

Development of Sm-153 Chitosan for Radiosynovectomy

Ali Bahrami-Samani¹, Amir Reza Jalilian¹, Hassan Yousefnia¹, Mehdi Akhlaghi²,
Mohammad Mazidi¹, Mohammad Ghannadi-Maragheh¹

¹Radiopharmaceutical Research and Development Laboratory, Nuclear Science and Technology Research Institute, Atomic Energy organization of Iran, Tehran, Iran.

²Research Institute for Nuclear Medicine, Tehran University of Medical Sciences, Tehran, Iran.

(Received 2 November 2009, Revised 23 November 2009, Accepted 4 December 2009)

ABSTRACT

Introduction: Developing new radiosynovectomy agents is of great importance due to the aging of human populations around the world and increasing the incidence of inflammatory diseases. In this work, Sm-153 chitosan agent was developed for the first time in our country and preparation and quality control of the compound is described.

Methods: Sm-153 chloride was obtained by thermal neutron flux ($4-5 \times 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$) of natural Sm_2O_3 sample, dissolved in acidic media. ^{153}Sm -samarium chloride (370 MBq) was used in preparation of ^{153}Sm -chitosan complex followed by quality control using MeOH: H₂O: acetic acid (4: 4: 2) as mobile phase. The complex stability and viscosity were checked in the final solution up to 2 days. The complex solution and $^{153}\text{Sm}^{3+}$ (80 $\mu\text{Ci}/100 \mu\text{l}$) were injected intra-articularly into male rat knee joint followed by scarification studies 6 d post injection.

Results: Sm-153 chitosan was prepared successfully with high radiochemical purity (>99%, ITLC) at room temperature after 10-30 min followed by autoclave sterilization. The complex was stable at room temperature and 37°C up to 2 days. No significant leakage of dose from injection site and its distribution in organs were observed up to 6 days for ^{153}Sm -chitosan.

Conclusion: Approximately, more than 90% of injected dose remained in injection site after 6d. The complex is a dedicated agent for radiosynovectomy. The experience from this study would lead to the development of more sophisticated radiosynovectomy radiopharmaceuticals for human use in the country.

Keywords: Chitosan, Radiosynovectomy, Samarium-153, Biodistribution

Iran J Nucl Med 2010;18(1):22-31

Corresponding author: Dr Amir R Jalilian, Radiopharmaceutical Research & Development Laboratory, Nuclear Science and Technology Research Institute, AEOI, Tehran, Iran
E-mail: ajalilian@nrcam.org

INTRODUCTION

In the treatment of rheumatoid arthritis, a surgical, chemical or radiation synovectomy (RSV) may be applied. Surgical synovectomy suffers from the risks of surgery and anesthesia, the need for hospitalization and a prolonged period of rehabilitation remained, albeit to a minor degree (1). In chemical synovectomy highly toxic agents like osmic acid, alkylating substances like nitrogen mustards, methotrexate and cobra venom were used initially but were then abandoned because of possible joint tissue damage (2, 3).

Chitosan, a natural and biodegradable polysaccharide with wide range applications in bio-pharmaceutics, agriculture and water treatment purposes (4), is an excellent molecule for intra-cavitary therapy. Sm-153 chitosan ($^{153}\text{Sm-CHITO}$) complex is reported for internal radiation therapy for treatment of diseases such as hepatocellular carcinoma and rheumatoid arthritis (5).

Also, the effectiveness of other lanthanide compound, Ho-166 chitosan, for malignancies such as gliomas and prostate cancer (6) has been successfully approved.

Many beta-emitters such as Sm-153, Lu-177 and Ho-166 can be produced in reasonable amounts using (n, gamma) reactions. ^{153}Sm ($T_{1/2} = 46.7$ h) usually prepared by neutron activation of natural and/or enriched $^{152}\text{Sm}_2\text{O}_3$ (7), can be an excellent therapeutic radionuclide, due to its medium energy beta

particles for targeted therapy modalities, for developing radiation therapy compounds using small ligands (8, 9). In this research, $^{153}\text{Sm-CHITO}$ complex production is described in details, followed by determination of complex radiochemical purity, stability and biodistribution (after intra-articular injection) in wild-type male rats.

METHODS

Production of ^{153}Sm was performed at the Tehran Research Reactor (TRR) using ^{152}Sm (n, γ) ^{153}Sm reaction with ^{152}Sm in purity of 98.7% (ISOTEC Inc.). Chitosan (medium molecular weight, MW=400 kDa, DDA=%85) was obtained from Fluka (Bucks, Switzerland). Chromatography paper, Whatman No. 1 was obtained from Whatman (Maidstone, UK). Radiochromatography was performed by using a bioscan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC, USA). A high purity germanium (HPGe) detector coupled with a CanberraTM (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activity in mice organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). Calculations were based on the 103 keV peak for ^{153}Sm .

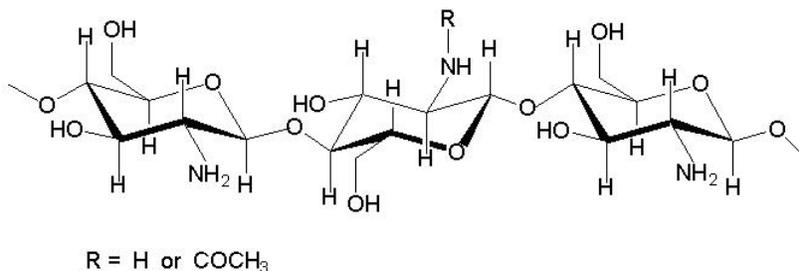


Figure 1. Chemical formula for chitosan

All values were expressed as mean \pm standard deviation (Mean \pm SD) and the data were compared using student T-test. Statistical significance was defined as $P < 0.05$. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edn. All of rats were purchased from Pasteur Institute of Iran, weighing 180-220 g ($n=5$) and were kept at routine day/night light program and were kept under common rodent diet pellets.

Production and quality control of $^{153}\text{SmCl}_3$ solution

The ^{153}Sm was produced by neutron irradiation of 100 μg of enriched $^{152}\text{Sm}_2\text{O}_3$ according to reported procedures (10) at a thermal neutron flux of $5 \times 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$ for 5 days. Specific activity of the ^{153}Sm was 27.75 GBq/mg.

The irradiated target was dissolved in 200 μl of 1.0 mol/L HCl, to prepare $^{153}\text{SmCl}_3$ and diluted to the appropriate volume with ultra pure water, to produce a stock solution.

The mixture was filtered through a 0.22 μm biological filter and sent for use in the radiolabeling step. Radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy and HPGe spectroscopy to detect various interfering beta and gamma emitting radionuclides.

The radiochemical purity was also checked by Whatman No.1 chromatography paper, and developed in a mixture of 10 mmol/L DTPA solution and also %10 ammonium acetate:methanol (1:1) as mobile phases.

Preparation of ^{153}Sm chitosan complex

^{153}Sm Samarium chitosan complex was prepared by dissolving of chitosan (35 mg) in 3.5 ml of 1% acetic acid aqueous solution following the addition of ascorbic acid (15 mg) and the mixture was stirred at room temperature till a transparent solution was

formed. To the above mixture 296-370 MBq (in 0.5 ml) of ^{153}Sm samarium chloride was added followed by stirring for 5 minutes and standing for 30 minutes at room temperature. For sterilisation, the 0.22 micron sterile filtration was not successful due to viscosity of the solution. Thus the radiolabeled mixture was autoclaved for 20 min at 120°C. The effect of the autoclave on the stability of the complex was also studied using ITLC. For kit preparation, the starting chitosan, ascorbic acid and acetic acid solution containing preservative amounts of benzyl alcohol was lyophilized under sterile conditions and kept in fridge up to 60 days.

Stability testing of the radiolabeled compound in final formulation

Stability of ^{153}Sm -chitosan in final preparation was determined by storing the final solution at 4°C and 25°C for 2 days and performing frequent ITLC analysis to determine radiochemical purity. ITLC analysis of the conjugated product was performed to monitor for degradation products or other impurities. After subsequent ^{153}Sm -labeling of the stored product, both labeling efficiency and radiochemical purity were determined.

Stability testing of the radiolabeled compound at 37°C

Final ^{153}Sm -CHITO solution (200 μCi) was incubated in presence of freshly prepared human serum (300 μl) and kept at 37°C for 2 days. The complex stability was assessed by size exclusion chromatography on a Sepharose column (1 x 30 cm). The column was equilibrated with PBS and eluted at a flow rate of 0.5 mL/min at room temperature; 1 mL fractions were collected and their activities were determined in a dose calibrator, also the presence of serum proteins in each fraction was determined by Folin-Colcitateu[®] method. The control samples were applied to the column separately, including Sm^{3+} cation, ^{153}Sm -

CHITO complex and human serum sample for retention time determination.

Quality control

For measuring radiochemical purity and radiolabeling yield, a 1 μ L sample of the [¹⁵³Sm]samarium chitosan complex was spotted on a chromatography paper (Whatman No. 1), and developed in a mixture of methanol/water/acetic acid (4:4:2) as the mobile phase. The R_f values of free Sm-153 and [¹⁵³Sm]samarium chitosan complex were 0.45 and 0.0, respectively.

Biodistribution of ¹⁵³SmCl₃ in male wild-type rats

To determine the biodistribution of free ¹⁵³SmCl₃, in case of any radioisotope leak from the injection site, the cation in normal saline solution was administered to wild-type rats. A volume of ¹⁵³SmCl₃ stock solution (50-100 μ L) was evaporated at 50°C and N₂ flow. To the residue, sterilized normal saline solution (according to appropriate specific activity) was added. For each animal, appropriate amount of ¹⁵³SmCl₃ activity (150 \pm 5 μ Ci in 50-100 μ L) was injected intravenously to rats through their tail vein. The animals were sacrificed at the exact time intervals (4, 24, 48 and 96 h), and the specific activity of different organs was calculated as percentage of injected dose per gram using an HPGe detector.

Biodistribution of radiolabeled chitosan wild-type rats after intra-articular administration

To determine the accumulation of radiolabeled chitosan in the intraarticular cavity radiolabeled chitosan solution was carefully administered to wild-type rats. A volume (50 μ L) of final radiolabeled chitosan solution containing 60 \pm 2 μ Ci radioactivity was injected intraarticularly to rats. The animals were sacrificed 4 and 6 d post injection. The specific activity of different organs was calculated as percentage of urea

under the curve of 103 keV peak per gram using an HPGe detector.

RESULTS AND DISCUSSION

Production and quality control of ¹⁵³Sm

The radionuclide was prepared in a research reactor according to regular methods with a range of specific activity 25.9-27.7 GBq/mg for radiolabeling use, after counting the samples on an HPGe detector for 5 hours, very slight amount of impurities were recorded and shown to be Eu radionuclides as shown in Table 1.

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

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

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

The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated for obtaining the desired pH and volume followed by sterile filtering. The radiochemical purity of the Sm-153 solution was checked in two solvent systems, in 10mM DTPA, Sm³⁺ cation is complexed to more lipophilic ¹⁵³Sm-DTPA form and migrates to higher R_f, while small radioactive fraction remains at the origin which could be related to other Sm ionic species, not forming ¹⁵³Sm-DTPA complex, such as SmCl₄, etc. and/or colloids.

On the other hand, %10 ammonium acetate:methanol (1:1) mixture was also used for the determination of radiochemical purity. The fast eluting species was possibly the ionic Sm-153 cations other than Sm^{3+} and the remaining fraction at $R_f.0.05$ was a possible mixture of Sm^{3+} and/or colloids. Due to existence of 1% impurity in both cases the existence of colloids is unlikely (Figure 2).

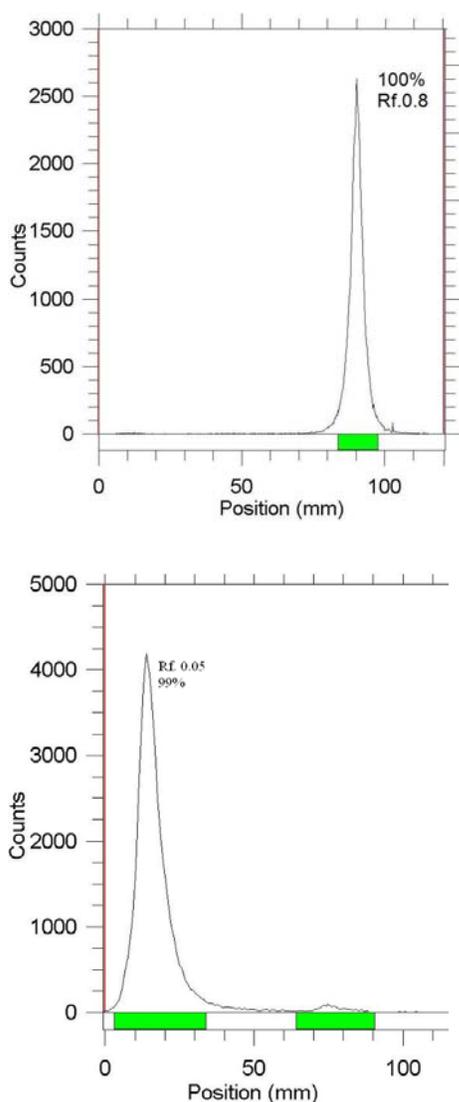


Figure 2. ITLC chromatograms of $^{153}\text{Sm-SmCl}_3$ solution in DTPA solution (pH. 5) (above) and 10% ammonium acetate:methanol (1:1) solution using Whatman no. 2 paper.

Preparation of [^{153}Sm]samarium chitosan complex

The effect of various factors on the labeling yield of [^{153}Sm]samarium chitosan were studied. In higher concentration no significant difference exists on labeling yield for added [^{153}Sm] samarium chloride activity (1110 MBq). The chitosan which had a molecular weight of 400 kDa was used to investigate the effect of chitosan concentration on labeling yield at pH=3.5.

Labeling yield increased with increasing chitosan concentration and reached above 98% when the concentration reached 35 mg/3 ml. The highest labeling yield was achieved at pH=2.8-3.2 while decreased beyond this range. The labeling yield of 99% was achieved after 30 minutes. The effect of absence and presence of ascorbic acid (at various concentrations) as a complex stabilizer were also studied.

ITLC using a mixture of methanol, water and acetic acid showed that the complex is majorly prepared in 30 min with 99% radiochemical purity; the remaining 1% is possibly attributed to other Sm ionic species which can not react with chitosan (Figure 3).

Based on the obtained results, the optimal procedure for the preparation of [^{153}Sm]samarium chitosan complex with a high labeling yield is as follows. 35mg of chitosan (MW=400 kDa) was dissolved in 3.5 ml of 1% acetic acid aqueous solution. The acidity of obtained solution was adjusted to pH=3 by addition of 0.5 M NaOH solution and followed by addition of [^{153}Sm]samarium chloride solution. Finally the total volume was adjusted to 4 ml by addition of deionized water.

Stability studies of [^{153}Sm]samarium chitosan complex

The stability of prepared [^{153}Sm]samarium chitosan complex was checked up to 48 hours after preparation. The complex was stable in acidic media (pH=3.5) and its radiochemical purity was above 99% even

48 hours after preparation. The radiochemical purity of [^{153}Sm]samarium chitosan complex was decreased to 99% after 72 hours in phosphate buffer solution. Also the stability of the complex was determined at 37°C for 48 h and the data were almost consistent with the final solution stability.

Although the complex is injected intra-articularly and is not in direct contact with serum, stability test was developed for the complex in presence of human serum at 37°C. The mixture was then passed through a sephadex column followed by the elution with PBS and each fraction was checked for radioactivity and presence of serum proteins by colorimetric method.

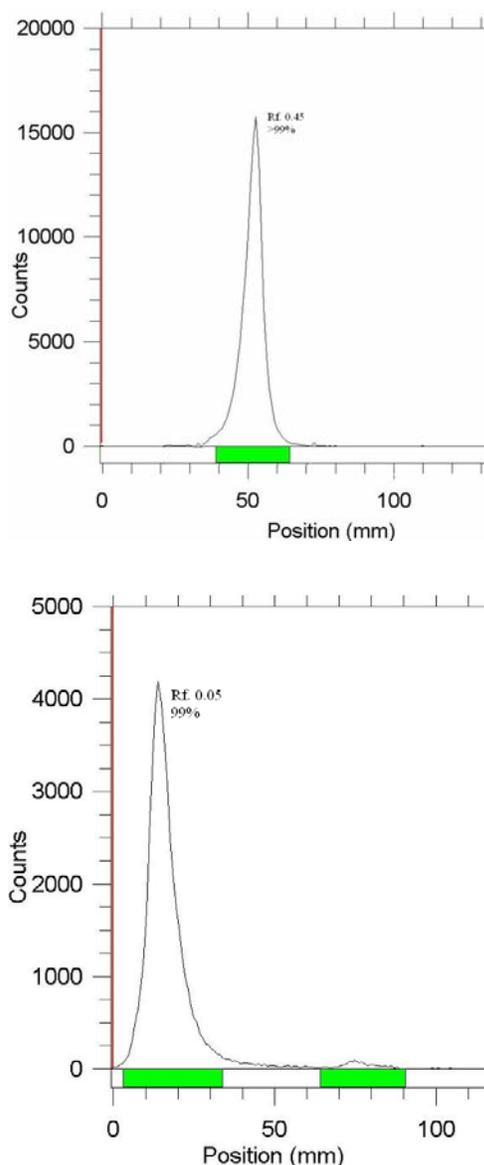


Figure 3. ITLC chromatograms of $^{153}\text{Sm-SmCl}_3$ (above) and $^{153}\text{Sm-chitosan}$ solution (below) on Whatman no. 1 paper using methanol: water: acetic acid (4:4:2) mixture.

Biodistribution studies for Sm^{3+} cation in rats

The animals were sacrificed by CO_2 asphyxiation at selected times after injection (4, 24 48 and 96h). Dissection began by drawing blood from the aorta followed by removing heart, spleen, muscle, brain, bone, kidneys, liver, intestine, stomach, lungs and skin samples. The tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g) (Figure 4).

For ^{153}Sm cation, the biodistribution was mainly in the liver, kidney and bone. The free cation is mainly soluble in water and it can be excreted *via* urinary tract. Since the metallic ^{153}Sm is transferred in plasma in protein-bond form, the major final accumulation was showed to be liver. After 48 h the metabolites and/or free cation were excreted from liver into intestines *via* hepatobiliary tract resulting significant activity in this organ, but it was not significant at 96h. Trace accumulation was also observed in spleen.

Biodistribution studies after intra-articular administration of Sm^{3+} cation in rats

The distribution of injected dose in rat organs up to 4d after intra-articular injection of [^{153}Sm]samarium chloride (60 $\mu\text{Ci}/100\mu\text{l}$) solution was determined for control studies. Based on these results, it was concluded that the most part of injected activity was extracted from the circulation and distributed in rat organs which similar to free Sm^{3+} distribution while administered intravenously (Figure 5).

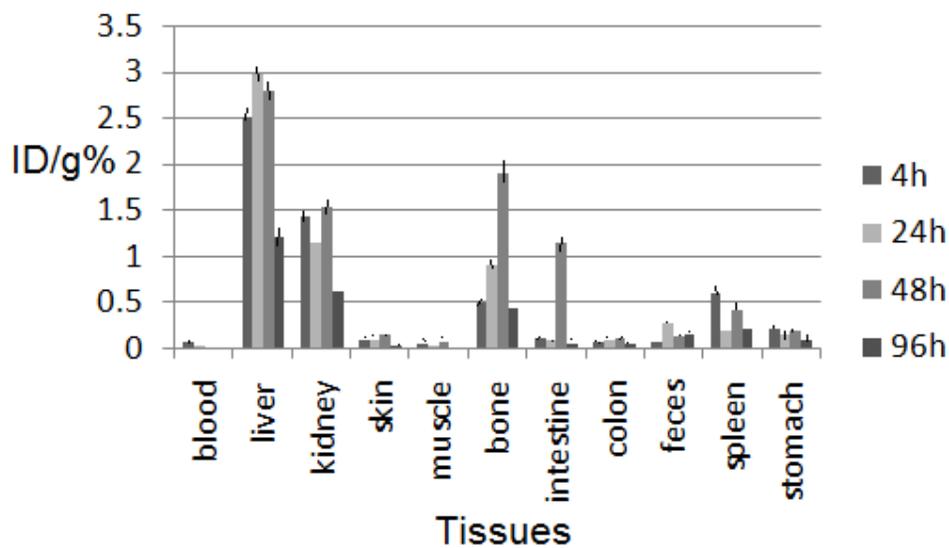


Figure 4. Percentage of injected dose per gram (ID/g %) of $^{153}\text{SmCl}_3$ in rat tissues at 4, 24, 48 and 96 h post injection.

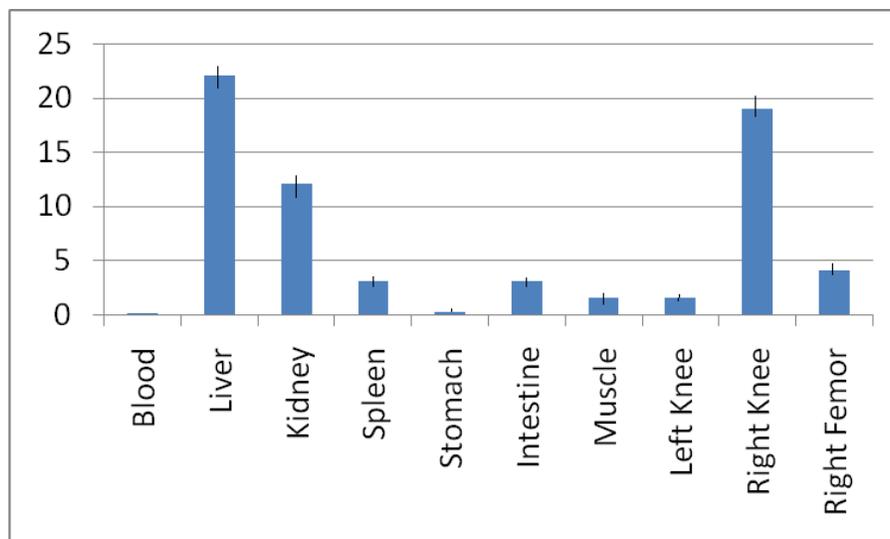


Figure 5. Distribution of $^{153}\text{SmCl}_3$ in wild-type male rats, 4 d after intra-articular injection of 60 μCi of compound. %ID-percentage of injected dose. Each bar presents mean \pm SD (n=3).

Biodistribution studies after intra-articular administration of ^{153}Sm -chitosan cation in rats

Figure 6. presents the distribution of injected dose in the rat organs at various time intervals after intra-articular injection of $60 \mu\text{Ci}/100\mu\text{l}$ of ^{153}Sm samarium chitosan complex as percentage of injected dose. In case of any leak from the joint, the complex would accumulate in reticuloendothelial (RE) system due to high molecular weight of the complex, unless the complex would dissociate at serum pH and Sm^{3+} cation would be formed.

A very small amount of activity was observed in spleen and liver, which are two important RE organs, indicating no major complex leak has occurred. Very negligible liver and kidney uptakes are observed which are possibly caused by Sm-153 cation release from the injected joint and not the radiolabeled complex uptake.

Figure 7 demonstrates the biodistribution of the compound among the tissues excluding the injected knee data in order to better understand the biodistribution of the leaks from the knee.

The distribution of the radioactivity among tissues after removing knee joint

accumulation data demonstrates a typical Sm^{3+} cation biodistribution among the tissues. It is believed that free Sm cation is the only radiochemical species escaping from knee joint and not $^{153}\text{Sm-CHITO}$ complex is found in circulation.

Since Sm-153 is a radiolanthanide practically produced by the research reactors in high specific activities using natural and enriched target, this work was designed for the research purposes of developing new radiolabeled chitosans such as Ho-166 chitosan for intracavitary therapy. The preparation of these compounds are feasible and due to the interesting biological properties of chitosan the human studies in most of east Asian countries has been started vastly used in hepatocellular carcinomas and radiosynovectomy (11, 12). The side effects usually imposed by iron macroaggregates are mostly a problem in clinics such as iron toxicity and painful organs, while in this case are rare.

The use of radionuclides in radiosynovectomy is usually a function of half life and beta particle range leading to the specification of any agent to a specific inflamed organ.

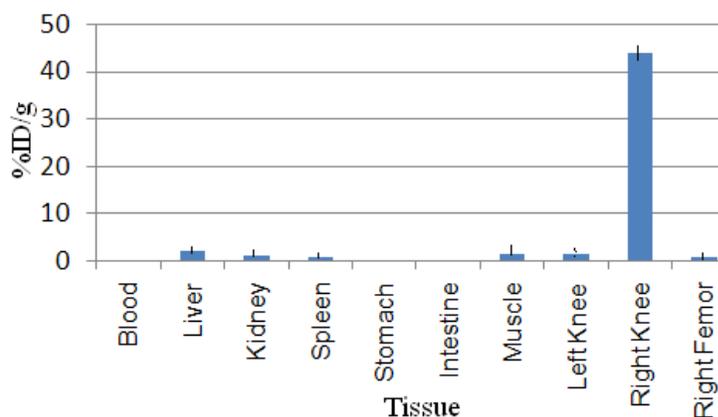


Figure 6. Distribution of ^{153}Sm -chitosan in wild-type male rats, 4 d after intra-articular injection of $60 \mu\text{Ci}$ of compound. %ID-percentage of injected dose. Each bar presents mean \pm SD (n=3).

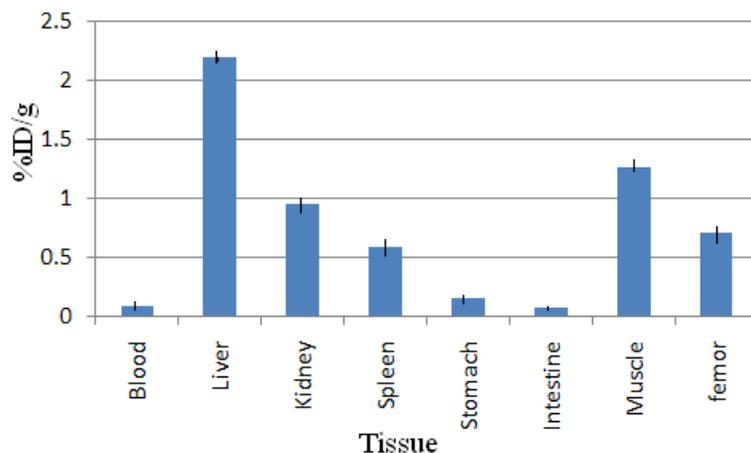


Figure 7. Distribution of [^{153}Sm]-chitosan in wild-type male rats excluding injected knee data at 6d after intra-articular injection of 60 μCi of compound. %ID-percentage of injected dose. Each bar presents mean \pm SD (n=3).

CONCLUSION

The [^{153}Sm]Samarium chitosan complex was prepared with high radiochemical yield (>99 %) in the optimized condition; 35mg/3ml of chitosan concentration in diluted acetic acid solution (pH=3). The prepared complex was stable in the final solution at room temperature, 37°C and presence of human serum, and can be used even 48 hours after preparation. Intra-articular injection of [^{153}Sm]samarium chitosan complex in male wild-type rats and investigation of leakage of activity in the body showed that most of injected dose has remained in injection site 6 d after injection. Finally a kit formulation was developed for the *in-situ* preparation of the radiopharmaceutical in distant clinical centers.

REFERENCES

1. Kampen WU, Brenner W, Czech N, Henze E. Intraarticular application of unsealed beta-emitting radionuclides in the treatment course of inflammatory joint diseases. *Curr Med Chem Anti*

Inflamm Anti Allergy Agents 2002;1(1):77-87.

2. Sledge, C.B. Correction of arthritic deformities in the lower extremities and spine, arthritis and allied conditions, 9th edition. Philadelphia: Lea and Febiger; 1979.
3. von Reis G, Swensson A. Intra-articular injections of osmic acid in painful joint affections. *Acta Med Scand* 1951; suppl. 259: 27-32.
4. Rinaudo M. Chitin and chitosan: properties and applications. *Prog Polym Sci* 2006;31:603–632.
5. Shin BC, Park KB, Jang BS, Lim SM, Shim CK. Preparation of ^{153}Sm -chitosan complex for radiation synovectomy. *Nucl Med Biol.* 2001;28(6):719-725.
6. Seong SK, Ryu JM, Shin DH, Bae EJ, Shigematsu A, Hatori Y et al. Biodistribution and excretion of radioactivity after the administration of ^{166}Ho -chitosan complex (DW-166HC) into the prostate of rat. *Eur J Nucl Med Mol Imaging.* 2005;32(8):910-917.
7. Firestone RB, Shirley VS, Baglin CM, Zipkin J. Table of isotopes, 8th edition (CDROM), Version 1.0. New York: Wiley-Interscience; 1996.

8. Misra SN, Gagnani MA, M ID, Shukla RS. Biological and clinical aspects of Lanthanide coordination compounds. *Bioinorg Chem Appl.* 2004;155-192.
9. Kostova I. Lanthanides as anticancer agents. *Curr Med Chem Anticancer Agents.* 2005;5(6):591-602.
10. Manual for reactor produced radioisotopes, IAEA, Vienna, 2003, IAEA-TECDOC-1340, ISBN 92-0-101103-2, ISSN 1011-4289, © IAEA, 2003, pp.71, Printed by the IAEA in Austria, January 2003.
11. Suzuki YS, Momose Y, Higashi N, Shigematsu A, Park KB, Kim YM et al. Biodistribution and kinetics of holmium-166-chitosan complex (DW-166HC) in rats and mice. *J Nucl Med.* 1998;39(12):2161-2166.
12. Huh R, Park YS, Lee JD, Chung YS, Park YG, Chung SS et al. Therapeutic effects of Holmium-166 chitosan complex in rat brain tumor model. *Yonsei Med J.* 2005;46(1):51-60.