Nuclear Engineering Progress **Research Article**

Cited in: https://nep.nstri.ir/
Received: 17 January 2021, Accepted: 25 April 2021, Published: 1 August 2021

Preparation of 191Os–phytate, an in-vivo radionuclide generator, for radiosynovectomy application

L. Moghaddam*[1], A. Jalilian[2], N.Salek[1], M. Jamre[3]

AFFILIATIONS

[1]Nuclear Fuel Cycle School, Nuclear Science and Technology Research Institute (NSTRI), 14155-1339 Tehran, Iran
[2]Radioisotope Products and Radiation Technology Section, Department of Nuclear Sciences and Applications, International Atomic Energy Agency (IAEA), Vienna, Austria
[3]Faculty of Nuclear Engineering and Physics, Amirkabir University of Technology, Tehran, Iran

* **Author to whom correspondence should be addressed:** lmoghaddam@aut.ac.ir

ABSTRACT

191Os is a parent radionuclide with a 15.4 d half-life. It decays by beta emission to 191mIr, which is a radionuclide with a 4.96s half-life. It decays by the isomeric transition to stable 191Ir, emitting a 129-keV gamma photon. In this study, 191Os–phytate was developed into an in-vivo radionuclide generator for simultaneous radiosynovectomy and imaging. 191Os-hexachloroosmate was used to prepare 191Os–phytate (100 µCi/50 µl) using reaction condition optimization followed by an intraarticular injection to rat knee joints. Also, its distribution and stability were assessed. The imaging of 191Os cation and 191Os–phytate was performed by SPCET. The 191Os–phytate complex was obtained at pH=5.5 with normal saline at room temperature. Radio-TLC showed an overall radiochemical yield of 95-98%. The complex was injected into the rats' knees, and the whole injected dose remained at the injection site even three days after injection. Due to the stability and retention of the complex in joints approved by biodistribution and imaging studies, the complex is a potential in vivo generator for cavital radiosynovectomy of minor joints.

Keywords: Radiosynovectomy, Phytate, Osmium-191, Biodistribution, Imaging

I. INTRODUCTION

Radiosynovectomy (RSV) has been proposed as a potent palliative therapy around the world for the last two decades [1] and several radio-pharmaceuticals have been developed and used in RSV, including 166Ho-macroaggregates [2] and Ho-166 phytate complex [3]. Phytate is the salt form of phytic acid (Fig.1). It is known that hexaphosphoric acid of inositol (Phytic Acid) is active in the absorption of calcium and phosphorous by the body and soluble in water in the form of sodium salt. However, it forms insoluble complexes with other metals such as Ca, Fe, and Zn. In the blood, phytic acid binds with calcium ions forming colloidal particles captured by the reticuloendothelial system, principally by Kupffer cells in the liver [4]. The intravenous administration of 99mTc-Phytate is used for hepatic gamma scintigraphy. It retains its capacity to react in vivo with calcium ions in the blood-forming 99mTc-phytate, a colloid engulfed by the

reticuloendothelial system's cells, allowing the organ to be easily visualized. However, it is also possible to observe the spleen and bone marrow [5]. Many phytate complexes labeled with beta-emitters such as 166Ho [6], $^{188\text{-}186}$Re [7], and ^{169}Er [7] have been developed for radiosynovectomy (RSV). 191Os (Eβ- max = 313 keV, T1/2 = 15.4 d), (Fig. 2), is one of the potential radionuclides for developing therapeutic radiopharmaceuticals usually prepared for 191Os/191mIr generator production.

191Os/191mIr generator is suitable for the first-pass radionuclide angio-cardiography developed to prepare repeated delusions of 4.96-sec 191m Ir from its 15.4-day 191Os parent for injection [8]. Recently, potential therapeutic tumor-avid 191Os-labeled bleomycin has been developed for ultimate therapeutic evaluation [9].

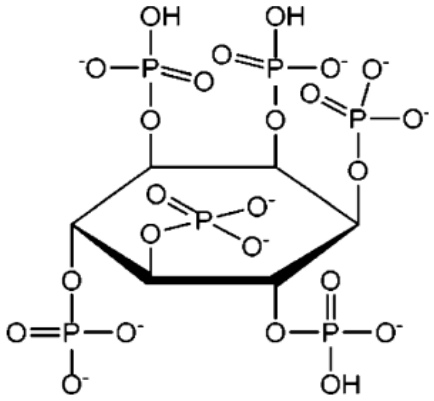

Fig. 1. Chemical formula for phytate

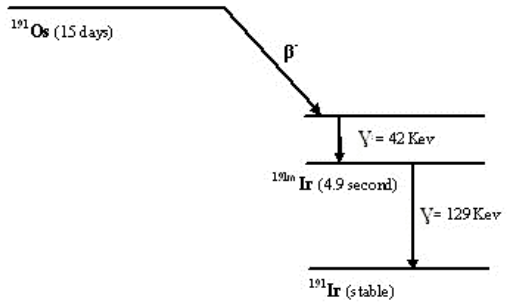

Fig. 2. Salient characteristics of osmium-191 decay

Due to the potential characteristics of 191Os as a beta emitter and phytate complex applications for radiosynovectomy, in this research, 191Os-phytate complex production is described in detail, followed by the determination of difficult radiochemical purity, stability, and bio-distribution (after intravenous and intraarticular injection) in wild-type rats.

II. MATERIALS AND METHODS

Isotopically enriched granulated metallic Osmium with a purity of >90% was obtained from commercial vendors. Phytate complex was prepared using a commercial phytate kit (Pars Isotope Co., Tehran, Iran, stannous chloride free). Chromatography paper, Whatman No. 2, was obtained from Whatman (Maidstone, UK). Radio-chromatography was performed using a BioScan AR-2000 radio TLC scanner instrument (Bioscan, Washington, DC, USA). A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activity in rat organs. All other chemical reagents were purchased from Merck (Darmstadt, Germany). All values were expressed as mean ± standard deviation (Mean± SD) and the data were compared using the student T-test. Statistical significance was defined as $P<0.05$. Animal studies were performed following the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd ed. All of the 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 standard rodent diet pellets.

A. Osmium-191 Production

^{191}Os was prepared by neutron irradiation of isotopically enriched (90%) granulated metallic ^{190}Os in the Research Reactor of Tehran with subsequent fusion in a mixture of KOH-KNO_3 [10]. After fusion, the irradiated target is dissolved in water to give a 0.4 N KOH solution of potassium perosmate (VIII), $K_2[0sO_4(OH)_4]$ (Fig. 3), which is mixed with two volumes of ethanol to reduce the Os (VIII) to Os (VI). After 10 minutes, five volumes of concentrated hydrochloric acid are added quickly, and the solution is heated in a boiling water bath for 30 minutes. The solution is then evaporated to dryness and the brick-red precipitate of K_2OsCl_6 dissolved in 0.9% NaCl-0.01N HCl [11].

B. Labeling of Phytate

The ^{191}Os-K_2OsCl_6 solution in a 2ml vial with 0.7-2.2 mCi activity was adjusted to pH=1 by HCl 1 M and NaOH 1M. The vial solution was added to the kit vial containing 10 mg sodium phytate (commercial phytate kit, Parsisotope Co., Tehran, Iran). The complex pH was adjusted to the pH=5.5 in normal saline and was shaken for 10 min at room temperature. ITLC checked the radiochemical purity of the kit. For measuring radiochemical purity and radiolabeling yield, a 5µl of the sample of the ^{191}Os-Phytate was spotted on Whatman No. 2 chromatography paper and developed in a mixture of 10mM DTPA solution as a mobile phase to discriminate free Osmium from the radiolabeled compound.

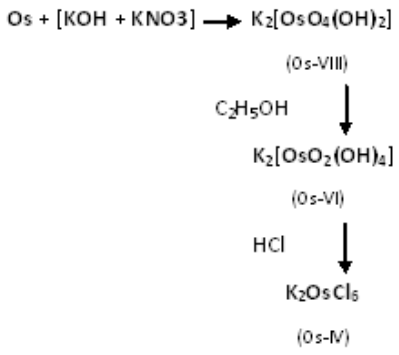

Fig. 3. Preparation of K_2OsCl_6

C. Stability of ^{191}Os-Pytate Complex in the Final Product

A sample of ^{191}Os-Phytate (18-180 MBq) was kept at room temperature for 48h while checked by ITLC every 2h. A micropipette sample (5 µL) was taken from the shaking mixture and the ratio of free radio-osmium to ^{191}Os-Phytate was checked by ITLC in a mixture of 10 mM DTPA solution as a mobile phase to discriminate free Osmium from the radiolabeled compound. A sample of ^{191}Os-phytate was kept at room temperature for 24 h while checked by RTLC at time intervals 5min, 2h, 4h, and 24h.

D. Bio Distribution

To determine the accumulation of ^{191}Os-K_2OsCl_6 and ^{191}Os-Phytate in the intraarticular cavity as well as after intravenous injection, the isotonic solution was carefully administered to wild-type rats of 200g average weight each. A volume (50 µl) of the final radiolabeled solution containing 100 µCi radioactivity was injected intraarticularly to rats. 5 groups were killed 30min, 4h, 24h, and 72h after injection of the radio-pharmaceutical. Samples of 13 organs including blood, liver, lung, heart, muscle (related to injected knee), spleen, stomach, kidney, thyroid, intestine, injected knee, uninjected knee, and tail were excised, weighed wet, and counted by NaI (Tl) well counter. The absolute tissue concentrations were expressed as a percentage of the administered dose per gram of the wet tissue.

E. Spec Imaging of ^{191}Os-K_2OsCl_6 and ^{191}Os-Phytate in Wild-type Rats

^{191}Os-K_2OsCl_6 and ^{191}Os-phytate solutions in appropriate buffers were injected (50µCi to each rat) intravenously through its tail vein. The images were taken at 24, 48, and 72 hours (for ^{191}Os-

K_2OsCl_6) and 4h, 24, 48, and 72 hours (for ^{191}Os-phytate) after administration of the labeled compound by a single head gamma camera. The distance of rat to high energy septa was 12 cm. The useful field of view (UFOV) was 540 mm×400 mm. The spatial resolution was 10 mm FWHM at the CFOV. Sixty-four projections were acquired for 30 seconds per view with a 64×64 matrix.

III. RESULTS AND DISCUSSION

A. Radionuclide Purity

The radionuclide purity was obtained by counting the samples on an HPGe detector (Gamma 2000\spectra\SPCANL \7790.dat) for 2 h, and two major photons (129.27 keV and 64.96 keV) were observed (Fig. 4). The 129.27keV is assigned to ^{191m} Ir decay and 64.96keV is assigned to x-ray emission of ^{191}Os.

B. Labeling

The radiochemical yields were determined by ITLC. At optimized condition, total labeling and formulation of ^{191}Os-Phytate took about 24h, with a yield of >98%. The radiolabeled complex was stable in an aqueous solution for at least 72h and no significant amount of other radioactive species was detected by ITLC. The trace amount of ^{191}Os-K_2OsCl_6 (≈2%) was detected by RTLC which showed that radiochemical purity of the ^{191}Os-Phytate was higher than 97% (Figures 5, 6).

Radiochemical impurities in the ^{191}Os-K_2OsCl_6 sample used in the radiolabeling step were checked by a 10mM DTPA solution as a mobile phase on Whatman No.2 paper (pH.3). The ^{191}Os-K_2OsCl_6 cation in $^{191}Os^{4+}$ form was chelated with the polydentate eluting leading to the migration of the cation in ^{191}Os-DTPA form to higher R_f (R_f.0.8). Any other ionic species would lead to the observation of new radiopeaks, especially at the origin (R_f.0.02-0.05) (Figure 5).

Because of the relatively high molecular weight of the colloid formed by the phytate monomers at the reaction pH, the phytate complex remains at the origin of ITLC (R_f 0.0) (Figure 6.).

In order to obtain the highest specific activity in the shortest possible time, a quantitative study was designed using different amounts of phytate for a specific amount of radioactivity (5 mCi of ^{191}Os for instance) while 25ºC was a considered suitable temperature (Table 1.). A satisfactory labeling yield of 94-98% was obtained at room temperature using 10 mg of phytate.

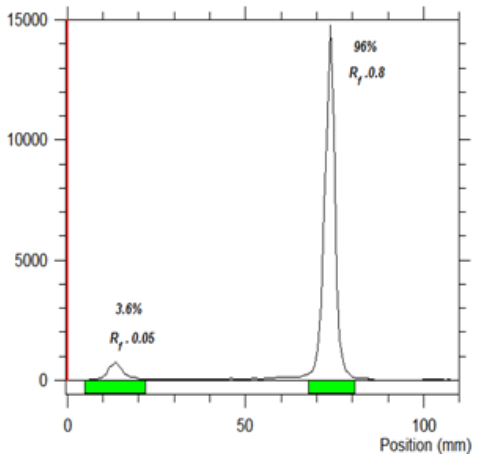

Fig. 5. Radiochoromatograph diagram of ^{191}Os-K_2OsCl_6

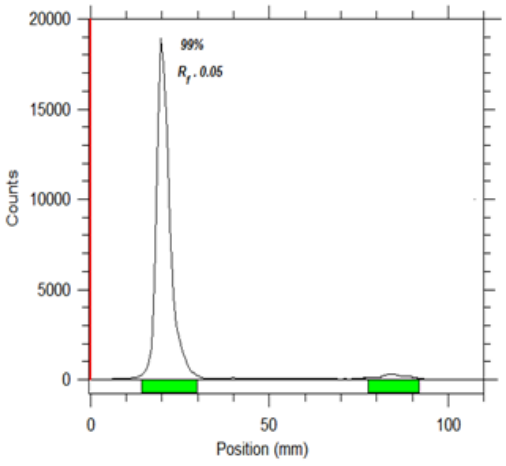

Fig. 6. Radiochoromatograph diagram of ^{191}Os-Phytate

Table 1

Percentages of Radiochemical Species in Radiolabeling Mixture Using Various Amounts of Sodium Phytate, 30 min, 25ºC and 5 mCi of ^{191}Os (n=3)

Reaction	^{191}Os - phytate (%)	^{191}Os-K_2OsCl_6 (%)	Sodium phytate (mg)
1	84±2	16±1.4	2.5
2	93±1	7±1.1	5
3	>98	<2	10

C. Biodistribution for free Os Cation

In order to investigate the biodistribution of ^{191}Os-phytate in wild-type rats, we had to obtain the biodistribution data for free osmium cation for 4-72h post-injection. The biodistribution of the cation was checked in various vital organs. The average percent dose per unit weight (%ID g^{-1}) of selected tissues from ^{191}Os-K_2OsCl_6 is demonstrated in Fig. 7. The liver uptake of Osmium is higher than other organs and final liver delivery looks like the possible route of accumulation.

For free ^{191}Os the radioactivity was mainly located in the heart, lung, liver, spleen, and kidney as previously shown by other researchers [12]. Heart uptake is naturally related to myocardial uptake as the daughter has been widely used as an angiographic agent in cardiology. Due to the resemblance of the Os cations especially at higher oxidation states to iodine anion, thyroid uptake was observed as shown in figure7 and this biokinetics was also shown for pertechnetate anion due to the charge/size resemblance. Also, as a free metallic cation, Osmium is carried through the circulation in protein-bound form and finally is accumulated in the liver. On the other hand, the water solubility, as well as negatively charge complex cation, is excreted through kidneys too.

D. Biodistribution After Intra-articular Administration ^{191}Os-Phytate

Figure 8 represents the distribution of the knee injected dose in the rats' organs at various time intervals after injection of 100 µCi/50 µl of the ^{191}Os-phytate complex as a percentage of injected dose. In case of any leak from the joint, the complex would accumulate in the reticuluendothelial (RE) system because of the high molecular weight of the complex, unless the complex dissociated at serum pH and Os cation was formed. Almost no detectable amounts of activity were observed in the spleen and lung, which are two important organs, showing no complex leak occurred. Very negligible kidney uptakes were observed, which was possibly caused by ^{191}Os cation release from the injected joint and not the radiolabeled complex uptake. Figure 9 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 demonstrated a typical Osmium cation biodistribution among the tissues. It is believed that free Osmium cation is the only radiochemical species escaping from the knee joint, and no ^{191}Os-phytate complex was found in circulation.

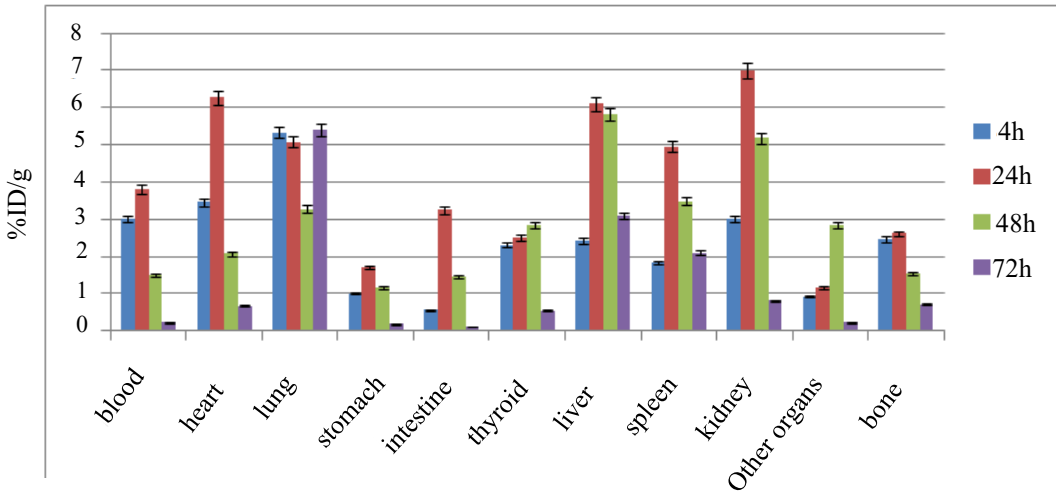

Fig. 7. Percentage of injected dose per gram of K_2OsCl_6 in wild-type rat tissues at 4, 24, 48, 72 h post injection (n=3).

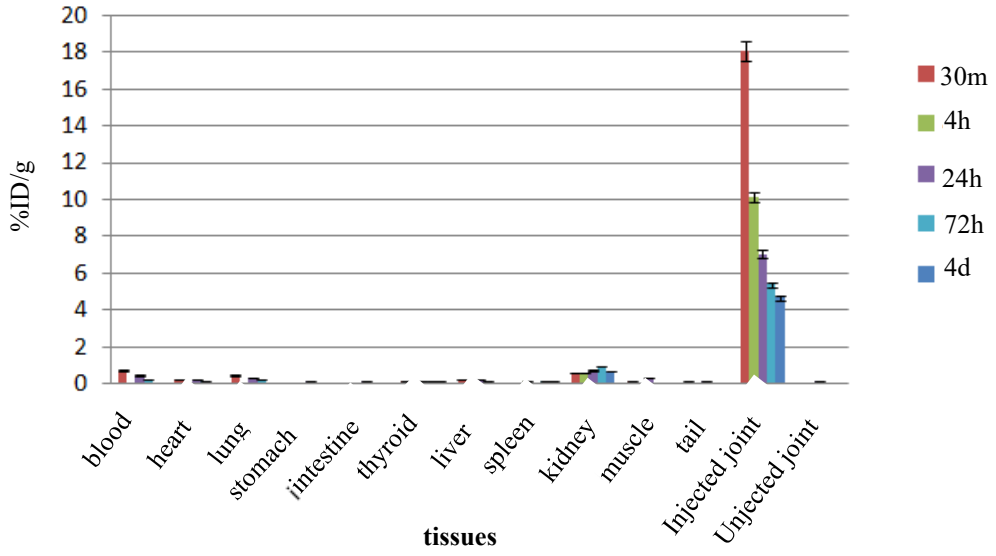

Fig. 8. Distribution of ^{191}Os-phytate in wild-type rats, 0.5, 4, 24, 72 h and 14d after intraarticular injection (n=3)

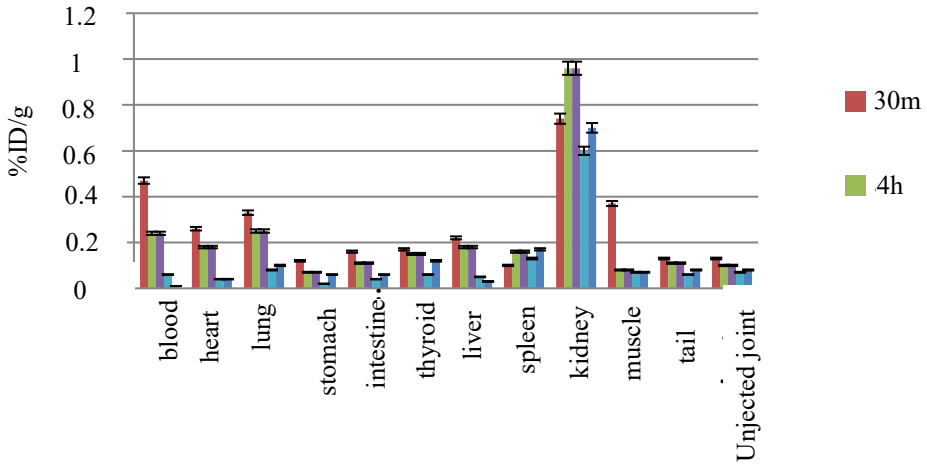

Fig. 9. Distribution of ^{191}Os-Phytate in wild-type rats excluding injected knee data at 0.5, 4, 24, 72 h and 14d after injection (n=3)

E. Imaging Studies

Due to the decay of ^{191}Os to the daughter gamma emitter (^{191m}Ir), an in vivo generator is formed after a colloidal accumulation of the formulation giving imaging opportunity for detecting the injection site activity. For the imaging studies of the free cation and the labeled compound wild type rats were used. In 24h free cation is majorly found in the heart while after 48h most the activity is excreted through the kidneys showing high activity in the bladder. After 72h most of the acidity is removed from the body, this is in accordance with other reported studies of the free cation in animals and humans (Figure 10).

Figure 11 shows images of ^{191}Os-phytate incidence in wild-type rats 4, 24, 48, and 72h after knee intra-articular injection. Also, as already shown by dissection studies, the complex is majorly accumulated at the injection site and no detectable leaks observed after 72h post intraarticular injection.

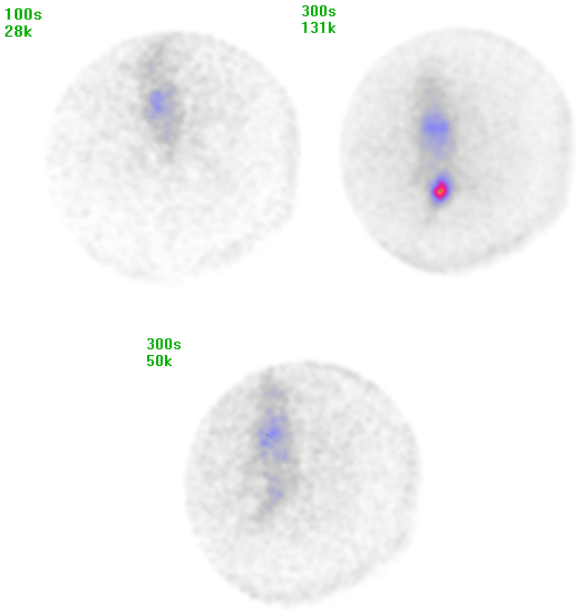

Fig. 10. Incidence images for [^{191}Os] K_2OsCl_6 uptake in normal rats, 24, 48 and 72h (from left to right) post i.e., injection.

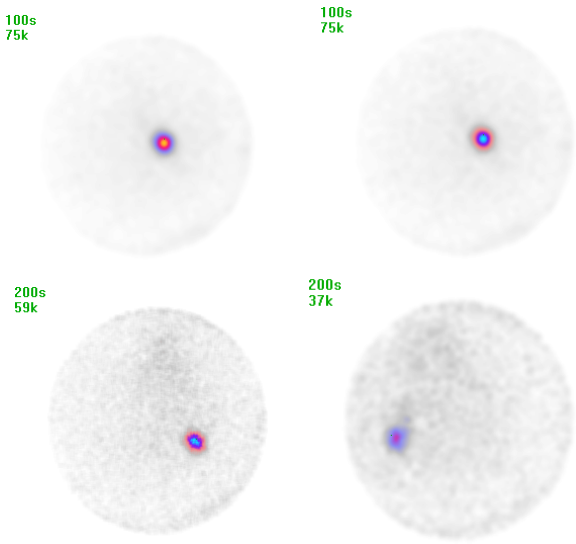

Fig. 11. ^{191}Os-phytate incidence images in wild-type rats 4, 24, 48 and 72 h (from left to right) after knee intraarticular injection.

IV. CONCLUSION

The ^{191}Os-Phytate complex was prepared with a high radiochemical yield (>98 %) in the optimized condition, the prepared complex was stable in the final solution at room temperature, 37°C, and presence of human serum, and can be used even 72 h after preparation. Intraarticular injection of the ^{191}Os-Phytate complex to male wild-type rats and investigation of leakage of activity in the body showed that most of the injected dose remained in the injection site 72 h after Injection. Due to the decay of ^{191}Os to the daughter gamma emitter (^{191}Ir), an in vivo generator is formed after the colloidal accumulation of the formulation giving imaging opportunity for detecting the injection site activity.

The rationale for using ^{191}Os for the possible radiosynovectomy studies is coming from its high similarities to the physical characteristics of ^{169}Er. Erbium-169, a soft beta-emitter (half-life 9.5 days, maximum energy 0 34 MeV) has been long used in the radiosynovectomy of carpal and metacarpal joints since the late 1970s and has been accepted

for trials by the European Society of Nuclear Medicine [1].

Unfortunately, ^{169}Er is produced in research reactors at high prices due to the use of the enriched ^{168}Er material giving a high final price for most countries even in EU states. On the other hand, the aging population of the world is increasing with arthritis as well as related problems giving more demand to the less expensive radiosynovectomy agents. The search for new radionuclides with soft beta emission is a vital part of the radionuclide/radiopharmacy field.

According to Table 2 ^{191}Os is also a soft beta emitter (0.37 MeV) and the half-life is comparable to the ^{168}Er. The maximum beta energies are very close (0.34 and 0.37 for ^{169}Er and ^{191}Os respectively). On the other hand, the near range in the tissue is also observed. The only consideration is a longer half-life of about 25% which can even be an advantage for the import/export as well as more secure transportation to the remote centers.

Thus, according to the physical data ^{191}Os-phytate can be a suitable candidate for small size joint radiosynovectomy such as meta-carpophalangeal, metatarsophalangeal, digital interphalangeal joints [1].

Table 2

The Physical Characters of Some Radionuclides Used in Various Joint Sizes for Radiosynovectomy

Radionuclide	Half life	beta maximum energy (MeV)	Range in tissue (mm)	Joint size	Ref
Yttrium-90	64.1 h	2.28	2.5	Large	[10]
Rhenium-186	89.3 h	1.077	4.5	Medium	[11]
Erbium-169	9.5 d	0.34	0.3	Small	[12]
Osmium-191	15.4 d	0.37	0.4	Small *	[13]

REFERENCES

1. G. Clunie and M. Fischer, *EANM procedure guidelines for radiosynovectomy*, EJNMMI **30**, pp. BP12-BP16, (2003).
2. M. Kropacek, et al., *Preparation of Holmium-166 Labelled Macroaggregates for radionuclide synovectomy*, Nucl Med Rev Cent East Eur **6**, pp. 1-4, (2003).
3. Y. S. Suzuki, et al., *Biodistribution and kinetics of holmium-166-chitosan complex (DW-166HC) in rats and mice*, JNM **39**, pp. 2161-2166, (1998).
4. E. W. Lee, et al., *Yttrium-90 selective internal radiation therapy with glass microspheres for hepatocellular carcinoma: current and updated literature review*, KJR **17**, p. 472, (2016).
5. I. Ikeda, et al., "Evaluation of 99mTc-phytate as radiopharmaceutical," Radioisotopes, **25**, p. 651, (1976).
6. A.R. Jalilian, et al.*, Development of 166Ho-phytate Complex for Radiosynovectomy*, NMMI **45**, pp. 87-92, (2011).
7. H. Palmedo, et al., *Painful Multifocal Arthritis: Therapy with Rhenium 186 Hydroxyethylidenedi-phosphonate (186Re HEDP) after Failed Treatment with Medication—Initial Results of a Prospective Study*, Radiology **221**, pp. 256-260, (2001).
8. C. Cheng, et al., *A new osmium-191 leads to iridium-191m generator*, JNM: official publication, Society of Nuclear Medicine, **21**, p. 1169, (1980).
9. M. Jamre, et al., *Development of an in vivo radionuclide generator by labeling bleomycin with 191 Os*, JRNC **290**, pp. 543-549, (2011).
10. C. Brihaye, et al., *New osmium-191/iridium-191m radionuclide generator system using activated carbon*, NMMI. (United States) **3**, (1986).
11. M. IAEA-TECDOC, 1340. Manual for reactor produced radioisotope, Vienna: International Atomic Energy Agency, (2003).
12. K. Kairemo, et al., *191mIr: distribution and retention in animal experiments*, Nuklearmedizin, **34**, p. 115, (1995).

How to cite this article
Moghaddam et al., *Preparation of 191Os–phytate, an in-vivo radionuclide generator, for radiosynovectomy application,* Nuclear Engineering Progress **1** (1): 9-17 (2021), DOI: 10.24200/nep.2021.1001