IAEA-TECDOC-1114

Optimization of production and quality control of therapeutic radionuclides and radiopharmaceuticals

Final report of a co-ordinated research project 1994–1998

INTERNATIONAL ATOMIC ENERGY AGENCY

IAEA

September 1999

The originating Section of this publication in the IAEA was:

Industrial Applications and Chemistry Section
International Atomic Energy Agency
Wagramer Strasse 5
P.O. Box 100
A-1400 Vienna, Austria

OPTIMIZATION OF PRODUCTION AND QUALITY CONTROL OF THERAPEUTIC RADIONUCLIDES AND RADIOPHARMACEUTICALS
IAEA, VIENNA, 1999
IAEA-TECDOC-1114
ISSN 1011–4289

Printed by the IAEA in Austria
September 1999

FOREWORD

The 'renaissance' of the therapeutic applications of radiopharmaceuticals during the last few years was in part due to a greater availability of radionuclides with appropriate nuclear decay properties, as well as to the development of carrier molecules with improved characteristics. Although radionuclides such as ^{32}P, ^{89}Sr and ^{131}I were used from the early days of nuclear medicine in the late 1930s and early 1940s, the inclusion of other particle emitting radionuclides into the nuclear medicine armamentarium was rather late. Only in the early 1980s did the specialized scientific literature start to show the potential for using other beta emitting nuclear reactor produced radionuclides such as ^{153}Sm,^{166}Ho, ^{165}Dy and $^{186-188}Re$.

Bone seeking agents radiolabelled with the above mentioned beta emitting radionuclides demonstrated clear clinical potential in relieving intense bone pain resulting from metastases of the breast, prostate and lung of cancer patients. Therefore, upon the recommendation of a consultants meeting held in Vienna in 1993, the Co-ordinated Research Project (CRP) on Optimization of the Production and quality control of Radiotherapeutic Radionuclides and Radiopharmaceuticals was established in 1994. The CRP aimed at developing and improving existing laboratory protocols for the production of therapeutic radionuclides using existing nuclear research reactors including the corresponding radiolabelling, quality control procedures; and validation in experimental animals.

With the participation of ten scientists from IAEA Member States, several laboratory procedures for preparation and quality control were developed, tested and assessed as potential therapeutic radiopharmaceuticals for bone pain palliation. In particular, the CRP optimised the reactor production of ^{153}Sm and the preparation of the radiopharmaceutical ^{153}Sm-EDTMP (ethylene diamine tetramethylene phosphonate), as well as radiolabelling techniques and quality control methods for the preparation of ^{153}Sm-hydroxyapatite, ^{153}Sm and ^{166}Ho-microspheres and ^{166}Ho-ferric-hydroxy-macro-aggregates for the treatment of rheumatoid arthritis.

The laboratory protocols included in the report were developed and thoroughly assessed and tested by the participants during the course of the CRP. These procedures are considered effective as well as simple to follow by anyone wishing to embark on the production and preparation of the above therapeutic radionuclides and radiopharmaceuticals for bone pain palliation and synovectomy applications.

The IAEA wishes to thank all the participants in the CRP for the valuable work and scientific contributions. The IAEA officer responsible for this CRP was H. Vera Ruiz of the Division of Physical and Chemical Sciences.

EDITORIAL NOTE

In preparing this publication for press, staff of the IAEA have made up the pages from the original manuscript(s). The views expressed do not necessarily reflect those of the IAEA, the governments of the nominating Member States or the nominating organizations.

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CONTENTS

1. INTRODUCTION

As radionuclide carriers, radiopharmaceuticals used for therapy are designed to deliver high doses of radiation to selected sites in target organs or tissues with minimized radiation to the surrounding vital healthy tissues. An array of radionuclide carriers with improved characteristics for therapeutic purposes, such as chelating agents, monoclonal antibodies, peptides, biodegradable particles, colloids, etc. have been developed. Alpha and beta emitting radionuclides are tagged on to those carriers. New ones are continually reported in the specialized literature. A greater availability of therapeutic radionuclides has given a tremendous impulse to therapeutic nuclear medicine in the past few years.

In particular, bone-seeking radiopharmaceuticals labelled with beta emitters to relieve intense bone pain resulting from metastases from breast, prostate and lung cancer, have shown to be clinically useful. The prevalence of metastatic bone disease in all countries, both developed and developing, creates a large demand for these new palliative agents.

Radiopharmaceuticals labelled with reactor-produced beta particle emitting radionuclides such as dysprosium-165, samarium-153, rhenium-186, rhenium-188, holmium-166 and others, are currently under intense clinical evaluation in developed countries for palliation of bone pain from metastatic bone cancer. The search for newer and better radiolabelled compounds also continues at a rapid pace because of the promising clinical results already reported in the literature.

Of particular significance is the fact that the above mentioned radionuclides can be produced in medium sized nuclear research reactors available in several developing Member States. Moreover, in many of these countries there already exist appropriate facilities and excellent experience in the field of preparation and quality control of radiopharmaceuticals for diagnostic studies that can be tapped for the preparation of radiotherapeutic agents.

The scope of the co-ordinated research project (CRP) focused on the optimization of reactor production protocols of beta emitting radionuclides as well as on the development of radiolabelling and quality control procedures of radiopharmaceuticals as therapeutic agents. In particular, special emphasis was given to the reactor production and quality control of radiotherapeutic agents for palliation of bone pain resulting from metastatic bone cancer and radiation synovectomy for the treatment of rheumatoid arthritis. The beta emitting radionuclides included in the investigations covered by this CRP were ^{165}Dy , ^{153}Sm, ^{166}Ho and ^{186}Re.

Keeping in mind that the development of simple, reliable and economical procedures and techniques suitable for adoption to local conditions prevailing in developing countries, the programme concentrated on achieving the following concrete objectives:

- To optimize and develop reliable reactor irradiation procedures for the production of ^{153}Sm, ^{165}Dy, ^{166}Ho and ^{186}Re using natural and isotopically enriched target materials.
- To develop reliable and economical radiolabelling procedures for the preparation and quality control of therapeutic radiopharmaceuticals for bone pain palliation and treatment of rheumatoid arthritis.
- To evaluate their potential as radiotherapeutic agents in animal models.

It was anticipated that the results of this CRP would lead to a strong programme for indigenous production of high quality radiotherapeutic agents in developing Member States operating nuclear research reactors, and encourage their clinical use.

Ten experienced scientists from laboratories in both developing and developed countries participated in the CRP organized during the period 1994–1998. The participants from Pakistan and Greece have joined the programme later. Research agreements and research contracts were signed with the following Member States: Argentina, Australia, Brazil, China, Finland, Greece, Indonesia, Malaysia, Pakistan and Thailand.

During the course of the CRP three Research Co-ordination Meetings (RCMs) were held in Sydney, Australia, 17–19 October 1994; São Paulo, Brazil, 9–12 December 1996; and Jyväskylä, Finland from 20–24 April 1998.

The successful development of these production methods with many types of reactors having different neutron flux and operational schedules can be very helpful for Member States of the IAEA. The data presented in this publication can be easily used as a quick reference to evaluate the possibilities of producing useful quantities of ^{153}Sm, ^{166}Ho, ^{165}Dy and ^{186}Re. It was found that these radionuclides can be produced in reasonable quantities even with lower flux reactors with relatively short irradiation times using enriched targets.

The data presented by the participating organizations show that under different reactors and laboratory conditions, high purity and clinically useful ^{153}Sm-EDTMP can be produced. Many participants reached routine production levels with regular supplies to the medical community in their respective areas. The large and detailed data on the preparation of ^{153}Sm-EDTMP will be found useful by many scientists in Member States who wish to explore the potential production of this radiopharmaceutical.

Moreover, in line with the recommendations of the Second RCM (São Paulo), all participants either continued or commenced work on the development of particulate radiopharmaceuticals for radiation synovectomy for the treatment of rheumatoid arthritis. Radiation synovectomy has also been proposed for treatment of haemophilia patients to control synovial inflammation Several radioisotopes, including ^{153}Sm, ^{165}Dy and ^{166}Ho have been suggested for this application. During the course of the investigations, ^{153}Sm and ^{166}Ho were found to be the recommended radioisotopes for the preparation of this type of radiopharmaceutical due to their convenient half-life, appropriate beta energy and high reactor production yields. The short physical half-life of ^{165}Dy (2.33 h) presents formidable logistical problems for distribution.

Several particle formulations have been investigated under the auspices of the CRP. These include hydroxyapatite, albumin and FHMA. Despite the difficulty of injecting particles in suspension into the knee joint of small animals, successful biodistribution studies have been performed by the participants. These animal studies showed little leakage from the joint. No disease models have been studied. At the present time, research is continued to optimize and more accurately control the size of the particles for synovectomy. Currently, Finland is the only participant routinely producing an agent for synovectomy (^{166}Ho-FHMA).

Particulate radiopharmaceuticals have potential for other therapeutic applications where appropriate delivery mechanisms are not available. The Australian report describes the development and pre-clinical evaluation of ^{166}Ho labelled microspheres for the therapy of liver metastases.

Although clinical investigations using these agents were not part of this CRP, all the participants, with the exception of Greece and Malaysia, after having completed the corresponding pre-clinical investigations, have supplied the bone seeking agent (^{153}Sm-EDTMP) to nuclear medicine physicians for clinical trials. Moreover, some participants in the ongoing clinical CRP on Efficacy and Toxicity of ^{153}Sm-EDTMD in the Palliative Treatment of Painful Bone Metastases are currently being provided with ^{153}Sm-EDTMP developed and prepared under the auspices of this CRP.

Comments on clinical results are beyond the scope of the CRP. However, the strong demand for these radiopharmaceuticals reported by the participants suggests an excellent degree of acceptance by the nuclear medicine community. It is estimated that in the CRP participating countries, more than 4000 patients have thus far been treated with ^{153}Sm-EDTMP with good therapeutic response for bone pain palliation. Several developing Member States operating nuclear research reactors have initiated programmes towards the preparation of therapeutic radiopharmaceuticals, many of those with the support of the Technical Co-operation Programme of the IAEA.

This publication contains highlights of the scientific achievements of the CRP, the participants' extended summary reports of their individual work, reactor irradiation protocols for the production of ^{153}Sm, ^{166}Ho, ^{165}Dy and ^{186}Re, production and quality control procedures for the preparation of ^{153}Sm-ethylenediaminetetramethylenephosphonate (^{153}Sm-EDTMP), ^{153}Sm-Hydroxyapatite (^{153}Sm-HA), ^{166}Ho-microspheres and ^{166}Ho-ferric-hydroxy-macroaggregates (^{166}Ho-FHMA), developed and tested under the auspices of the CRP. It is hoped that these procedures will be valuable information for laboratories in Member States considering or initiating the production of therapeutic radionuclides and radiopharmaceuticals for clinical use, based on the above mentioned reactor produced radionuclides.

2. SUMMARY REPORTS OF PARTICIPATING COUNTRIES

2.1. ARGENTINA

Research Agreement No.:	ARG/7406/CF
Title of the project:	Production and quality control of radiotherapeutics and radiopharmaceuticals
Chief scientific investigator:	M.G. Argüelles
Collaborators:	G.A. Rutty Solá, D.L. Bottazzini

The original goals of the project were as follows:

- Optimization of production protocols and quality control procedures for the routine production for ^{153}Sm-EDTMP.
- Preparation of two ^{153}Sm-synovectomy agents.

2.1.1. Main developments

The project was focused on the following:

- Production and quality control of ^{153}Sm-EDTMP for medical use.
- Biological evaluation of this agent.
- Preparation of albumin microspheres labelled with ^{153}Sm.
- Preparation of hydroxyapatite particles labelled with ^{153}Sm.
- Animal evaluation of these radiosynovectomy agents.

2.1.1.1. Production of ^{153}Sm

^{153}Sm chloride was produced in RA-3 reactor (Centro Atómico Ezeiza) by irradiation of 98.7% $^{152}Sm_2O_3$ via $^{152}Sm(n,\gamma)^{153}Sm$. The target material was dissolved in diluted nitric acid to a concentration of 5 mg/mL. It was then put inside a quartz ampoule and carried to dryness by heating under dry nitrogen flow. The sealed ampoule was irradiated for 36 hours, at a thermal neutron flux of 7.10^{13} n.cm^{-2}.s^{-1}. Irradiated target was dissolved in HCl 0.1 N to get it as chloride, with a specific activity about of 5.55–11.10 GBq (150–300 mCi) ^{153}Sm/mg Sm_2O_3.

2.1.1.2. Quality control

The gamma ray spectrum was obtained using a HPGe detector associated to a multichannel analyzer.

2.1.1.3. Labelling

The irradiated material (1 mg, 5.55–11.10 GBq) was dissolved in 3–4 mL of EDTMP solution (60–300 mg) in saline (pH8–9). After a twenty minutes resting period, a water dilution was performed. It was then heated up to 75°C for 45 minutes and afterwards diluted to the final volume of 15 mL. After membrane filtration (0.22 μm) and autoclaving dispensing was carried out. The activity concentration in the final solution was 740 to 925 MBq/mL having a pH between 7 and 8.

2.1.1.4. Quality control

Radiochemical control

The radiochemical purity was determined by thin layer chromatography, using ITLC/SG and water as the solvent. The complex runs together with the solvent front (R_f = 0.8–1.0) while the Sm^{3+} remains on the origin (R_f = 0). The radiochemical purity was in all cases higher than 97%.

Pharmacological studies

Toxicity, pyrogen and sterility tests were performed following known procedures.

2.1.1.5. Biodistribution

Biodistribution was performed in Wistar rats, 0.2 mL (74 MBq) of complex were injected in the penis vein of 200–250 g male rats. The rats were housed in metabolic cages till they were killed. At the predetermined times the rats were killed by thoracic incision. Samples of blood were taken by cardiac puncture and the tissues of interest were dissected. The tissues were counted in a dose calibrator, calculating the % of the injected dose in each tissue. The effective dose was estimated measuring the syringe before and after injection.

Rats were chosen for biodistribution studies instead of mice since their basal metabolism is lower than in mice. Among the different periods of time we selected the two h as representative for the behaviour of the complex (Table I).

TABLE I. UPTAKE (% ID) OF ^{153}Sm-EDTMP IN RATS 2 h AFTER INJECTION

	Urine	Kidneys	Liver + Spleen	Stomach + Intestine	Blood/g	Femur	Lungs
X	43.9	1.0	0.40	0.50	0.010	1.57	0.029
s.d.	8.6	1.7	0.09	0.23	0.007	0.19	0.018

Wistar rats: body wt.: 200–250 g; n = 8; mean ± s.d. of the % injected dose/organ are given.

2.1.1.6. In vitro stability

The in vitro stability was studied by chromatography between two hours and five days. After the five days period the complex showed stability. Biodistribution studies were

performed in rats using the complex 24 and 48 h after labelling. The results showed no difference as regards the initial compound.

2.1.1.7. Labelling optimization

Different molar ratios EDTMP/Sm were studied. No variation in radiochemical purity was detected. An important tissue is the liver since the complex composition modifies hepatic uptake. Even when the radiochemical purity of the compound is not affected by the molar ratios chelating agent/radioisotope, the in vivo behaviour makes the change evident. Consequently the chosen molar ratio should be higher than 20/1 (EDTMP/Sm moles).

A remarkable difference is noticed when the EDTMP concentration is modified in the final solution. When this concentration is increased the hepatic concentration of the complex diminishes. For EDTMP concentration a minimum of 10 mg/mL was established. Results are shown on Table II.

TABLE II. FEMUR/LIVER + SPLEEN RATIOS VS [EDTMP]

[EDTMP] (mg/mL)	Liver + Spleen	Femur	Femur/Liver + Spleen
2	3.11 ± 0.50	1.14 ± 0.06	0.37 ± 0.04
4	1.79 ± 0.40	1.66 ± 0.19	0.95 ± 0.22
6	0.82 ± 0.20	1.71 ± 0.32	2.12 ± 0.25
12	0.40 ± 0.09	1.57 ± 0.19	3.94 ± 0.53
18	0.41 ± 0.11	1.63 ± 0.22	4.10 ± 0.82

Wistar rats; body wt.: 200–250 g; mean ± s.d. of the % injected dose are given; n = 8;
molar ratio EDTMP: Sm = 20:1.

2.1.1.8. In vivo stability

Long term biodistribution tests were performed in order to study the radiopharmaceutical behaviour. Groups of five Wistar rats were injected and the biodistribution was carried out as in the previous cases. The rats were killed 24 and 48 h after injection. During these periods the rats were kept in metabolic cages having free access to standard diet and water.

The dose measured 2 h post administration of 90% was observed in femur 24 h later, with a slight increase in hepatic uptake. The complex was rapidly excreted into the urine, reaching 40% of the initial dose 2 h post administration. At that moment the amount of circulating ^{153}Sm activity is negligible.

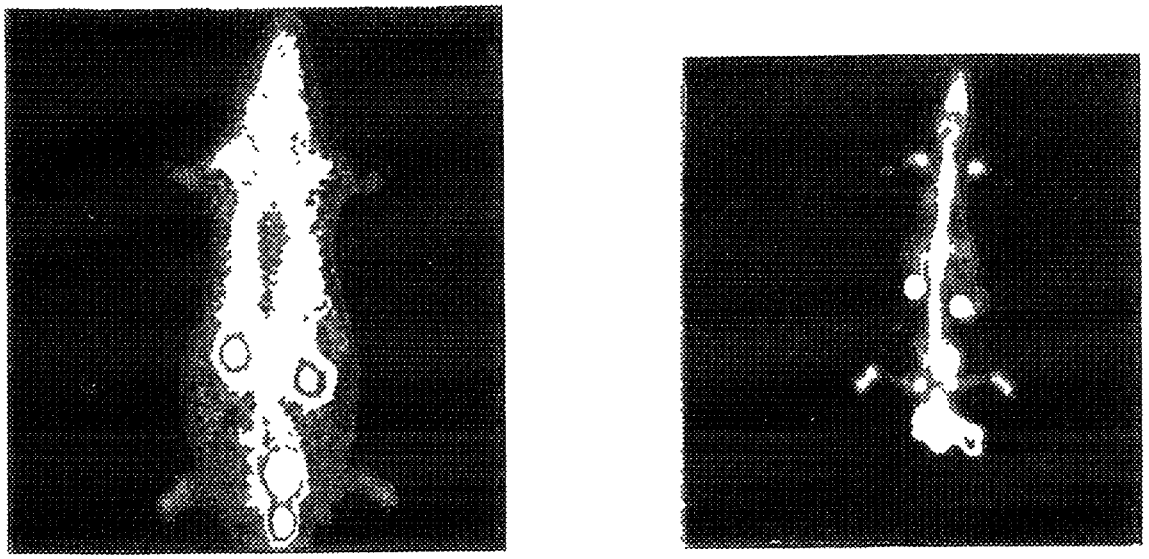
As regards initial hepatic uptake, an important decrease is observed 48 h post administration, an 80% of the maximum uptake remaining in femur. At the same time, almost complete clearance of non-osseous tissue is observed.

2 1 1 9 Dynamic studies in gamma camera

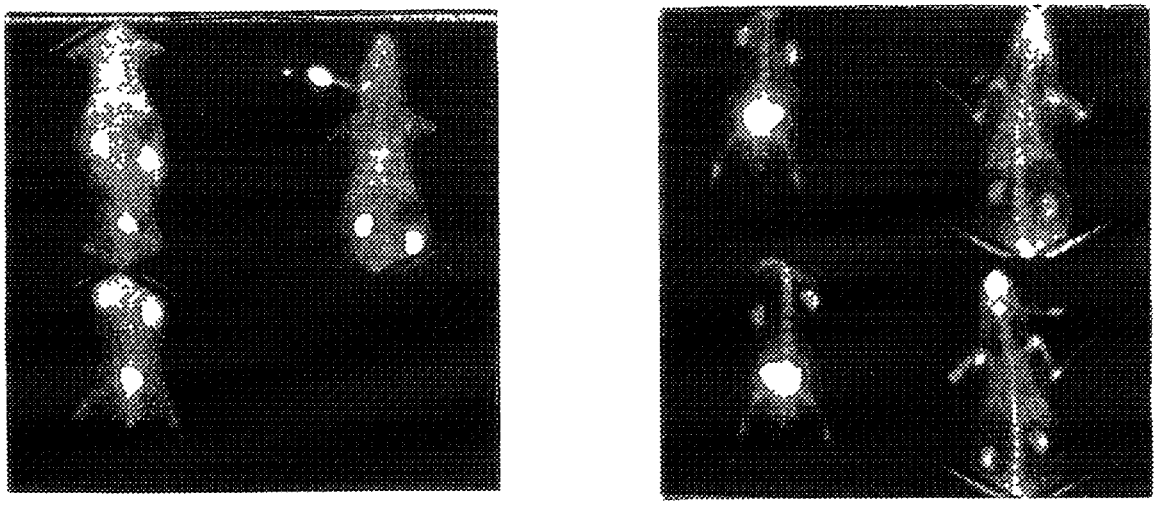
The studies were performed in Wistar rats and New Zealand rabbits. Rats were anaesthetized with Urethane (intraperitoneal injection 1 g/kg body weight), the complex (0 2 mL: 37 MBq) injected into the penis vein and then they were imaged with gamma camera Images were obtained five min to two h p.i using a high resolution collimator (500 000 counts were measured with a 128 × 128 pixels matrix).

Male and female rabbits weighing about 4 kg were used. All of the animals were anaesthetized twice A Ketamine (35 mg/kg) + Xilacine (5 mg/kg) dose was used first and Ketamine (10 mg/kg) was injected afterwards.

Intravenous injection of complex (0 2-0 3 mL, 185–222 MBq) was performed and images obtained with a gamma camera using the method previously described from 5 min to 2 h post administration.



A) rats, 5 min p i (left) and 120 min p i (right)



B) rabbits, 5 min p i (left) and 90 min p i (right)

FIG 1 Skeletal gamma camera images obtained with ^{153}Sm-*EDTMP*

The images are shown in Fig. 1. Imaging evaluation shows an excellent skeleton uptake. Analysis of the long term post administration image demonstrates blood clearance and excretion into the urine.

2.1.1.10. Human clinical trials

Up to this moment more than 75 doses of this radiopharmaceutical have been delivered mainly to the Roffo Oncology Department and, in lower amounts, to the Nuclear Medicine School in Mendoza.

The results of the studies carried out in the Roffo Institute as informed by the hospital researchers, are as follows: Thirty-two (32) patients were studied by bone scintigraphy evaluation and laboratory data at the beginning of treatment and weekly during the subsequent three months. The patient's ages were from 40 to 82 years, and a 22 years old one. Fifteen prostate cancer male patients, one lung cancer male patient, thirteen female suffering from breast cancer, one lung cancer female patient and one melanoma cancer female patient were examined. The 22 years old male patient suffered from cavum cancer. All kind of therapeutic treatments tried on them had been exhausted and they were refractory to analgesic treatments. They were administered a 18.5 MBq/kg-37.0 MBq/kg (0.5–1.0 mCi/kg) body weight dose. Response signs to treatment appeared within the first five days pi., showing a remarkable recovery from pain symptoms in 70% of the male patients, probably due to the fact that nor radiotherapy neither chemotherapy treatments had been undergone by these patients. The fifteen female patients and the young male patient, having received radiotherapy and/or chemotherapy treatments previously, showed a lower and variable analgesic response.

Duration of response was variable, between fifteen days to six months. In laboratory controls decreases in hemathologic levels were observed in some patients, but they were temporary. Representative results are given in Table III.

The nuclear medicine school in Mendoza informed that the radiopharmaceutical was administered in three patients. One female patient suffering from breast cancer was injected a 18.5 MBq/kg (0.5 mCi/kg) body weight dose and the obtained response was negligible. other

TABLE III. CLINICAL TRIALS

Age (sex)	Tumour type	Previous treatment	mCi/kg	Pain palliation	Duration of response
50–80 (M)	Prostate	Hormonetherapy	0.5–1.0	73% (11/15)	2–6 months
80 (M)	Lung	Chemotherapy	0.5	100% (1/1)	1 month
22 (M)	Cavum	Chemotherapy	0.5	0% (1)	
40–82 (F)	Breast	Surgery, chemotherapy, Radiotherapy	0.5–1.0	61% (8/13)	0.5–4 months
74 (F)	Lung	Radiotherapy	0.5	100% (1/1)	3.4 months
80 (F)	Melanoma	Surgery, radiotherapy	1.0	0% (0/1)	

two were male prostate and lung cancer patients. A good response was obtained injecting a 37 MBq/kg (1 mCi/kg) body weight dose.

2.1.2. Albumin microspheres labelled with ^{153}Sm

2.1.2.1. Preparation of microspheres

The microspheres were obtained by heat denaturalization of a human serum albumin (HSA) emulsion in vegetable oil. A 10% human albumin solution and olive oil were used.

- The HSA solution was added, drop by drop, into the olive oil stirring vigorously.
- The emulsion was heated up to 140–160°C for one hour.
- The suspension was cooled and diluted with n-hexane. It was filtrated with mesh 200 in order to discard the particles over 75 μm. The supernatant was filtered thorough membrane filter. The microspheres were rinsed with acetone and dried.

2.1.2.2. Particle size measurement

It was performed using a optical microscope with micrometric ocular. The size distribution is shown on Table IV.

TABLE IV. SIZE DISTRIBUTION OF MICROSPHERES

% of microspheres	Size (μm)
14	5–15
49	15–25
23	25–35
10	35–45
4	45–55

2.1.2.3. Labelling with ^{153}Sm

Labelling was done in two steps:

- ^{153}Sm-citrate was prepared by adding sufficient citric acid to the $^{153}SmCl_3$ solution to give a concentration of 15 mg/mL citric acid in 0.1 N HCl.
- The radioactive solution was added to the particulate suspension (20 mg) stirring continuously (30 min, 37°C).

Radiolabelled particles were rinsed with saline and separated by centrifugation (5 min at 1000 rpm) and labelling efficiency was determined. The microspheres were resuspended in 2 mL of saline.

2.1.2.4. Labelling efficiency

The radioactive mixture was transferred to a centrifuge tube using 4 mL of saline to rinse, centrifuged at 1000 rpm for 5 minutes. The supernatant was then transferred to another tube. Measurements of radioactivity were made and labelling efficiency was calculated as percentage of initial activity.

Labelling did not depend on the presence of emulsifiers or particles pre-treatment. Although, it was high dependent on complex mass as can be seen on Table V.

The labelling efficiency was greater than 75% when the Sm-citrate mass was about 2.5μg/20 mg of microspheres. When this quantity increases, the labelling efficiency decreases, so it is less than 20% when Sm-citrate amount is higher than 15 μg.

TABLE V. EFFECT OF Sm-CITRATE MASS ON LABELLING EFFICIENCY

Sm-citrate mass	Labelling efficiency
0.8 ± 0.1	70.1 ± 2.6
2.5 ± 0.5	78.1 ± 2.6
5.0 ± 0.2	68.6 ± 1.5
7.6 ± 0.1	51.2 ± 0.8
15.8 ± 0.2	18.9 ± 0.5

n = 10: microspheres mass = 20 mg.

2.1.2.5. In vitro stability

Stability of the labelled particles was studied in normal saline and 1% albumin solution, at 36°C over 48 h. Albumin microspheres retained 80% of the initial activity after 48 h incubation.

2.1.3. Hydroxyapatite labelled with ^{153}Sm (^{153}Sm-HA)

2.1.3.1. Preparation

Hydroxyapatite particles were prepared from the reaction of calcium nitrate and ammonia phosphate at high pH; 0.33 MOL of $Ca(NO_3)_2$ was dissolved in 300 mL of water. The solution was adjusted at pH12 by addition of concentrated ammonia and diluted to 600 mL. A $(NH_4)_2HPO_4$ solution (0.2 MOL in 500 mL, similarly brought to pH12 and diluted to 800 mL) was added, drop by drop, stirring vigorously. A voluminous precipitate was formed. The reaction mixture was gently boiled for 10 min. The precipitate was allowed to settle and the supernatant solution was separated by decantation. The precipitate was rinsed with hot water, dried at 150°C and heated for an hour at 240°C to remove the ammonium nitrate. By strong heating at 800°C for an h, the product becomes largely anhydrous and hardened. The yield of the hydroxyapatite synthesis was always greater than 80%.

2.1.3.2. Particle size measurement

Particle size range was studied using light microscopy with micrometric ocular. A process of sieving using sieves of 200 and 400 mesh was carried out. The portion of the sample retained on sieve 200 mesh (range over 75 μm) was discharged. It was difficult to estimate size bellow 5 μm (Table VI).

TABLE VI. SIZE DISTRIBUTION HA-MICROPARTICLES

% of microparticles	Size (μm)
18	5–15
35	15–25
26	25–35
14	35–45
5	45–55
2	55–65

2.1.3.3. Labelling

It was done in two steps using the method previously described in Section 2.1.2.2.

2.1.3.4. Labelling efficiency

It was done using the method previously described in Section 2.1.2.4.

Labelling did not depend on the presence of emulsifiers or microparticle pre-treatment. Though, it was high dependent on complex mass as can be seen on Table VII. The labelling efficiency was greater than 99% when the ^{153}Sm-citrate quantity was less than 7 μg/10 mg of particles. On the other hand, the labelling efficiency was less than 50% when ^{153}Sm-citrate mass was 75 μg.

TABLE VII. EFFECT OF Sm-CITRATE MASS ON LABELLING EFFICIENCY

Sm-citrate mass (μg)	Labelling efficiency
6.4 ± 0.1	99.3 ± 0.2
9.4 ± 0.1	97.5 ± 0.5
12.8 ± 0.2	95.0 ± 0.4
19.0 ± 0.1	93.2 ± 1.1
26.0 ± 0.3	79.9 ± 0.8
38.0 ± 0.1	77.2 ± 0.5
75.0 ± 0.3	49.7 ± 0.7

n = 11; microparticles mass = 10 mg.

2.1.3.5. In vitro stability

In vitro stability studies were performed by incubating particles in normal saline and 1% human serum albumin solution over 6 days at 37°C with agitation. At different times radiolabelled particles were centrifuged at 1000 rpm for 5 min and activity in the particles and supernatant was measured.

Labelled particles showed to be stable more than 6 days. No dissociation of activity from the particles was observed. HA particles retained more than 99% from the original labelling at the studied conditions. No changes in particle size were observed. The sedimentation velocity was lower when the particles were dispersed in 5% glucose solution. This is an important factor because if the sedimentation occurs very fast, many particles remain into the syringe and the administration is difficult.

2.1.3.6. Animal model

The biodistribution studies were performed in Wistar rats and New Zealand rabbits. Prior the administration microparticles were resuspended in 5% and autoclaved for 20 min at 121°C. Each rat was injected intra-articularly with 18.5 MBq (0.5 mCi) of ^{153}Sm-HA. Animals were kept in metabolic cages over periods of 3 and 6 days. At the end of the period the animals were killed and organs were removed and counted. The per cent injected dose in blood, urine and different organs was calculated. Extra-articular leakage was calculated as the sum of all activities in major organs, total urine excreted and the activity remaining in the circulating blood at the time of sacrifice.

To determine the biological fate of radionuclide that is injected intra-articularly but is not bound to HA, control studies were performed in Wistar rats. Each rat was injected intra-articularly with 18.5 MBq (0.5 mCi) of ^{153}Sm-citrate. Animals were kept in metabolic cages over periods of 3 and 6 days. The total urine excreted within 3 days was collected. At the end of the period the animals were killed and organs were removed and counted. The per cent injected dose in blood, urine and different organs was calculated. Extra-articular leakage was calculated as the sum of all activities in major organs, total urine excreted and the activity remaining in the circulating blood at the time of sacrifice.

The extra-articular leakage of radionuclide following injection of ^{153}Sm-citrate into knee joints of rats is summarized on Table VIII. Samarium-153-citrate exhibited a total of only 5%

TABLE VIII. EXTRA-ARTICULAR CUMULATIVE LEAKAGE*

	3 days n = 6	6 days n = 6
^{153}Sm-HA	0.14 ± 0.13	0.32 ± 0.20
^{153}Sm-citrate	37.2 ± 1.2	42.3 ± 1.0

* Blood, liver, kidney, lung, bone, spleen, urine.

leakage in urine and blood. 32% of the activity was retained in the body, of which a significant amount (10%) was found in bone. These control studies show that more than 50% of the ^{153}Sm activity is retained within the joint space.

The extra-articular leakage of ^{153}Sm-activity following injection of ^{153}Sm-HA particles into the knee joint is summarized on Table VIII. Total cumulative leakage in rats is 0.14% in 3 days and 0.32% in 6 days. All organs showed insignificant accumulation of ^{153}Sm activity.

Normal rabbits were used as models to evaluate in vivo stability of radiolabelled HA particles. The studies were performed in New Zealand rabbits. Male and female rabbits weighing about 4 kg were used. Each rabbit was injected intra-articulary (into the left posterior knee joint) with 0.2 mL containing 37 MBq (1 mCi) of ^{153}Sm-HA. Images were obtained with a gamma camera using a high-resolution collimator (500 000 counts were measured with a 128 × 128 pixels matrix). The per cent-injected dose in blood, urine and different organs was calculated daily over a period of 6 days. After that the animals were killed and the tissues were counted.

No extra-articular localization of activity was detected by whole-body scans. All organs showed insignificant accumulation of ^{153}Sm activity. The principal observed fact over the whole study, was the permanence of the injected product in the joint.

2.1.3.7. Histopathology

Histopathology of rabbit knee was performed 6 weeks after particles administration. These studies did not reveal any changes into the joint.

Acknowledgements

The authors would like to thank E.A. Torres, R. Amor, I.S. Luppi Berlanga, A. Mender, J. Arashiro, and J.C. Furnari for technical assistance.

Report

ARGUELLES, M.G., LUPPI BERLANGA, I.S., TORRES, E.A., RUTTY SOLA, G.A., RIMOLDI, G., "Preparation and biological behaviour of samarium-153-hydroxyapatite particles for radiation synovectomy", Modern Trends in Radiopharmaceuticals for Diagnosis and Therapy, IAEA-TECDOC-1029, IAEA, Vienna (1998) 531–537.

2.2. AUSTRALIA

Research Agreement No.:	AUL/7395/CF
Title of the project:	Pre-clinical development of holmium 166 microspheres for therapy of hepatic metastases
Chief scientific investigator:	E.L.R. Hetherington

2.2.1. Main developments

Because of the ^{153}Sm-EDTMP patent situation in Australia, work on development of this agent was not continued as part of the co-ordinated research project. This agent is now produced by The Australian Nuclear Science and Technology Organisation (ANSTO) under license from Dow Chemical Company and marketed as Qudramet™.

ANSTO has continued the development of therapeutic agents based on beta emitting radionuclides including ^{165}Dy, ^{186}Re, ^{188}Re and ^{166}Ho. The work reported here describes the development, preclinical studies, production and quality control of ^{166}Ho microspheres for therapy of liver metastases and has been performed in collaboration with the Departments of Nuclear Medicine and Oncology at Freemantle Hospital, Western Australia.

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Introduction

Radiolabelled microspheres administered by intra-arterial injection are used for therapy of hepatic metastases. The beta emitter yttrium-90 is the most commonly used isotope. This paper describes the production and preclinical evaluation of resin based microspheres with a particle size of 13 ± 2 mm labelled with ^{166}Ho. Unlike ^{90}Y , the distribution of ^{166}Ho in the liver can be accurately imaged using its 80.5 keV photon emission as an aid to radiation dosimetry. The microspheres are non-toxic, stable in vitro and after administration, show negligible leakage or concentration of radioactivity other than in the liver. Biodistribution studies were used to obtain preliminary radiation dose estimates.

In 1960 Grady first reported the use of ^{90}Y labelled particles for therapy of hepatic metastases with administration by intra-arterial injection. Developments of this technique have been reported by a number of authors. Radiolabelled microspheres are used, as an alternative to external beam therapy or chemotherapy. Hepatic metastases usually develop

from carcinoma of the colon and only 5 per cent of patients have respectable tumours. The median survival time is 10.6 months. Chemotherapy can slightly improve survival time and can improve a patient's quality of life. External beam therapy is often not a viable option since irradiation of the metastases with an appropriate dose is difficult without a giving a high dose to healthy liver tissue. Microspheres have also been investigated for therapy of hepatocellular carcinoma.

The rationale for the use of radiolabelled microspheres has been outlined by Ehrhardt and Day. Most of the blood supply to normal liver is via the portal vein. The metastatic tumours receive blood via the hepatic artery. Thus administration of radiolabelled particles of the appropriate size through the hepatic artery provides the means for selective irradiation of the metastases with sparing of normal liver. The size of the microspheres used is of the order of 13–30 mm. ^{90}Yttrium microspheres have been produced by ANSTO for a number of years. Glass based ^{90}Yttrium microspheres, TheraSphere™, are available commercially from Nordion.

The success of hepatic metastases therapy depends on choice of radionuclide, particle size, appropriate method of administration, selectivity of tumour uptake, in vivo stability of the product and accurate radiation dosimetry. This report outlines the development and preclinical assessment of ^{166}Ho labelled resin microspheres.

The radioactive decay scheme of ^{166}Ho is shown in Table IX. It was considered suitable for internal therapy based on radiolabelled microspheres because of the convenient half-life, the maximum range of the beta emissions (0.84 cm), and the presence of a low energy (80.5 keV) gamma emission. This energy allows imaging of the distribution of the microspheres in the liver with a gamma camera and suitable collimator.

TABLE IX. PRINCIPAL EMISSIONS IN THE DECAY SCHEME OF HOLMIUM-166 (HALF-LIFE = 26.8 h) [6]

Radiation Type	Energy MeV	Intensity (%)
Auger and IC	0.0055	25.700
	0.0231	10.600
	0.0780	24.500
Beta (E_{max})	0.1910	0.304
	0.3900	0.950
	1.7730	48.000
	1.8540	51.000
Gamma	0.0805	6.200
	1.3790	0.930
	1.5810	0.183
	1.6620	0.121

Although the ^{166}Ho beta emissions have a shorter range than those of the commonly used ^{90}Y (~1.0 cm), this is unlikely to be therapeutically significant even under point source irradiation conditions. The dose profiles of ^{90}Y and ^{166}Ho are very similar up to a distance of approximately 3 mm from a point source. Beyond this distance both profiles fall off very rapidly.

This report describes the preclinical development of resin based ^{166}Ho microspheres including radiolabelling, stability studies, animal biodistribution and toxicity studies and preliminary radiation dosimetry.

2.2.1.1. Radioisotope production and labelling

^{166}Ho is produced at ANSTO by irradiation of holmium oxide (Ho_2O_3) in a thermal neutron flux of 5.0×10^{13} n·cm^{-2}·s^{-1} in the HIFAR reactor. The activation cross section of ^{165}Ho is 61.5 barns. The target mass is typically 5–10 mg, adjusted together with the irradiation time, to meet final activity requirements for pre-clinical experiments or clinical use. Irradiation of the dry oxide yields an ex-reactor activity close to that calculated for a given mass and irradiation time in the above neutron flux. After irradiation the target is dissolved in 32% HCl, evaporated to dryness and re-dissolved in Water for Injection (WFI) in preparation for labelling the microspheres.

The ^{165}Ho resin microspheres (200 mg per patient dose + 100 mg for quality control and retention samples) with a particle size of 13 ± 2 mm are prepared by a process which commences with the thorough mixing of the active ^{165}HoCl solution with Aminex® A-5 action exchange resin (BIO-RAD Inc. Hurcules CA, USA). This resin has sulphuric acid functional groups attached to divinylbenzene copolymer lattices.

After mixing, the microspheres are separated by filtration and washed three times with WFI with filtering after each wash. The microspheres are then treated with a 10% sodium tri-sodium phosphate solution and washed and filtered three times. After the final wash, the suspension is centrifuged and supernatant in excess of 6 mL per patient dose plus the volume required for Q.C. and retention removed. The pH of the final suspension is in the range 10–11. A single patient dose uses 200 mg of resin or approximately 2×10^8 spheres (by calculation and measurement with a haemocytometer). Following radiolabelling, the microspheres are autoclaved at 132°C for 7 minutes and treated in an ultrasonic bath for 30 minutes. See Annex for summary of irradiation, labelling and quality control procedures. The microspheres for preclinical evaluation were also prepared in this way.

2.2.1.2. In vitro stability studies

A series of experiments was performed to establish the in vitro stability of the microspheres. Three batches of microspheres were prepared following the above procedure using a ^{166}Ho activity of 20 MBq per batch. Each batch was mixed with 5 mL human blood plasma and incubated at 37°C. at selected time points after incubation the suspension was shaken and then centrifuged at 2500 rpm for 5 minutes. An aliquot of the supernatant was taken and the ^{166}Ho present measured in an ionization chamber. Leaching of activity was calculated as the total supernatant activity expressed as a per centage of the total batch activity. This procedure was repeated at 5 time points up to 120 hours post incubation. It was found that 98 per cent of the ^{166}Ho activity was retained on the resin for the 120 hour period (Fig. 2).

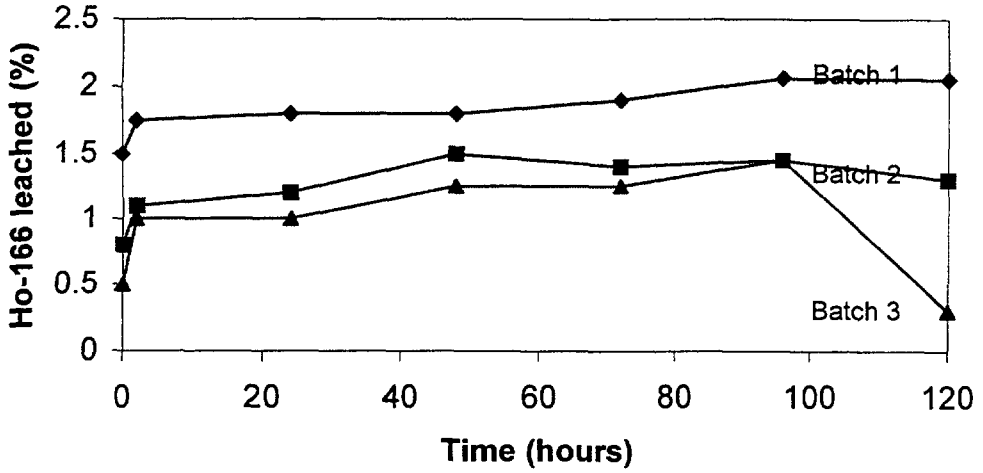

FIG. 2. In vitro human plasma leakage stability study of holmium-166.

2.2.1.3. Biodistribution

To determine the leakage of activity from the liver after administration of ^{166}Ho microspheres, a biodistribution study was performed on AAW/Wistar male rats. Since the livers of the animals had no tumours, and administration via the hepatic artery proved to be very difficult, the microspheres were injected via the portal vein. The microspheres were injected under direct vision in anaesthised rats. At pre- determined time points of 3 h, 24 h, 48 h, 72 h, 96 h and 120 h the rats were sacrificed, dissected and organ activities determined by

TABLE X. WHOLE BODY BIODISTRIBUTION IN RATS AFTER INTRA PORTAL VEIN INJECTION OF HOLMIUM-166 MICROSPHERES EXPRESSED AS A PERCENTAGE OF ADMINISTERED ACTIVITY AT 6 TIME POINTS

	Mean organ activity concentration at given time points					
Time	3 h	24 h	48 h	72 h	96 h	120 h
Organ						
Liver	99.57	99.51	99.96	96.24	94.64	94.33
Spleen	0.0	0.01	0.02	0.02	0.04	0.05
Kidney	0.02	0.05	0.08	0.13	0.2	0.18
Skin	0.17	0.02	0.26	0.4	0.33	0.53
Bone	0.17	0.36	0.52	2.88	4.44	4.6
Lungs	0.01	0.01	0.02	0.02	0.03	0.04
Heart	0.0	0.0	0.01	0.01	0.03	0.01
Blood	0.02	0.03	0.05	0.06	0.12	0.1
Urine	0.01	0.0	0.01	0.02	0.02	0.01
Bladder	0.0	0.0	0.0	0.01	0.02	0.02
Stomach	0.03	0.01	0.02	0.15	0.05	0.04
Small int.	0.0	0.0	0.01	0.01	0.02	0.02
Large int.	0.0	0.0	0.01	0.01	0.02	0.01
Pancreas	0.0	0.0	0.01	0.01	0.01	0.02

gamma counting. Three rats were used for each time point. Mean organ activities, as per centages of injected activities, were calculated for all time points. The biodistribution is shown in Table X.

The biodistribution study showed that 94 per cent of the injected activity was retained in the liver up to 120 hours post injection with approximately 4.6 per cent accumulating in the bone after several half-lives indicating suitability for human administration. The results were also used for a preliminary estimation of the liver and other organ radiation doses.

2.2.1.4. Radiation Dosimetry

Radiation dose estimates for the organs of a 70 kg adult from the administration of ^{166}Ho microspheres were estimated using the biodistribution data in Table X. The results were normalized to a total of 100 per cent for each time point to correct for activity retained in syringes during injection and other small experimental losses. For times greater than 120 hours, it was assumed that there was no loss of activity from any organ other than by radioactive decay.

TABLE XI. RESIDENCE TIMES (CUMULATED ACTIVITY PER UNIT ACTIVITY ADMINISTERED) FOR HOLMIUM MICROSPHERES IN THE ORGANS OF MALE RATS

Organ	Residence Time (Hours)
Liver	36.64
Spleen	0.043
Kidney	0.028
Skin	0.075
Cortical bone	0.21
Trabecular bone	0.21
Lungs	0.0073
Heart wall	0.0016
Heart contents	0.0017
Urine	0.0047
Stomach	0.012
Upper L. intestine	0.0023
Lower L. intestine	0.0023
Small intestine	0.0031
Brain	0.0009
Pancreas	0.0015
Red marrow	0.0007
Remainder of body	0.011

Dose calculation used MIRDOSE version 2 with decay and dose data for ^{166}Ho added. Organ residence times were calculated as the sum of two components. The residence times for the 0–120 hour period were obtained by integration of the time point data using a computer program RESTIME. Residence times post 120 hour were calculated from the decay of the activity present at that time. Since the microspheres were administered directly to the liver it was assumed that the initial activities in the other organs was zero. Residence times are given in Table XI. Activity in the blood was assigned to a number of organs as suggested by Cloutier and Watson [9] using the percentages of the total blood in those organs [10]. Activity not assigned to individual organs was assumed to be uniformly distributed in the body. The dose calculation assumed that human residence times were identical to those obtained for the rat. The calculated organ doses are given in Table XII.

Activity not assigned to individual organs was assumed to be uniformly distributed in the body. The dose calculation assumed that human residence times were identical to those obtained for the rat. The calculated organ doses are given in Table XII.

TABLE XII. RADIATION DOSE ESTIMATES FOR THE 70 kg REFERENCE ADULT MALE DETERMINED FROM BIODISTRIBUTION STUDIES IN RATS

Organ	Radiation Dose (MGy/MBq)	Radiation Dose (Rad/mCi)
Adrenals	0.013	0.048
Gallbladder wall	0.026	0.095
Stomach	0.014	0.05
Kidneys	0.049	0.17
Liver	7.74	26.64
Red marrow	0.04	0.148
Bone surfaces	0.045	0.167
Spleen	0.096	0.355
Lungs	0.009	0.033
Total body	0.206	0.762

There was no significant host response to microspheres in any organ and apart from the lungs and serosa of the duodenum, no microspheres were observed in any organ. Red cell blood counts and platelet counts did not change from baseline values, nor did they differ from values of control animals in any of the three species. Isolated neutrophilia in one rabbit and one guinea pig was attributed to preoperative infection. No other significant change was seen in leukocyte counts and the blood film morphology remained unchanged from that of control studies.

In a separate study at Fremantle Hospital histopathological examination of liver tissue at 2, 3, 7 and 14 days post injection of ^{166}Ho microspheres showed the expected distribution in interlobular spaces with no evidence of inflammatory liver response or clumping. Autoradiographic examination of the tissue samples indicated that activity was confined to the vicinity of the microspheres. Background activity in the liver macrophage was negligible, confirming the absence of leaching of radioactivity from the microspheres.

2.2.2. Conclusion

The method described is suitable for the preparation of biologically stable ^{166}Ho microspheres. They are now being evaluated in a Phase 1–2 clinical trial at Fremantle Hospital, WA. To date nine patients have participated in the trial. Results are not yet available for publication.

Acknowledgements

V. Papazian, A. Donald, J. Chapman and V. Nguyen of ANSTO, and R.J. Clancy and M.F. Leahy of Fremantle Hospital contributed to the development and pre-clinical evaluation of ^{166}Ho microspheres.

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Annex
Procedure for Production and Quality Control of Holmium-166 Microspheres

Target irradiation

5–10 mg Ho_2O_3 irradiated in a thermal neutron flux of 5.0×10^{13} $n \cdot cm^{-2} \cdot s^{-1}$ in the HIFAR reactor to produce specified activity. Typically 40–50 GBq (single patient dose plus activity for QC and retention).

Microsphere preparation

(1) Digest Ho_2O_3 in 2 mL of 32% HCl at 300°C.
(2) Evaporate to dryness and dissolve in 6 mL of water for injection (WFI).
(3) Measure activity and dispense required amount for patient, QC and retention.
(4) Mix with 300 mg Aminex® A-5 resin and stir for 30 minutes.
(5) Wash and stir with 50 mL WFI and remove waste water (to 20 mL) by filtration.
(6) Repeat **5.** three times.
(7) Add 50 mL 10% tri-sodium phosphate.
(8) Stir for 30 minutes and remove liquid in excess of 20 mL by filtration.
(9) Wash and stir with 50 mL WFI and remove liquid in excess of 20 mL by filtration (3 times).
(10) Centrifuge at 2500 RPM for 5 minutes and remove liquid in excess of 9 mL.
(11) Agitate and dispense into 3 vials containing 6 mL, 2 mL and 1 mL for patient dose, retention sample and QC respectively.
(12) Autoclave at 132°C for 7 minutes.
(13) Sonicate for 30 minutes.

Quality control

(1) Perform LAL test for endotoxins. Limit is 175 EU/per patient dose.
(2) Test pH. Required range is 9–11.
(3) Determine radionuclidic purity by gamma spectrometry.
(4) Confirm particle size as 13 ± 2 mm.
(5) Measure unbound ^{166}Ho in liquid. To be not greater than 1 per cent.

2.3. BRAZIL

Research Contract No.:	BRA/7407/RB
Title of the project:	Production of ^{153}Sm - EDTMP
Chief of Scientific Investigation:	J. Mengatti
Others Investigators:	H.T. Gastiglia, M.F. Barboza, N.S. Pereira, C.P.G. Silva

2.3.1. Main developments

2.3.1.1. Production of ^{153}Sm

The general procedure for the production of ^{153}Sm and ^{153}Sm-EDTMP is given in Fig. 3.

Irradiation

^{153}Sm (samarium-153) was obtained by neutron irradiation under a thermal flux of 1.3–1.5 × 10^{13} n.cm^{-2}.s^{-1} during 40 h. Natural $^{152}Sm_2O_3$ was dissolved in 1N HNO_3 in order to obtain a solution of 10–15 mg/mL. From this stock solution an amount containing 10 or 15 mg of Sm was put into a quartz vial and evaporated to dryness, the vial is flame-sealed and encapsulated into an aluminium container. After the end of irradiation the target was opened and then dissolved with 0.1N HCl or with 3 × 1 mL 0.9% NaCl at 85°C.

2.3.1.2. Kit formulation and labelling

Lyophilized form and labelling procedure

Kit formulation in lyophilized form was prepared in sterile form at pH10.5, containing 50 mg EDTMP from ICN-Biochemicals, per vial (stable for 12–18 months). A solution of ^{153}Sm in 0.9% NaCl, 111 to 222 MBq/mL (3–6 mCi/mL) was added into an EDTMP kit, the volume (15–17 mL) was adjusted with 0.1N HCl and/or 0.05 M phosphate buffer with a final pH7.5–8.0. The influence of different pH values from 6.5 to 11.0 was evaluated, as well as the molar ratio EDTMP/Sm, from 0.7 to 40 (Fig. 4, Tables XIII and XIV). These parameters were evaluated during the development of the research.

Liquid form and labelling procedure

216 mg/8 mL EDTMP solution, pH = 7.8–8.0, prepared in sterile form, filtered through 0.22 μm membrane filtration and autoclaved. The EDTMP solution was kept at room temperature, which is stable for 12 months. A solution of ^{153}Sm in 0.9% NaCl, 37 000 MBq/mL (1000 mCi/mL) was put into a EDTMP solution vial, the volume (30 mL) was adjusted with 0.18 M Buffer Phosphate with a final pH7.5–8.0. The reaction time was 30 min. The following parameters were unaltered in all samples; molar ratio EDTMP/Sm = 15, EDTMP concentration = 14.7 mg/mL and Sm concentration = 0.333 mg/mL. This formulation was adopted for routine production.

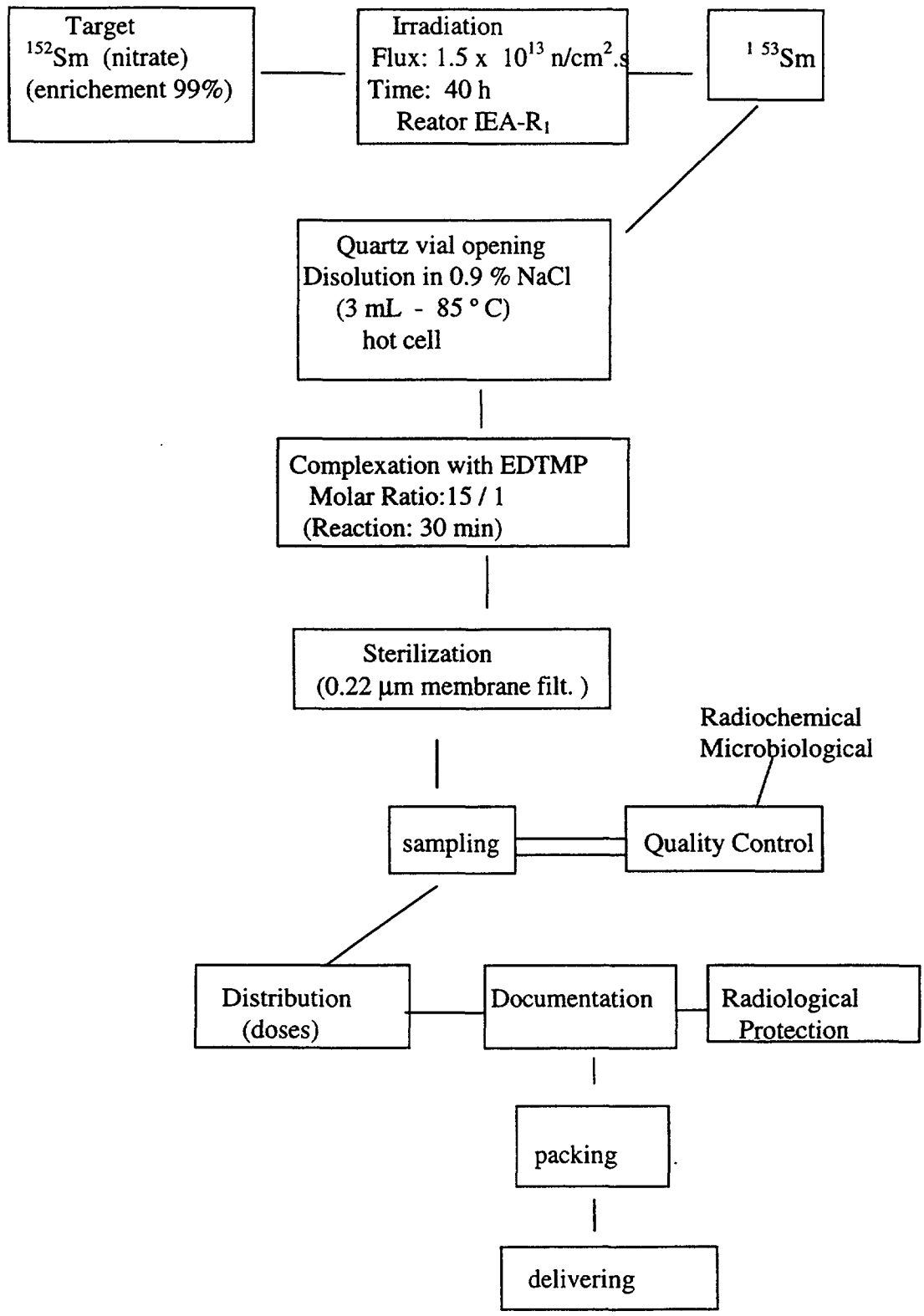

FIG. 3. Diagram of ^{153}Sm-EDTMP production.

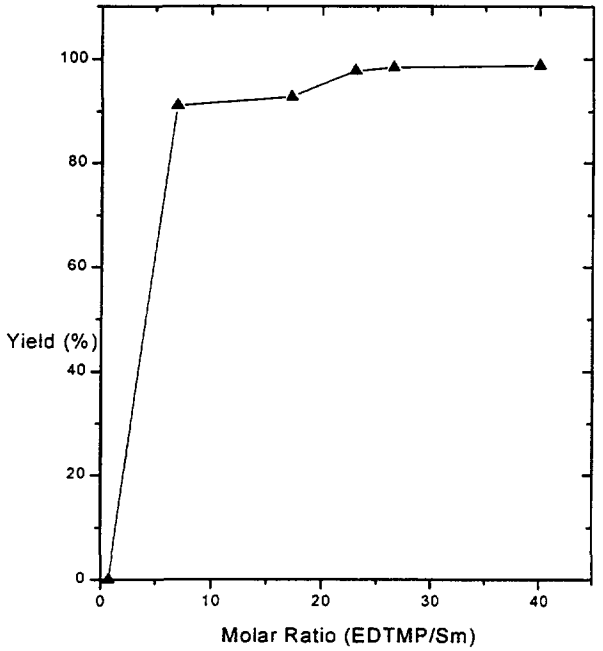

FIG. 4. Influence of molar ratio EDTMP/Sm.

TABLE XIII. LABELLING YIELD OF ^{153}Sm-EDTMP (%) AS A FUNCTION OF pH

Solvents	pH		
	6.5	7.5–8.0	10.5–11.0
1	91.62	98.35	98.44
2	95.38	98.85	98.80
3	97.65	99.85	99.33

Molar ratio = 26.5.

Solvents:

1. Pyridine:EtOH:H_2O (1:2:4)
2. NH_4:EtOH:H_2O (0.1:2:4)
3. NH_4:MeOH:H_2O (0.2:2:4)

TABLE XIV. LABELLING YIELD OF ^{153}Sm-EDTMP AS A FUNCTION OF TIME AND MOLAR RATIO

Molar ratio	Time (h)			
(EDTMP/Sm)	0.5	24	72	96
6.9	90.70	91.10	90.32	90.20
17.24	91.14	92.73	91.45	90.84
23.00	96.03	97.73	96.40	95.10
26.50	97.50	98.30	97.91	96.70
40.00	98.91	98.80	98.75	98.64

pH = 7.5–8.0.

2.3.1.3. Quality control

Radiochemical purity

Radiochemical purity was assayed by:

- Whatmann 3 MM paper chromatographic system (10 × 1 cm) in different solvents:
  1. Pyridine:EtOH:H2O (1:2:4)
  2. NH4OH:EtOH:H2O (0.1:2:4)
  *3. NH4OH:MetOH:H2O (0.2:2:4)
- Sephadex C-25 (Pharmacia) column (2–3 cm height). ^{153}Sm-EDTMP was eluted with 0.9% NaCl (30 mL), and $^{153}Sm^{+3}$ and $^{153}Sm(OH)_3$ were retained in the column (Table V).
- The highest radiochemical purity (99.85%) was obtained at pH = 7.5–8.0 with solvent NH_4OH:MetOH:H_2O (0.2:2:4).
- The R_f values obtained with different solvents were presented in the Table XV. ^{153}Sm-EDTMP solution kept stable for 5 days as shown in Table XVI.

TABLE XV. PAPER CHROMATOGRAPHY SYSTEM (WHATMAN 3 MM) R_f VALUES IN DIFFERENT SOLVENTS

Solvents	$^{153}SmCl_3$	^{153}Sm-EDTMP
1. Pyridine:EtOH:H_2O (1:2:4)	0.0	0.7–0.8
2. NH_4:EtOH:H_2O (0.1:2:4)	0.0	0.8–0.9
3. NH_4:MeOH:H_2O (0.2:2:4)	0.0	0.9–1.0

TABLE XVI. RADIOCHEMICAL PURITY (%) OF ^{153}Sm-EDTMP AS A FUNCTION OF TIME (h)

0.5	24	120
98.71	98.52	98.25
99.05	97.35	98.45
97.85	98.53	97.65
98.65	98.41	97.95

* With solvent No. 3.

Radionuclidic purity

Radionuclidic impurities were determined using a gamma detector, Mod. GEM-10175-P (ORTEC). The Ortec program (MAESTRO II) was used to identify and quantify the activities of the different contaminants. The values (%) are presented in Table XVIII.

TABLE XVII. RADIOCHEMICAL PURITY BY SEPHADEX C- 25 CHROMATOGRAPHIC SYSTEM

Production (number)	Radiochemical purity (%)
1	99.9
2	99.6
3	99.4
4	98.7
5	99.5
6	99.6
7	98.7
8	99.8
9	99.7
10	99.0

TABLE XVIII. RADIONUCLIDIC IMPURITY OF ^{153}Sm-EDTMP

^{152}Eu	$t_{1/2}$ = 9.3 h (%) 6.8×10^{-4}
^{152}Eu	$t_{1/2}$ = 4865.5 d (%) 4.1×10^{-6}
^{154}Eu	$t_{1/2}$ = 3212 d (%) 8.0×10^{-6}
^{155}Eu	$t_{1/2}$ = 1810.4 d (%) 2.9×10^{-5}
^{156}Eu	$t_{1/2}$ = 15.2 d (%) 5.8×10^{-6}
^{153}Gd	$t_{1/2}$ = 242 d (%) 5.5×10^{-5}

TABLE XIX. BIODISTRIBUTION OF ^{153}Sm-EDTMP IN NORMAL RATS EXPRESSED IN % DOSE/ORGAN

Organs	Time (h)		
	2	24	72
Liver	0.49	0.58	0.33
Kidney	0.46	0.35	0.56
Lung	0.07	0.03	0.02
Spleen	0.04	0.03	0.02
Stomach	0.05	0.02	0.01
Muscle	0.13	0.10	0.01
Heart	0.01	0.01	0.01
Marrow	0.02	0.01	0.01
Femur	1.69	1.89	1.95
Blood	0.03	0.02	0.01
Skeletal	42.25	47.25	48.75

Biodistribution

The results of biodistribution studies using experimental animals (normal rats) are shown in Table XIX, at 2, 24 and 72 hours post injection.

Biological control

Micro-organisms (aerobic, anaerobic, fungus and yeast), were determined in several cultures medium: Thioglicolate (Merck), Triptone Soya Broth (Oxoid) and Sabouraud Broth 2% glucose (Merck) at room temperature and 37°C during 10 days. Pyrogen (a fever-producing bioproduct of gram-negative bacteria) was evaluated by LAL test (Limulus Amaebocyte Lysate reagent) which is the most sensitive and specific way to detect pyrogen at 37°C in 1 h.

The microbiological and Pyrogent test made in all samples showed the sterility and non-pyrogenicity of ^{153}Sm-EDTMP solution.

Hot-cell

The hot-cell of ^{153}Sm-EDTMP was finished on 22 November 1995, when we started its routine production and its delivering to hospitals and nuclear medicine centres in Brazil.

2.3.1.4. Clinical application

The routine production of ^{153}Sm-EDTMP started in November 1995. About 146 GBq was distributed to Hospital and Nuclear Clinics in São Paulo in that year. The total ^{153}Sm-EDTMP activities distributed in 1996 and 1997 were 1286 and 1247 GBq, respectively. During this period of two years approximately 860 patients were treated.

2.3.1.5. Routine production of ^{153}Sm-EDTMP

As a result of the studies carried out, a protocol of routine production is presented:

Reagents:

- 0.18M Buffer phosphate - pH7.5.
- Dissolve 0.499 g $NaH_2PO_4.2H_2O$ and 2.146 g Na_2HPO_4 in 50 mL distilled water, adjust pH to 7.5 and complete the volume to 100 mL.
- EDTMP Solution pH7.7–7.8.
- Dissolve 648 mg EDTMP in approximately 2.3 mL 2N NaOH. Add 5 mL H_2O, adjust pH to 7.7–7.8 with 2NaOH and complete the volume to 12 mL.with bidestilled H_2O.

Note: Both solutions were filtered through 0.22 μm membrane filtration and sterilized at 120°C for 20 minutes.

Labelling procedure

- Wash abundantly the burette with sterile distilled water.
- Open the quartz vial.
- Remove ^{153}Sm from the quartz vial with 3 × 1.0 mL 0.9% NaCl by heating it at 85°C and transferring it into a 15 mL glass vial.
- Check the activities of the quartz vial.
- Add EDTMP solution (648 mg/12 mL) to the vial containing ^{153}Sm.
- Stir the solution and let it stand for 30 min.
- Add 25 mL of Buffer Phosphate pH = 7.5:0.9% NaCl (6:19), then stir.
- Filter the solution through 0.22 μm membrane filter into a 50 mL evacuated vial.
- Clean the burette with sterile saline solution containing 1% benzilic alcohol, and then with distilled water.
- Sampling of:
  a) 1 mL for activity determination (radioactive concentration; MBq/mL).
  b) 2 drops for radiochemical control.
  c) 1 drop for microbiological control.
- Calculate the total activity, after filtration through Millipore, assuming 40 mL as the total volume.
- Distribution of doses according to the schedule.

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2.4. CHINA

Research Agreement No.: CPR/7372/CF

Title of the project: Optimization of the production and QC of ^{153}Sm-EDTMP

Chief scientific investigators: Jin Xiaohai, Du Jin

2.4.1. Main developments

- The reactor irradiation conditions as a function of neutron flux and target enrichment.
- The effect of target chemical forms and external volume on the specific activity of ^{153}Sm.
- The quality control of ^{153}Sm-EDTMP.
- ^{153}Sm-EDTMP stability studies in vitro and vivo.
- HPLC challenge combination analyses on the ^{153}Sm-EDTMP with BSA, mouse serum and cysteine.

2.4.1.1. Preparation of target in a different chemical form

- Low neutron flux reactor (light water swimming pool reactor ($\phi = 2–3 \times 10^{13}$ n.cm^{-2}s^{-1}).
- Liquid target ($Sm(NO_{3)3)}$.
- Solid target (coated tube of $Sm(NO_3)_3$) was prepared with a minus pressure.
- Thin slice target ($Sm(NO_3)_3$) was prepared with a hand-press machine.
- High neutron flux reactor (heavy water reactor, $\phi = 1 \times 10^{14}$ n. cm^{-2}s^{-1}).
- Solid target (enrich target or natural target).
- Solid target including thin slice target, slab target and power of Sm_2O_3.
- Moulding of Sm_2O_3 power to produce pellets, tablets or pills can be achieved by means of a hand press machine.
- $Sm(NO_3)_3$ liquid target.

  Based on the normal reaction of Sm_2O_3 and HNO_3, 100 mg of enriched target was added to a small cup, dissolved by addition of ultra pure nitric acid (1.73 mL), (1 MOL/L), heating for 5 min (100 voltage) under the little minus pressure, the solution then appears transparent, if necessary, the solution was diluted to a concentration of 30 mg Sm/mL with deionized water, and shaken well. The solution was passed through a 0.22 μm sterilization filter. Based on the requirements, the solution was dispensed into a

pyrogen-free 10 mL penicillin vial, then closed with rubber stopper and sealed with aluminium cap. The vials were autoclaved at 120°C for 30 min, after cooling down, the vials were labelled and stored for preparing target, which will be dispensed into a quartz vial and sealed with fire ready for irradiation.

- Solid target of $Sm(NO_3)_3$

Based on irradiation requirements, dispensing into the quartz vials (in a small volume parts (about 20 mg Sm_2O_3)), and then put them a minus pressure container with a suitable heating condition (here, be careful for heating and pressure, otherwise the liquid will be sprayed out, and the $Sm(NO_3)_3$ will be backed to Sm_2O_3). In this case, $Sm(NO_3)_3$ will be coated on the wall of the vial.

2.4.1.2. The optimization of reactor irradiation conditions of target

- Neutron flux as high as possible.
- Liquid target concentration is not so high.
- Irradiation time: 2 days for high flux reactor, 4–5 days for low flux reactor.
- $^{152}Sm(NO_3)_3$ coated tube target is only suitable for low flux reactor.

Two years ago, the heavy water research reactor was shut down, so most of the irradiation conditions of target were carried out with swimming pool reactor.

2.4.1.3. Effect of the amount of irradiated target, chemical forms of target on the specific activity

TABLE XX. EFECT OF THE AMOUNT OF IRRADIATED Sm_2O_3 TARGET ON THE SPECIFIC ACTIVITY OF ^{153}Sm

Amount of Sm_2O_3 (mg)	Irradiation time (d)	Theoretical yield (GBq)	Experimental yield (GBq)	Specific activity (GBq/mgSm)
20	3	161	91	5.3
50	3	410	204	4.8
80	3	642	294	4.3
150	3	1204	294	3.2
200	3	1603	433	2.9
250	3	2004	559	2.5
300	3	2409	630	2.4

TABLE XXI. EFFECT OF Sm CONCENTRATION OF LIQUID TARGET ON THE SPECIFIC ACTIVITY OF ^{153}Sm

Concentration of $Sm(NO_3)_3$ (mg/mL)	$Sm(NO_3)_3$ volume and activity (μL) and (GBq) 175 μL	75 μL	50 μL	37 μL	30 μL	Specific activity (GBq/mg Sm)
80	18.6	12.4	7.9	6.8	5.0	2.3
60	16.4	10.4	7.4	4.6	4.2	2.5
40	16.2	9.1	6.4	4.3	4.0	3.1
20	12.3	5.4	4.8	3.5	1.6	5.0
10	7.7	3.7	2.6	1.9	1.6	5.9

TABLE XXII. EFFECT OF THE SOLID AND SLICE TARGET OF THE SPECIFIC ACTIVITY OF ^{153}Sm

Form of target	Quantity of target (mg)	Irradiation time (d)	Neutron flux ($n.s^{-1}cm^{-2}$)	Yield (GBq)	Specific activity (GBq/mg Sm)
thin slice	3 × 50	5	7.6×10^{13}	1033	6.9
thin slice	3 × 100	5	7.6×10^{13}	1013	3.4
thin slice	3 × 200	5	7.6×10^{13}	996	1.7
powder	100	5	7.6×10^{13}	287	2.9
powder	200	5	7.6×10^{13}	539	2.7

TABLE XXIII. SPECIFIC ACTIVITY OF LIQUID TARGET COMPARED WITH THAT OF SOLID TARGET

Quantity of target (mg) solid/liquid	Neutron flux ($n.s^{-1}cm^{-2}$) solid/liquid	Actual yield (GBq) solid/liquid	Specific activity (GBq/mg Sm) solid/liquid
$12.7/123.2 \times 10^{13}$		16.3/61.1	1.1/2.6
3.9/1.3*	3.2×10^{13}	36.1/34.2	9.2/2.6
5.0/5.0*	3.0	3.2×10^{13}	9.9/32.8
16.5/16.0	3.2×10^{13}	13.3/41	0.8/2.6
18.3/18.0	3.2×10^{13}	13.7/47.7	0.8/2.6
$14.6/24.0.2 \times 10^{13}$		54.6/164	11/33
av. natural target	3.2×10^{13}	–	0.9/2.6
av. enriched target	3.2×10^{13}	–	11/30

* Enriched target (^{152}Sm >97.60%).

TABLE XXIV. RESULTS FROM THE DIFFERENT ANALYSIS METHODS

Method	column[1],	solvent-1	solvent-2	solvent-3	HPLC
AT[2]	5	120–150	60–80	30–40	30
R.P[3]	99.0 ± 0.1	98.0 ± 7.5	97.8 ± 2.3	98.1 ± 0.5	98.7 ± 0.0

[1]: AG-50 × 8W, 0.5 × 0.5cm, [2]: Analysis time, [3]: Radiochemical purity.

Solvent-1: pyridine/ethanol/water = 1:2:4 pH: 7-14,
Solvent-2: hydroxide ammonium/methanol/water = 0.1:2:4 pH: 11–14,
Solvent-3: hydroxide ammonium/acetone/water = 0.2:0.5:4 pH: 7–9.

2.4.1.4. Quality control of ^{153}Sm-EDTMP

- Analysis of nuclear purity.
- Analysis of radiochemical purity.
- Study on the radiochemical of ^{153} Sm-EDTMP.
- Paper chromatography of ^{153}Sm-EDTMP in solvent-3 with different pH.
- HPLC analysis of ^{153}Sm-EDTMP.
- Total samarium content measurement.
- Standard spectrum of Sm-EDTMP, EDTMP and Sm.
- Sm-Arsenazo III absorption spectrum and working curve.

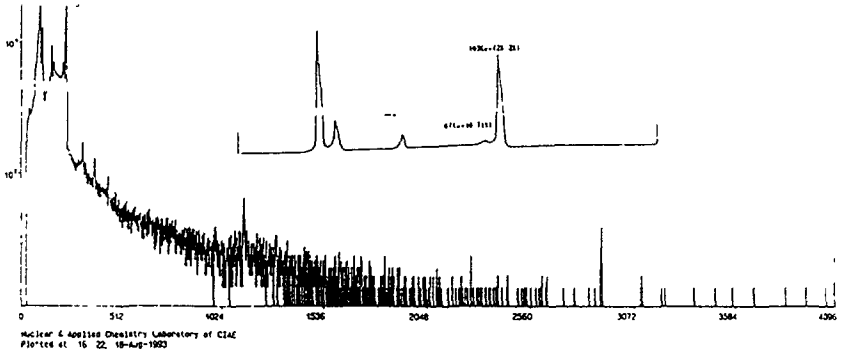

FIG. 5. Analysis of nuclear purity.

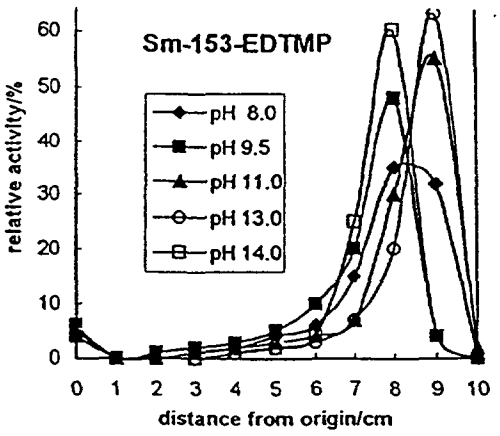

FIG. 6. Paper chromatography of ^{153}Sm-EDTMP in solvent-3.

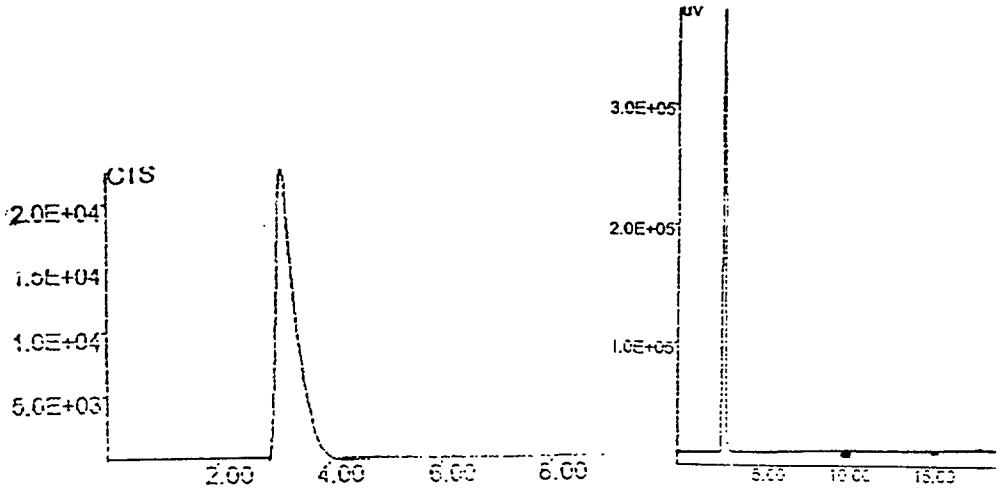

FIG. 7. HPLC chromatography of ^{153}Sm-EDTMP.

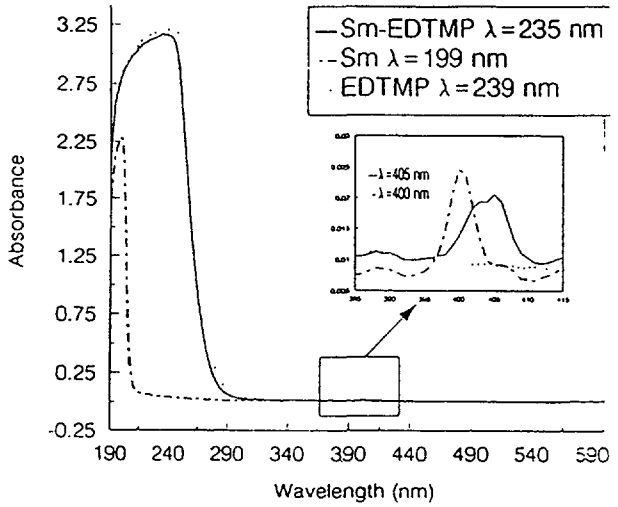

FIG. 8. Standard spectrum of Sm-EDTMP, EDTMP and Sm.

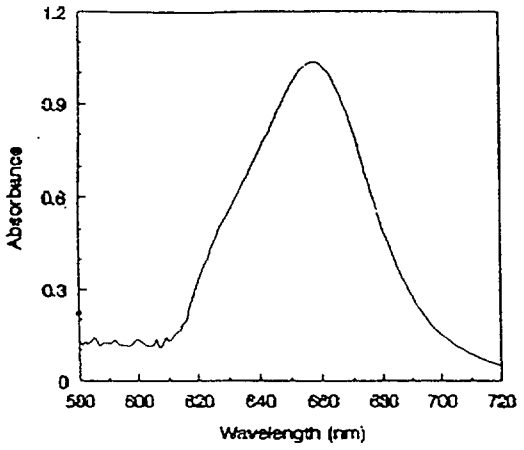

FIG. 9. Sm-Arsenazo III absorption spectrum.

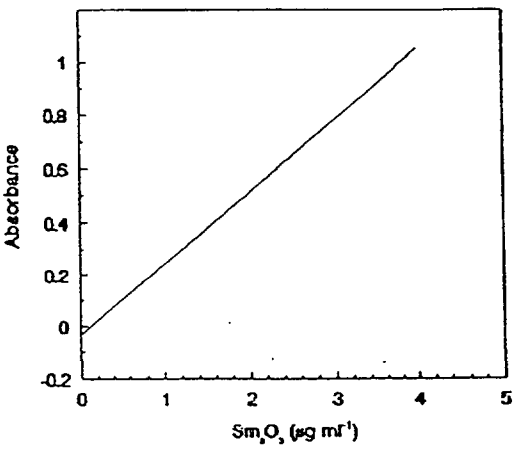

FIG. 10. Working curve

2.4.1.5. Biological control

TABLE XXV. BACTERIAL ENDOTOXIN TESTS OF ^{153}Sm-EDTMP

Freeze-dried LAL 0.1 mL	Bacterial endotoxin test water 0.1 mL	Mixture incubation at 37°C for 1 h	Use as a negative control
Plus control standard endotoxin 0.1 mL	0.1 mL	Mixture incubation at 37°C for 1 h withstand 180° inversion of the tube	Use as a positive control
Plus dilution sample of ^{153}Sm-EDTMP 0.1 mL	0.9 mL	Mixture incubation at 37°C for 1 h withstand 180° inversion of the tube	Negative or positive control

TABLE XXVI. BIODISTRIBUTION OF ^{153}Sm-EDTMP IN RATS (N = 3, % ID)

h	0.5	1.0	3.0	24	48
Blood	2.16 (0.35)	0.11 (0.03)	0.04 (0.05)	0.03 (0.05)	0.03 (0.02)
Heart	1.28 (0.43)	0.30 (0.12)	0.09 (0.02)	0.12 (0.02)	0.08 (0.05)
Lung	0.76 (0.70)	0.29 (0.02)	0.12 (0.02)	0.13 (0.06)	0.08 (0.04)
Liver	1.20 (0.43)	0.43 (0.03)	0.40 (0.07)	0.55 (0.39)	0.28 (0.06)
Spleen	0.40 (0.35)	0.13 (0.02)	0.08 (0.00)	0.10 (0.08)	0.11 (0.05)
Kidney	9.02 (3.02)	1.21 (0.05)	1.15 (0.28)	0.85 (0.06)	0.82 (0.33)
Bone	17.00 (2.21)	25.26 (3.84)	48.71 (8.00)	36.89 (10.10)	32.34 (6.96)

2.4.1.6. Stability study on ^{153}Sm-EDTMP in vitro

- Effect of the liver uptake of ^{153}Sm-EDTMP on the various molar ratios (EDTMP/Sm).
- For the enriched target: (specific activity: 500–1000 mCi/mg Sm).
- For the natural abundance target: (molar ratio = 6:1, specific activity: 20–50 mCi/mg Sm).
- Effect of molar concentration on the acidity of preparation under the identical radiation doses.
- For the enriched target.
- For the natural abundance target.
- Effect of the radiation doses on the radiochemical purity and property of preparation.
- For the natural abundance target.
- For phosphate buffer solution.
- HPLC analysis of ^{153}Sm-EDTMP under the cysteine or BSA serum challenge conditions (37°C) with ultrahydrogel 120 μm, 7.8 × 100 mm column.

- BSA challenge analysis of ^{153}Sm-EDTMP.
- Fresh mouse serum challenge of ^{153}Sm-EDTMP.
- HPLC analysis of ^{153}Sm-EDTMP in urine.
- HPLC comparative analysis of ^{153}Sm-EDTMP in serum and cysteine.
- ^{153}Sm-EDTMP instability analysis on radiation (after 2, 3 half-lives and 2 months).

TABLE XXVII. EFFECT OF THE DIFFERENT MOLAR RATIOS ON LIVER UPTAKE

No.	pH	R.P.(%)	liver uptake (% ID							
			6*	10*	20*	50*	100*	150*	200*	250*
1	7.8	99	13.4	3.6	3.0	2.8	0.50	0.21	0.16	0.10
2	7.9	99	17.1	4.1	3.1	3.1	0.48	0.19	0.15	0.20
3	8.0	99	10.3	3.7	3.5	1.9	0.44	0.13	0.16	0.18
4	7.7	99	8.8	4.2	3.2	2.7	0.21	0.18	0.14	0.15
5	.9	99	11.5	3.4	3.2	2.8	0.11	0.19	0.17	0.19

* EDTMP/Sm molar ratio.

TABLE XXVIII. LIVER UPTAKE AT 3 H POST-INJECTION OF ^{153}Sm-EDTMP

No.	Injected dose (MBq)	Liver uptake (% ID)
1	3.7	0.61
2	3.7	0.62
3	3.7	0.43

TABLE XXIX. EFFECT OF MOLAR RATIOS ON THE pH OF ^{153}Sm-EDTMP* SOLUTION

Molar ratios (EDTMP/Sm)	pH of ^{153}Sm-EDTMP			R.P. (%)		
	1(d)	2(d)	3(d)	1(d)	2(d)	3(d)
10:1	8.2	7.1	6.8	99	99	99
40:1	8.0	7.3	6.9	99	99	99
60:1	8.1	7.4	6.9	99	99	98
100:1	7.9	7.4	7.2	99	99	98
200:1	8.0	7.3	7.1	99	99	99

* The pH determined with acidimeter.

TABLE XXX. EFFECT OF MOLAR RATIOS ON THE pH OF ^{153}Sm-EDTMP SOLUTION

Molar ratios	Concentration of Sm (mg/mL)	Concentration of EDTMP (mg/mL)	pH of ^{153}Sm-EDTMP solution 1(d)	2(d)	3(d)
1:1	0.86	2.50	8.0	7.8	7.8
3:1	0.86	7.50	8.1	7.9	7.8
5:1	0.86	12.50	8.0	7.9	7.7
6:1	0.86	15.00	7.9	7.8	7.8
10:1	0.86	25.00	7.9	7.8	7.8

TABLE XXXI. EFFECT OF TOTAL RADIATION DOSES ON THE pH AND R.P. OF ^{153}Sm-EDTMP

Total activity (GBq)	Total doses (Gy/h)	pH changes 1(d)	2(d)	R.P. (%) 1(d)	2(d)	Property of solution
1.2	5.43	8.1	8.0	99	99	colourless
4.0	18.30	8.0	7.9	99	99	colourless
12.0	54.30	7.9	7.2	99	99	light brown
24.0	109.00	8.1	6.9	99	99	deep brown
36.0	163.00	8.0	6.4	99	88	deep brown

TABLE XXXII. EFFECT OF TOTAL IRRADIATED DOSES ON THE pH AND R.P. OF ^{153}Sm-EDTMP (ENRICHED TARGET) IN THE PHOSPHATE BUFFER SOLUTION* MEDIUM

Total irradiated dose (Gy/h)	pH change 1(d)	2(d)	3(d)	R.P. (%) 1(d)	2(d)	3(d)
5.4	7.5	7.5	7.5	99	99	99
18.3	7.5	7.5	7.5	99	99	99
54.3	7.5	7.5	7.5	99	99	99
109.0	7.5	7.5	7.5	99	99	99
163.0	7.5	7.5	7.5	99	99	99

* pH = 7.5, 0.18 MOL/L phosphate buffer solution, molar ratio = 100:1.

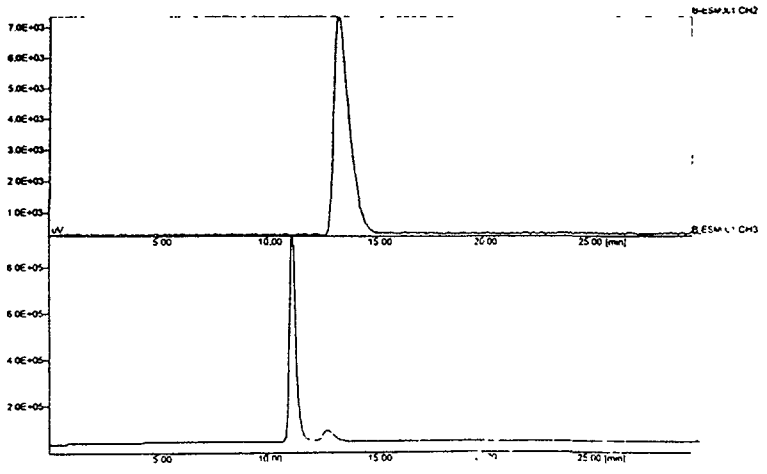

FIG. 11. BSA challenge analysis of ^{153}Sm-EDTMP.

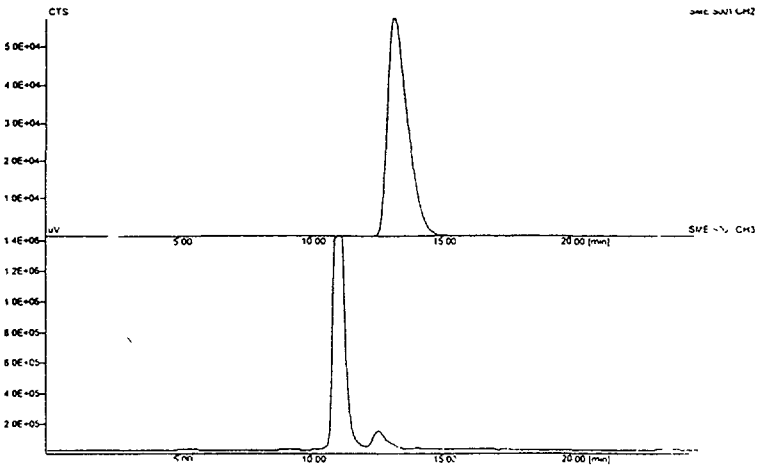

FIG. 12. Fresh mouse serum challenge of ^{153}Sm-EDTMP.

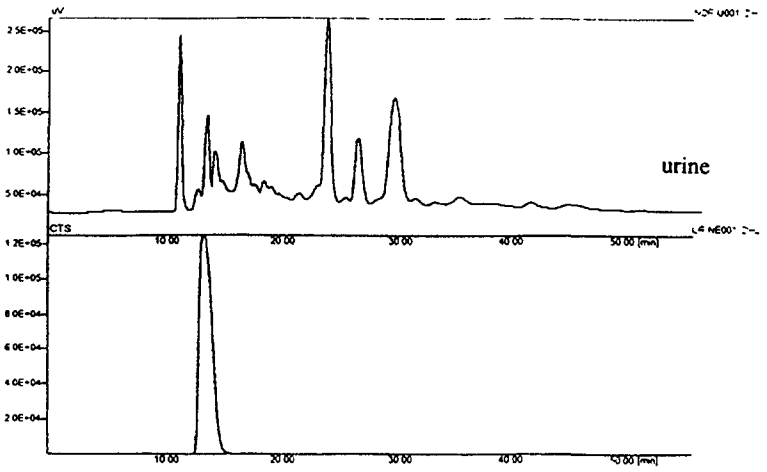

FIG. 13. HPLC analysis of ^{153}Sm-EDTMP in urine.

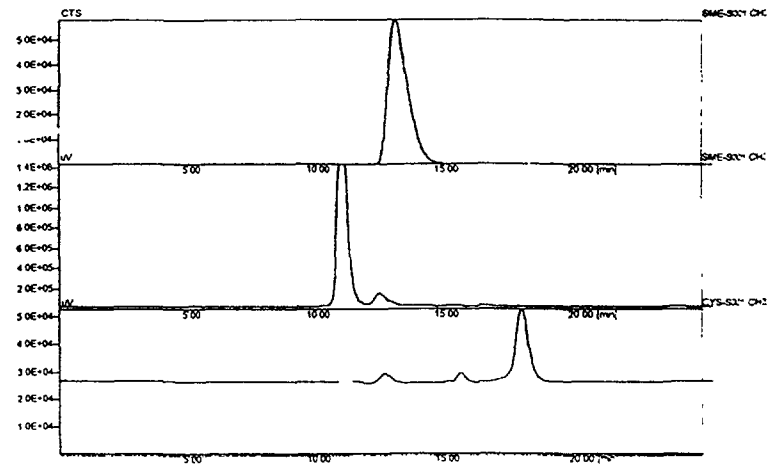

FIG. 14. HPLC comparative analysis of ^{153}Sm-EDTMP in serum and cysteine.

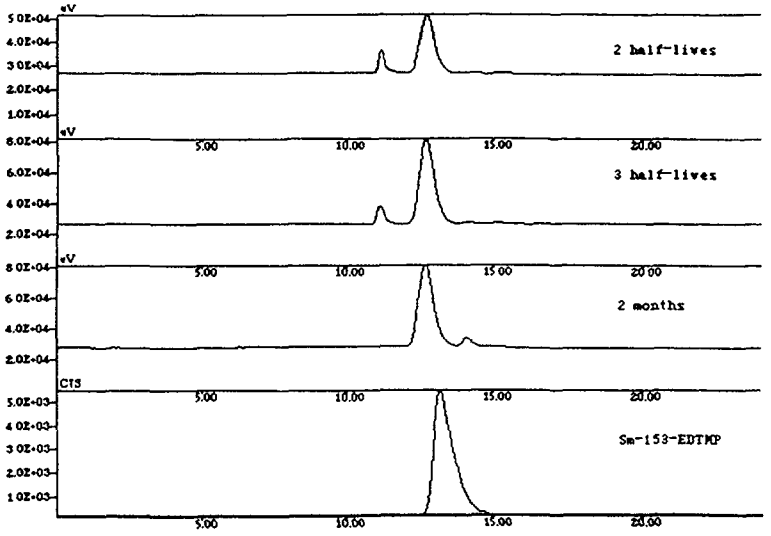

FIG. 15. ^{153}Sm-EDTMP instability analysis on radiation (after 2, 3 half-lives and 2 months).

2.4.1.7. Conclusion

- Based on our experiment, the liver uptake level was acceptable for natural target. S.A: 1.11–3.7 GBq/mg Sm, when the molar ratio of EDTMP/Sm was equal to 6:1, the result of ECT imaging was also excellent.

- In order to obtain the same excellent ECT imaging for enriched target (^{152}Sm > 98%, S.A: 18.5–37 GBq/mg Sm), the molar ratio was more than 80.

- Under the same irradiated doses, not only the pH of solution was affected by total irradiation doses, but also affected by molar ratio for the enriched target, although no any R.P. change was observed and for natural target, the same situation were existed, the change of pH was not so big.

- The phosphate buffer can reduce pH resulting from irradiation doses.

- The more radiation concentration increasing, the more radiation decomposition was observed.

- Instability of EDTMP (pH = 8.0–8.4) may be resulted from autoclave (at 120°C for 30 min).

- Incubation challenge tests of ^{153}Sm-EDTMP with BSA, cysteine and serum (at 37 ± 0.5°C for 4 h, molar ratio: 100:1) demonstrated that ^{153}Sm-EDTMP was very stable, no any changes were observed.

- Serum can bound with $^{153}SmCl_3$ under the incubation, but cysteine cannot.

- A much more heavy weight molecular than EDTMP appeared post-autoclave, but it was not stable , several days later, it will disappear, a light weight molecular than EDTMP resulted from radiation degradation.

2.4.2. Co-operation with participating laboratories

- Nuclear Chemistry Division, Pakistan Institute of Nuclear Science & Technology.
- Radioisotope Production Center, PPR-BATAN, Kawasan Puspiptek.

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2.5. FINLAND

Research Agreement No.:	FIN/7373/CF
Title of the project:	Optimization of the production and quality control of radiotherapeutic radionuclides and radiopharmaceuticals
Chief scientific investigator:	J. Hiltunen
Contributors at MAP:	P. Penttilä, J. Haukka, T. Nikula

2.5.1. Main developments

Since MAP has produced ^{153}Sm-EDTMP routinely since 1990, there was no need within this CRP to perform basic development for production of radioisotope, or development of radiolabelling and product formulation. However some product optimization has been performed.

MAP is not having its own reactor, nor it has larger hot cells attached to reactor site. For this reason we focused our efforts to improve the handling of irradiated targets. Firstly we wanted to get rid of handling of dry $^{152}Sm_2O_3$, which is difficult to dissolve. $^{152}Sm_2O_3$ was converted to nitrate form since it is more easily dissolved after irradiation. Conversion is made by dissolving the oxide to small amount of strong nitric acid, diluted and then it is dispensed to 9 and 12 mg targets quartz glass ampoules. The liquid is evaporated to dryness with gentle heating and nitrogen flow. The dried ampoules are closed by welding.

We had to change the reactor used for ^{153}Sm production and therefore we tested different reactor conditions and times of production according the following Table. The last three all used depending of their operational hours.

Neutron flux	Irrad time	Sm-EDTMP prep	Spec act at prod (GBq/mg)
1.0×10^{13} n.s^{-1}.cm^{-2}	15 h	immediate	1.5
2.0×10^{13} n.s^{-1}.cm^{-2}	40 h	24 h	3.0
1.5×10^{14} n.s^{-1}.cm^{-2}	10 h	24 h	4.0
1.5×10^{14} n.s^{-1}.cm^{-2}	20 h	70 h	4.0

Production protocol for ^{153}Sm-EDTMP

The production is done weekly usually on Tuesdays.

- 9 mg of enriched ^{152}Sm as nitrate is irradiated to get 16 to 50 GBq (at production time).
- Target dissolved to 1 mL of isotonic saline (pH ca. 4).
- The above solution added to 180 mg of EDTMP solution (10.5 mL buffered to pH 7.5).
- Incubation 30 to 40 minutes at room temperature.

- 5 mL of pH7.5 phosphate buffer (0.18 M) added and saline added to make 0.5 GBq/mL at time of calibration.
- Sterile filtration through 0.22 μm membrane.
- Dispensed according orders to sterile vials to
- Autoclaving.

Quality control for ^{153}Sm-EDTMP

Sterility and apyreginicity is tested routinely. Sterility is tested with thioglycollate and casein culture medias with incubation samples in 37°C pH is checked of the final QC sample, and it should be 7.5. Radiochemical purity is verified with Sephadex C-25 system explained in detail in Table 4 in Chapter 3. Additionally since MAP buys the irradiated material from external source we make a positive identification of ^{153}Sm with gammaspectroscopy.

^{166}Ho-FHMA production

Along with the goals of this CRP, MAP has chosen ^{166}Ho-FHMA as a product for testing since our previous good experience with ^{165}Dy-FHMA and because ^{166}Ho shows more ideal properties for synoviorthesis than ^{153}Sm. Production method was developed and optimized for ^{166}Ho-FHMA (holmium-166 ferric hydroxide macro aggregate) including quality control methods.

1. Target solution (for 5 patient doses)

   - dissolve in 3% nitric acid if solid target;
   - take 1.1 mL of (30 mg/mL) ^{166}Ho solution;
   - add 3.5 mL (4 mg/mL of Fe) $FeSO_4$ solution.

2. Preparation of doses

   - place 1.1 mL NaOH (0.4 N) and 2.2 mL isotonic saline into centrifuge tubes (test tubes);
   - take 0.9 mL of target solution and add to tubes each representing one patient dose;
   - add 1.1 mL polyvinyl pyrrolidone (PVP; 16 mg/mL) into each tube;
   - mix thoroughly and centrifuge with 1400 rpm for 3 min;
   - discard the supernatant;
   - add 6 mL of pH8.5 PVP (16 mg/mL), mix thoroughly and centrifuge with 1200 rpm for 3 min;
   - discard the supernatant, add 6 mL of isotonic saline, mix thoroughly, and centrifuge at 1200 rpm for 3 min;
   - discard the supernatant and add wanted volume of isotonic saline to the tube and transfer it to vial;
   - sterilize closed vial by autoclaving 20 min at 120°C.

Quality control for ^{166}Ho-FHMA

- Check of pH of the final solution (should be less than 9).
- Check of sterility with culture media test tubes.
- Radiochemical purity check: TLC with saline or EDTA solution

or

make one extra dose and after thorough centrifugation take a 3 mL (of 6 mLs of liquid) sample of supernatant carefully (to avoid contamination from suspension) and measure the activity in sample, multiply by factor of two, and measure the activity of suspension compare the measured activities:
radiochemical purity = P
activity of suspension minus activity of supernatant sample = S
activity of supernatant 2 = I

$$P\,\% = \frac{S}{I+S} \times 100\%$$

- or use 0.22 μm sterile filter for a small sample with suspension.
  Measure the total activity and measure the filtered activity.

In vitro and in vivo testing of ^{166}Ho-FHMA

The particle size distribution was tested by filtering the prepared product through set of different porosity filters starting. The batch-to-batch consistent particle size distribution is shown below. However in reality the particle size is slightly bigger since filtering requires some pressure and it breaks some particles

Part. size distribution (μm)	<1.2	1.2–3	3–5	5–8	8–12	>12
%	0	7	45	30	13	5

The particle size distribution remains constant for at least four days after preparation, as well as radiochemical purity.

The in vivo biodistribution studies were made with six rabbits. 60–78 MBq were injected into left knee joint of the animal. Two were sacrificed after 24 h p.i., two at 48 h p.i. and two at 72 h p.i.. The animals were kept in metabolical cages. The vital organs were counted at well type counter. Only kidneys and urine showed clear increase in activity. The leakage is presented below (as % of injection dose):

	24 h p.i.	48 p.i.	72 h	Total
Urine	0.002	0.026	0.023	0.051
Kidneys	0.001	0.012	0.018	0.026
Total				0.077

The results showed extremely good retention in the knee. Thus we entered to clinical patient therapies.

2.5.2. New contributions

Important new clinical data with the use of ^{153}Sm-EDTMP for treating bone lesions caused by osteosarcoma and metastasized cancers was collected. Our clinical partners in Kuopio University Hospital, Tampere University Hospital and Radiumhospital in Oslo, Norway have particularly contributed to the new data. New clinical data is suggesting the usefulness of this radiopharmaceutical also in bony tumour regression therapy. These findings will be further studied in separate clinical trial.

Well documented and clinically proven production protocol were submitted to many other centres for development and production of ^{153}Sm-EDTMP.

The tested and optimized production method with relevant quality control measures was developed for ^{166}Ho-FHMA.

The first in vitro and in vivo animal biodistribution testing of ^{166}Ho-FHMA was performed and reported. Additionally first ever patient treatments have been performed with haemophiliac patients with excellent initial results.

2.5.3. Co-operation with participating laboratories

A collaboration by changing information has been with practically all institutions, which have participated the program, accumulated common knowledge to every participant. This is clearly recognized.

Particularly at IPEN in Brazil ^{166}Ho-FHMA in vitro and animal biodistribution has been produced together. Also there was collaboration on production of ^{153}Sm-HA (hydroxy apatite).

We offered our facility to visits and joint development with Thai, Chinese and Greek scientists.

Reports and Publications

AHONEN, A., et al., ^{153}Sm-EDTMP in bone metastases, J. Nucl. Biol. and Med. **38** Suppl. 1 (1994) 123–127.

PENTTILÄ, P., MÄKELÄ, O., HILTUNEN, J., HAUKKA, J., KANCKOS, S., KOMULAINEN, R., Animal Biodistribution and Initial Human Experience of ^{166}Ho-FHMA for Radiosynoviorthesis, III Nordic Congress of Nuc. Med., 11–12 June 1998.

2.6. GREECE

Research Agreement No.:	GRE 8357/CF
Title of the project:	Synthesis and radiolabelling with ^{153}Sm and ^{186}Re of bone seeking agents as therapeutic radiopharmaceuticals
Chief scientific investigator:	E. Chiotellis
Collaborators:	I. Pirmettis, P. Bouziotis, A. Varvarigou

The original goals of the project were the optimization of production protocols and quality control procedures of ^{153}Sm-EDTMP.

2.6.1. Main developments

The program was focused on the following:

- Production and quality control of ^{153}Sm-EDTMP for medical use.
- Establishment of the quality control method, which is validated for analyzing $^{153}Sm^{3+}$, ^{153}Sm-EDTMP, $^{153}Sm_x(OH)_y$.
- Synthesis and biological evaluation of other ^{153}Sm-phosphonate complexes.

2.6.1.1. Production and quality control of ^{153}Sm-EDTMP for medical use

Synthesis of EDTMP

The synthesis of the ligand EDTMP is accomplished according to Moedritzer and Irani, with minor modifications, as follows:

In a suitable reaction vessel equipped with a magnetic stirring bar and dropping funnel were added phosphorous acid (33.66 g) and degassed water (35 mL). Dissolution of the phosphorous acid was achieved by stirring, and then concentrated hydrochloric acid (33.44 g) was added. The dropping funnel was charged with ethylenediamine (5 g) and which was added in a dropwise fashion to the acidic solution. When addition was complete, the solution was refluxed for one hour. After the one hour reflux period, solid paraformaldehyde was added, in small increments, over a one hour period with continued heating to maintain reflux during addition. After the completion of the addition of the formaldehyde, reflux was maintained for an additional two hours. After overnight cooling at room temperature, the reaction mixture was added, in very small quantities, to an excess of absolute ethanol. Vacuum filtration followed by washing with cold ethanol and dry ether gave ethylenediamine tetramethylenephosphonic acid.

Investigation of the purified product showed all findings (melting point, IR, NMR, elemental analysis) according to literature.

Preparation of EDTMP solution (150 mg/10.5 mL)

(1) A 2N NaOH solution is prepared by dissolving 8 g NaOH in 100 mL water for injection.

(2) 3.57 g EDTMP are weighed in a beaker.

(3) 200 mL water for injection are added.

(4) 2N NaOH is added until pH7.5.

(5) The solution is transferred to a 250 mL volumetric flask, and water is added to the mark.

(6) The solution is sterilized by filtration through a 0.22 μm sterile and pyrogen-free filter membrane, and distributed in sterilized 30 mL vials. Each vial contains 10.5 mL of EDTMP (150 mg) solution.

(7) The vials are capped with rubber septums and sealed with aluminum capsules.

(8) The vials are autoclaved at 121°C for 20 min.

(9) The vials are labelled and samples are sent for quality control.

Expiration Date: 12 months after production date.

Preparation of Sm-153

(1) 52.2 mg of enriched (98.7%) Sm-152 oxide are weighed into a 10 mL volumetric flask.

(2) 1.5 mL 4N HNO_3 is added to dissolve the $^{152}Sm_2O_3$.

(3) Water is added to 10 mL.

(4) The solution is sterile filtered, capped, capsulated and autoclaved.

(5) Either 0.1 or 0.2 mL of the solution are placed in a quartz ampoule, and then the ampoule is sealed (each target contains either 3 or 6 mg ^{152}Sm) and welded in an aluminum can.

(6) The can is irradiated in a 5.0 MW reactor, at a flux of 4.81×10^{13} neutron.cm^{-2}.s^{-1}. Irradiations were carried out for 4 h, where a 3 mg sample of enriched Sm yielded 162 mCi ^{153}Sm (specific activity 54 mCi/mg, 86% yield).

(7) After cooling, the can is opened in a hot cell. The quartz vial is removed and transferred to a glove box, where the tip is broken.

(8) The solution of ^{153}Sm is diluted and transferred to a sterile glass vial. One to two mL of saline are added to ensure the quantitative recovery of the ^{153}Sm from the quartz ampoule.

Preparation of ^{153}Sm-EDTMP

The resulting ^{153}Sm solution is transferred to a 10.5 mL solution of EDTMP containing 150 mg EDTMP. After 30 minutes of incubation, 2 mL of pH7.5, 0.18 M phosphate buffer is added to the preparation. The whole solution is filtered by a 0.22 μm sterile filter and dispensed into sterile vials. A sample of 1.0 mL is dispensed into a vial for quality control.

2.6.1.2. Quality control

Radiochemical control methods

The following solutions were prepared:

- ^{153}Sm at pH1–3 (free samarium). This solution was either the target solution or drops from the target solution diluted with saline and acidified by HNO_3 if required.
- ^{153}Sm at pH7.5 (samarium hydroxides). This solution was prepared by the addition of PBS buffer 0.18M pH7.5 to a sample of target solution. (Note: a white gelatin precipitate was formed a few hours later.)
- ^{153}Sm-EDTMP (pH7.5). The solution from the standard preparations.

The examined chromatographic systems and the behavior (R_f) of each form of samarium are presented in Table XXXIII.

TABLE XXXIII. CHROMATOGRAPHIC METHODS FOR THE QUALITY CONTROL OF ^{153}SM-EDTMP

Chromatographic system	Eluent	153Sm at pH <3	153Sm at pH7.5	153Sm-EDTMP (pH7.5)
COLUMN C-25	Saline	retain	retain	pass
Millipore 0.22 μm	Saline	pass	retain	pass
Whatman no. 1	NH_4:MEOH:H_2O 2:20:40	0	0	0.9–1
Whatman no. 1	NH_4:H_2O 1:25	0	0	1
Whatman no. 3	NH_4:MEOH:H_2O 2:20:40	0	0	0.8–1
Whatman no. 3	NH_4:ACETONE:H_2O 2:10:30	0	0	1
Whatman no. 3	NH_4:H_2O 1:25	0	0	0.9–1
ITLC-SG	Water	0–0.5	0	1
ITLC-SG	NH_4:H_2O 1:25	0	0	1
ITLC-SG	Acetone	0.5–1	0	0
ITLC-SG	Acetone:HNO_3 10:1	1	split	0
TLC-SG	NaOH 0.2N	—	—	0.9–1
TLC-SG	NH_3:MEOH:H_2O 2:20:40	—	—	1

The samarium at pH7.5 remains in the origin of any of the tested chromatographic systems. It is also retained on the C-25 column (>99.5%) and the 0.22 μm membrane filter (≈90%). This behaviour of samarium at pH7.5 is very important, because this is the final pH of the production of ^{153}Sm-EDTMP. Thus, in case where the labelling yield is not high most of the samarium impurities (hydroxides) will be removed during the sterilization by the 0.22 μm sterile filter.

The efficiency of the C-25 column was checked and it was found that the column binds completely the free samarium as well as the hydroxides.

The chromatography on Whatman paper showed that the samarium at pH<3 or at pH7.5 remains in the origin, while ^{153}Sm-EDTMP migrates in the front with any of the three developing solvents (Table XXXIII).

The ITLC-SG/Water chromatography showed a migration of free samarium with R_f of 0–0.5 (most of the activity at the origin). The hydroxides also remain at the origin while the ^{153}Sm-EDTMP ascends with the eluent.

The separation between free samarium and samarium hydroxides was achieved by the ITLC-SG/Acetone system. This system gives a $R_f = 0$ for hydroxides and a $R_f = 0.5–1$ for free Sm. With this system the ^{153}Sm-EDTMP remains at the origin.

A clear $R_f = 1$ for free Sm and $R_f = 0$ for ^{153}Sm-EDTMP was measured by ITLC-SG/Acetone:HNO_3 10:1 system, but with this system a reliable R_f for hydroxides was not measured. Usually a splitting of the activity on the ITLC was observed (probably due to the formation of free Sm from the action of HNO_3 on the hydroxides during the chromatography).

Stability of the final product

Stability tests for one week were carried out for one week using either Whatman no. 1 or ITLC-SG eluted with NH_4:H_20 1:25. One can see high radiochemical purity (>97%) for at least one week (Table XXXIV).

TABLE XXXIV. QUALITY CONTROL — STABILITY OF ^{153}SM-EDTMP

Time after preparation	Whatman no. 1 NH_4:H_20 1:25	ITLC-SG NH_4:H_2O 1:25
1 h	99.0	99.1
24 h	98.9	99.3
48 h	99.4	99.2
72 h	99.5	98.8
144 h	98.6	97.1

Microbial testing

(a) Sterility:
(i) Thioglycolate medium.
(ii) Soybean-Casein digest medium.

(b) Pyrogenicity:
Limulus Amoebocyte Lysate (LAL) test.

Biodistribution studies

The in vivo biodistribution of the ^{153}Sm-EDTMP was evaluated in mice, with particular focusing on blood clearance, urine elimination and liver and bone uptake (Table XXXV).

The biodistribution study showed that the complex is localized in the skeleton (46 and 43% at 2 and 4 hours), while the remaining activity was excreted via the urinary system. No significant activity was to be found elsewhere.

2.6.1.3. Clinical trials

Pilot production of ^{153}Sm-EDTMP has begun and the radiopharmaceutical is available upon request. Clinical trials are not yet under way, since both the Nuclear Medicine Departments of the collaborating hospitals, as well as N.C.S.R. «Demokritos» are awaiting the

TABLE XXXV. BIODISTRIBUTION OF ^{153}SM-EDTMP IN MICE AT 2 AND 4 HOURS P.I (% DOSE PER ORGAN)

Organ	2 hours	4 hours
Blood	0.05 ± 0.03	0.01 ± 0.00
Liver	0.77 ± 0.03	0.48 ± 0.13
Heart	0.02 ± 0.00	0.01 ± 0.00
Kidneys	0.46 ± 0.07	0.40 ± 0.06
Stomach	0.11 ± 0.04	0.30 ± 0.13
Intestines	0.32 ± 0.04	0.43 ± 0.05
Spleen	0.01 ± 0.00	0.00 ± 0.00
Muscle	0.24 ± 0.10	0.33 ± 0.01
Lungs	0.03 ± 0.00	0.03 ± 0.00
Bone	46.37 ± 1.67	43.66 ± 1.02
Urine	49.81 ± 1.87	52.95 ± 1.42

final approval which is to be granted by the Greek National Drug Organization. The data which will be gathered from the clinical trials will be used for the marketing authorization of the product in Greece.

2.6.1.4. Further work

An attempt was made to investigate the biodistribution of two other phosphonate chelates determine whether the ^{153}Sm-EDTMP distribution profile could be improved.

Synthesis of tetraazacyclododecane tetramethylene phosphonic acid (DOTMP)

The ligand is prepared according to Lazar, et al., with a slight modification regarding the acquisition of the final product, as follows:

2.5 g of 1, 4, 7, 10- tetraazacyclododecane and 4.46 g of anhydrous phosphorous acid were dissolved in 4.76 mL H_2O, and 1.73 mL concentrated hydrochloric acid were added. The mixture was heated to reflux, and then 1.23 g dry, solid paraformaldehyde were added in small quantities over a one hour period. After addition of the paraformaldehyde, the mixture was refluxed for an additional hour. The product does not precipitate upon cooling the reaction mixture to room temperature, so a method similar to the one used for the acquisition of EDTMP is employed. Specifically, the reaction mixture was added in very small quantities to an excess of absolute ethanol, with vigorous stirring, and we finally managed to get a precipitate, which was identified as being DOTP, with IR, NMR and elemental analyses.

Preparation of ^{153}Sm-DOTMP

0.3 mL of ^{153}Sm solution (7.5 mCi/0.225mg Sm^{3+}) added to an alkaline solution of DOTMP, containing 15 mg DOTMP (0.027 mmol). After 30 minutes of incubation, 1 mL of pH7.5, 0.18 M phosphate buffer is added to the preparation. The whole solution is filtered by a 0.22 μm sterile filter and dispensed into a sterile vials.

Synthesis of Triethylenetetramine Hexamethylene Phosphonic Acid (TTHMP)

A Mannich-type reaction is employed to synthesize the ligand, as described by Moedritzer and Irani, with minor modifications regarding the synthesis and acquisition of the product, as shown below:

A quantity of 8.8 g of triethylenetetramine was added in a dropwise fashion to 33.59 g of phosphorous acid dissolved in 36 mL degassed water, after the addition of 40.32 mL concentrated HCl. After the addition of the amine, the solution was refluxed for one hour. At the end of the one hour reflux period, 11.34 g solid paraformaldehyde was slowly added over a one hour period, maintaining reflux. After all the HCHO is added, the reaction mixture is stirred under reflux for an additional two hours, then allowed to cool. After cooling, the reaction mixture was added, in very small quantities, to an excess of absolute ethanol. Excess absolute ethanol precipitated the product from the reaction solution.

TABLE XXXVI. QUALITY CONTROL — STABILITY OF ^{153}SM-DOTMP AND ^{153}SM-TTHMP

	^{153}Sm-DOTMP		^{153}Sm-TTHMP	
	Whatman no. 1 NH_4:H_2O 1:25	ITLC-SG NH_4:H_2O 1:25	Whatman no. 1 NH_4:H_2O 1:25	ITLC-SG NH_4:H_2O 1:25
1 h	99.1	98.2	97.2	97.3
24 h	99.1	97.9	98.7	97.5
48 h	99.3	98.7	98.9	97.0
72 h	99.4	98.9	99.0	96.5
144 h	99.6	99.7	92.2	95.2

TABLE XXXVII. BIODISTRIBUTION OF ^{153}SM-DOTMP AND ^{153}SM-TTHMP IN MICE AT 2 AND 4 HOURS P.I. (% DOSE PER ORGAN)

	^{153}Sm-DOTMP		^{153}Sm-TTHMP	
	2 hours	4 hours	2 hours	4 hours
Blood	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Liver	0.08 ± 0.00	0.09 ± 0.00	1.66 ± 0.41	1.50 ± 0.18
Heart	0.01 ± 0.00	0.00 ± 0.00	0.03 ± 0.00	0.01 ± 0.00
Kidneys	0.67 ± 0.14	0.49 ± 0.04	1.46 ± 0.72	0.53 ± 0.04
Stomach	0.06 ± 0.00	0.05 ± 0.03	0.08 ± 0.01	0.15 ± 0.10
Intestines	0.34 ± 0.12	0.68 ± 0.07	0.32 ± 0.02	0.48 ± 0.11
Spleen	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Muscle	0.39 ± 0.14	0.28 ± 0.10	0.93 ± 0.09	0.35 ± 0.14
Lungs	0.03 ± 0.00	0.01 ± 0.00	0.05 ± 0.02	0.03 ± 0.00
Bone	39.13 ± 1.42	38.24 ± 1.42	43.76 ± 1.77	42.05 ± 0.60
Urine	57.31 ± 1.82	58.79 ± 0.67	49.70 ± 1.52	53.36 ± 0.59

Preparation of ^{153}Sm-TTHMP

0.3 mL of ^{153}Sm solution (9.5 mCi/0.285mg Sm^{3+}) added to an alkaline solution of TTHMP, containing 10 mg TTHMP (0.014 mmol). After 30 minutes of incubation, 1 mL of pH7.5, 0.18 M phosphate buffer is added to the preparation. The whole solution is filtered by a 0.22 μm sterile filter and dispensed into a sterile vial.

Stability of the final products

Stability tests were carried out for one week using either Whatman no. 1 or ITLC-SG eluted with NH_4:H_20 1:25. One can see high radiochemical purity (>97%) for at least one week (Table XXXVI).

Biodistribution studies of ^{153}Sm-DOTMP and ^{153}Sm-TTHMP

Biodistribution studies were performed on mice. The results obtained (Table XXXVII) indicate that both products show skeletal concentration comparative to ^{153}Sm-EDTMP, with low liver uptake and elimination through the renal system.

2.6.1.5. Co-operation with participating laboratories

MAP Medical Technologies Oy, Finland. Provided training at their facilities for the production of ^{153}Sm-EDTMP and other radiopharmaceuticals (IAEA Fellowship GRE/97001P).

Publications

CHIOTELLIS, E., PIRMETTIS, I., BOUZIOTIS, P., VARVARIGOU, A., "Preparation and experimental evaluation of a series of samarium-153 complexes" (presentation at the 1997 European Association of Nuclear Medicine Congress, Glasgow), Eur. J. Nucl. Med. **24** 8 (1997) 932.

BOUZIOTIS, P., et al, Initial assessment of Sm-153-TTHMP as a bone palliation agent, Eur. J. Nucl. Med. (in press).

2.7. INDONESIA

Research Contract No.:	INS/8589/RB
Title of the project:	Synthesis and evaluation of ^{153}Sm-ethylenediamine tetramethylene phosphonate (EDTMP) bone pain palliative agent and ^{153}Sm-hydroxyapatite, albumin microspheres and ferric hydroxide macroaggregates
Chief scientific investigator:	S.R. Tamat
Other Investigators:	W. Wydiastuti, B. Purwadi, Djoharly, L. Indriastuti, Y. Musdja

The original goals of the project were as follows:

- Optimization of the neutron irradiation with the 30 MW nuclear reactor using natural and enriched targets of samarium.
- Development of target radiochemical and quality control procedures of ^{153}Sm.
- Optimization of the synthesis, purification and characterization of ethylenediamine tetra-methylene phosphonic acid (EDTMP).
- Development of EDTMP radiolabelling procedures with ^{153}Sm, and establishment of quality control method, and animal biodistribution studies.
- Optimization of the synthesis of hydroxyapatite, human serum albumin microspheres and ferric hydroxide macroaggregates, labelling with ^{153}Sm and purification.
- Improvement of quality control methods based on chromatography system.

2.7.1. Main developments

2.7.1.1. Irradiation of Sm_2O_3

- 1 g of natural Sm_2O_3 was placed in a small screw capped aluminum can, and then irradiated using an irradiation can in the centre thimble of the TRIGA MARK II 1 MW reactor with 1.8×10^{13} n.cm^{-2}.s^{-1} for 60 h. The irradiated target was dissolved in 1 mL of 1N HCl solution and then diluted to 5 mL or 10 mL with water. Average specific activity produced was 0.35 GBq/mg Sm. Diluted solution was used in experiments.
- 100 mg of natural Sm_2O_3 or 5–10 mg of enriched Sm_2O_3 (98.7% ^{152}Sm) in an aluminum foil was placed in an irradiation can, then was irradiated in 30 MW RSG-GAS reactor with 1.2×10^{14} n.cm^{-2}.s^{-1} for 100 h yielded about 10 GBq/mg Sm from the natural, and of 40 GBq/mg Sm from the enriched targets. Similarly, the irradiated target was dissolved in 1 mL of 1N HCl solution and diluted to 5 mL with water. Diluted solution was used in experiments.

2.7.1.2. Preparation, quality control and evaluation of ^{153}Sm-EDTMP

- EDTMP has been synthesized by modified Mannich reaction with an average yield of 44.4%. However, a commercial product from TCI (Japan) was later successfully used.
- A required radioactivity of $^{153}SmCl_3$ solution in 0.1 N HCl was adjusted to pH4.0 with 0.5 M NaOH solution. A carrier solution was occasionally used containing 5 mg of natural Sm_2O_3 in 10 mL of 0.1N HCl solution.
- Maximum of 1 mL of the above $^{153}SmCl_3$ solution was slowly added while stirring into a solution of 75 mg EDTMP in 0.5 M NaOH which was adjusted to pH8.0. The ^{153}Sm-EDTMP product was purified through 300 mg of Chelex-100 resin filled in a 2.5 mL syringe. However, purification was usually not necessary to produce pure compound. Preparation of ^{153}Sm-EDTMP with EDTMP:Sm molar ratio of 250:1 yielding a final volume activity of 0.5 GBq/mL.
- The product was then sterilized by membrane filtration.
- The radiochemical purity of ^{153}Sm-EDTMP was determined by TLC on cellulose sheets with pyridine:ethanol:water (1:2:4) as solvent. R_f of ^{153}Sm-EDTMP = 0.85 and of ionic ^{153}Sm = 0, and no other radioactive spot on the TLC. The radiochemical purity was determined also using 300 mg of Chelex-100 column.
- Biodistribution studies in 20–25 g Swiss white mice were carried out by intravenous injection of 0.2 mL (9.25 MBq) of ^{153}Sm-EDTMP solution. Mice were sacrificed after a certain time post injection and organ tissues were collected. Radioactivity in tissues were counted and calculated against a standard. Bone uptake was highest (55% ID) at 2 h post injection, then decreased slowly over 24 h to 47% ID.
- Clinical trial involving 12 terminally ill patients in three hospitals was performed.

Typical formulation of 153Sm-EDTMP for hospital use

Radioactive concentration:	7.4 GBq/mL on Friday 9.00 a.m.
Total activity:	7.4 GBq
EDTMP concentration:	3.31 mg/mL
Sm_2O_3 content:	0.143 mg/mL
Final pH:	7.5
Radiochemical purity:	more than 97% as ^{153}Sm-EDTMP complex
Patient dose is:	20–40 MBq/kg body weight at the time of injection.

2.7.1.3. Synthesis of hydroxyapatite

- Synthesis of hydroxyapatite has been carried out based on Chinol et al. (1993) method, using 0.5M $Ca(NO_3)_2$ solution and 0.5M $(NH_4)_2HPO_4$ solution at pH12 and boiling for 10 min. Commercially available hydroxyapatite suspension and hydroxyapatite cellulose crystals were also used in experiments.
- 10 mL of 0.5M $Ca(NO_3)_2$ solution was adjusted to pH12 by addition of concentrated ammonia. An equivalent of 0.5M $(NH_4)_2HPO_4$ solution (similarly brought to pH12 with ammonia) was added dropwise to the above solution while stirring vigorously.

- The reaction mixture then was gently boiled for 10 min and the precipitate was allowed to settle, and the supernatant was discarded by decantation.
- The precipitate was rinsed with hot water, dried at 150°C and heated for an hour at 240°C to remove the ammonium nitrate.

Preparation of ^{153}Sm-hydroxyapatite (HA)

- Labelling was car of the required radioactivity of $^{153}SmCl_3$ solution in 0.1N HCl was added to 2 mL of 0.05M Sm-citrate carrier solution.
- The above ^{153}Sm-citrate solution was added to 200 mg of hydroxyapatite or hydroxyapatite cel ried out according to the Chendaming (1997) method.
- A small volume lulose suspension in 0.1 M phosphate buffer pH6.8 (or normal saline solution) while shaking continuously for 5 min in a water bath, then cooled at room temperature and centrifuged at 1200 rpm for 10 min.
- Radiolabelled particles was rinsed once with 5 mL of normal saline solution, separated by centrifuge for 10 min at 1200 rpm, then was resuspended in 5 mL of normal saline solution.

2.7.1.4. Quality control of ^{153}Sm-HA

- The particle size of ^{153}Sm-HA was determined by optical microscopy technique using haemocytometer. The particle sizes were between 5 μm to 50 μm (Fig. 15(a), Fig. 15(b) and Table XXXVIII).
- The labelling efficiency was determined by centrifugation and decantation. Labelling efficiency was higher than 85%.
- The radiochemical purity was determined on the resuspended particles by centrifugation or by TLC on silica gel G60 and 80% methanol as solvent. The radiochemical purity was always greater than 95% for all types of hydroxyapatite.
- *In vitro* stability was determined for several days on the resuspended particles in saline or 1% albumin solution, and determined the radioactivity released into the solution.

Biological evaluation of ^{153}Sm-HA

Biological evaluation of the ^{153}Sm-HA are continuing by injection of the colloids in the normal hind leg joint of rats or rabbits. Evaluation in a simulated inflammation of hind leg joint is under investigation. There should be no release of radioactivity into the circulation.

2.7.1.5. Preparation of albumin microspheres

- Preparation of albumin microspheres was carried out based on Mumper et al. (1992) method.
- 0.8 mL of 20% HSA solution was added dropwise into 100 mL of olive oil while stirring vigorously at 1150 rpm for 45 min.
- The suspension then was heated slowly to 140–160°C and kept at the temperature for one hour.

- The suspension was cooled and diluted with 100 mL of hexane, filtered with Whatman no. I paper, washed with 50 mL of hexane, dried and purified through a mesh 200 sieve to remove particles >75 μm. Typical particle sizes was 95% between 5 to 50 μm (Fig.15(d) and Table XXXVIII).

Formulation of ^{153}Sm-albumin microspheres

- Labelling with ^{153}Sm was carried out according to the Argentinean (1996) method.
- 15 mg of citric acid was added to a required radioactivity (in 1.0 mL) of $^{153}SmCl_3$ solution in 0.1N HCl. The solution then was added to 10 mg of the microsphere, and warmed (37°C) on a water bath while shaking continuously for 30 min.
- Supernatant was discarded and the radiolabelled particles was rinsed once with 5 mL of normal saline solution and separated by centrifuge (5 min at 1000 rpm), then was resuspended in 5 mL of normal saline solution. However, ^{153}Sm labelling efficiency was always lower than 15%. Effort to prepare ^{153}Sm-albumin microspheres was discontinued at this point.

Formulation of ^{99m}Tc-albumin microspheres

- Labelling of the microspheres with ^{99m}Tc as described for the ^{99m}Tc-MAA.
- 12 mg (in 0.15 mL) of $SnCl_2.2H_2O$ solution was added to 1 mL solution of 120 mg pyrophosphate in water, then adjusted to pH= 2 with 0.1N HCl.
- 0.1 mL of the above solution was added to 10 mg of the albumin microspheres, incubated for 10 min, then was heated on a water bath for 15 min and cooled.
- The microspheres was washed twice with 2 mL of 0.4% of polysorbate-80 solution, with 5 mL of normal saline solution, resuspended in 2 mL of normal saline solution and pH was then adjusted to 4.0 with 0.1N HCl solution.
- 148 MBq of ^{99m}Tc-pertechnetate solution was added to the above solution and incubated for 10 min. The labelled microspheres was purified from the free TcO_4^- by centrifugation at 2000 rpm for 20 min and if necessary washed with 5 mL of normal saline solution.

2.7.1.6. Quality control of 153Sm-microspheres and 99mTc-microspheres

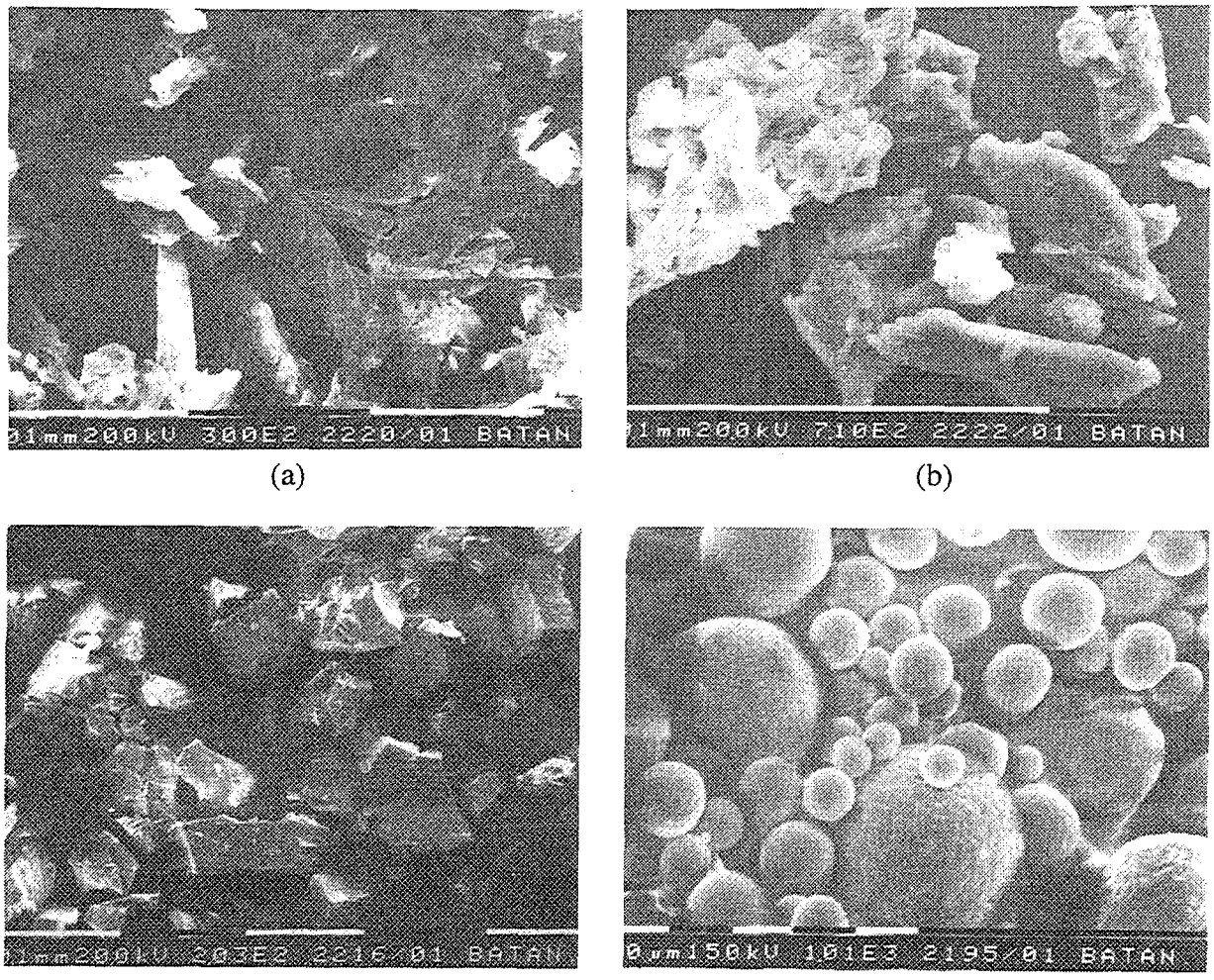
- Similar to the ^{153}Sm-HA, the particle size were determined by optical microscopy with a haemocytometer and micrometric ocular. It was recorded also by an electron microscope. Most of the particles (>90%) was between 5 to 50 μm (Fig. 15(d) and Table XXXVIII).
- The labelling efficiency was determined by centrifugation at 2000 rpm for 20 min. Labelling efficiency was about 50%.
- The radiochemical purity was determined on the resuspended particles by centrifugation or by TLC on silica gel G60 using 80% methanol as solvent.
- *In vitro* stability was determined for several days on the resuspended particles in saline solution or 1% albumin solution, and measured the radioactivity released into the solution.

Biological evaluation of ^{153}Sm-microspheres

Biological evaluation of the ^{153}Sm-microspheres is continuing by injection of the microspheres in the normal hind leg joint of rats or rabbits. Evaluation in a simulated inflammation of hind leg joint is under consideration. There should be no release of radioactivity into the circulation.

TABLE XXXVIII. TYPICAL PARTICLE SIZE DISTRIBUTION OF FHMA, ALBUMIN MICROSPHERES AND HYDROXYAPATITE

Size Range (μm)	% FHMA	% Microspheres	% Hydroxyapatite
<5	..... <1	..... <1	..... <1
5–10	 19	 76	 3
10–25	 55	 15	 21
25–50	 23	 5	 75
50–100	 2	 3	..... <1
>100	..... <1	..... -	..... -



(a) (b)

(c) (d)

FIG. 16. SEM image of: (a) and (b) Hydroxyapatite cellulose; (c) FHMA; and (d) Albumin Microspheres.

Preparation and quality control of 153Sm-ferric hydroxide macroaggregates (FHMA)

- The ^{153}Sm-FHMA was prepared in a single step by coprecipitation of ^{153}Sm in the formation of $Fe(OH)_3$ precipitate, as described in the Argentinean (1996) method.
- To a required radioactivity (in 0.25 mL) of ^{153}Sm-chloride solution was added 0.5 mL of 0.1M $Sm(NO_3)_3$ solution.
- 4 mL of 0.1M $FeSO_4$ solution was added dropwise to the radioactive solution and stirred for one min. The solution then was adjusted to pH7.5 with 1M NaOH solution and 1 mL of 1% PVP solution was added. A sufficient amount of water was added to a final volume of 12.5 mL, stirred at 1000 rpm for 15 min and then centrifuged at 1200 rpm for 10 min.
- Supernatant was discarded and the macroaggregates were rinsed with 5 mL of 1% PVP solution to remove particles smaller than 5 μm, then rinsing was repeated if necessary. The macroaggregates then was resuspended in 10 mL of normal saline solution.

Quality control of 153Sm-FHMA

- The particle size was determined similarly as above. The particles should be ≥90% of 5–50 μm, and less than 1% of <5 μm, as determined by optical microscopy (Fig. 15(c) and Table XXXVIII).
- The radiochemical purity was determined by TLC on silica gel G60 sheet with normal saline solution or 1% EDTA solution as solvent. Radioactivity should be ≥95% as ^{153}Sm-FHMA. R_f of ionic Sm is 0.8 and R_f of particles is 0.0.
- Labelling efficiency was always higher than 90%, and = 95% pure labelled compound can be obtained after washing once with 1% PVP solution.
- *In vitro* stability of the labelled particles was examined by resuspension of the particles in 2 mL of normal saline solution or 2 mL of 1% albumin solution and kept at room temperature for several days. Every day, the suspension was centrifuged, supernatant was collected and counted to determine the release of $^{153}Sm^{3+}$ radioactivity into the solution. ^{153}Sm-FHMA prepared at pH7.5 using 1 mL or 2 mL of 1% PVP solution were stable for four days.

Biological evaluation of 153Sm-FHMA

Biological evaluation of the ^{153}Sm-FHMA is continuing by injection of the colloids in the normal hind leg joint of rats or rabbits. Evaluation in a simulated inflammation of hind leg joint is under consideration. There should be no release of radioactivity into the circulation.

Reports and Publications

TAMAT, S.R., et al., Preparation of samarium-153 ethylenediamine tetramethylene phosphate and its biodistribution studies in mice, Hasil Penelitian Pusat Produksi Radioisotop **2** (1995) PPR-BATAN 107–132.

TAMAT, S.R., et al., "Preparation and quality control of ^{153}Sm-radiopharmaceutical" (Proc. 1997 Workshop on the Utilization of Research Reactors, 1997), JAERI-Conf 98-015, 339-348.

2.8. MALAYSIA

Research Contract No.:	MAL/8358/RB
Title of the project:	Development and production of the radiotherapeutic agents of ^{153}Sm and ^{166}Ho
Chief scientific investigator:	Rehir Dahalan
Collaborator:	Wan Anuar Wan Awang

2.8.1. Main developments

2.8.1.1. Preparation and irradiation of target for ^{153}Sm production

Two different forms of Sm_2O_3 were used as target materials, initially Sm_2O_3 of natural abundance from Sigma Chemical Company and later 98.7 per cent enriched Sm_2O_3 from Isotec Inc. USA were used. A range of 10–20 milligrams of the natural abundant Sm_2O_3 and only about 3 milligram of the enriched Sm_2O_3 was used for irradiation. The oxide was dissolved in 1.0 M nitric acid and placed in a quartz ampoule and dried with a gentle flame. The target was placed in an aluminium capsule and irradiated in the dry-tube irradiation facility at one of the in core position at the Malaysian Institute for Nuclear Technology Research (MINT) research reactor for 6 hours. The target was irradiated in the dry-tube position at neutron flux at the estimated about 0.6×10^{13} $n.cm^{-2}.s^{-1}$. The target was allowed to cool overnight and the quartz ampoule measured for total activity by placing it in the dose calibrator. The ^{153}Sm was recovered using 2 mL of 0.9% saline and the activity of ^{153}Sm in solution was again measured in the dose calibrator.

2.8.1.2. Irradiation of dry and liquid form of samarium nitrate

1.0 mg of samarium oxide was dissolved in 0.1, 0.2, 0.3 and 0.4 mL of 1.0 M nitric acid and sealed in 8.0 mm internal diameter quartz ampoule. Another 1.0 mg of samarium oxide was dissolved in 0.2 mL of 1.0 M nitric acid in the 8.0 mm internal diameter quartz ampoule and dried with a gentle flame as above. Each of these ampoules was irradiated for 6 hours in the same dry tube position for 6 hours, in the same neutron flux. The total yield and amount recovered using 0.9% saline solution from the above irradiation were measured.

2.8.1.3. Labelling of ^{153}Sm to the EDTMP ligand and quality control

The ligand, EDTMP was obtained from Tokyo Chemical Company Limited, Japan and molar ratios of ligands: Sm of between 3–10 were initially used. The EDTMP, dissolved in distilled water was added to the ^{153}Sm solution and the pH was adjusted to about 7.5 and the mixture, incubated at various time intervals at room temperature. The results obtained from this experiment showed that 30 minutes was more than sufficient as incubation period, which was then used for subsequent labelling procedures. Quality control on the complex were done using thin layer paper chromatography using ITLC-SG paper and 1.0 M ammonia solution or NH_4OH:methanol:water (0.2:2:4) as solvent system. QC analysis, were carried out daily for 12 days, to assess the stability of the formed complex. The biodistribution of ^{153}Sm-EDTMP complex were studied by injecting it into male rats weighing 100–150 grams and images at various time intervals acquired using an ADAC gamma camera. Several groups of rats were also injected and sacrificed at various times.

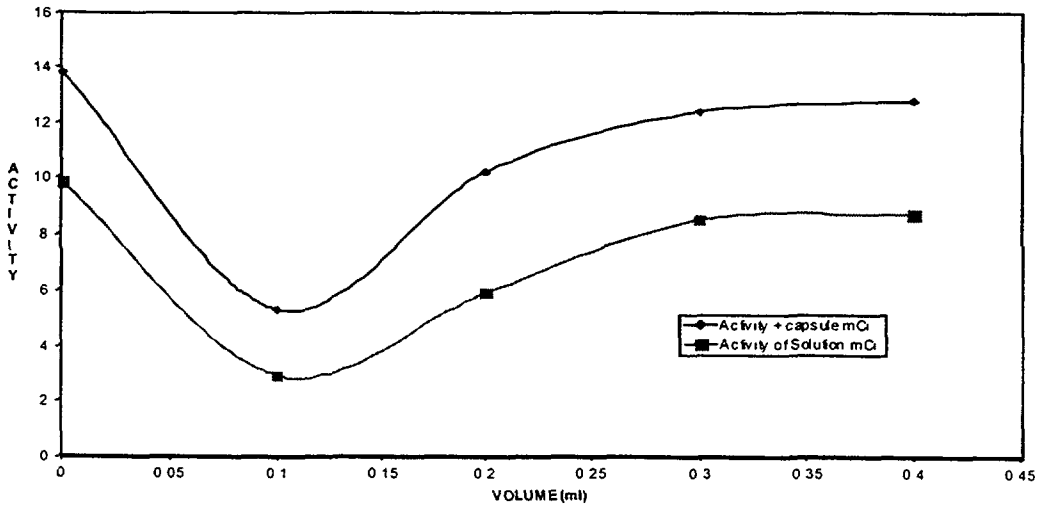


FIG. 17. Irradiation of 1.0 mg samarium nitrate for 6 hours.

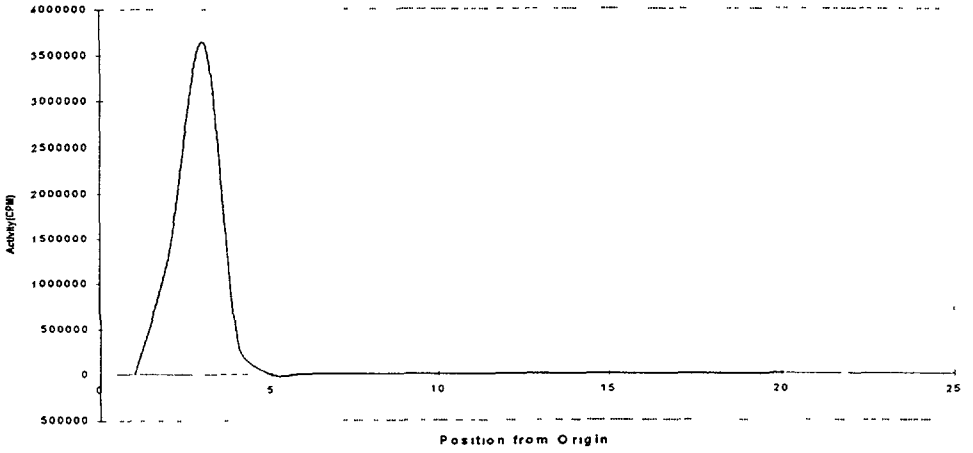


FIG. 18. ^{153}Sm on ITLC-SG (ammonia 1M).

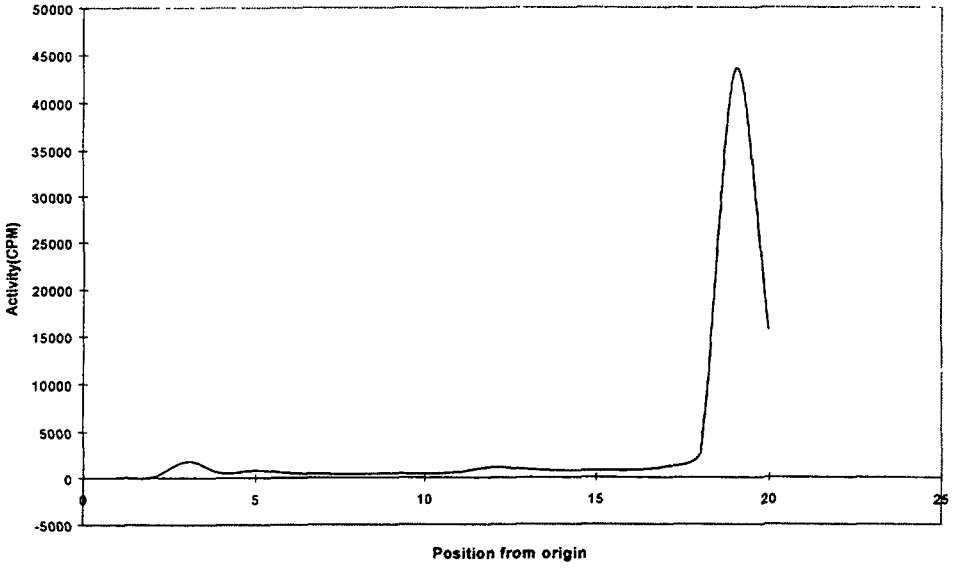


FIG. 19. ^{153}Sm-EDTMP on ITLC-SG (ammonia 1M).

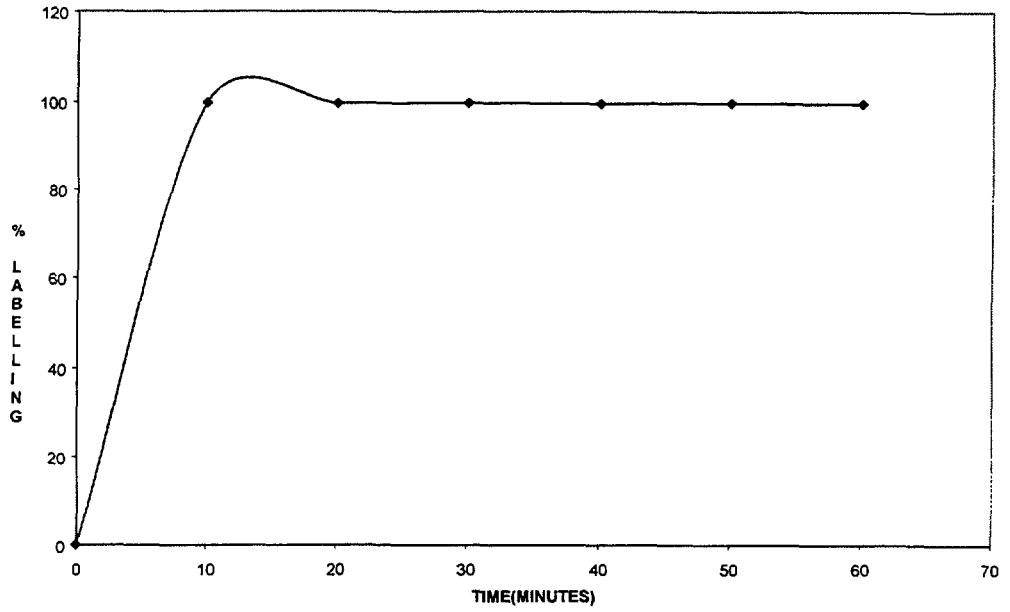


FIG. 20. Rate of formation of ^{153}Sm-EDTMP.

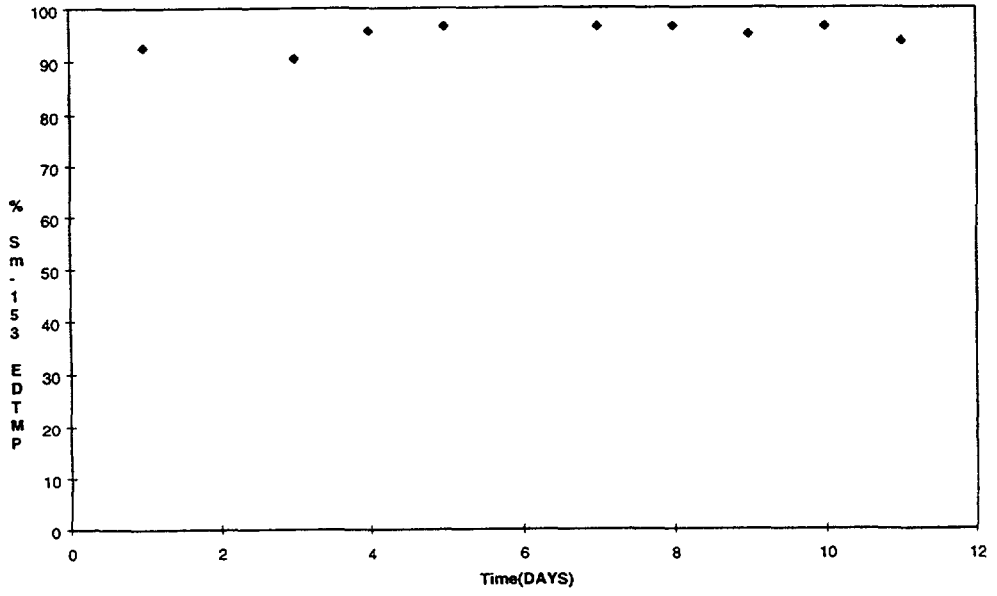


FIG. 21. Labelling efficiency of ^{153}Sm-EDTMP.

2.8.1.4. Preparation of albumin microspheres

The microspheres were obtained by heat denaturation of human serum albumin emulsion in vegetable oil. About 30 milliliters of 10% human serum albumin solution was added to 50 milliliters of corn oil. Emulsification was achieved using an IKA homogenizer with a suitable generator head, at various stirring rpms. The emulsion was heated up to 150°C

for 30 minutes. The suspension was then cooled and diluted with n-hexane. The whole solution was filtered through a sintered glass filter of mesh number 200 to get rid of the larger particles. The microspheres were rinsed several times with acetone, collected and dried.

2.8.1.5. Particle size measurement

This was initially done using an optical microscope with a micrometric ocular. Later, a small portion of the microspheres were resuspended in distilled water at pH5.5, at 30°C, reduced with 50 μg of stannous chloride and labelled with ^{99m}Tc. The labelled microspheres are passed through a cascade of filters of various sizes and the filters counted in a well counter.

2.8.1.6. Observations

There was no significant advantage of irradiation in the form of the liquid target. Average specific activity of natural Sm target were about 0.12 GBq/mg Sm_2O_3 and for the enriched Sm target about 0.64 GBq/mg Sm_2O_3. The dry form probably was spread over a significant surface area, thus enhancing the geometry and thus a higher activation was observed, similar to liquid target. This was further verified, using a bigger internal diameter quartz ampoule and higher volume of liquid.

Low pH will cause the EDTMP complex to precipitate out, therefore proper control of the pH at about 7.5 is necessary. The rate of the formation of the ^{153}Sm-EDTMP complex was shown to be spontaneous. More than 90 per cent labelling efficiency was obtained within 10 minutes, therefore the rest of the experiments were performed using 30 minutes incubation time, which was more than sufficient to achieve a high labelling yield. Labelling efficiency did not improve with increased ligand ratio. Labelling efficiency was more than 95 per cent at ligand ratios of 2 and 10. The free ^{153}Sm remained at the origin for both TLC solvent systems and the ^{153}Sm-EDTMP complex traveled to about R_f 0.7–0.9. The ^{153}Sm-EDTMP complex were stable up to 12 days studied.

The image of injected ^{153}Sm-EDTMP complex in rats showed that with lower molar ratio of 2.5 and 5 there appears to be activity of ^{153}Sm in the liver, with significant activity in the bone. At higher molar ratio of 10 most of the ^{153}Sm activity concentrates in the bone structure within 1 hour and remain true for 24 hours or more.

At ligand to radionuclide ratios of 2.5 and 5, injection into the rats showed some localization of significant amount of ^{153}Sm activity in the liver within 1 hour and remains until 72 hours. However, at higher ratio of 10, most ^{153}Sm activity were in the bone tissues only, this probably suggest that at low ligand ratios there might be dissociation of the complex resulting in free ^{153}Sm finding its way to the liver either directly or via some other complexing process.

The yield for ^{153}Sm could be further improved by irradiating at the central thimble position and for longer irradiation time when the situation warrants it, particularly when we are ready to work with higher specific activity and for preparations to be used in patients. However, the expected maximum yield is yet to be determined. If the need does arise we could also rely on a higher flux from a neighbouring country for higher specific activity ^{153}Sm.

Currently, the preparations are performed with terminal sterilization, by filtration. This method have ensured that the final product is aseptic and suitable for injection into patient as part of the data gathering in our application for the registration with the Drug Control Authority of the Ministry of Health Malaysia and the eventual clinical trial.

The sizes of the HSA microspheres using the specified emulsion generators were consistent irrespective of speed of stirring. However, labelling of ^{166}Ho to the microspheres failed to produce any consistent results.

2.8.2. New scientific contributions

The results have shown the possibility of using the low flux research reactor (0.6×10^{13} n.cm^{-2}.s^{-1}) running on restricted time schedule to produce suitable amount of ^{153}Sm for possible use in radiotherapy.

2.8.3. Co-operation with participating laboratories

The department of radiotherapy of the National University Hospital (Hospital Universiti Kebangsaan Malaysia) in Kuala Lumpur, has agreed to proceed with the initial clinical trial of the ^{153}Sm-EDTMP.

Reports and Publications

DAHALAN, R., et al., ^{153}Sm-EDTMP (Ethylene Diamine Tetramethylene Phosphonic Acid), A Radiotherapeutic Agent for the Alleviation of Bone Pain in Advanced Bone Cancers, Federation of Asian Pharmaceutical Association (FAPA) Seminar: New Horizon in Pharmacy, Kuala Lumpur, Malaysia. 17–21 November 1996.

DAHALAN, R., et al., ^{153}Sm-EDTMP (Ethylene Diamine Tetramethylene Phosphonic Acid), A bone seeking radiotherapeutic agent, Nucl. Sci. J. of Malaysia (in press).

2.9. PAKISTAN

Research Contract No.:	PAK/8668/RB
Title of the project:	Preparation and evaluation of radioisotopes for therapeutic applications
Chief scientific investigator:	M.M. Ishfaq
Collaborators:	A. Mushtaq, M. Jawaid

2.9.1. Main developments

The use targeted therapy using β emitting radiopharmaceuticals has become increasingly popular in the field of oncology, endocrinology and rheumatology. Recent reports suggested the use of ^{186}Re-HEDP and ^{153}Sm-EDTMP complexes for the palliative treatment of skeletal metastases and, ^{153}Sm and ^{166}Ho particles for the treatment of rheumatic arthritis. During the last two years work has been done to optimize the production yields of ^{186}Re, ^{153}Sm and ^{166}Ho radionuclides in the Pakistan Atomic Research Reactor (PARR-1) at a thermal flux of 1×10^{14} n. $cm^{-2}.s^{-1}$ and the studies on the formulation of ^{186}Re-HEDP, ^{153}Sm-EDTMP and ^{153}Sm-MDP complexes including their biodistribution. The work on the separation of ^{188}Re from irradiated natural tungsten has also been carried out along with the preparation of ^{153}Sm-HMA and ^{153}Sm-FHMA particles.

Irradiation protocol

Irradiation protocol for the production of ^{186}Re, ^{153}Sm and ^{166}Ho radionuclides were developed to obtain optimum production yields. Appropriate amounts of Re (metal), Sm_2O_3 (powder) natural as well as enriched in ^{152}Sm (99.06%), $Sm(NO_3)_3$ powder, $Sm(NO_3)_3$ solution (0.2 mL) and Ho_2O_3 (powder) were sealed in quartz ampoule which were then cold welded in Al containers for irradiation in PARR-1 for different time periods. The absolute activity of the samples were measured with precalibrated HPGe detector (Canberra) which was used to adjust calibration factor of ionization chamber (Capintec). The results are tabulated in Tables XXXIX–XXXXI.

TABLE XXXIX. VARIATION IN THE SPECIFIC ACTIVITY OF ^{186}Re RADIONUCLIDE WITH THE AMOUNT OF Re METAL

Amount of Re (mg)	Irradiation time (h)	Activity mCi/GBq	Specific activity/mg mCi/GBq
5	24	230/8.5	46/1.7
5	48	466/17.2	93.2/3.4
5	72	634/23.4	126.8/4.7
10	72	1088/40.3	108.8/4.1
30	72	2911/107.8	97.03/3.6
100	72	7878/291.8	86.78/2.9

TABLE XXXX. SPECIFIC ACTIVITY OF Sm LIQUID TARGETS COMPARED WITH Sm SOLID TARGETS (IRRADIATION TIME 72 h)

Form of target	Quantity of Sm Mg	Yield mCi/MBq	Sp. Activity/mg mCi/MBq
$Sm(NO_3)_3$ film	6.5	988/36556	152/5624
$Sm(NO_3)_3$ liquid	6.5	1749/64713	269/9955
$Sm(NO_3)_3$ powder	7.0	628/23236	90/3320
$^{152}Sm(NO_3)_3$film*	6.0	9198/340326	1533/56721
$^{152}Sm(NO_3)_3$ liquid*	6.0	15330/567210	2555/94535
$^{152}Sm_2O_3$ powder*	6.2	6336/234432	1022/37811

* Enriched target ^{152}Sm >99%.

TABLE XXXXI. THE ACTIVATION YIELD OF ^{166}Ho IN PARR-1 FOR VARIOUS IRRADIATION TIMES AND AMOUNTS OF Ho

Amounts of Ho (mg)	Irradiation time (h)	Activity mCi/GBq	Specific activity/mg mCi/GBq
5	1	65/2.4	13/0.5
5	10	570/21.1	114/4.2
5	24	1020/37.3	204/7.5
5	48	1796/66.5	359/13.3
10	48	3393/125.6	339.3/12.6
20	48	6586/243.9	329.3/12.2
40	48	12379/458.5	309.5/11.4

The irradiated Re targets were oxidized with 5 mL of hydrogen peroxide (35%) and incubated for a period of one h. Then 5 mL 25% ammonia solution was added slowly to the reaction mixture. After evaporating the solution, the resultant ammonium perrhenate was dissolved in 5 mL of water.

The irradiated targets were dissolved in 5M HCl and evaporated to dryness. The residue was taken up in saline solution.

The following may be concluded from these tables:

(i) 5 mg of Re metal is optimum for the production of 4.6 GBq/mg of ^{186}Re.

(ii) Specific activity of ^{153}Sm increases in the order Sm_2O_3 powder $<Sm(NO_3)_3$ film $<Sm(NO_3)_3$ liquid.

(iii) 6 mg of $^{152}Sm(NO_3)_3$ liquid target is optimum for the production of 94 GBq/mg of ^{153}Sm.

(iv) 5 mg of Ho target as Ho_2O_3 is optimum for the production of 13 GBq/mg of ^{166}Ho.

Synthesis and labelling

A. Synthesis of 1-hydroxy-ethylidene-1, 1-diphosphonic acid (HEDP)

Hydroxy ethylidene diphosphonic acid (HEDP) was prepared by the method of Costronovo. The HEDP was converted to Na_2-H_2-HEDP by titrating HEDP with NaOH and purified by crystallization using water-absolute alcohol system.

B. Formulation of ^{186}Re-HEDP, ^{153}Sm-EDTMP and ^{153}Sm-MDP complexes

^{186}Re-HEDP complex was prepared by taking 10 mg of HEDP as disodium salt, 4 mg of stannous chloride dehydrate and 3 mg of gentisic acid (antioxidant). After labelling with 0.15 mL (0.15 mg) of ^{186}Re solution, pH of the solution was adjusted to 6.5 with 0.5M sodium acetate solution. Stability of the complex was found to be more than 97% for eight days.

Similarly ^{153}Sm-EDTMP was obtained at pH 7.5 with 1:5 (Sm:EDTMP) molar ratio incubated for a period of 20 min at room temperature. Stability of complex was checked up to 8 days, which remained >99%. The effect various variables were studied to optimize the complexing yield of ^{153}Sm-EDTMP complex which are plotted in Figs 21–23.

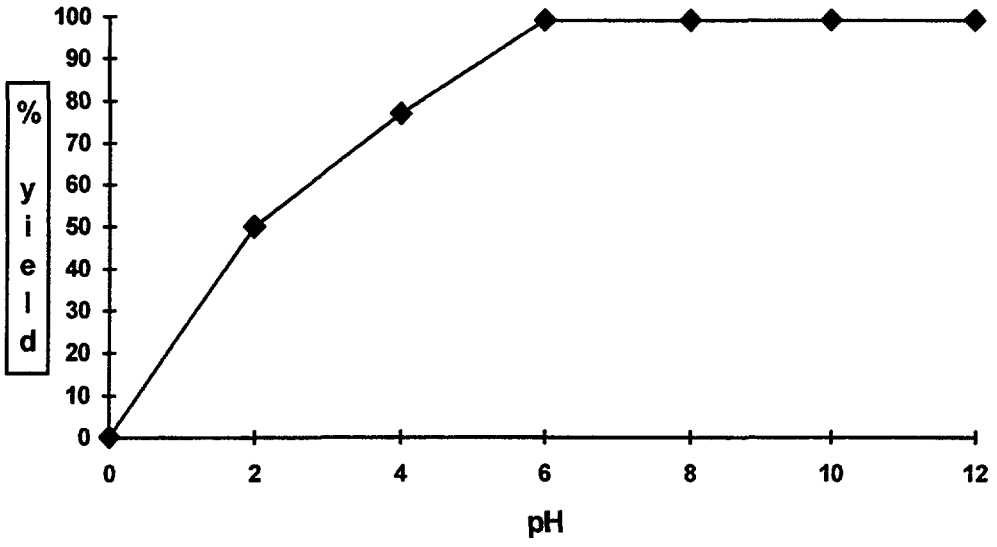


FIG. 22. The effect of the pH on the complexing yield after 20 min incubation, molar ratio Sm:EDTMP = 1:5.

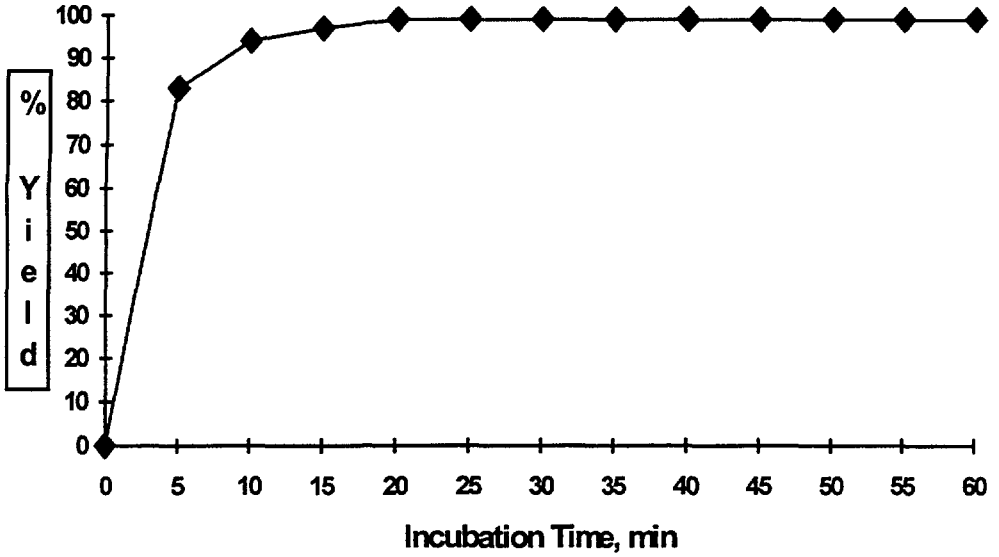


FIG. 23. The effect of incubation period on the complexing yield, molar ratio Sm:EDTMP = 1:5.

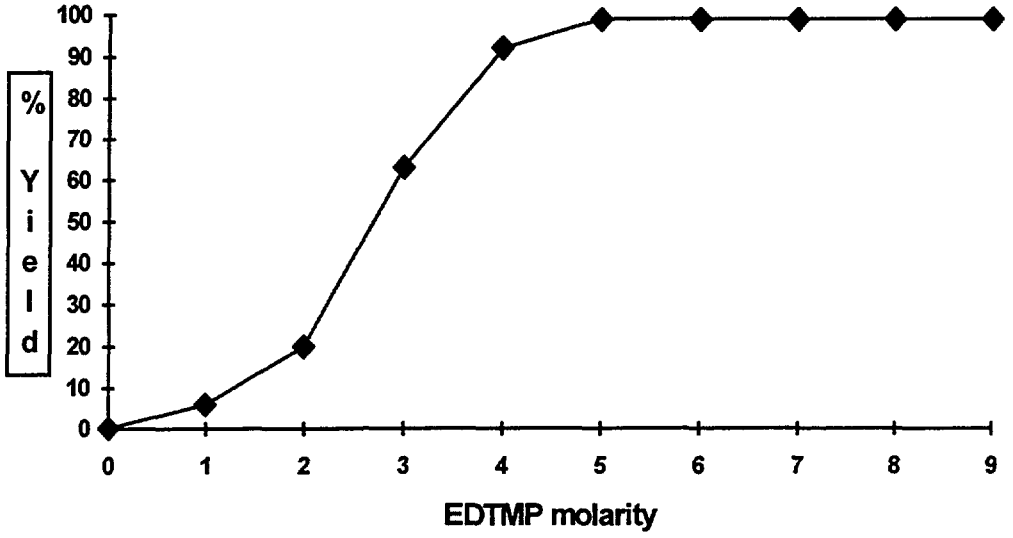


FIG. 24. Complexation of ^{153}Sm EDTMP, Sm molarity = 1, incubation period = 20 min.

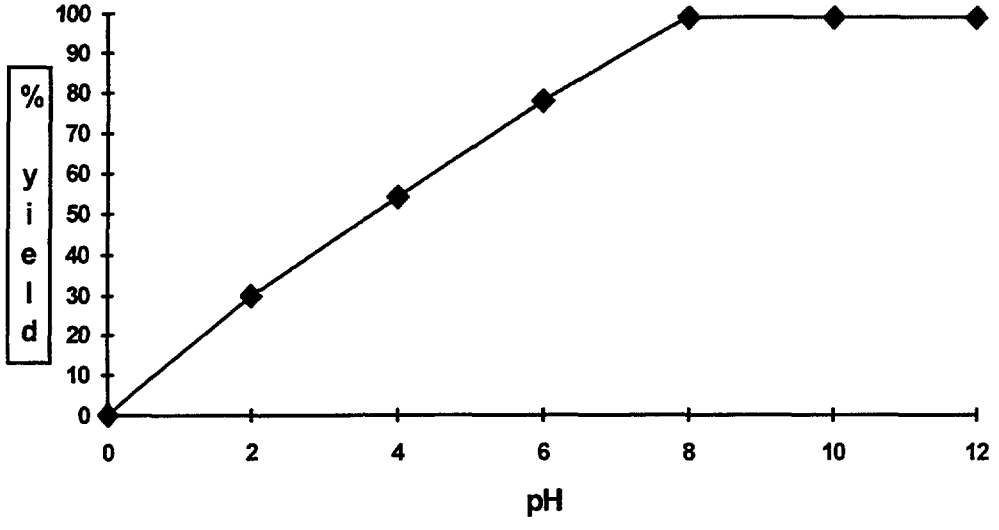


FIG. 25. The effect of pH on the labelling yield of ^{153}Sm-MDP.

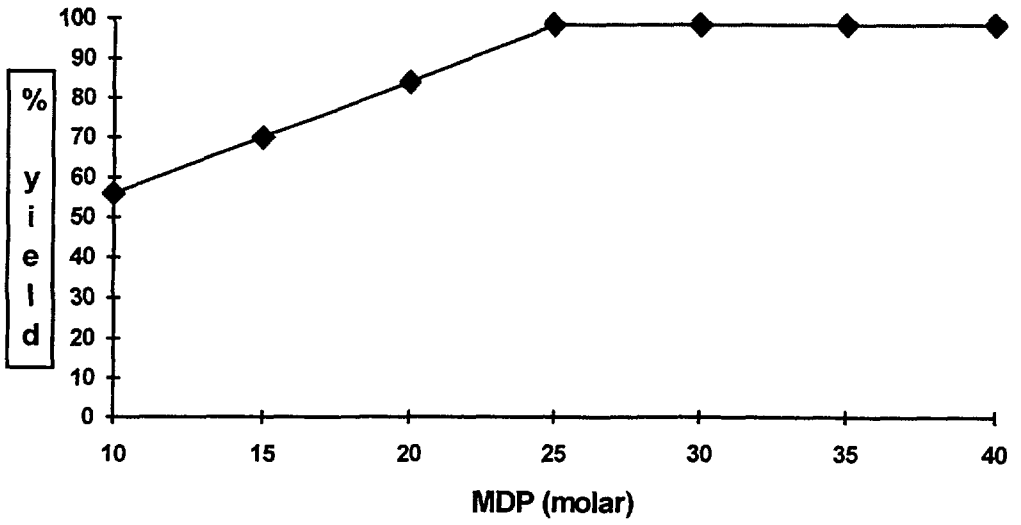


FIG. 26. The effect of molar ratio of MDP on the labelling yield of ^{153}Sm-MDP complex, pH = 8, Sm = 1 mg.

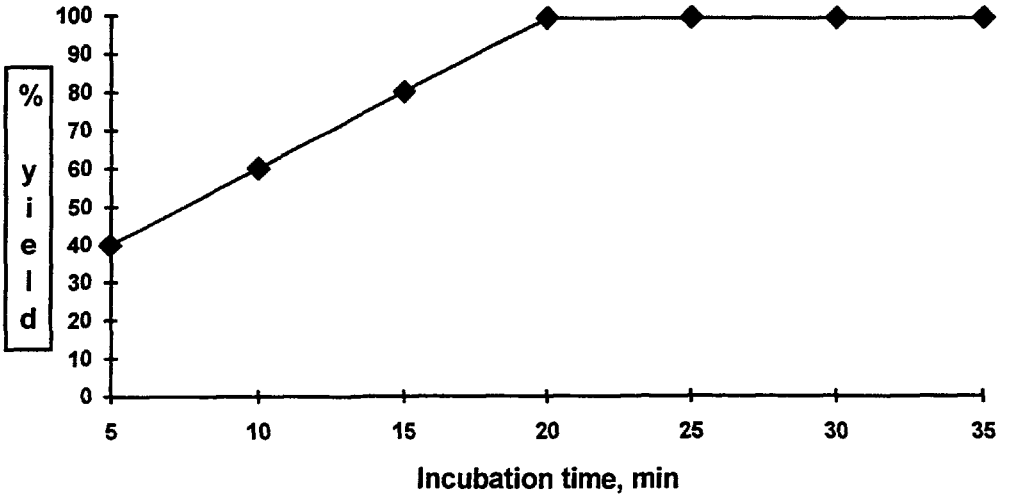


FIG. 27. The effect of incubation time (in boiling water) on the labelling yield of ^{153}Sm-MDP complex.

^{153}Sm -MDP complex was prepared by taking 25 mg MDP (5 mL) and 1 mg of ^{153}Sm (1 mL) at pH8 and incubating the mixture for 20 min in boiling water bath. The complex was showed stability more than 98% for a period of five days. The effect of various variables like pH, molar ratio and incubation period for the labelling of ^{153}Sm-MDP complex are shown in Figs 24–26.

C. *Separation of ^{188}Re from irradiated natural tungsten*

No-carrier-added production of ^{188}Re is possible through reactor irradiation of tungsten by the double neutron capture cross-section of ^{186}W.

$$^{186}\mathrm{W}\ (n,\gamma)\ ^{187}\mathrm{W}\ (n,\gamma)\ ^{188}\mathrm{W} \xrightarrow[69\ \mathrm{d}]{\beta} {}^{188}\mathrm{Re} \xrightarrow[17\ \mathrm{h}]{\beta,\gamma} {}^{188}\mathrm{Os}$$

A target of Na_2WO_4 (1 g) was irradiated in PARR-1 reactor for 72 h. After 10 days it was dissolved in water (10 mL). W was then precipitated as tungsten (VI) oxide and digested at room temperature for four h. The ppt. were separated by centrifugation, washed with 10 mL distilled water, and then dissolved in 0.1 N NaOH and placed under shielding to generate ^{188}Re by the decay of ^{188}W. After five days, it was again precipitated and supernatant solution was collected (solution 1) whereas the ppt. was again dissolved in 0.1 N NaOH (solution 2). Aliquots were counted on a MCA calibrated Canberra series 85 coupled to a Ge (intrinsic) detector for ^{188}Re (155 keV) and ^{187}W.(480 keV). The results indicate that solution 1 contains about 98% of ^{188}Re with <2% of ^{187}W whereas solution 2 shows >98% of ^{187}W with <2% of ^{188}Re. The effect of digestion time of the precipitation on the recovery of ^{188}Re was studied in ordered to work out efficiency of the separation procedure. The results are shown in Table XXXXII.

Amount of ^{188}Re recovered after 15 min digestion time was 88% which increased to >98% after 3 h digestion. Similarly amount of tungsten precipitated after 15 min incubation period was 80% which increased to 99% when the incubation period was 3 h. Similar results were obtained after incubation period of 4 h. The solution was finally purified to remove the traces of tungsten by passing it through alumina column.

Quality control

Radiochemical purity ^{186}Re-HEDP complex was measured by Whatman 3MM paper using acetone for perrhenate (R_f 0.9-1) and labelled product (R_f 0) and using saline for reduced rhenium (R_f 0) and labelled product (R_f 0.9–1).

Similarly the labelling yield of ^{153}Sm-EDTMP complex was determined using the following four systems:

System 1:	solvent;	pyridine:ethanol:water (1:2:4).
System 2:	solvent;	ammonia:ethanol:water (0.1:2:4).
System 3:	solvent;	ammonia:methanol:water (0.2:2:4).
		Whatman 3 MM paper strips 1.5 × 11 cm were used as support for above mentioned system.
System 4:	solvent;	1M ammonium hydroxide.
		Support 1TLC Silica gel (Gelman) strip 1 × 6 cm.

Table XXXXIII shows the chromatographic behaviour of ^{153}Sm-EDTMP compared with that of $^{153}SmCl_3$. ITLC, silica gel chromatography gave results within 2 minutes of time while system 3 took half an hour and system 1 and 2 more than one hour.

The complexing yield of the ^{153}Sm-MDP was carried out by the following chromatographic methods:

Method 1:	solvent:	pyridine:ethanol:water (1:2:4).
Method 2:	solvent:	ammonia:ethanol:water (0.1:2:4).
Method 3:		1M ammonia hydroxide.

Whatman 3 MM paper strips 1 × 10 cm were used as support for methods 1 and 2 whereas TLC Silica gel (Gelman) strips 1 × 6 cm were applied for method 3. Method 1 & 2

TABLE XXXXII. THE EFFECT OF DIGESTION TIME ON THE RECOVERY OF ^{188}Re RADIONUCLIDE

Time of digestion	Amount of W precipitated (%)	Amount of ^{188}Re recovered (%)
15 minutes	80	88
30 minutes	85	91
1 hour	93	94
2 hours	98	98
3 hours	99	98
4 hours	99	98

TABLE XXXXIII. CHROMATOGRAPHIC BEHAVIOUR OF $^{153}SmCl_3$ AND ^{153}Sm-EDTMP

System	Developing time (min)	$^{153}SmCl_3$ (R_f)	^{153}Sm-EDTMP (R_f)
1	75	0	0.7–0.8
2	75	0	0.9–1
3	30	0	0.9–1
4	1	0	1

showed R_f values 0.8 and 0 for ^{153}Sm-MDP complex and $^{153}SmCl_3$ respectively. The method 3 indicated ^{153}Sm-MDP at R_f 0.9 and $^{153}SmCl_3$ at R_f 0.

Animal study of ^{153}Sm-EDTMP complex

Biodistribution of these complexes were performed in ~200 g Sprague Dawley rats. Two hundred microlitres of the complex was injected into the tail veins of rats and each rat was killed by cervical dislocation at appropriate time. A 1 mL sample of blood was drawn from the heart and weighed immediately after killing. The rat was then weighed and subsequently dissected with special care being taken to separate the blood and urine on the kill papers, the tissue washing and the urine collected from the cages. Counting was performed using Capintec dose calibrator. The results on biodistribution of ^{153}Sm-EDTMP complex is shown in Table XXXXIV.

TABLE XXXXIV. BIODISTRIBUTION OF ^{153}Sm-EDTMP IN RATS % INJECTED DOSE/ORGAN, (S.D) OF 3 RATS

Organ	20 min	2 h	24 h
Blood 6.5% of body wt	6.0 (0.5)	0.04 (0.02)	0.008 (0.002)
Liver	0.90 (0.10)	0.30 (0.04)	0.15 (0.04)
Spleen	0.06 (0.008)	0.006 (0.003)	0.005 (0.003)
Large intestine	0.06 (0.05)	0.10 (0.03)	0.05 (0.01)
Small. intestine	0.80 (0.08)	0.80 (0.06)	0.08 (0.02)
Kidneys	1.70 (0.40)	0.30 (0.04)	0.30 (0.04)
Muscle	8.75 (0.90)	0.30 (0.05)	0.10 (0.05)
Skeleton femur × 25	47.6 (3.6)	57.8 (4.5)	55.5 (4.0)

Preparation ^{153}Sm-particles.

A. Preparation of ^{153}Sm-hydroxide macroaggregates (HMA)

Preparation of ^{153}Sm-hydroxide macroaggregate (HMA) particles were carried out as follows:

(i) One mL of samarium chloride (30 mg Sm) and 3 mL of saline were mixed and 3 mL of 0.2 N NaOH was added in a tube which was centrifuged with 1400 rpm for 3 min. The supernatant was discarded and the samarium hydroxide was resuspended in 4 mL of saline. The tube was again centrifuged with 1200 rpm for 3 min. After discarding the supernatant, 4 mL of saline was added with thorough mixing and transferred and sealed in 10 mL serum capped vial. This vial was sterilized by autoclaving at 120°C for 20 min. The final pH of the ^{153}Sm-HMA suspension was in the range 9.5–10.5. The size distribution of particles in the suspension of ^{153}Sm-HMA was determined by serial filtration through Millipore filters.

(ii) ^{153}Sm-HMA suspension of pH range 9.5–10.5 contains >80% of particles in the range 5–25 μm with few particles less than 0.45 μm.

B. Preparation of ^{153}Sm-ferric hydroxide macroaggregates

The preparation of ^{153}Sm-ferric hydroxide macroaggregates (FHMA) was obtained by the following method:

(i) 0.5 mL of $FeSO_4$ solution (2 mg of Fe^{+2}/mL) and 5 mL of saline were mixed and 0.5 mL of ^{153}Sm solution (0.5 mg/mL in dilute HCl) was added into the vial which was shaken for 1 min. 2 mL of NaOH (0.1N) was then added and the vial was again shaken for 0.5 min. The vial was centrifuged for 5 min at 1500 rpm. The supernatant was removed with 10 mL syringe (18 gauge needle) and the precipitate resuspended in 6 mL of PVP solution (16 mg/mL). The vial was centrifuged for 3 min at 1200 rpm. The supernatant containing fine particles was removed with a 18 gauge needle with 10 cc syringe and the precipitate was resuspended for injection in 0.7 mL saline.

(ii) The labelling efficiency was found to be >90%. The results indicated that about 4% of the particles were less than 0.45 μm in size and 5% were more than 25 μm.

2.9.2. Participation of other laboratories

Department of Isotope, China Institute of Atomic Energy where one Pakistani collaborator has obtained training on the preparation of ^{153}Sm-EDTMP complex under IAEA programme.

2.9.3. New contributions

- Quality control procedures developed for ^{153}Sm-EDTMP and ^{153}Sm-MDP complexes.
- Separation of ^{188}Re from ^{188}W via precipitation method.

Reports and Publications

MUSHTAQ, A., JAWAID, M., ISHFAQ, M.M., Preparation of ^{153}Sm-EDTMP: its quality control and biodistribution, Chung Hua Society, China, Nucl. Sci. J. **34** (1997) 196–202.

ISHFAQ, M.M., MUSHTAQ, A., JAWAID, M., Preparation and evaluation of radioisotopes for therapeutic applications-1 Preparation of Re-186-HEDP complex and its radiochemical quality control, Publications International, Lahore, Pakistan, Sci. Int. (Lahore) **9** (1997) 131–134.

ISHFAQ, M.M., MUSHTAQ, A., JAWAID, M., "Optimization on the production of ^{186}Re, ^{153}Sm and ^{166}Ho radionuclides in a reactor for radiotherapy", Proc National Chemistry Conf., Lahore, 1997), Institute of Chemists, Lahore (1997) 67–73.

2.10. THAILAND

Research Contract No.: THA/7374/RB

Title of the project: Optimization of the production and quality control of samarium-153 and their labelled compounds

Chief scientific investigator: N. Virawat

2.10.1. Main developments

The optimization of ^{153}Sm(samarium-153) production using the research reactor TRIGA mark III

The ^{153}Sm production using Sm_2O_3 (enriched >98%) as the liquid target to improve specific activity by dissolving Sm_2O_3 with 4M HNO_3, and filtered by 0.22 micron membrane filter, irradiation time about 10 h, neutron flux about 2×10^{13} n·cm^{-2}s^{-1}, the specific activity is about 2 times of solid target (Table XXXXV).

TABLE XXXXV. SPECIFIC ACTIVITY OF ^{153}Sm

Weight of Sm_2O_3 (mg) solid/liquid	Activity (mCi) solid/liquid	Specific activity (mCi/mgSm) solid/liquid
11.20/10.10	288/425	29.77/48.72
15.60/10.14	293/443	21.75/50.59
7.40/10.00	157/374	24.57/43.31
5.60/10.01	103/411	21.30/47.59
	Average	24.35/47.55

Production of ^{153}Sm-EDTMP (samarium-153-ethylenediaminetetramethylenephosphonate) scheme for bone pain palliation is as follow:

A. Preparation of $SmCl_3$ (samarium chloride)
After irradiation, cooled for 1d, evaporated the irradiated target until dry, added 2 mL saline, pH of the final $SmCl_3$ solution about 4.

B. Preparation of EDTMP solution
Na-EDTMP, dissolved EDTMP (TCI Japan) by 2M NaOH, made volume with saline solution. The final solution was 20 mg EDTMP/mL, pH about 7.5–8.

Ca-EDTMP, dissolved $CaCl_2$ in boiled water then added to EDTMP solution by the molar ratio Ca:EDTMP = 1:1, made volume by saline. The final solution was 80 mg EDTMP/mL, pH about 7.5–8.

C. Labelling EDTMP solution with $^{153}SmCl_3$.
^{153}Sm:Na-EDTMP molar ratio = 1:10.
^{153}Sm:Ca-EDTMP molar ratio = 1:100.

D. Quality control
Radiochemical purity (RCP) determined by Sephadex C-25 column method, prepared by swollen Sephadex C-25 in distilled water about 1 h then drop into 5 mL syringe until 2 cm height. Drop about 1 mCi of ^{153}Sm-EDTMP in to the column, eluted the column by 20 mL saline solution.

$$\text{RCP (\%)} = \frac{\text{activity in eluate}}{\text{activity in (eluate + column)}} \times 100$$

Both ^{153}Sm-Na-EDTMP and ^{153}Sm-Ca-EDTMP had radiochemical purity over 99% at the injection time.

Pyrogen test by LAL technique and Sterility test by inoculate the samples in to media.

Toxicity tests performed did not show any sign of abnormality in any rat.

E. Animal study
The biodistribution of ^{153}Sm-Na-EDTMP and ^{153}Sm-Ca-EDTMP were studied in rats. From Table II some of radioactivity in the liver could have been originated from the uptake of colloidal form of ^{153}Sm, percentage of recover dose in liver for ^{153}Sm-Ca-EDTMP was lower than ^{153}Sm-Na-EDTMP about two times. Percentage of recover dose in bone not quite different in both types. Sm-EDTMP highly localized in bone within 2 h post injection and long retention which was the most advantage. The blood activity was completely cleared within two hours post injection and also nonosseous activity was rapidly cleared, the excretion of Sm-EDTMP in to urine was completely excreted in 24 h post injection in both types.

TABLE XXXXVI. % RECOVER DOSE IN ORGANS AT VARIOUS TIME POST INJECTION IN RATS OF ^{153}Sm-Na-EDTMP AND ^{153}Sm-Ca-EDTMP

Organ	2 h		24 h		48 h	
	1:10	1:100	1:10	1:100	1:10	1:100
Liver	0.74	0.45	0.845	0.445	0.93	0.575
Kidney	0.795	0.625	0.46	0.535	0.535	0.53
Muscle	0.785	0.89	0.335	0.18	0.205	0.015
Bone	53.905	47.885	54.24	50.935	64.275	66.44
Blood	0.115	0.295	0.01	0.025	0	0
Urine	21.385	27.64	19.95	21.845	0.435	0.11
Stomach	0.17	0.185	0.045	0.055	0.055	0.145
Tot. GI	1.57	2.78	2.225	3.525	0.465	0.425

F. Clinical trial

The results of clinical trials performed by nuclear medicine centres were informed as follows:

- ^{153}Sm-EDTMP doses were administered to 23 patients without problems. The ratios of the lesion quantification were tabulated in Table XXXXVII. Lesion to normal bone ratios for ^{153}Sm-EDTMP and ^{99m}Tc-MDP were 7.1 ± 4.6 and 9.7 ± 8.9, respectively. Lesion to soft tissue ratios were 15.8 ± 10.2 for ^{153}Sm-EDTMP and 18.8 ± 12.7 for ^{99m}Tc-MDP. Normal bone to soft tissue ratios 2.9 ± 1.6 for ^{153}Sm-EDTMP and 2.9 ± 1.4 for ^{99m}Tc-MDP. Significant correlation between the accumulation of these two agents in bone cancer lesions were observed ($p < 0.001$). The data were similar to those of previous studies which indicated that ^{153}Sm-EDTMP was as sensitive as ^{99m}Tc-MDP for identifying bony lesions and the soft tissue localization was minimal.

- Pain relief was assessed every 2 weeks after treatment for 16 weeks. Of the 16 available patients, complete pain palliation was reported in 8 cases and partial response was reported in 6 patients. No response was found in 2 patient. Pain relief occurred within two weeks after administration of ^{153}Sm-EDTMP .

TABLE XXXXVII. CORRELATION BETWEEN SKELETAL LOCALIZATION OF ^{153}Sm-EDTMP AND ^{99m}Tc-MDP

Ratio	^{153}Sm-EDTMP .	^{99m}Tc-MDP	Correlation coefficient (r)	P
Lesion to normal bone (n = 35)	7.1 ± 4.6	9.7 ± 8.9	0.86	<0.001
Lesion to soft tissue (n = 32)	15.8 ± 10.2	18.8 ± 12.7	0.86	<0.001
Normal bone to soft tissue (n = 27)	2.9 ± 1.6	2.9 ± 1.4	0.90	<0.001

All data is mean ± 1s.d.

G. Product specification

- % labelling >99.5
- pH7–7.5
- sterile, pyrogen free

Production of ^{153}Sm-HA (samarium-hydroxyapatite) scheme for radiation synovectomy is as follow:

A. Preparation of ^{153}Sm-citrate

Added citric acid monohydrate to $^{153}SmCl_3$ (in saline solution) by the mole ratio 2:1. Let it stand for 30 min at room temperature, the final solution was ^{153}Sm-citrate solution.

B. *Incubation of ^{153}Sm-citrate with HA particles*

Mixed HA with 750 µL water, added 250 µL of ^{153}Sm-citrate solution , sealed vial, rotated swirl at room temperature about 30 min transferred radiolabelled particles to 15

mL centrifuge tube, rinsed with 4 mL saline solution, centrifuged at 1000 rpm 8 min collected precipitate (labelled HA particles) and supernatant (free ^{153}Sm), the percentage of labelling was calculated.

$$\text{Percentage of labelling} = \frac{\text{activity of precipitate}}{\text{activity of precipitate + activity of supernatant}} \times 100$$

Added 2 mL saline solution to precipitate, autoclave 30 min.

Radiolabelling of HA particles with ^{153}Sm is simple to perform and provides good yield of labelled particles.

The factors that affect the properties of ^{153}Sm-HA are such as pH, ^{153}Sm-specific activity, lower pH and specific activity of Sm decreases % labelling of ^{153}Sm-HA, HA quantity, higher HA quantity increases % labelling of ^{153}Sm-HA. The ^{153}Sm-HA showed high in vitro stability in saline solution up to several half-life of ^{153}Sm at pH 4–6.

C. Joint leakage study

Joint leakage study was performed in 3 groups of Spraque Drawley rats at 1, 3 and 6 day periods. Each rat in each period was injected intra-articular with ^{153}Sm-HA activity 15–40 μCi, volume 0.15 mL. Rats were kept in metabolic cages and the total urine excreted within 1, 3 and 6 days post injection were collected in containers. At the end of 3 periods post injection, rats were sacrificed and dissected. Samples from each organ were weighed and counted in MCA counter. The radioactivity uptake in organs were calculated.

D. Control study

Control study was performed as the joint leakage study as above but using ^{153}Sm-citrate instead of ^{153}Sm-HA.

The control study performed with ^{153}Sm compound not bound to HA particles showed the distribution of radioactivity in rats that leaks from the joint to organs. For ^{153}Sm-HA, the leakage of ^{153}Sm up to 6 days post intra-articular injection is lower (Table XXXXVIII).

E. Product specification

- ~20 mCi/dose/2 mL
- particle sizes 10–40 μ
- pH4–6
- % labelling >95
- sterile, pyrogen free

2.10.2. New contributions

- Due to low specific activity of ^{153}Sm, to limit the EDTMP content not to high in labelled compound ^{153}Sm-Ca-EDTMP, the molar ratio of ^{153}Sm:Ca-EDTMP should be 1:100.
- Added Ca to EDTMP.

TABLE XXXXVIII. % ID OF ^{153}Sm-HA AND ^{153}Sm-CITRATE DISTRIBUTION IN NORMAL RATS

	^{153}Sm-HA			^{153}Sm-citrate		
Days post injection	1	3	6	1	3	6
Liver	0.05	0.08	0.10	8.56	8.69	5.29
Spleen	0.00	0.00	0.15	0.22	0.10	0.19
Kidney	0.00	0.00	0.15	0.63	0.39	1.13
Muscle	0.00	0.00	0.00	14.36	1.77	15.94
Skin	0.00	0.00	0.00	0.00	0.00	0.00
Bone	0.00	0.00	0.27	4.94	3.51	7.46
Lung	0.02	0.06	0.00	0.20	0.11	0.26
Heart	0.00	0.00	0.01	0.19	0.03	0.27
Blood	0.00	0.00	0.00	0.45	0.09	0.58
Urine	0.00	0.01	0.82	0.47	0.11	1.76
Stomach	0.01	0.00	0.20	0.20	0.00	0.30
Tot. GI	0.10	0.08	0.09	1.70	0.34	0.58
Tail	0.04	0.00	0.04	0.36	0.30	0.66
Knee joint	8.54	19.55	3.85	26.70	43.05	24.43
Cumulative extra-leakage	0.22	0.23	1.83	32.28	15.44	34.42

2.10.3. Co-operation with participating laboratories

- Department of Radiology and Medicine, Siriraj Hospital, Faculty of Medicine, Mahidol University, Bangkok.
- Department of Radiology, Chulalongkorn Hospital, Faculty of Medicine, Chulalongkorn University, Bangkok.
- Department of Radiological Technology, Faculty of Medical Technology, Mahidol University, Bangkok.
- Pramong Kutklao Hospital.

Reports

KULLAPRAWITHAYA, U., VIRAWAT, N., CHINGJIT, S., LAOHAWILAI, S., NGAMPRAYAD, T., Optimization of the Production and quality control of samarium-153 and ^{153}Sm-EDTMP, 32nd Annual Scientific Meeting of the Radiological Society of Thailand and the College of Radiologists of Thailand, Bangkok, Thailand (26–28 January 1995) 120.

VIRAWAT, N., KULLAPRAWITHAYA, U., CHINGJIT, S., LAOHAWILAI, S., NGAMPRAYAD, T., Optimization of the Production and quality control of samarium-153 and ^{153}Sm-EDTMP, 6th Conference on Nuclear Science and Technology, Bangkok, Thailand (2–4 December 1996) 461–475.

AUNGURARAT, A., VIRAWAT, N., CHINGJIT, S., PHUMKEM, S., Optimization of samarium-153 labelled Hydroxyapatite Particles as Therapeutic Agent for radiation synovectomy, 35th Annual Scientific Meeting of the Radiological Society of Thailand and the Royal College of Radiologists of Thailand, Bangkok, Thailand (26–28 March 1998) 70.

3. PRODUCTION PROTOCOLS

3.1. PRIMARY RADIONUCLIDES

TABLE IXL. TARGET MATERIALS AND IRRADIATION PARAMETERS FOR THE PRODUCTION OF ^{153}Sm, ^{166}Ho, ^{165}Dy and ^{186}Re FOR LABELLING THERAPEUTIC RADIOPHARMACEUTICALS

Radioisotope	Target Isotope	Enrichment per cent	Target Chemical Form	Neutron Flux $n \cdot cm^{-2} \cdot s^{-1}$	Specific Activity
^{153}Sm	^{152}Sm	~99	$^{152}Sm(NO_3)_3$ (1) $^{153}Sm_2O_3$	5.0×10^{13} (2)	1–40 GBq/mg (3)
^{166}Ho	^{165}Ho	Natural	$Ho(NO_3)_3$ Ho_2O_3	$>1.0 \times 10^{13}$	1–40 GBq/mg
^{165}Dy	^{164}Dy	Natural	$Dy(NO_3)_3$ (1) Dy_2O_3	$<1.0 \times 10^{13}$ (4)	To be supplied
^{186}Re	^{185}Re	~99	^{185}Re metal	$>1.0 \times 10^{13}$	(5)

(a) Participants have used both solid and liquid targets to suit irradiation and processing requirements. Some participants reported improved irradiation yield with liquid targets.

(b) A neutron flux as low as 6.0×10^{12} $n \cdot cm^{-2} \cdot s^{-1}$ has been used to produce useful activities of ^{153}Sm.

(c) The CRP participants investigated a range of specific activities. Animal and other data reported suggest that a lower specific activity than given may be used to label EDTMP. The combined effects of specific activity and the Sm/EDTMP molar ratio is dealt with in several of the participants reports.

(d) Despite the reduction in irradiation yields of ^{165}Dy, a neutron flux less than $<1.0 \times 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$ must be used to minimize contamination of the radionuclide with ^{166}Dy–^{166}Ho, which is produced at a rate proportional to the square of the neutron flux by activation of ^{165}Dy.

(e) See the report from Pakistan for preliminary work on ^{186}Re. The consensus view is that ^{186}Re and ^{188}Re are less suitable than ^{153}Sm for development as a radionuclide for bone pain palliative radiopharmaceuticals. The relatively long half–life ^{186}Re in particular probably restricts production to reactors which operate continuously for at least one week per cycle. Extensive chemical processing is usually required to produce agents with acceptable radiochemical purity.

3.2. ^{153}Sm-EDTMP

3.2.1. Radiolabelling

Preparation of ^{153}Sm-EDTMP

(a) Irradiate 9 mg of enriched ^{152}Sm as nitrate to get 16 to 50 GBq (at production time).

(b) Dissolve the target in 1 mL of isotonic saline solution (pH ca. 4).

(c) Add the above solution to 180 mg of EDTMP solution (10.5 mL buffered to pH7.5).

(d) Incubate the resulting preparation for 30 to 40 min at room temperature.

(e) Add 5 mL of pH7.5 phosphate buffer (0.18 M) and also add saline added to give 0.5 GBq/mL at the time of calibration.

Comments

(a) When the target is dissolved in saline solution, the pH of the solution should be carefully monitored. The optimum pH of the resulting solution should be approximately 4, in order to avoid the unwanted formation of samarium hydroxides.

(b) Labelling should be carried out at pH above 7.0, to obtain a stable complex.

(c) Labelling of EDTMP with Sm can be performed equally well using a wide range of EDTMP : Sm molar ratios (from 5:1 to 250:1). A labelling yield of over 99% is obtained.

(d) The EDTMP used should have a purity of at least 99% to avoid the formation of non-bone specific compounds. A variety of commercial and locally produced sources have been tested, and no major differences have been noted. Emphasis must be made on the testing of new EDTMP sources with validated quality control methods (labelling yield, stability tests, and biodistribution studies).

(e) During the labelling procedure, there is a possibility of pH decline. After the 30 min incubation period, the addition of phosphate buffer is recommended as a safety precaution, to reduce the possibility of $^{153}Sm^{+3}$ release into the solution, resulting in the unwanted precipitation of $Sm(OH)_3$ (optimum pH: 7.5).

(f) Sterile filtering is also recommended after the labelling procedure, for the removal of any samarium hydroxides, which are possibly formed.

3.2.2. Quality control

Radionuclidic purity

Radionuclidic purity of ^{153}Sm-EDTMP complex should be carried out by gamma spectrometry using MCA coupled with pre-calibrated detector.

Radiochemical purity

It has been observed that a number of quality control procedures for the determination of radiochemical purity of ^{153}Sm-EDTMP complex have been used by each country. Those have already been given in section (2). The possible impurities in the complex are free Sm and $Sm(OH)_3$. A more detailed study on the measurements of radiochemical purity of the complex has been carried in Greece where thirteen different systems have been tested. It is more useful for practical purposes to select a method that is less time consuming and capable of resulting accurate picture of radiochemical purity of the complex. An extensive data on the radiochemical purity of the complex given in each Country's report indicate the use of Sephadex C-25 column as a primary method which retains free Sm as well as $Sm(OH)_3$. The other method which give quick results is the use of ITLC SG with 1M NH_4OH or NH_4:H_2O (1:25) as solvent.

Biological purity

The presence of microorganisms (aerobic, anaeorbic, fungus and yeast) in ^{153}Sm-EDTMP complex can effectively be determined by Thioglycollate, Triptone Soya Broth and Sabouraud Broth 2% glucose at room temperature and at 37°C. The microorganisms can finally be removed from the product by membrane filtration technique before dispensing that not only retains bacteria but also $Sm(OH)_3$. The use of membrane filter may cause lowering of radioactivity which may be the outcome of the retention colloidal form of Sm on the filter. Pyrogens can be measured by LAL test within one hour at 37°C.

Stability

The ^{153}Sm-EDTMP complex must be stable for more than five days. It has been observed that if its radioactive concentration of ^{153}Sm-EDTMP is high, it may show yellow colour. However, the yellow colouration in the product doesn't effect its radiochemical purity. This yellow colouration may be reduced either by diluting the product or by dispensing a single in one vial for hospitals. It is more useful to keep the product in frozen condition using dry ice to avoid yellow colouration. Stability of the product will be better if its shipment is carried out in dry ice.

Calibration of ^{153}Sm dose

For calibrating ^{153}Sm dose it is more appropriate to use dose calibrator which contains calibration factor. Alternatively, secondary calibration sources may be used to work out the calibration factor of the ionization chambers available in hospitals or laboratory. Similarly, using the results of the efficiency curves of precalibrated MCA for absolute measurements a calibration factor for dose calibration could be adjusted.

3.3. RADIOLABELLED PARTICLES

Three new radiopharmaceuticals (^{153}Sm-HA, ^{166}Ho-microspheres and ^{166}Ho-FHMA) which have been tested also in animal biodistribution studies have been developed by few participating centres. They all show good in vitro and in vivo stability as evaluated with animal experiments.

The preferred properties for radiosynovectomy agents have been studied in some detail by other groups, particularly by Johnson with his co-workers (L.S. Johnson, J.C. Yanch, et al., (1995)) and R.J. Mumper and co-workers (R.J. Mumper, B.J.A. Mills, et al., (1992)). According that short half-life of less than two days, and high enough beta energy (around 2 MeV) of the nuclide to allow needed range are preferred characteristics for the nuclide. Thus properties of ^{166}Ho are very close to optimal, but ^{153}Sm decays with lower energy betas, which might be more suitable to treat small joints rather than knees and other bigger joints. High stability of particles in vivo is a natural demand, but also optimal particle size range have been defined to be between 3 and 15 μms.

^{153}Sm-HA and ^{166}Ho-FHMA are planned to be used in radiosynovectomy with local injection into inflamed joint synovium. In this CRP particle size of ^{166}Ho-FHMA has been analyzed to be in optimal size range. The particle size distribution of ^{153}Sm-HA has not been optimized, but is in bigger range. However the particles can easily selected to reach closer to optimal size range. Both radiopharmaceuticals show very high in vitro stability and in vivo stability when injected into knee joints of rats or rabbits. ^{166}Ho-FHMA is in clinical trials stage.

The production of both ^{166}Ho-FHMA and ^{153}Sm-HA is fast and straight forward with relatively high yields. The radiochemical quality control is easily performed by sentriguating the precipitate and by removal of supernatant. The activity of both precipitate and supernatant are measured. Supernatant represent non-bound activity.

One practical issue for these radiopharmaceuticals is that HA particles deposit very rapidly to the bottom of the vial or syringe and this causes practical problems in injections to deliver intended dose to patient. This could be improved to some degree when using more viscose media. ^{166}Ho-FHMA do not have those problems. For more details please see the enclosed country reports, for ^{166}Ho-FHMA see Finnish report and for ^{153}Sm-HA. there are several reports.

^{166}Ho-microspheres are designed to treat hepatic metastases and thus their optimal target size is somewhat bigger than what is needed in radiosynovectomy. The in vitro and in vivo studies showed high stability and thus clinical patient studies are ongoing. For further details please see the Australian country report.

In total there is considerable amount of new data collected within this CRP and clinical studies have been initiated particularly with both ^{166}Ho based particles.

3.4. BIODISTRIBUTION STUDIES

Biodistribution studies should be performed in rats which gave good data results. But dogs also recommended as a perfect model if available. The first observation should be 2 or 3 hours post injection that bone uptake could be seen , the next observation should be 24, 48 hours or longer to see the blood clearance and the excretion of radioactivity in to urine and the retention of Sm-EDTMP in bone. The interested organs should include bone, liver, kidney, muscle and as many organs as practicable. Data should be reported in % Recover dose, %ID or %ID per g.

4. SUMMARY OF PRODUCTION AND QUALITY CONTROL DATA

TABLE L. ^{153}Sm PRODUCTION

Country	Reactor flux/ irradiation time ($n \cdot cm^{-2} \cdot s^{-1}$)/time	Type of target	Obtained specific activity (GBq/mg)	Batch act. (GBq)	Frequency of production	Sm-152 Enrichment (%)
Argentina	7×10^{13}/36 h	$Sm(NO_3)_3$ solid	14	14	weekly	>98.7
Australia	5×10^{13}/6 d	Sm_2O_3 solid	26	130	weekly	>99
Brazil	2×10^{13}/40 h	$Sm(NO_3)_3$ film	2.5	80	weekly	98.3
China, P.R.	8×10^{13}/3 d	$Sm(NO_3)_3$ solid	30	1740	twice/month	>98.7
Finland	2×10^{13}/40 h 1.5×10^{14}/20 h	$Sm(NO_3)_3$ solid	3.0 5.0	50 70	weekly	>99
Greece	4.81×10^{13}/4 h	$Sm(NO_3)_3$ liquid	1.5	experiment	experiment	>98.7
Indonesia	1.2×10^{14}/100 h	Sm_2O_3 solid	40	experiment	experiment	>98.7
Malaysia	0.6×10^{13}/12 h	$Sm(NO_3)_3$ film	0.64	3.2	experiment	>98.7
Pakistan	1.2×10^{14}/72 h	$Sm(NO_3)_3$ solid	94	experiment	experiment	>99
Thailand	2×10^{13}/11 h	$Sm(NO_3)_3$ liquid	3	15	twice/month	98.3

TABLE LI. PREPARATION OF ^{153}Sm-EDTMP

Country	Sm:EDTMP per batch	Molar ratio EDTMP:Sm	Incub. time /temp. °C	Final vol. activity (GBq/mL)	Calibr/exp After prep.	Source of EDTMP	Method of sterilization
Argentina	1:60	20	45 min/75	0.9	24/48 h	in house	Autoclaving
Australia	1:1166*	60	Not required	2	24/48 h	Dow, USA	Autoclaving
Brazil	1:43	15	30 min/25	1.0	24/24 h	ICN, USA	Sterile filter
China	1:150	100	1 h/70	1.2	48/48 h	TCI, Japan	Sterile filter
Finland	1:20	7	30 min/20	0.5	24/48 h	TCI,Japan	Autoclaving
Greece	1:25	7	30 min/20	0.5	18/24 h	in house	Autoclaving
Indonesia	1:717	250	30 min/30	0.5	4/6 d	TCI,Japan	Sterile filter
Malaysia	1:30	10	30 min/30	0.4	24/8 h	TCI,Japan	Sterile filter
Pakistan	1:15	5	20 min/25	1	24/48 h	TCI,Japan	Sterile filter
Thailand	1:300	100	50 min/30	0.7	24/30 h	TCI,Japan	Sterile filter

* to be confirmed.

TABLE LII. ^{153} Sm-EDTMP, GENERAL INFORMATION

Country	Initial clinical work	Number of patients	Dose/patient (MBq/kg)	No. of hospitals involved	Registration/ legal acceptance
Argentina	1996	75	20–40	2	under consideration
Australia*	1988/1996	123	37	1	♣
Brazil	1995	860	37	47	under consideration
China	1993	2000	20–37	30	under consideration
Finland	1990	1250	20–60	30	registered
Greece	No				under consideration
Indonesia	1996	12	20–40	3	under consideration
Malaysia	No				pending
Pakistan	1998	3	37	2	under consideration
Thailand	1995	50	37	2	under consideration

* Original ANSTO product.

♣Dow Chemical Company ^{153}Sm-EDTMP(Quadramat) has been registered in Australia and marketed by Australian Radioisotopes.

TABLE LIII. QUALITY CONTROL METHODS

Country	QC Method 1	QC Method 2	RCP release limit
Argentina	ITLC SG water	Sephadex G-25	>97%
Australia	Ion exchange chromatography	HPLC to be implemented	*
Brazil	Sephadex C-25		>98%
China	Sephadex C-25	NH_3/MeOH/H_2O 0.2:0.5:4	>99%
Finland	Sephadex C-25	ITLC SG in saline	>98%
Greece	Whatman no. 1 NH4:H2O, 1:25	ITLC SG NH4:H20, 1:25	>99%
Indonesia	TLC, cellulose	Chelex-100	>97%
Malaysia	ITLC SG, 1M ammonia	ITLC SG NH_3/MeOH/H_2O	>99%
Pakistan	ITLC SG, 1M ammonium hydroxide	PC NH_3:MeOH:H_2O 0.2:2:4	>99%
Thailand	Sephadex C-25	TLC, pyridine	>99%

* not supplied.

Sephadex C-25 method — A column with two cm height of water incubated Sephadex C-25 is prepared. 100 MBq of Sm-153 EDTMP is put to the column. Measure the activity with the dose calibrator. Let the solution go through and rinse with 15 to 20 mL of saline. Measure the activity of the column. The percentage of column activity after rinsing divided by the initial activity gives the percentage of impurities.

ITLC SG (water/saline) — The hydroxide and free Sm remain at origin, when Sm-EDTMP ascends with the eluent.

TLC cellulose — Cellulose plate with eluent (pyridine : EtOH : water, 1:2:4). Free Sm remains at the bottom while EDTMP passes through.

Chelex-100 — A column is prepared with 600 mg pretreated with saline. Free Sm is retained in the column, while Sm-EDTMP is passed through.

ITLC SG ammonia — 1 N ammonia in water is used as eluent. Sm stays at the bottom while Sm-EDTMP goes with eluent.

Whatman MM — Elution mixture (ammonia : MeOH : water, 0.1:2:4) Whatman 3 MM paper strips are used with. Free Sm stays in the bottom, while Sm-EDTMP goes with eluent.

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