Country report: Serbia

Development, Preparing and Quality Assurance of Radiopharmaceuticals Based on ^{188}Re and ^{90}Y for Radionuclide Therapy: The Possibilities for their Production in Laboratory for Radioisotopes, Ins «Vinča»

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Time period covered: 2008-07-1 to 2009-12-31

The main object of the research planed for this project was to optimize the procedures for the ^{90}Y and ^{188}Re labelling of different compounds as well as their in vitro and *in vivo* evaluation. The work has been involved setting up the facilities, standardization of preparing protocols, and improving existing quality assurance/quality control (QA/QC) procedures in order to supply reliable products to the national nuclear medicine community.

The planed program of work (Research Contract No. 14827/R_0, for the period 2008-04-01 to 2009-03-31) was:

1. Radiolabelling of carrier molecules such amino acids and peptides with Y-90 and Re-188;

2. Development of Y-90 particulates;

3. Evaluation of the labelled molecules in vitro, *in vivo* and collect data for clinical trial of promising radiotracers.

The IAEA renewed the Contract (Contract No. 14827/R_0 for the period 2009-03-17 to 2010-03-16) with the following modifications of the Program of work:

1. Development of Sr-90/Y-90 electrochemical generator and QC of Y-90;

2. Preparation of Y-90 colloids for radiosynovectomy.

This presentation highlighting the work done through the Research contract No: 14827 (14827/R_0, 14827/R_1) during the first 18 months.

This work could be presented in tree separated parts:

1. ^{90}Y and $^{186/188}$Re complexes for tumor therapy and bone palliation:

1) ^{90}Y complexes of phosphonate ligands of HEDP, MDP and DPD;

2) ^{90}Y complexes of DMSA;

2. ^{90}Y-particulates

1) ^{90}Y-colloids for hepatocelular carcinoma: ^{90}Y-Sb_2S_3 and ^{90}Y-Sn colloids;

2) ^{90}Y-colloids colloids for radiosynovectomy: ^{90}Y-HA without or with presence of stabilizers like phosphonates HEDP, MDP, DPD;

3. Development of Sr-90/Y-90 generator: electrochemical $^{90}Sr/^{90}Y$ - generator and QC of ^{90}Y.

1. ^{90}Y complexes for for tumor therapy and bone palliation

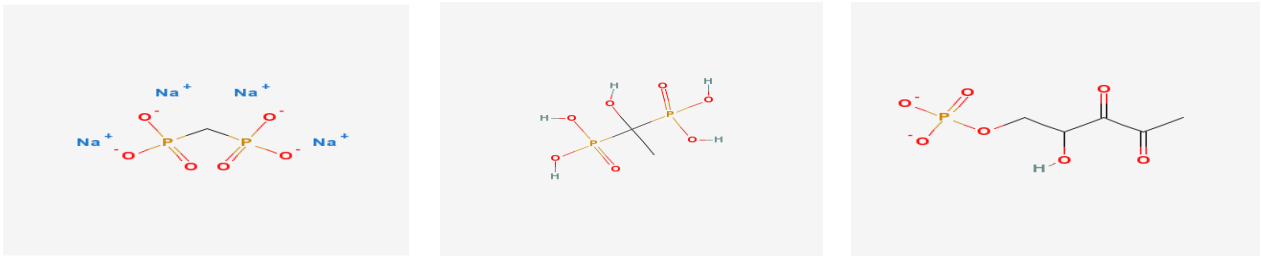
The use of the therapeutic radiopharmaceuticals that localize selectively at the metastasis sites is found to be an effective treatment for the palliation of pain. Radionuclides decaying by the emission of β-particles like yttrium-90 are preferred in most of this applications.^{90}Y have favorable properties for therapy, Table 1:

Table 1: Phisical characteristic of radionuclide Y-90

Radionuclide	Half life (days)	Emission	Max. range (mm) (air/tissue)
Y-90	2.67	β-	12/3.9

1) ^{90}Y complexes of HEDP, MDP and DPD

The object of these studies was to research the possibility for ^{90}Y-complexion of polyphosphonate ligands trihydroxy (phosphonomethyl) phosphanium (MDP), (1-hydroxy-1-phosphonoethyl)phosphonic acid (HEDP) and (2 hydroxy-3,4-dioxopentyl) phosphate (DPD). Molecular structures of polyphosphonate ligands MDP, HEDP and DPD are presented in Fig.1.



MDP HEDP DPD

Fig.1. Molecular structures of polyphosphonate ligands HEDP, MDP and DPD.

The labelling of polyphosphonate ligands was carried out by varying experimental parameters such as: ligand concentration, pH values, time and temperature of the reaction. Analysis of the complexes enclosed the radiochemical quality control methods (ITLC, paper chromatography and HPLC), in vitro stability studies of the ^{90}Y-complexes, as well as physiological distribution studies per organ of experimental animals (health male Wistar rats).

The preparation of ^{90}Y-HEDP, ^{90}Y-MDP and ^{90}Y-DPD

^{90}Y-labelling: A direct labeling method was optimized by varying HEDP, MDP and DPD concentration and pH in the labeling mixture, as well as the reaction temperature and the reaction time. The stock solution of polyphosphonate ligands was prepared by dissolution the desired ligand concentration (0.01–10 mg/ml) in double distilled water. An appropriate amount of the ^{90}Y-chloride solution (~370 MBq per vial) was edded. The pH values of the resulting reaction mixtures were adjusted to 4.0 and/or 6.5. The total reaction volume in each vial was maintained at 3 ml. Ascorbic acid (10 mg) was used as radiolytic stabilizer in all samples;

Radiochemical purity: ITLC-SG – 0.9 % NaCl or CH_3OH (Rf=0.9-1.0 for free $^{90}Y^{3+}$ and R_f=0.0-0.2 for ^{90}Y-complex);

Serum stability studies: The stability of ^{90}Y–HEDP, ^{90}Y–MDP and ^{90}Y–DPD in human serum was assessed by measuring the release of ^{90}Y from the complex at 37 0C over a 10-day period.

Organ distribution studies: the experiments were done as distribution per organ of animals (health male Wistar rats).

The results: obtained results have shown that radiochemical purity was > 95 % for all ^{90}Y-complexes. The serum stability result have shown that the complex was quite stable at the studied conditions up to 10 days. No significant dissociation of activity from the complex was observed, e.g. the percentage of ^{90}Y released from this complex at 10 days was <2.0%.

The results for organ distribution studies of ^{90}Y-complexes were presented in Table 2.

Table 2. Organ distribution studies of ^{90}Y–MDP, ^{90}Y–HEDP and ^{90}Y–DPD% (id/g ± SD)

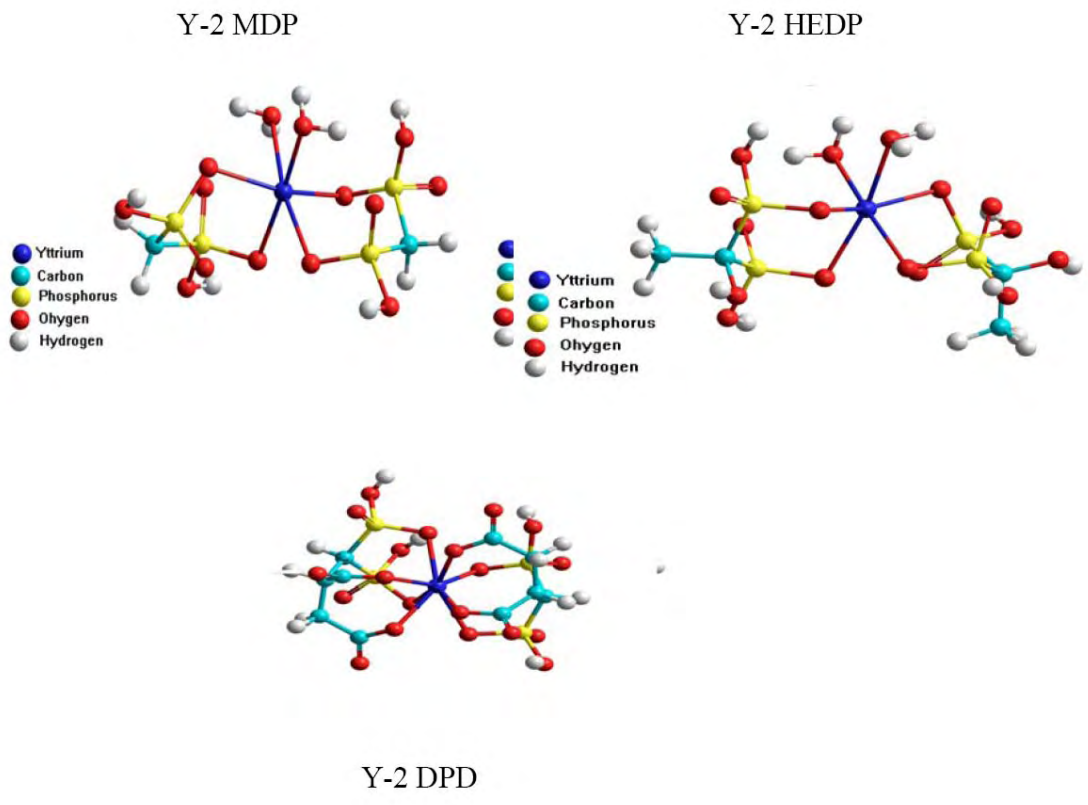
^{90}Y–complexes	^{90}Y–MDP (pH=6.5÷7.5)	^{90}Y–HEDP (pH=6.5÷7.5)	^{90}Y–DPD (pH=6.5÷7.5)
Organ	1 h / 24 h	1 h / 24 h	1 h / 24 h
Blood	0.206 ± 0.065 0.057 ± 0.031	0.469 ± 0.039 0.038 ± 0.006	0.251 ± 0.037 0.115 ± 0.014
Heart	0.172 ± 0.065 0.132 ± 0.044	0.251 ± 0.033 0.086 ± 0.014	1.848 ± 0.672 0.389 ± 0.078
Lung	0.242 ± 0.062 0.124 ± 0.054	0.242 ± 0.077 0.075 ± 0.011	1.022 ± 0. 343 0.049 ± 0.012
Liver	5.424 ± 0.544 3.225 ± 0.517	0.253 ± 0.036 0.182 ± 0.070	0.174 ± 0.058 0.153 ± 0.022
Spleen	1.072 ± 0.360 1.424 ± 0.765	0.139 ± 0.049 0.128 ± 0.054	1.680 ± 0.734 0.105 ± 0.055
Kidney	0.317 ± 0.065 0.500 ± 0.191	0.825 ± 0.151 0.331 ± 0.083	1.337 ± 0.0425 0.581 ± 0.187
Intestines	0.054 ± 0.011 0.029 ± 0.011	0.060 ± 0.006 0.017 ± 0.002	0.055 ± 0.011 0.044 ± 0.009
Stomach	0.417 ± 0.205 0.199 ± 0.071	0.204 ± 0.007 0.114 ± 0.061	0.253 ± 0.068 0.161 ± 0.043
Muscle	0.170 ± 0.011 0.144 ± 0.014	0.227 ± 0.060 0.111 ± 0.020	0.092 ± 0.015 0.024 ± 0.008
Bone	2.589 ± 0.334 6.972 ± 1.438	4.840 ± 0.805 5.067 ± 0.589	13.984 ± 1.126 11.339 ± 1.097

The organ distribution study of ^{90}Y-HEDP (Table 2) has shown that complexes were localized in the skeleton. With high skeletal uptake no significant activity was to be found elsewhere.

The satisfactory results of ^{90}Y–MDP organ distribution in healthy test animals (Table 2) were obtained 24 h after iv application: high skeletal uptake. but also a significant activity in liver and spleen.

The more favorable organ distribution results were obtained for ^{90}Y–DPD (Table 2): the uptake in bone was 11–13% ID/g after 24 e.g. 1 h. With high skeletal uptake, low uptake in soft tissues and rapid blood clearance the ^{90}Y–DPD complex proved to be an excellent candidate for tumor therapy as well as in bon palliation.

The molecular modeling studies: Thermodynamic stability of radiopharmaceuticals is of the crucial interest, because the loss of radiometal may result in accumulation of radioactivity in non-target organs. Therefore, the selected chelators have to form a complex with high thermodynamic stability to retain its chemical integrity in competition with natural chelators present in a blood stream. Chemical stability of compounds may be judged according to molecular energy data. So, application of molecular modeling as a computation tool is useful for preliminary theoretical analysis of chemical compounds without resorting to laboratory testing. The molecular modeling studies have been carried out for structural analysis of the ^{90}Y–HEDP. ^{90}Y–MDP and ^{90}Y–DPD complexes using software HyperChem TM release 6.03. version for Windows.



Conclusion: The experimental results have shown that ^{90}Y-complexes of different polyphosphonate ligands MDP, DPD and HEDP were obtained in high radiochemical purity and with favorable organ uptake, so they may have potential for use in the palliative treatment of bone metastases. Since one of objects was to explore the potential of the therapeutic analogue of ^{99m}Tc- complexion of polyphosphonate ligands MDP, DPD and HEDP, the above developments and encouraging findings with ^{90}Y-MDP, ^{90}Y-DPD and ^{90}Y-HEDP gave an impetus to undertake the present study.

2) ^{90}Y complexes of DMSA

The object of the study was also labelling of meso-dimercaptosuccinic acid (DMSA) with Y-90. In this way we try to obtain a labelled compound for therapy of bone malignancies, as

well as for palliative of bone pain. The labelling with ^{90}Y was carried out by varying experimental parameters such as ligand concentration, pH, time and temperature of the reaction, in order to maximize the labelling yield. Analysis of the complexes enclosed the radiochemical quality control (ITLC, paper chromatography and HPLC), determination of pharmacokinetical parameters and serum stability, as well as organ distrbution study in health male Wistar rats. A series UV absorption spectra of yttrium (0.50 mM) solution with increasing *meso*-DMSA concentration were done too.

HPLC radiochromatograms of labelling mixture showed good separation of ^{90}Y-DMSA from free ^{90}Y: R/T was 5.629 min and 6.552 min respectively. The stability of the ^{90}Y-DMSA complexes was studied at various time points. After preparing (without and with ascorbic acid. with adjusted the pH to 8.0) the ^{90}Y-DMSA was incubated for 24 h at room temperature and radiochemical purity was analised. ^{90}Y-DMSA with ascorbic acid as radiolysis stabilizer was found to retain radiochemical purity at as high as 95 % at 24-h incubation. The serum stability of ^{90}Y–DMSA was assessed by measuring the release of ^{90}Y from the complex at 37 0C over a 10-day period. The serum stability results for ^{90}Y–DMSA at different time points showed that the complex prepared with ascorbic acid was quite stable at the studied conditions up to 10 days. No significant dissociation of activity from the complex was observed (the percentage of ^{90}Y released from this complex at 10 days was <5.0%).

The potential structure of ^{90}Y-DMSA was proposed according the molecular modelling results. The molecular modeling studies were carried out for structural analysis of the complexes using software HyperChemTM release 6.03 version for Windows.

Our spectrophotometric investigations (unpublished data) suggest that the complex formation between $^{90}Y^{3+}$ at very low concentration (10^{-7} to 10^{-9} mol dm^{-3}) and DMSA in great excess, depending of the pH value, favoured complexes with metal to ligand mole ratio 1:2, The tetradentate ligand meso-DMSA has been defined as H_4L in protonated form with four protonation sites (two sulfur's atoms in the thiolate moiety and two oxygen's atoms in the carboxylic acid groups). The proposed conformations of meso-DMSA in solution upon increasing pH value are defined as: H_2L^{2-} (I), HL_3- (II) and L^{4-} (the linear structure III). The proposed structure for ^{90}Y-DMSA complex predicted $[^{90}Y(LH_2)_2(H_2O)_2]$- complex, which include two six-membered chelate rings via sulfur and oxygen atoms from meso-DMSA. Upon the addition of the second OH^- equivalent to HL^{3-}, another hydrogen bond in internal six-member ring is broken and L^{4-} species are predominant in solution. The representative and energetically favourable conformation $[^{90}Y(LH)_2(H_2O)_2]^{3-}$, which include two five-member chelate rings via sulfur atoms (Fig.5 and Fig 6).

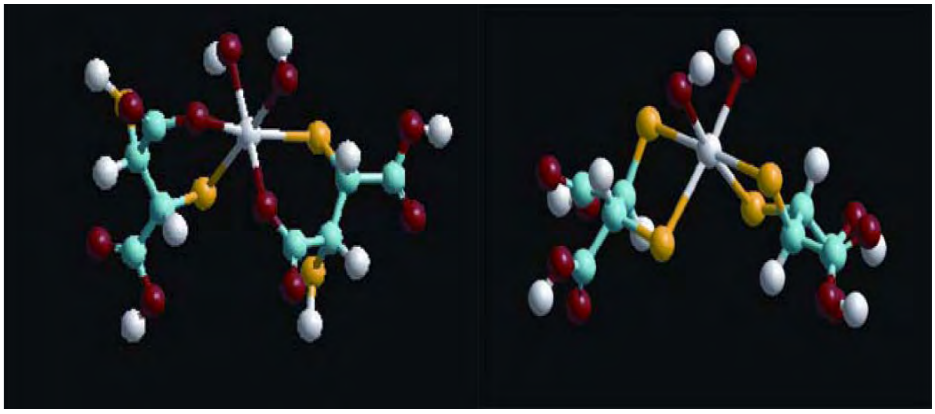


Fig.5. Fig.6.

Complete results of radiochemical purity conrtol as well as organ distribution study confirmed that ^{90}Y-DMSA could be obtained with high radiolabelling yeald, with high radiochemical purity and with satisfactory organ distribution study. Therefore, ^{90}Y-DMSA could be also a candidate radiopharmaceuticals for tumour therapy an the palliative treatment of bone metastases.

2. ^{90}Y-particulates

1) ^{90}Y-colloids for hepatocelular carcinoma: ^{90}Y-Sb_2S_3 and ^{90}Y-Sn colloid

Radiocolloids as diagnostic and therapeutic agents play an important role in nuclear medicine. The properties of a radiocolloid dispersion, characterized by particle size, shape, charge and stability, are very significant parameters that determine its organ distribution *in vivo*. In our recent study we try to explore the factors influencing the labeling yield and particle size distribution of ^{90}Y-labeled antimony trisulfide and tin colloid. Photon correlation spectroscopy (PCS), transmission electron microscopy (TEM) and filtration analysis were used to determine the particle size distribution. The labeling efficiency of ^{90}Y-labeled colloid particles under various conditions was investigated.

The results of our research have shown that both ^{90}Y-labeled colloids can be prepared in high yields under optimized conditions. Labeling efficiency of ^{90}Y-labeled antimony trisulfide and tin colloid was >95 and >88%, respectively. Under well-standardized conditions of the preparation, the reproducibility of the particle size and its distribution is very good, within 7-23 and 85-103 nm for ^{90}Y-antimony trisulfide and ^{90}Y-tin colloid, respectivelly. TEM is a desirable size-measuring technique because individual particles can be observed and measured. A good agreement between TEM and filtration analysis data was found for the particles of both ^{90}Y-labeled colloids.

Preparation of Y-^{90} colloids for radiosynovectomy

Radiosynovectomy or radiosynoviorthesis is a method for treatment of some joint disorders, often of chronic nature and relatively common in any society. One of the most common causes is rheumatoid arthritis. It is a long suffering from pain, deformity and disability, Radiosynovectomy is a type of radiotherapy used to relieve pain and inflammation from rheumatoid arthritis.

Radiosynovectomy involves local intra-articular injection of suitable β -emitting radionuclides in the form of radiocolloids or radiolabelled particulates into the affected synovial joints. These β-emitting radionuclides penetrate only from fraction of a millimeter to a few millimeters and destroy the inflammatory tissue and thus reduce swelling and pain.

The use of radioparticles for radiosynovectomy are characterised by the radionuclide and the the particulate carrier. The ideal radionuclide is pure β -emitter having a short half-life, or β -emitter with minimal γ- emissions, which has a range of between 2 and 10 μm with low cost, high chemical purity and non-toxic. An ideal particulate carrier must be taken up by the synovial tissue and form a stable complex with radionuclide. It has to be prepared easily and reproducibly, and be non-toxic and non-allergenic. Many radionuclides, namely, ^{166}Ho, ^{153}Sm, ^{90}Y, ^{32}P, ^{198}Au, ^{186}Re, etc. have been identified as potential radionuclides for radiation synovectomy in various particulate forms. Some new radiopharmaceuticals could also be used for radiosynovectomy.

Preparation of ^{90}Y-HAp

Introduction

In our researches hydroxyapatite (HAp), a natural constituent of bone, was studied as a particulate carrier for beta-emitting radionuclides in radiation synovectomy. Particles were radiolabelled with ^{90}Y and their *in vivo* safety was studied following intra-articular injection into knees of normal rats. The aim of our researches was to explore the factors influencing the labelling yield and particle size distribution of ^{90}Y-labelled calcium hydroxyapatite particles (HAp). We examined the influence of different phosphonates like diphosphonates 1-hydroxy ethylidene-1,1-diphosphonate (HEDP) as chelator in the yttrium-90-labelled calcium hydroxyapatite particles (HAp) too. In the experiments we used HAp with different particle sizes in order to investigate their biological behavior. Organ distribution studies are performed via two different methods of drug administration, intravenous or intra-articular application.

Materials and methods

Micro hydroxyapatite powders were synthesized in Laboratory for radioisotopes. Vinča Institute of Nuclear Sciences. Hydroxyapatite powder is precipitated via wet precipitation method. Aqueous solutions of calcium hydroxide, Ca $(OH)_2$, and ortho-phosphoric acid (H_3PO_4, 85%), both of analytical grade, were used as reactants for the preparation of HAp particles, with different sizes.

The labelling was carried out by use of ^{90}Y in form of $^{90}YCl_3$ (in 0.05 mol/dm-3 HCl), supplied from Polatom, Poland. ^{90}Y was added to 5 mg of HAp in 0.5 ml sterile water into a conic glass veal. The reaction was mixed for 60 min at 37 ºC. The final suspension for injection was made in sterile saline with pH=7.0. Radiolabelling yield was determined by centrifugation (3500 rpm for 5 min) and supernatant was cheerfully separated and radioactivity was measured in both supernatant (free ^{90}Y) and pellet (particles of HAp labelled with ^{90}Y). Radiochemical purity was determined by paper chromatography with ITLC-SG strips in 80% methanol and saline. Particle size was analyzed in undiluted samples, at 20ºC, using a light-scattering photon correlation spectroscopy (PCS) instrument Zetasizer Nano ZS (Malvern Instruments Ltd, England, U.K.), which measure particles in the size range 0.6 nm to 6 μm. The in vitro stability of ^{90}Y-labelled particles ^{90}Y-HAp as well as with introducing diphosphonate like HEDP as chelators were studied in saline and in 1% human serum at 37ºC. About 0.3 ml aliquots of ^{90}YHEDP-HAp or ^{90}Y-HAp were dispensed in 1ml of saline and human serum. At the end of 24h (48, 96… h), the suspension was vortexed thoroughly and centrifuged at 3500 rpm for 5 min. The supernatant was removed and counted for any leaked out radioactivity from the particles. Organ distribution was studied in Wistar rats under anesthesia, after intravenous or intra-articular injection of ^{90}Y-labelled particles.

Results and discusion

a) Radiolabelling yield and radiochemical purity

Radiolabelling yields of 97-99 % was achieved in all particles preparations. Radiochemical purity of labelled HAp particles was confirmed with ITLC-SG strips in 80% methanol and saline as mobile phases with R_f $^{90}YCl_3$=1, R_f ^{90}Y-HEDP=0, R_f ^{90}Y-HEDP-HAp=0, R_f ^{90}Y-HAp=0 and R_f $^{90}YCl_3$=1, R_f ^{90}YHEDP=1, R_f ^{90}YHEDP-HAp=0, R_f ^{90}YHAp=0, respectively. Radiocnemical purity was > 99 %.

b) In vitro stability studies

The stability of ^{90}Y-labelled particles was studied in saline as well as in 1% human serum at 37ºC. About 0.3 ml aliquots of ^{90}Y-HAp as well as ^{90}Y-HEDP-HAp were dispensed in 1ml of saline and human serum. At the end of 24h, 48h,…up to 5 days, the suspension was vortexed thoroughly and centrifuged at 2000 rpm for 5 min. The supernatant was removed and counted for any leaked out radioactivity from the particles. The experimental results have showen that the ^{90}Y-labelled particulates showed excellent in vitro stability, > 98% in both media at 37ºC during 5 days.

c) Particle size analysis

The size distribution of synthesized HAp particles (PCS method), presented at Fig. 1. have shown that 75.2% of particles were with diameter of 1.37 μm and 24.8% were with 5.23 μm (Z-Average 2.73 μm).

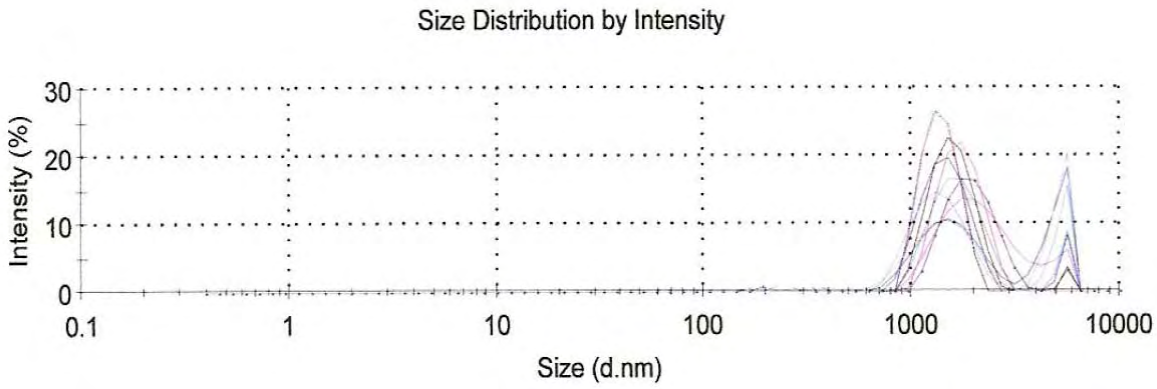


Fig. 1: The size distribution of synthesized HAp particles (PCS method)

d) Biological studies

Organ distribution was studied in rats, after intravenous injection of ^{90}Y-labelled particles ^{90}Y-HAp or ^{90}Y-HEDP-HAp. These results were presented at Fig. 2. a) and b) respectively.

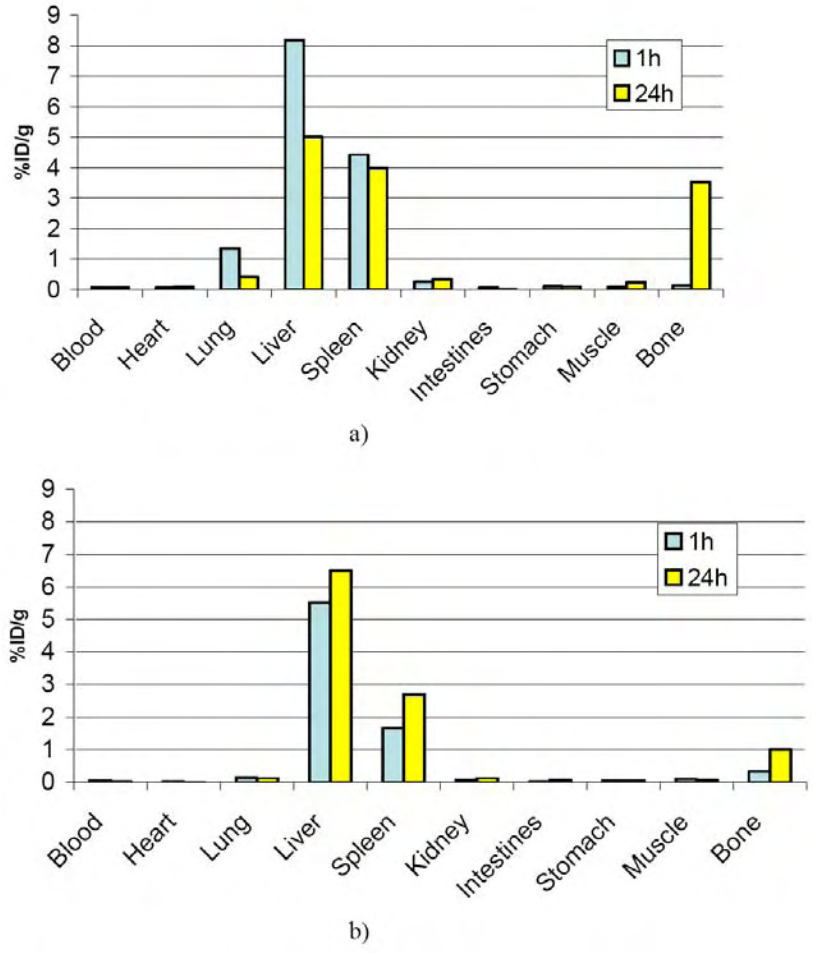


Fig. 2. Organ distribution study of ^{90}Y-HAp (a) and ^{90}Y-HEDP-HAp (b) after intravenous application

The results pointed at the fact that ^{90}Y-HEDP-HAp was stable *in vivo* with 1% of leaching $^{90}Y^{3+}$ accumulated in bone after 24 h. ^{90}Y-HAp is less stable with 3.5% of leaching activity in the bone.

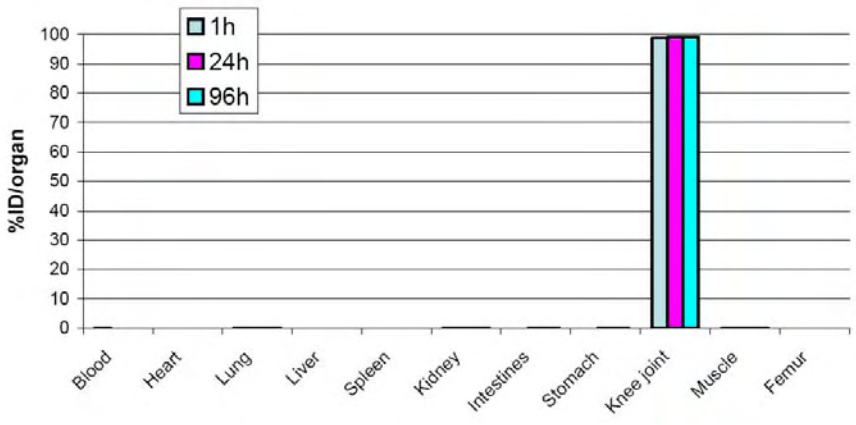


Fig. 3. Organ distribution study of ^{90}Y-HAp after intra-articular application

Organ distribution in rats was studied also after intra-articular injection of ^{90}Y-labelled particles ^{90}Y-HAp as well as ^{90}Y-HEDP-HAp. The results of intra-articular injected ^{90}Y-HAp were presented at Fig. 3. The similar organ distribution results were obtained for intra-articular injected ^{90}Y-HEDP-HAp.

The presented results have shown the dependence of organ distribution of radiolabelled particles on a way of drug administration After intravenous application of radiolabelled particles, greater uptake of ^{90}Y-HAp and ^{90}Y-HEDP-HAp was in liver and then in spleen. The results of organ distribution after intra-articular application of radiolabelled particles in rats confirmed that almost 99.1% of radioactive particles. ^{90}Y-HEDP-HAp as well as ^{90}Y-HAp, localized in the synovium for at least 96 h, with no detectable activity in the other organs.

Conclusion

The present study has shown that ^{90}Y-labelled HAp particles could be prepared in high yield as well as with excellent radiochemical purity. HAp particles used for labelling were synthesized and characterized in Laboratory for radioisotopes. The labelled particulates have shown high in vitro stability at 37ºC. Biological studies carried out in Wistar rats confirmed complete retention of intra-articular injected radioactivity within the synovial cavity of normal animals for up to 96 h post-injection. Stability of ^{90}-yttrium-HAp complexes increased with introducing diphosphonate HEDP as chelator.

3. Development of Sr-90/Y-90 electrochemical generator and QC of Ittrium

90 (90/Y) has many favourable features which recommend its application in radionuclide therapy. It has a half-life (64.1 hr) consistent with the rate of antibody accumulation in tumor. and no accompanying gamma-ray radiation in its decay. Beta rays have intermediate energy of 0.9367 MeV (β-max =2.3MeV) and a stable daughter (^{90}Zr). The important feature is that yttrium – 90 can be attached for many chelate molecules. Furthermore, the considerable path length in tissues of its β- particles (r95 = 5.9 mm) represents a major advantage in solid tumors. Y-90 exists in secular equilibrium with its parent isotope strontium-90, which is a product of fission reaction. There are many impurities which must be removed and pure yttrium – 90 need to convert in appropriate form to be ready for application in medicine therapy. As the demands for radionuclide purity are so high, the obtaining and refinement of ^{90}Y are very important steps in radionuclide therapy.

A ^{90}Sr/^{90}Y generator system based on an electrochemical separation technique is in development in cooperation with the group from India (M. Venkatesh, Bhabha Atomic Research Center, Mumbai, India) and by use the protocol for the electrochemical separation process developed under the CRP: Therapeutic Radionuclide Generators: ^{90}Sr/^{90}Y and ^{188}W/^{188}Re (IAEA, Technical Reports Series No. 470).

The object of this work was:

1. preparation of ^{90}Sr-^{90}Y generator with useful activity of 3.7 GBq (100 mCi);
2. electrochemical separation of ^{90}Y;
3. developmet of the methods for determination of other chemical and radionuclide impurity;
4. transformation solution of ^{90}Y in appropriate form.

1) Preparation of ^{90}Sr-^{90}Y generator

The equipment for electrochemical separation was completed during a period March-July 2009. A potentiostat unit Potentiostat/Galvanostat/ZRA, Series G 750 (Gamry Instruments, inc) was obtained together with software license FC 350 (Gamry Instruments, inc).

The electrolysis cell is a three electrode system housed in a glass cells fitted with an acrylic cap, made in laboratory of the Faculty of Technolgicy and Metallurgy, University of Belgrade. Two electrodes, an anode and a cathode, dimensions 10x100mm, sealed in glass holder, are high purity platinum plats electrodes made by The Institute for Mining and Metallurgy Bor, Serbia. As a reference electrode saturated calomel electrode Gamry Instruments, inc. was used (Fig. 1). The anode and cathode are fully immersed in solution facing each other. High purity argon gas (from local supplier) was passing through a glass tube, which dipped into the electrolysis solution.

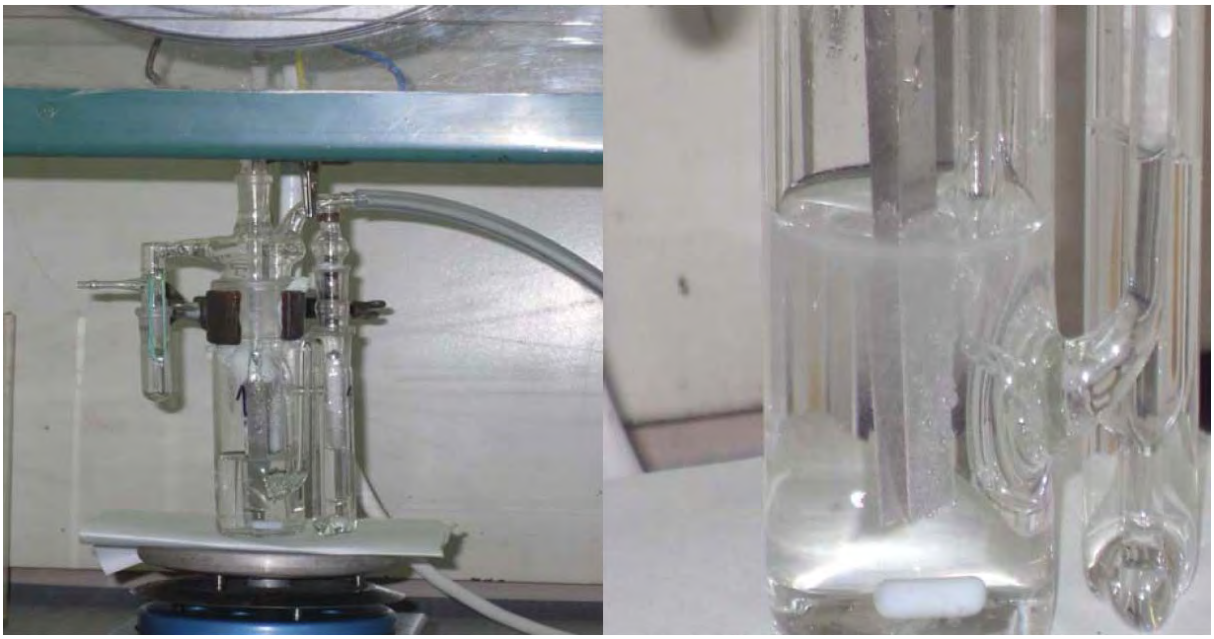
a) b)

Fig.1. The equipment for electrochemical separation (Laboratory for radioisotopes, Institute “Vinca”) (a) with supplying of argon gas (b)

Fig.2. Stable electrolytic potential at platinum cathode

2) Electrochemical separation of ^{90}Y

Sr-90/Y-90 electrochemical generator developed in Serbia is based on electrolysis of a mixture of ^{90}Sr and ^{90}Y in nitrate form at pH 2-3 at a potential of –2.5V. with 100-200 mA current. ^{90}Y deposited from 3 mM HNO_3 on the platinum electrode, which is used like a cathode. During the second electrolysis, ^{90}Y is removed from the platinum electrode. In this step, the cathode from the first electrolysis containing ^{90}Y is used as anode. Upon electrolysis, ^{90}Y is leached and is deposited to the fresh cathode which is taken out and by dipping in acetate buffer dissolved in a small volume of acetate solution, to obtained ^{90}Y-acetate, suitable for labelling. For measuring we used dose calibrator (Capintec CRC 15R, USA) which contains calibration factor. For calibrating ^{90}Y dose secondary calibration sources will be used.



a) b)

Fig.3. The electrolytic cell completed in Laboratory for radioisotopes, Institute “Vinča” (a) and electrolytic separation of gas H_2 (b).

3) Developmet of the methods for determination of other chemical and radionuclide impurity

^{90}Sr breakthrough is the major problem often encountered with $^{90}Sr/^{90}Y$ generator. Because ^{90}Sr is a bone seeker, the upper limit of ^{90}Sr in ^{90}Y solution for human use is 74 kBq (2 mCi). In order to provide data concerning ^{90}Sr contamination, developmet of the methods for determination of chemical and radionuclide impurity was necessary.

The radionulcidic purity of the ^{90}Y solution was analyzed by paper and ITLC chromatography. Chromatography paper Whatman Nº 1 (18 x 2 cm) and ITLC SG strips (14 x 1 cm) and 0.9% saline solution was used for the analyses. During the chromatography, ^{90}Sr moved with the solvent front, while ^{90}Y stayed at the origin.

In order to determine the radionulcidic purity of the ^{90}Y solution we also involved in our experiments so cold “BARC technique”. This method as a combination of solvent extraction and paper chromatography (extraction paper chromatography EPC) was suggested as a sensitive and accurate analytical technique for estimation of the purity of ^{90}Y. Whatman Nº 1 (18 x 2 cm) paper chromatography strip impregnated with 2-ethyl hexyl phosphonic acid

(KSM-17) at the point of spotting is used. Upon development with normal saline ^{90}Sr moves to the solvent front leaving ^{90}Y completely chelated and retained at the point of spotting. As in the first experiments the mixture of ^{90}Sr and ^{90}Y with low activity, at the megabecquerel level, was used, the activity at the solvent front was estimated by use of dose calibrator (Capintec CRC 15R, USA) which contains calibration factor and compared with the total spotted activity, Fig.4.

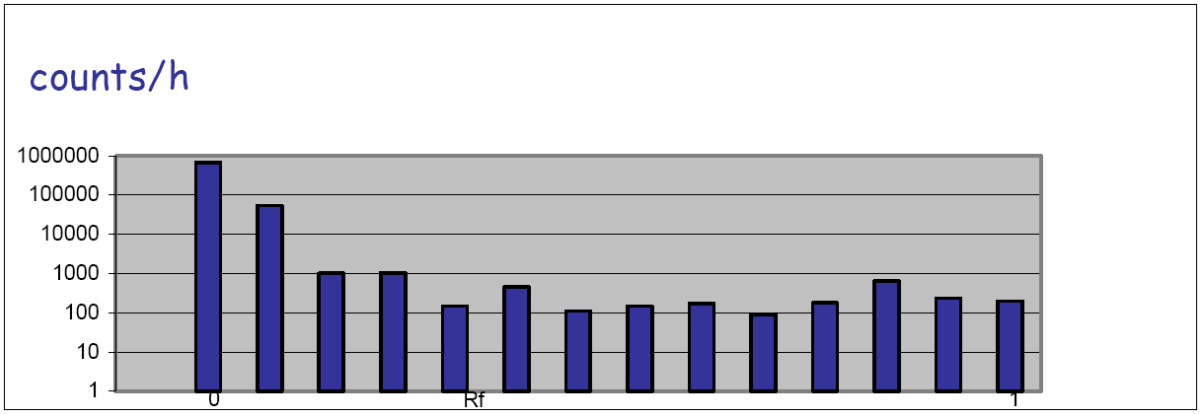


Fig. 4. extraction paper chromatography (EPC) of a sample of ^{90}Y from the electrochemical generator

In our first experiments the mixture of ^{90}Sr and ^{90}Y with low activity (only few mCi) was used. Therefore this work involved setting up the facilities, standardization of preparing protocol and improving existing quality control (QC) procedures in order to supply reliable products to the national nuclear medicine community. These preliminary results are the confirmation that we successfully completed the equipment for preparing of Sr-90/Y-90 electrochemical generator, established the electrochemical separation technique as well as QC of ^{90}Y. In next step, after supplaying of some quantities of ^{90}Sr (200 mCi), we could involve production of ^{90}Sr/^{90}Y generator in order to supply this product to the national nuclear medicine community in Serbia.

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