FORMULATION AND QUALITY CONTROL STUDIES OF EDTMP FREEZE-DRIED KIT FOR THE PREPARATION OF $^{153}$Sm-EDTMP

FORMULASI DAN KAJIAN KAWALAN KUALITI KIT RADIOFARMASEUTIKAL EDTMP BAGI PENYEDIAAN 153SM-EDTMP

Khong Khei Choong, Mohd Rodzi Ali, Yahaya Talib, Ng Yen, Siti Selina Abdul Hamid, Manisah Saedon, Azahari Kasbollah, Rahimah Abdul Rahim, Muhammad Hanaffi Mohamad Mokhtar, Wan Hamirul Bahrin Wan Kamal

Medical Technology Division, Malaysian Nuclear Agency, Bangi, 43000 KAJANG, MALAYSIA.

Corresponding author email: khong@nuclearmalaysia.gov.my

Abstract

As the third most common organ associated with advanced stage cancer, bone metastasis is relatively common in patients diagnosed with breast, lung and prostate cancer. The spread of the cancerous cell to bone may lead to skeletal related events like bone pain which may impair mobility and quality of life. Conventionally, bone pain is managed by localised external radiotherapy and administration of analgesics. Utilisation of bone-targeted radionuclide therapy has been poor due to its availability and cost. This study highlighted the preparation and quality control studies of freeze-dried ethylenediaminetetramethylene phosphonic acid (EDTMP) kit, suitable for the convenient preparation of $^{153}$Sm-EDTMP as a promising radiopharmaceutical in bone pain palliation. A batch of 25 lyophilised EDTMP kits was prepared using EDTMP, NaOH and anhydrous CaCO$_3$. Each produced kit vial contained a lyophilised mixture of 35mg EDTMP, 10.2mg CaCO$_3$ and 9.6mg NaOH. The $^{153}$Sm-EDTMP was prepared by incubating the cold-kit with sodium chloride 0.9% and $[^{153}$Sm]$^{3}$SmCl$_3$ solution at room temperature for 15 minutes with pH regulated in between 7-8.5. Labelled compound was evaluated by radiochemical purity (RCP) test, stability study and also scintigraphic imaging in normal Sprague-Dawley rats. The $^{153}$Sm-EDTMP complexes were produced with good RCP followed by prolonged high stability in days. Whole body scintigraphic images of rats revealed good skeletal uptake of the $^{153}$Sm-EDTMP complexes and with major rapid renal clearance of unbound $^{153}$Sm-EDTMP. Further preliminary clinical investigation should be employed in facilitating its utilisation in clinical use.

Keywords/Kata kunci: bone pain palliation, EDTMP, $^{153}$Sm, $^{153}$Sm-EDTMP, bone targeted, radiopharmaceuticals

Abstrak

Tulang merupakan organ sasaran ketiga paling kerap dikaitkan dengan kanser peringkat metastasis. Antara jenis kanser yang lazimnya merebak ke tisu rangka adalah kanser payudara, peparu dan prostat. Penyebabannya boleh menyebabkan implikasi seperti kesakitan tulang dan secara langsung menyebabkan aktiviti harian serta kualiti hidup pesakit. Secara konvensional, keradangan tulang boleh dirawati dengan radioterapi dan pengambilan ubat tahan sakit. Penggunaan terapi radionuklid sasaran tulang adalah rendah berikut perkhidmatan yang terhad dan kos rawatan yang tinggi. Kajian ini memberi fokus kepada proses penyediaan dan kawalan kualiti kit radiofarmaseutikal ethylenediaminetetramethylene phosphonic acid (EDTMP) untuk penyediaan $^{153}$Sm-EDTMP dalam rawatan paliatif kesakitan tulang. Sebanyak 25 kit disediakan dengan EDTMP, NaOH dan anhydrous CaCO$_3$. Komposisi setiap kit adalah 35mg EDTMP, 10.2mg CaCO$_3$ and 9.6mg NaOH. $^{153}$Sm-EDTMP disediakan dengan campuran larutan sodium chloride 0.9% dan $[^{153}$Sm]$^{3}$SmCl$_3$ dalam kit EDTMP dalam keadaan suhu bilik selama 15 minit dengan penyelarasan pH campuran di antara 7-8.5. Kompleks $^{153}$Sm-EDTMP dikesikan dan dinilai dengan ujian radiochemical purity (RCP), ujian kestabilan kompleks dan pengimejan keseluruhan di tikus Sprague-Dawley. Keputusan kajian menunjukkan RCP yang baik dan kestabilan kompleks yang berpanjangan. Pengimejan gama tikus juga memaparkan imej pengambilan kompleks $^{153}$Sm-EDTMP yang memuaskan dalam sistem rangka serta perkumuhan $^{153}$Sm-EDTMP melalui buah pinggang tikus. Kajian kliniklanjutan perlu dijalankan bagi menyokong dan merealisasiakan penggunaannya di kalangan pesakit.
INTRODUCTION

Bone metastases in cancer patients signify an advanced and incurable state of the disease. Among the solid tumours originated from breast, lung and prostate, the development of bone metastases was reported in between 80-85% in these patients. Following lung and liver, bone secures as the third most common organ affected by cancerous metastases (Mundy 2002; Palma et al. 2001). These metastases in bone may deteriorate the disease with skeletal-related events like hypercalcaemia, pathological fracture, neurological deficit, immobility, bone pain and spinal cord compression (Coleman 2006; Pockett et al. 2010). Palliative treatment and care indirectly play an essential role in patients’ good quality of life (DeNardo 1998). The conventional treatment modalities include palliative chemotherapy, local radiotherapy or surgery and administration of bisphosphonates, steroids and both opioid and non-opioid analgesic (Glannakenas et al. 2004; Hoskin 2003). The combination of local and systemic treatment is usually employed as single therapy may be less effective over an extended period of time. Nevertheless, these approaches also resulted in numerous unwanted side effects (DeNardo 1998; Serafini 2001). Throughout the years, bone-targeted radionuclide therapy has emerged and utilised by the developed nations. These systemic radionuclide therapies offer superiority compared to the conventional treatment with reduced demand for the use of analgesics, radiotherapy and chemotherapy, improved pain palliation and quality of life in terminal stage cancer patients (Serafini 2001). Due to its restricted availability and cost of treatment, the bone-targeted radionuclide therapy is rarely seen in clinical use among patients in Malaysia. The present approved radionuclides for bone pain palliation can be categorized into 3 generations, first-generation phosphorus-32 (32P), second generation strontium-89 (89Sr) and third-generation samarium-153 (153Sm) and lutetium-177 (177Lu) (Sarto 2004). These radionuclide complexes act by binding to the actively dividing metastatic bone lesions and subsequently local doses of beta radiation is delivered to the cancerous lesions (Sarto 2003; Volkert 1999). The nature of comparatively high energy pure beta emitters of 32P [T½ = 14.26 days, Eβ(max) = 1.71 MeV, No γ] and 89Sr [T½ = 50.53 days, Eβ(max) = 1.49 MeV, No γ] has placed patients on higher risk of bone marrow suppression and furthermore impeded the concurrent pharmacokinetic and dosimetric evaluations (Chakraborty et al. 2008). In contrast, 153Sm [T½ = 46.27 hours, Eβ(max) = 0.81MeV, Eγ =103 keV (28%) and 177Lu [T½ = 6.73 days, Eβ(max) = 0.49MeV, Eγ = 208 keV (11%), 113 (6.4%)] are more preferable in clinical use. The shorter half-life of 153Sm may probably limit the widespread in the body (Banerjee et al. 2012; Das et al. 2013).

Ethylendiamine tetramethylene phosphonic acid (EDTMP) is nitrogenous organic polyphosphonic acid. It poses good chemical stability and thermal tolerance. It acts as a chelator that complexes with many radio-metal ions (Wilky et al. 2013). In clinical practice, EDTMP is labelled with 153Sm to form 153Sm-EDTMP (Fig. 1). 153Sm emits a low energy range of beta radiation at maximum energies of 0.64, 0.71 and 0.81 MeV with average beta energy of 0.23 MeV. Tissue penetration by these beta particles derives with an average of 2.5mm. The relatively short distance tissue penetration enables effective radiation doses to the bone lesions with minimum effect to the surrounding healthy tissue. Resulting from the beta decay, the gamma energy of 103 keV permits the scintigraphic imaging of the 153Sm-EDTMP uptakes and distribution in the body (Farhangi et al. 1992). During the uptake process, a phosphonate bridge is formed between EDTMP and calcium ions in the bone matrix and leads to the release of 153Sm. The samarium atoms form insoluble hydroxides linked to the hydroxyapatite matrix and thus are retained long-term in osseous tissues. These complexes uptake and retention is increased fivefold in cancerous lesions compared to healthy bone tissue. Upon administration, the circulating 153Sm-EDTMP is cleared rapidly from the blood via renal excretion with <1% remains in the circulation one hour post administration (Collins et al. 1993; Eary et al. 1993). 153Sm-EDTMP has been approved by the Food and Drug Administration of USA on March 28, 1987, for the clinical indication of metastatic bone pain relief (Smith 2011). The clinical recommended dose is 1.0 mCi/kg administered intravenously over 1 minute. Onset of pain relief ranges from 48 hours to 7 days and repeated doses may be administered at minimum interval of 6 to 8 weeks from previous dose subjects to patients’ responses (Collins et al. 1993; Holmes 1992).

Figure 1. (a) Chemical structure of EDTMP (Ranjbar et al. 2015) (b) Chemical structure of 153Sm-EDTMP complex (Weekes et al. 2016).
Throughout the years, various studies have been conducted and the results have indicated that pain relief in patients with metastatic bone pain was achieved from 62%-84% with $^{153}$Sm-EDTMP treatment (Sapienza et al. 2004; Serafini et al. 1998; Tian et al. 1999; Tripathi et al. 2006). Study of safety and efficacy of repeated doses of $^{153}$Sm-EDTMP has been established by Sator et al. (2007), decrement of pain scores was statistically significant at week 4 ($p < 0.002$) and week 8 ($p<0.003$) in addition, no serious adverse events were reported throughout the study (Sartor et al. 2007). The production of $^{153}$Sm-EDTMP in Malaysia for clinical use has yet to remain unanswered. In practice, there is solely one commercial product (Quadramet®) available for clinical use. Cost of treatment has always been a substantial contributor to the limited use of bone-targeted radionuclide therapy. Therefore, this research paper highlights the preparation and quality control studies of $^{153}$Sm-EDTMP.

MATERIALS AND METHODS

Materials

EDTMP was obtained from Santa Cruz Biotechnology (USA). Samarium oxide, Sm$_2$O$_3$ (> 98% enriched in $^{152}$Sm) as the target for neutron irradiation in reactor was procured from Russia. Other analytical graded chemicals used in the study were purchased from the reputed manufacturer, Calcium carbonate, CaCO$_3$ from Systerm (Malaysia), Sodium hydroxide, NaOH from Sigma-Aldrich (Germany), Hydrochloric acid, HCl from Friendemann Schmidt (USA), Methanol from Merck KGaA (India), Ammonia solution 25% from Merck KGaA (Germany).

Formulation of Lyophilised Freeze-Dried EDTMP Kits

A batch of 25 lyophilised freeze-dried EDTMP kits was prepared at strength of 35mg/ml. A total of 255 mg CaCO$_3$ was dispersed into 10 mL of highly-purified water followed by the addition of 875 mg of EDTMP and vigorously stirred on a magnetic stirrer at room temperature. An aqueous solution of 1 M NaOH was prepared by dissolving 2000 mg of NaOH in 50 mL of highly purified water. The NaOH solution was slowly added into the mixture of EDTMP and CaCO$_3$ with constant stirring. The addition of NaOH was withheld once the solution turned clear and pH ~ 7. The final volume of the solution was prepared up to 25 mL with highly purified water. Subsequently, the solution was filtered through a 0.22 μm hydrophilic PVDF membrane sterile filter (Bioflow, Malaysia). 1 mL (35mg of EDTMP) of the aliquots was dispensed into each glass vial. The vials were then subjected to the lyophilisation process (Lyodryer, Lyophilization Systems, USA).

Production and Radiochemical Processing of $^{153}$Sm

Sm-153 was produced by neutron bombardment of enriched Sm$_2$O$_3$ (98.44% in $^{152}$Sm) target at a thermal neutron flux of $1.49 \times 10^{12}$ n·cm$^{-2}$.s$^{-1}$ for a duration of 24 hours at the TRIGA PUSPATI reactor. The target was cooled for 10 hours after the end of bombardment. 0.1 M HCl was used to dissolve the target into solution form inside a lead-shielded area. The radioactivity was measured inside a well-type ion chamber dose calibrator (Aromlab 100, Biodex Medical Systems, USA). Radionuclide purity of $^{153}$Sm was ascertained by high-resolution gamma spectroscopy using a HPGe detector (Canberra 2000 Genie, Mirion Technologies, USA) coupled to a 16K multi-channel analyser system. The radionuclide purity was confirmed by the gamma emission at major energy peaks of 70 keV and 103 keV. Other additional properties of $^{153}$Sm were also found at 41 keV and 47 keV.

Preparation of $^{153}$Sm-EDTMP

Lyophilised EDTMP kit was reconstituted with 1 mL of sodium chloride 0.9% at room temperature and followed by the addition of SmCl$_3$ solution. The pH of reaction mixture was adjusted with 1 M NaOH to the range of 7 to 8.5 and the reaction vial was incubated for 15 minutes at room temperature.

Radiochemical Purity and Stability Study

The efficiency of radiolabelling of $^{153}$Sm-EDTMP was determined by using instant thin layer chromatography (ITLC) system. The stationary phase ITLC-silica gel sheet (Agilent Technologies, USA) was cut into 10 x 1 cm and dried in the oven prior to use. 2 μL of the reaction mixture was spotted at the origin of 1.5 cm from the one end of the paper strip. The strip was eluted by mobile phase mixture solution of NH$_4$OH:MeOH:H$_2$O (0.2:2:4) (Bahrami-Samani et al. 2009; Ranjbar et al. 2017). Subsequently, the eluted strip was dried and the yield of $^{153}$Sm-EDTMP was evaluated by TLC scanner (Bioscan AR-2000, Eckert & Ziegler, USA).
Scintigraphic Imaging Study

The biodistribution of $^{153}$Sm-EDTMP complex was observed by scintigraphic imaging in normal Sprague-Dawley rats. For \textit{in vivo} imaging study, the $^{153}$Sm-EDTMP complex (2mCi/0.2ml) was injected intravenously via the tail lateral vein of rats, each weighing 300-400 g. All rats were anaesthetised by a mixture of ketamine hydrochloride and xylazine hydrochloride prior and throughout the imaging process. Sequential scintigraphic images were acquired in the single-head gamma camera (Hamamatsu BH6602, Beijing Binsong Photon Techniques, China) at time intervals of 0 hours, 6 hours and day 1 until day 7. All images were captured by the $^{153}$Sm energy window of 103 keV for minimum counts of 500,000 using 1024 x 1024 matrix pixels.

RESULTS AND DISCUSSIONS

Formulation of Freeze-Dried EDTMP Kit

A batch of 25 lyophilised EDTMP kits was prepared using EDTMP, NaOH and anhydrous CaCO$_3$. Each produced kit vial contained a lyophilised mixture of 35 mg EDTMP, 10.2 mg CaCO$_3$ and 9.6 mg NaOH. The kits were stored at temperature between 2 – 8 ºC.

Production and Radiochemical Processing of $^{153}$Sm

$^{153}$Sm was generated with a specific activity of 26.71 Ci/g following the 24 hours irradiation of enriched Sm$_2$O$_3$ (98.44% in $^{153}$Sm) target with a thermal neutron flux of $1.49 \times 10^{12}$ n·cm$^{-2}$·s$^{-1}$. The radionuclide purity of $^{153}$Sm was confirmed by the distinctive gamma emission at major energy peaks of 70 keV and 103 keV via high-resolution gamma spectroscopy. A total radioactive concentration of $^{153}$SmCl$_3$ of 131mCi/1mL was produced whilst 118mCi/0.9mL was used in radiolabelling with EDTMP.

Preparation of $^{153}$Sm-EDTMP

$^{153}$SmCl$_3$ solution was sent to the Nuclear Pharmacy Facility of National Cancer Institute where radiolabelling of EDTMP with $^{153}$Sm was conducted. At point of the radiolabelling process, the reconstituted EDTMP solution was added to the $^{153}$SmCl$_3$ solution (110mCi/0.9ml) and pH was adjusted to 8 with 1.0 M NaOH solution. The final solution was filtered with 0.2 μm filter (Sartorius, Malaysia) with final yield activity of 93.5mCi (79% of the initial radioactive activity).

Radiochemical Purity and Stability Study

Both free $^{153}$Sm$^{3+}$ and $^{153}$Sm-EDTMP were evaluated by the ITLC system for its radiochemical purity. With the mobile phase mixture of NH$_4$OH:MeOH:H$_2$O, the free $^{153}$Sm$^{3+}$ remained at the origin while $^{153}$Sm-EDTMP complexes migrated to the solvent front region (Fig. 2).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{ITLC chromatograms of (a) $^{153}$SmCl$_3$ and (b) $^{153}$Sm-EDTMP complex on stationary phase ITLC-SG paper with mobile phase NH$_4$OH:MeOH:H$_2$O (0.2:2:4).}
\end{figure}
The stability analysis of $^{153}$Sm-EDTMP complex was done for up to 21 days with incubation of the reaction mixture at room temperature by employing the standard ITLC system mentioned in radiochemical purity study. It was shown that the $^{153}$Sm-EDTMP complex exhibited an initial excellent radiochemical purity of $>99\%$ and subsequently sustained good stability of $>98\%$ over the duration of 21 days (Fig. 3).

![Radiochemical Purity Study of 153Sm-EDTMP](image)

Figure 3. Radiochemical purity study of $^{153}$Sm-EDTMP complex with time. Initial radiochemical purity was found to be 99.72\% ($\pm$ 0.06) and on Day 21 the radiochemical purity diminished to 98.30\% ($\pm$ 0.17).

**Scintigraphic Imaging Study**

Scintigraphic imaging study was performed to determine the biodistribution of radioactive complex prepared with the EDTMP lyophilised free-dried kit. Normal Sprague-Dawley rats were injected with free $^{153}$Sm$^{3+}$ or $^{153}$Sm-EDTMP and images were acquired at day 1 and day 5 for free $^{153}$Sm$^{3+}$ (Fig. 4) while $^{153}$Sm-EDTMP (Fig. 5) was recorded at 0 hour, 6 hours and day 1 until day 7 of post injection. Significant accumulation at liver was observed in the image with administration of $^{153}$Sm$^{3+}$ in the rats. No significant uptake by other organs was demonstrated in these rats and $^{153}$Sm$^{3+}$ was remained detectable at the liver despite at time of 5 days post injection.

![Scintigraphic images](image)

Figure 4. Scintigraphic images of normal Sprague-Dawley rats injected with free $^{153}$Sm$^{3+}$ at different post injection time points (a) Day 1 and (b) Day 5.

Upon administration of $^{153}$Sm-EDTMP into the rats, the image clearly demonstrated a general distribution of the $^{153}$Sm-EDTMP complex throughout the body with significant accumulation at the bilateral kidneys and bladder. At 6 hours post injection, the accumulation of activity at the bilateral kidneys and bladder subsided with rapid uptake of $^{153}$Sm-EDTMP complex at the skeleton tissues. As the imaging proceeded with days, significant uptake of the $^{153}$Sm-EDTMP complex was observed in the skeleton and this accumulation was found to be retained up to 7 days post injection. No significant uptake by other organs was demonstrated in these rats.
Figure 5. Scintigraphic images of normal Sprague-Dawley rats injected with $^{153}$Sm-EDTMP at different post injection time points (a) 0 Hour, (b) 6 Hours, (c) Day 2, (d) Day 3, (e) Day 4, (f) Day 5, (g) Day 6 and (h) Day 7.

Attributable to its distinctive nuclear features, the use of $^{153}$Sm-EDTMP as a bone-targeted radionuclide therapy has been established in many parts of the world. However, due to several limitations, the clinical use of $^{153}$Sm-EDTMP has been restricted in local settings. With the capability of TRIGA PUSPATI reactor and Medical Technology Division of Malaysian Nuclear Agency, the production of $^{153}$Sm-EDTMP has been promising towards its utilisation in clinical practice. Therefore, in this study $^{153}$Sm was produced and subsequently the freeze-dried EDTMP kit was radiolabelled to form $^{153}$Sm-EDTMP complexes. Radiochemical purity and scintigraphic imaging studies were performed as part of the quality control evaluations.

$^{153}$Sm is a reactor-generated radionuclide that produces both beta and gamma emissions, which enable it functions as a concurrent therapy and imaging agent. Apart from the large-scale production with high specific activity by neutron irradiation of enriched Sm$_2$O$_3$, the short half-life of $^{153}$Sm permits the fractional dose regimes to be administered to patients (Ehrhardt et al. 1998). Formulation of EDTMP for $^{153}$Sm has been reported in a few
studies, the common concentrations of EDTMP described in the studies were ranged from 20 – 100 mg/mL (Ferro-
flores et al. 1996; Goeckeler et al. 1987). Ferro-Flores et al. (1996) concluded the molar ratio of EDTMP/Sm has a significant effect on the radiochemical purity of the $^{153}$Sm-EDTMP complex. The higher the ratio of EDTMP/Sm the better radiochemical purity achieved. Nonetheless, excessive EDTMP triggers ion chelation of Ca$^{2+}$, Na$^{+}$ and K$^{+}$ that have essential functions in myocardial cells. This could potentially cause unwanted adverse effects or harms to cardiac function. Accounting to that, the EDTMP freeze-dried kit was formulated with concentration of 35 mg/mL which composition was similar to the commercially available Quadramet. Owing to the chelating properties of EDTMP, the formation of $^{153}$Sm-Ca/Na-EDTMP complexes causes a reduction in serum Ca$^{2+}$ level. Thus, the addition of CaCO$_3$ in the formulation prevents the fall in serum Ca$^{2+}$ level additionally reduces toxicity of the formulation.

As a beta emitter, the radiation released internally during the decay of $^{153}$Sm to $^{153}$Eu has a great potential to kill the surrounding healthy cells. Hence, it is important to ensure the excellent quality of the radiochemical purity and stability of the $^{153}$Sm-EDTMP complexes in vivo. In this study, the average radiochemical purity obtained was more than 99% and the complex remained stable for up to Day 21 of the study.

Upon intravenous administration, $^{153}$Sm-EDTMP exhibited high affinity for the skeleton tissues with rapid renal clearance and excretion of unbound $^{153}$Sm-EDTMP. It was also reported that the $^{153}$Sm-EDTMP cleared from blood biexponentially with half-lives of 5.5 minutes, 65 minutes with complete urine excretion by 6 hours (Lantheus Medical Imaging Inc. 2017). Based on the scintigraphic images recorded, as soon as the compound was injected into the Sprague-Dawley rats, an intense uptake was observed in the bilateral kidneys and bladder apart from the skeleton tissues. Following the post injection of 6 hours, more profound bone uptake was observed while the uptake at kidneys and bladder diminished significantly. The selective accumulation of $^{153}$Sm-EDTMP in the skeleton was observed clearly throughout the imaging period with no significant accumulation of activity in any other major organs or tissues. Indubitably, the results highlighted the essential role of renal function in the elimination and excretion process. Thus, dose and administration of $^{153}$Sm-EDTMP should be optimised and use with cautions in patient with renal impairment.

**CONCLUSION**

Freeze-dried EDTMP kits with lyophilised composition of 35 mg EDTMP, 10.2 mg CaCO$_3$ and 9.6 mg NaOH were produced and radiolabelled with $^{153}$Sm up to 110 mCi. Radiochemical purity study showed a good radiolabelling efficiency of the $^{153}$Sm-EDTMP complex. Additionally, the preparation demonstrated high stability in vitro stability study over 21 days. Biodistribution studies revealed the complex has high affinity towards skeleton with rapid and major renal elimination. No significant uptake by other vital organs or tissues. The formulation of the lyophilised freeze-dried kit has forwarded a promising approach for cancer bone pain palliation in the local settings. Further preliminary clinical investigation should be employed in facilitating its utilisation in clinical use.

**REFERENCES**


