectrometry and was found to be between 99.98 and 99.99 % at 24 h post EOI, depending on the duration of irradiation. ^{177m}Lu [$T_{½} = 160.4$ days, $\beta^- + IT$, $E_\gamma = 128, 153, 228, 378, 414$ and 418 keV] was found to be the sole radionuclidic impurity.

Conclusion

Owing to the very high thermal neutron capture cross-section of ^{176}Lu and the presence of strong resonance near thermal region, the specific activity of (n,γ) produced ^{177}Lu should not be calculated by simply dividing the activity produced by mass of the target irradiated, unlike other (n,γ) produced medical radionuclides. As validated by ICP-AES measurements there is significant reduction in the Lu content in target during irradiation. Consequently, depending on the available thermal neutron flux, the duration of irradiation of Lu target to obtain maximum specific activity of ^{177}Lu needs to be determined by extensive theoretical calculation and optimization study as reported in the present article.

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