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PROPOSAL

A CYCLOTRON ISOTOPE PRODUCTION CENTER FOR BIOMEDICAL RESEARCH

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Abstract

We propose the development of a Cyclotron Isotope Production Center with enhanced features that may be used for Biomedical Physics research as part of the Legnaro INFN Laboratories. The cyclotron accelerator should feature a "biomedical" beam-line providing 70 MeV protons with a current of several hundreds of micro-amperes. This document will review the employment of innovative radionuclides in medicine and the features that a new cyclotron should have to produce them. We will also specify equipment and procedures for "targetry", irradiation, radiochemical processing, and labeling, which a production center should have. Quality control and radioprotection issues will also be addressed.

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We propose the development of a Cyclotron Isotope Production Center with enhanced features that may be used for Biomedical Physics research as part of the Legnaro INFN Laboratories. The cyclotron accelerator should feature a "biomedical" beam-line providing 70 MeV protons with a current of several hundreds of micro-amperes. This document will review the employment of innovative radionuclides in medicine and the features that a new cyclotron should have to produce them. We will also specify equipment and procedures for "targetry", irradiation, radiochemical processing, and labeling, which a production center should have. Quality control and radioprotection issues will also be addressed.

SUMMARY

PREAMBLE

New needs show up for original and innovative positron, beta$^-$ and alpha emitting, neutron poor radionuclides that may be produced by accelerators of the kind cyclotron. For this reasons the authors of this proposal, researchers of Medicine, Medical Physics, Health Physics and Nuclear and Radiochemistry of the University of Padua, University of Milano, University of Bologna, INFN-Milano (LASA-Segrate), the National Legnaro Laboratories (LNL-INFN), the IOV ("Istituto Oncologico Veneto"), and ASLs of the Regione Veneto, do strongly foster the development of a beam line and an Isotope Production Center addressed to Biomedical Research to be served by the state of the art 70 MeV Cyclotron that has been recently approved by the National Institution of Nuclear Physics (INFN) and is due to be installed at the National Laboratories of Legnaro.

1. INDRODUCTION

The deployment of the *Radiotracer Principle* in the 1920s by the Hungarian born radiochemist György von Hevèsy, Nobel Prize in Chemistry 1943 and titled as the "Father of Nuclear Medicine", demonstrated that natural and artificial radiotracers would be a powerful tool for investigating inorganic, organic and biological systems [1]. The powerfulness of the modern applications of this technique is based on the high *specific activity* of radiotracer itself (short half-life and low amount of either isotopic or molecular *carrier*) [2-8]. A very high A_S radiotracer (MBq·μg^{-1} to TBq·μg^{-1}) has the advantage that the system under investigation is not "perturbed" by the addition of radiotracer itself. This property provides particular benefits if the system under investigation is constituted of living organisms: cell cultures, animals, humans, leading to detailed information on biokinetics of uptake and release of different chemical species in diverse compartments or districts, without interfering with their natural metabolism. Finally, the addition of known amounts of isotopic or molecular *carrier* to the radiotracer allows the accurate investigation of effect *vs*. amount of substance relationships. In order to assure the reliability of the investigation, it is mandatory carrying out an accurate quality control/assurance on both the radionuclide and labelled species, that means the "experimental" determination of the following parameters: radionuclidic purity, radiochemical purity, chemical purity, specific activity, activity

concentration and - in the case of living organisms - biological purity as well. All previous parameters tend to spoil with time, and the experimental evaluation of these phenomena must be investigated too. For the last three decades, the majority of high A_S radiotracers and labelled compounds used in research, life sciences, bio- and nano-technologies, space research, industrial applications, environmental and cultural heritage studies, is produced artificially by fast ion accelerators and nuclear reactors in minor extent. The accelerator produced radionuclides belong to the *neutron poor* region of the Table of Nuclides (*red* side of beta stability valley), conversely the nuclear reactor produced radionuclides belong to the *neutron rich* one (*blue* side). For higher Z, a series of useful α emitters (*yellow* area) can be produced by accelerator irradiation too. A few radionuclides of very high Z, characterized by spontaneous fission decay (*green* area) find increasing applications in medicine (*e.g.* ^{252}Cf for neutron irradiation of coronary restenosis). The red nuclides (β^+ and EC decay) are used extensively for radiodiagnostics purposes onto humans (gamma-camera, SPET, PET), while the blue ones (β^- decay) are used more and more for the metabolic radiotherapy of tumours and to minor extent for other pathologies. In recent years the yellow radionuclides are being used for metabolic radiotherapeutic purposes and there are increasing investigations about the possibility to use low energy Auger emitters for hitting efficiently the DNA, with irreversible double and multiple strand breaks (DSB, MSB), after internalization into cell nuclei. To conclude, it must be perceived by people and governments that radiotracers and radiopharmaceuticals are used in large quantities in modern societies. In North America, every year are performed about 35 million investigations by nuclear medicine devices and 15 million of them are carried out by ^{99m}Tc, used for labelling a range of radiopharmaceutical compounds. Furthermore, several hundred thousand treatments of metabolic radiotherapy with unsealed radionuclides and labelled radiotracers are carried out annually in most developed countries and Italy too.

At the beginning of 2000, the introduction also in Italy of a few PET imaging centers (were only 4 in 2000 and 77 in 2007) with the "FDG" radiotracer (2-FDG, 2-[^{18}F]-fluoro-2-deoxy-*D*-glucopyranose, fluoro-deoxy-glucose) in the routine practice of nuclear oncology gave a new boost to the Nuclear Medicine. At the same time a substantial progress has been achieved in radionuclide therapy (metabolic radiotherapy), especially in radio-immunotherapy and radio-peptide targeted therapy. All these developments open large prospects both in radiodiagnostic imaging and radionuclide therapy with the availability of many carrier molecules (i.e. radiotracers, radiopharmaceuticals), which are currently

evaluated in preclinical and clinical studies. Presently, 2-FDG is mainly used in *oncology* (85-90% of total investigations in Italy), despite that it was developed in early times for neurological investigations (BNL, USA, 1976), for assessing in-vivo the metabolism of glucose. Beyond oncology, new innovative radiopharmaceuticals are expected to be validated in *cardiology* and *neurology* as well in the coming years.

This document will review innovative radionuclides in medicine, the possible research on this field, and which features a new cyclotron should have to produce them. We will also sketch the state of the art of the *radiochemical processing* of the activated target, which we need to separate the radionuclide of interest. Due to the high power density deposited by the accelerator beams in the target a new branch of accelerator technology (*targetry*) was strongly developed in the last decades. The radionuclides are then used for *labeling* of chemical species (*i.e.* radiotracers, radiopharmaceuticals) suitable for the investigation of body organs, or districts. All steps of production, radiochemical processing and labeling are controlled and followed with time by *quality control* (QC) investigations in order to optimize and upgrade the performances of final radioactive product to guarantee its safe administration onto humans. The presence of long-lived and highly radiotoxic impurities must be also assessed in order to prevent undesired dose to the medical and paramedical personnel and pollution of the environment by radwastes as well.

The main steps will be described in some details in the following: production, targetry, radiochemical processing, labeling and quality control.

2. INNOVATIVE RADIONUCLIDES IN DIAGNOSTICS AND THERAPY

2.1 PET and SPET imaging

Positron emitters (β^+) for PET imaging, currently used, are the short-lived "physiological radionuclides" carbon-11 ($T_{1/2}$ = 20 min), nitrogen-13 ($T_{1/2}$ = 10 min), oxygen-15 ($T_{1/2}$ = 2 min) and above all fluorine-18 ($T_{1/2}$ = 110 min). This latter is undoubtedly the radionuclide of choice in most practical cases, due to its favorable radio-physical characteristics (positron end point energy and half-life)) and chemical characteristics as well (F is a bio-mimetic of –H and –OH groups and a modulator of C chemical bonds in biomolecules). Beside, the well known 2-FDG, a range of novel carrier candidates (radiotracers), including FLT, F-MISO, FES, F-choline and F-DOPA, have been clinically evaluated and some of them could be approved for a routine use in the coming years. However, the short physical half-life of these radionuclides, including fluorine-18, the longest living, requires their production in a cyclotron located at short distance from user centre. That's why there is more and more interest for positron-emitting radionuclides with short half-lives but which can be produced in a generator and especially for gallium-68 (physical half-life: 68 minutes) whose father is **germanium-68** (with a long half-life of 271 days). Such a generator $^{68}Ge/^{68}Ga$ has the great advantage of being used for a few months in a nuclear medicine department, but germanium-68 needs to be produced in a cyclotron with a high intensity beam due to its low production yield.

Fluorinated molecules feature small size and consequently fast kinetics after intravenous injection, which is compatible with the relative short physical half-life of fluorine-18. However, for larger carrier molecules (biochemical vectors), such as antibodies or more generally immune-constructs, blood kinetics is much slower and maximal tumor accretion is observed relatively late, several hours or some days after intravenous injection. This time interval is not compatible with the 110 minutes half-life of fluorine-18. For this new imaging application, named immuno-PET, new radionuclides with longer half-lives are needed, like the:

Iodine-124, a positron-emitting radionuclide with a physical half-life of 4.2 days, which favorably fits with the blood kinetics of antibodies for immuno-PET imaging and metabolic radiotherapy;

Copper-64 (half-life: 12.7 hours), another positron-emitting radionuclide of great interest,

which is also considered for routine production for both PET imaging and negatron/positron metabolic radiotherapy as well.

Another clinical application that requires radionuclides with half-lives longer than that of fluorine-18, even for small molecules with fast blood kinetics, is the pre-therapeutic dosimetric calculation. For this application, the innovative approach consists in using pairs of positron- and negatron(beta-)-emitting radionuclides. Given the present clinical routine use of iodine-131 and yttrium-90 for the labeling of immuno-constructs and oligo-peptides, the favorite pairs of radionuclides are iodine-124-(β^+)/iodine-131-(β^-) and yttrium-86-(β^+) / yttrium-90-(β^-). However, the latter pair is not routinely used because of a high energy gamma ray, emitted at a substantial rate by yttrium-86 that would bring radioprotection issues.

Another highly requested pair of radionuclides is copper-64-(β^+,β^-)/copper-67-(β^-) due to the favorable characteristics of both of them.

In non oncology applications, the diagnosis of myocardial ischemia in cardiology may benefit from the radionuclide imaging. Thallium-201 (γ) and technetium-99m (γ) MIBI (Cardiolite®) have been used in SPECT practice for decades. However, the low energy of their emitted gammas requires an attenuation correction that bears some shortcomings, like a relative high percentage of false positive results, and consequently useless invasive coronarography procedures. For this reason today a new isotope is preferred for this kind of radio-diagnosis: the rubidium-82m (β^+) that is a positron-emitting radionuclide that behaves like thallium-201 and is taken-up by the myocardial muscle. The high energy (511 keV) annihilation photons allow a reliable attenuation correction, and the diagnostic specificity of rubidium-82m imaging is significantly higher than that of thallium-201 or technetium-99m as for MIBI SPECT imaging. Rubidium-82m has a very short half-life (75 s) and is produced in a generator by decay of **strontium-82** that has a 25.5 d half-life. The very short half-life of rubidium-82m allows both rest and stress imaging in less than 30 minutes as compared to a few hours for thallium-201 or technetium-99m MIBI SPECT. Strontium-82/rubidium-82m generators have been used in the USA for more than a decade, but, currently, the production capability of high activity strontium-82m is seriously limited. The proposed high energy/high intensity cyclotron would produce up to 600 generators a year.

Finally, **technetium-94m** ($T_{1/2}$ = 53 min) is another short-lived PET radionuclide, cyclotron produced, with high potentialities as a substitute of the SPET radionuclide ^{99m}Tc. Today, the production rate is low and does not meet the hospitals' needs.

Production of Mo-99/Tc-99m Generator. About 50% to 80% of the Nuclear Medicine tests are based on the isotope Tc-99m. The medical investigations employing this isotope are more than 15 million per year and the required activity is 10,000 Ci per week. Today there are only two large reactor production sites in the world: in North America (Canada) and Europe (The Netherlands). A recent accident of radioactivity loss and dispersion in the European plant (the 25/08/2008) shut down the Tc-99m production for three months causing a world-wide shortage of the isotope and delay or cancellation of a substantial fraction of the diagnostic activity. Smaller shortages have also happened in the past due to plants programmed maintenance. In spite of the production system vulnerability, no new sites are foreseen because of the serious security, safety and environmental risks. The sites employ 70 tons per year of HEU (High Enriched Uranium), military grade, irradiated by high flux density reactor neutrons. Subsequently, the molybdenum Mo-99, 66 hours half life, is separated from the uranium through radiochemical processing. Eventually Mo-99/Tc-99m generators are distributed to the hospitals for radiopharmaceutical labeling.
Security risks might come from HEU theft by criminal and terroristic groups, while the environmental issue consists of the disposal of high activity transuranic isotopes produced by high flux reactors on HEU.

To solve these issues and smooth the world production of Tc-99m, the two large reactor centers might be flanked by a network of regional sites that would cover a substantial part of the technetium overall demand by employing alternate procedures. Multiple decentralized production centers and alternate methods are being tried out in South Africa, Australia, and Brazil under IAEA supervision. The alternate innovative methods are: 1) Light Enriched Uranium (LEU) fission in low flux reactors; 2) LEU fission through subcritical Accelerator Driven Systems (ADS); 3) Mo-98 neutron capture through reactor n irradiation; 4) Ion irradiated Isotopic targets for producing technetium precursors like the Mo-99.

As for the last method, there are numerous detailed studies on proton induced fission on U (233, 235, 238) that show cross section of 1,550 mbarn at 80 MeV. This reaction may be employed to create Tc-99m in thick target. A further yet unexplored way is the Th-232 fission by 20-80 MeV protons with a cross section of 1,200 mbarn at 80 MeV and a yield of $7 \cdot 10^{-2}$ fissions per incident-proton on thick target. The capture of fission neutrons after adiabatic thermalization by Thorium should not generate transuranic isotopes like Np, Pu, contrary of what happens with high flux irradiated U-235.

The fragments mass distribution is peaked at A=100, quite close to Mo-99 (which has a production cross section of ~ 60 mbarn), so the fissioned material should present a quite high specific activity, a radiotoxicity smaller than the reactor irradiated targets, and the radiochemical rendering should be comparable with that of the HEU irradiation process.

2.2 Radionuclide Therapy and Metabolic Radiotherapy

Beta (β^-), alpha (α) and Auger emitters may be used for radiotherapy, which are either brought directly into the cancer by *brachytherapy* or intravenously conveyed by radiopharmaceuticals for *metabolic radiotherapy*.

The mostly used beta emitters are iodine-131(β^-) and yttrium-90(β^-), other than rhenium-186g, samarium-153, holmium-166 and lutetium-177g(β^-). Their negatron energy spectrum is suited for targeting tumors of different sizes as a function of beta end point energy. However, iodine-131 also emits a relatively large fraction of high energy gamma rays, which requires medical staff radiation safety constraints, including some confining of patients in shielded rooms for a few days. These constraints seriously limit the number of patients who could benefit of this therapy. Yttrium-90, a high energy (2.28 MeV) beta-emitter, is taken up by bone/bone marrow after release from its chelator coupled to the carrier molecule, resulting in marrow irradiation, which limits the allowed injected activity. Moreover, yttrium-90 does not emit gamma rays for pre-therapeutic imaging, which suggests the use of a demanding multiple labeling during the treatment with suitable either isotopic or isomorphous multi-gamma emitters like yttrium-86 and indium-111. For all these reasons, new isotopes are now proposed that are partially free of these drawbacks.

Copper-67 (β^-) ($T_{1/2}$ = 61.5 h) is a radionuclide with favorable radio-physical and biological characteristics that has been pre-clinically and clinically evaluated for more than 2 decades. Cu-67 outdoes iodine-131 and yttrium-90 as for therapeutic index in a few clinical studies. However, its industrial production has been limited by the lack of high energy (70 MeV), high intensity (a few hundreds of μA) cyclotrons for producing the large activities necessary in clinical studies.

Rhenium-186 (β^-, γ) ($T_{1/2}$ = 90 h, E(β-) ~ 0.35 MeV, E(γ) ~ 137 keV), produced by a cyclotron with reactions W-186(p,n) or (d,2n)Re-186, favorably compares with the higher beta energy Re-188 (β-, γ) ($T_{1/2}$ ~16 h, E(β-) ~ 2.2 MeV, E(γ) ~ 155 keV) obtainable through

the generator $^{188}W/^{188}Re$, obtained by high flux density reactor. The lower β- energy avoids dangerous irradiation of the marrow in bone treatment and the long half-life allows a direct transport from a cyclotron facility (no generator being available). Both isotopes emit gammas with energies exploitable for imaging. The possible reactor production through neutron capture on enriched Re-185 implies several shortcomings that the cyclotron production may avoid and in particular the low specific activity.

Palladium-103 (γ) ($T_{1/2}$ = 17 d, E(γ,X) = 21 keV), is used in prostate cancer and uveal melanoma brachytherapy. It proves sometimes more effective than I-125 as for rapidly proliferating and poorly differentiated tumors. The choice between the two is driven by the tumor growing rate (Gleason Index). Production of palladium-103 may be accomplished by either cyclotron p beam on a rhodium plated target or reactor by bombarding an enriched Pd-102 target with neutrons. In contrast to cyclotron production, nuclear reactor gives a Pd-103 that is not carrier free, is always mixed with Pd-102 and other contaminants, and has a specific activity that cannot be adjusted. Reactor-produced palladium-103 from enriched palladium-102 is also expensive because of the difficulty in enriching palladium-102 (only 1.02 % natural abundance) from palladium metal.

Finally alpha-emitting radionuclides are being more and more considered for use in therapy because of the large LET (Linear Energy Transfer) that gives a high killing effect especially for small clusters of malignant cells. A few alpha-emitting radionuclides are available, including astatine-211, lead-212/bismuth-212 (generator), actinium-225/bismuth-213 (generator), protoactinium-230. Unfortunately the proposed cyclotron would not provide the He ions, necessary for the astatine reaction, although this feature could be added later. However a proton cyclotron may produce the following isotopes:

Thorium-228 (α) ($T_{1/2}$ = 1.91 y, E(α) ~ 5.4 MeV) is employed to feed the Pb-212/Bi-212 generator through the intermediate Ra-224. Th-228 comes from the reaction Th-232(p,X) with a reasonable cross section ~ 60 mbarn (at 60 MeV).

Actinium-225 (α) ($T_{1/2}$ = 10 d, E(α) = 5.8 MeV) may be used directly or in a generator to give the Bi-213 ($T_{1/2}$ = 46m, E(β-) = 0.435 keV at 97.80 %, E(α) = 5.8 MeV at 2.20 %). It comes from the reaction on the radioactive radium Ra-226(p,2n) with high cs~800 mb (17 MeV) or on the Th-232(p,X) with a cross section ~ 3 mb (60 MeV).

Protoactinium-230 (α,β-) decays at 7.8 % into U-230 and gives E(β^-) = 150 keV, and, through U-230, E(α) = 5.8 MeV. Pa is produced by a reaction Th-232(p, 3n)Pa-230.

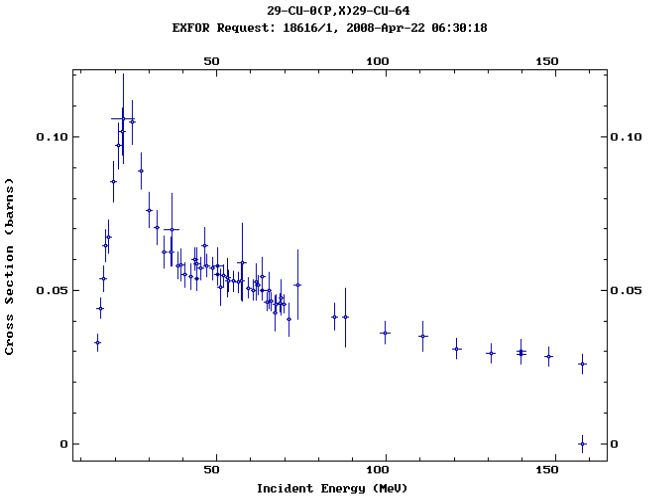
3 RADIONUCLIDE PRODUCTION CROSS SECTIONS

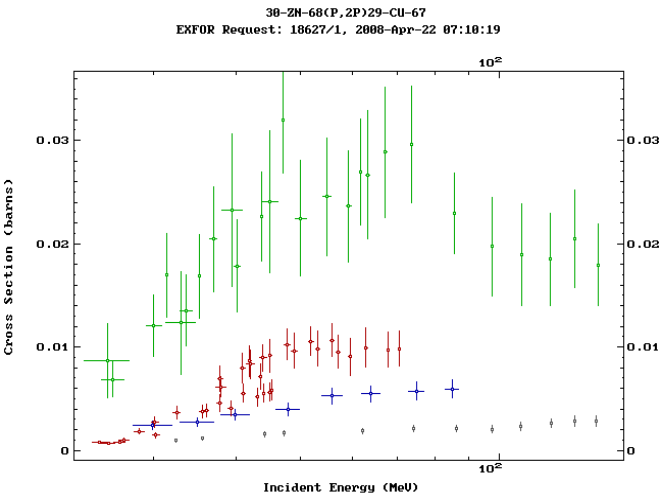
The following table shows the production cross-sections for the radionuclides provided by a proton-cyclotron that have been here suggested for medical applications. We have considered the energy (p-ener) that gives the largest cross-sections in the range 40-70 MeV or close (marked with *). The yields, i.e. the activity obtained per unit incident charge (1 C), look approximately acceptable for all the nuclides of the table, although their precise (and complex) determination, including targeting and radiochemical issues, and likewise the comparison with competing methods, is outside the scope of this report.

Radionu	Target	reaction	p-ener (MeV)	σ_{Max} (mbarn)
Cu-64	Ni	^{nat}Ni(p,n)	40	50
*Cu-64	Ni	^{64}Ni(p,n)	15	675
Cu-67	ZnO	^{68}Zn(p,2p)	70	25
Ge-68	Ga	^{69}Ga(p,2n)	45	100
*Ge-68	Ga	^{69}Ga(p,2n)	20	550
Sr-82	RbCl	^{nat}Rb(p,4n)	50	100
I-124	Te	^{nat}Te(p,n)	53	150
*I-124	Te	^{124}Te(p,n)	12	590
*Re-186	W	W(p,n)	10	17
*Pd-103	Rh	^{103}Rh(p,n)	10	500
Th-228	Th	^{232}Th(p,X)	70	60
Ac-225	Th	^{232}Th(p,X)	60	3
*Pa-230	Th	232Th(p,3n)	30	260

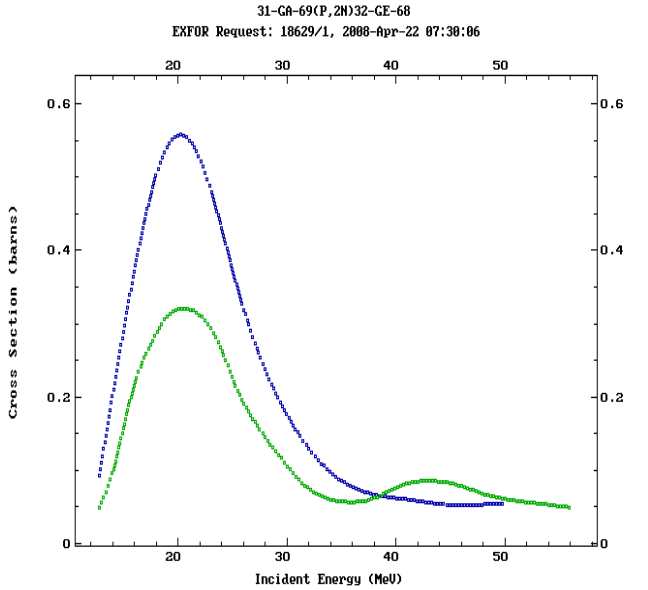
The following graphs show the radionuclides production cross sections as a function of the proton energy, as drawn from the EXFOR data base (*www.nndc.bnl.gov*).

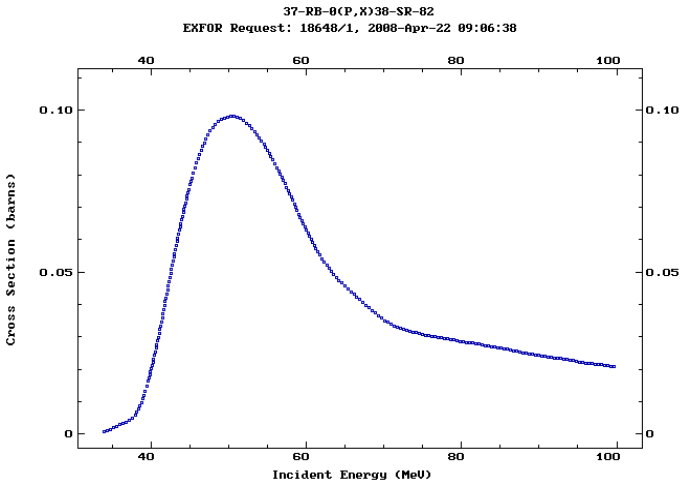
Cu-64, Cu-67



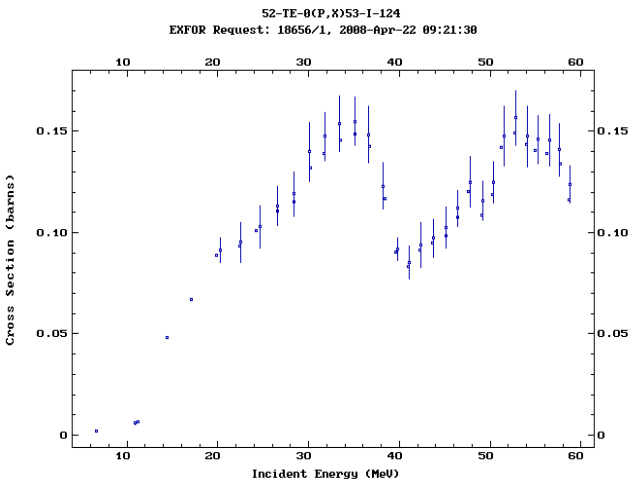


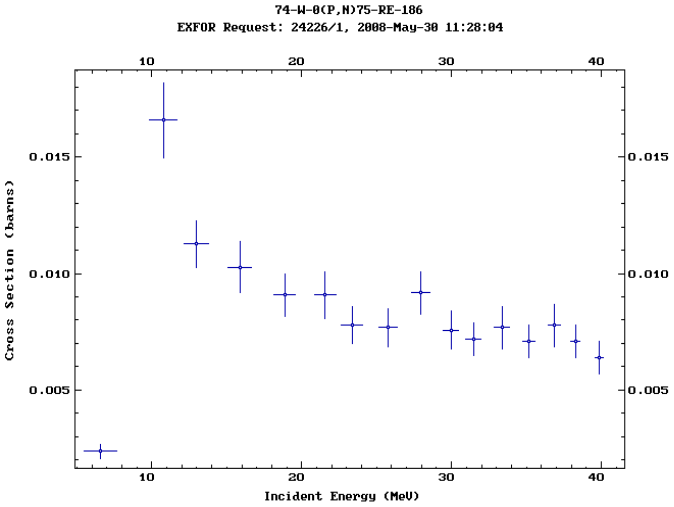
Ge-68, Sr-82



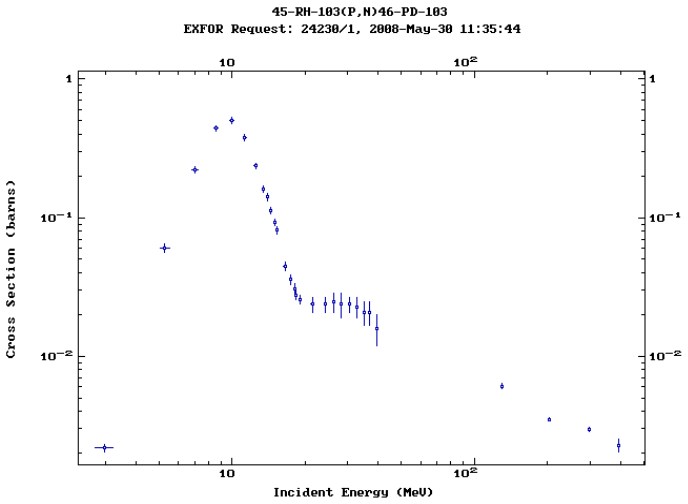


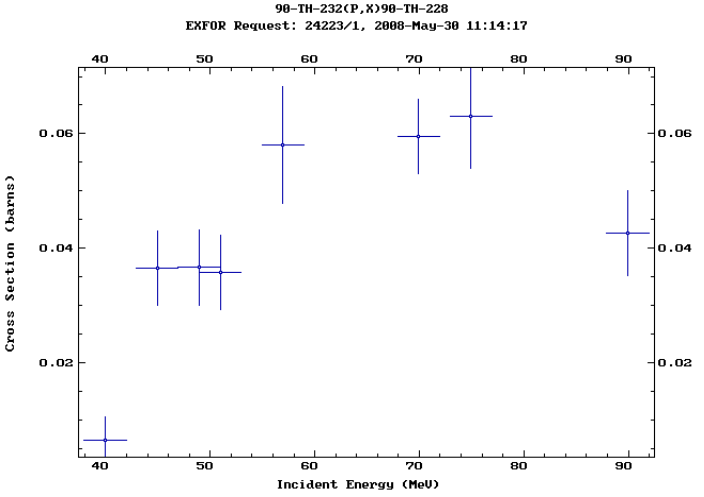
I – 124, Re-186



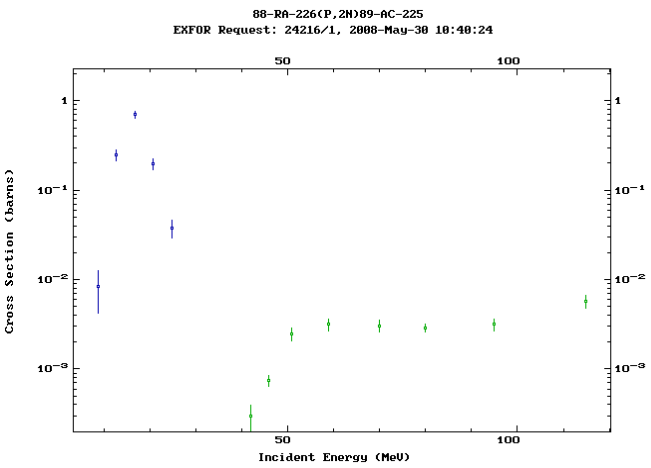


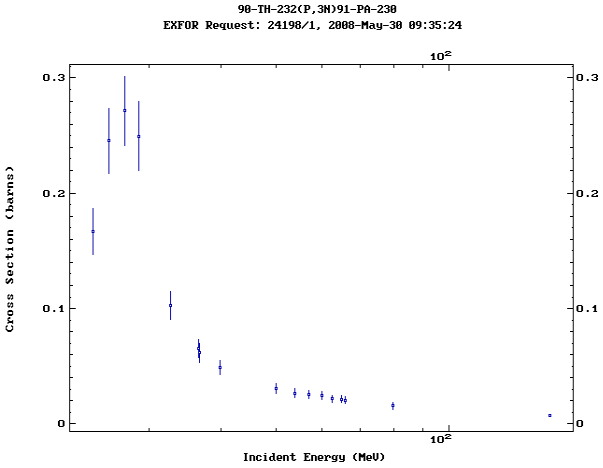
Pd-103, Th-228





Ac -225 [left: ^{226}Ra(p,2n), right: ^{232}Th(p,X)], Pa-230





4 RADIOCHEMICAL PROCESSING

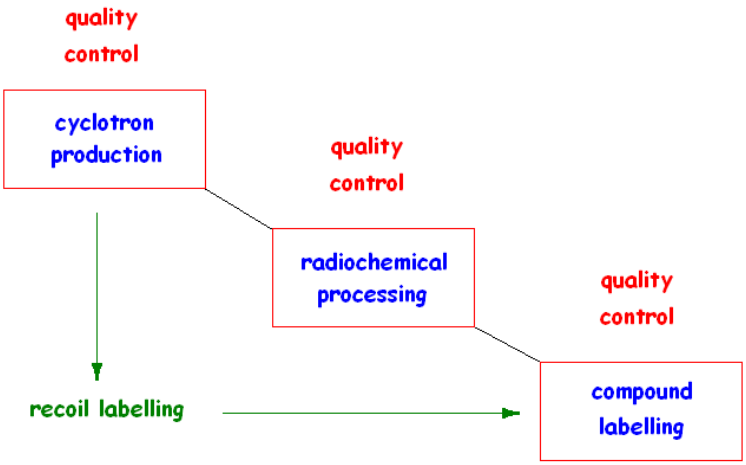
4.1 General Remarks

As a rule, the radionuclide produced by accelerator activation is diluted in a overwhelming amount of target material (and undesired often unavoidable chemical impurities) and cannot be used directly for any application onto living organisms (cells, animals, humans). The very small massic amount of radioactive species produced (the *nanocomponent*, produced at pg to ng amounts) is diluted in a large amount of target material (the *macrocomponent*, several mg to g amounts) and a typical ratio of the two specimens is of the order of several millions up to many trillions. The technology of separation of the nanocomponent from the macrocomponent is often named *sub-nanochemistry* or *ultratrace chemistry* and shows very particular features. The ratio between the initial amount of target material and the final one in the final preparation is named *decontamination factor* DF and ranges often from 10^5 to 10^8. Besides, the target is composed by either gas, liquid or solid specimen and in the different cases different approaches must be afforded. The radionuclidic impurities can be roughly classified as *isotopic* (same Z) and *non-isotopic* (different Z) with the radionuclide under production. While in principle all non-isotopic impurities can be effectively separated by a suitable radiochemical processing of the target, the isotopic impurities can be only minimized by an appropriate choice and optimization of irradiation conditions.

In classical radiochemistry (since the Curie's, through von Hevesy until the Seaborg era) it was considered mandatory the *intentional addition* to the radioactive mixture of an appropriate amount of chemical or physical species (carrier, hold-back carrier) able to carry on the nanocomponent and to facilitate and improve its *radiochemical separation yield* RCY from either the target or the decontamination from undesired impurities. In practice, with a few exceptions, the addition of a suitable isotopic carrier (same Z and same chemical form) or non-isotopic carrier (isomorphous, isodimorphous, any other) has the significant advantage of improving the overall yield and diminishing the manipulation time that is of great relevance in case of short-lived radionuclides, taking into account the exposition of personnel to radiation too (ALARA criterion). The radiochemical methods based on the use of carriers added to the target are named *carrier-added* (CA) methods, especially if the carrier is isotopic with the nanocomponent. In many practical cases the use of non-isotopic carriers is acceptable, only if followed by a further and often very difficult and time-

consuming purification step of the final product. The *no-carrier-added* (NCA) methods are presently used in most practical situations for applications in the life-sciences, in spite of the somewhat lower radiochemical yield RCY achievable.

In order to decrease the amount of carriers (undesired or accidentally added) and other stable impurities, the miniaturization of targets, equipment, tubing, processing vessels, chemicals, is mandatory and leads to a specific branch of radioanalitycal and synthetic sub-nanochemistry. At least the use of plastic equipment instead of glassware must be preferred in order to avoid undesired addition of metallic impurities.



To separate the NCA nanocomponent from the irradiated macrocomponent any chemical or physical method is suitable: precipitation and co-precipitation, ion-exchange and any other kind of chromatography techniques, wet- and dry distillation, termochromatography, liquid-liquid extraction. electrodeposition, mass separation, centrifugation, electrophoresis, gas-jet, others. The radionuclides produced in NCA form have the main advantage of a very high *specific activity* A_S (either massic or molar: activity to mass of isotopic carrier or mass of labelled compound) leading to a very high A_S of the final labelled product. Of course the A_S must not be interchanged with the *concentration of activity* C_A of the labelled species that – apart the completely different definition – has very much lower values (typical C_A are in the $MBq \cdot g^{-1}$ range compared to typical A_S of $GBq.g^{-1}$ to $TBq \cdot g^{-1}$). Operating in strictly NCA conditions it is often possible to reach the maximum theoretical value of A_S that is properly named *carrier-free* (CF) A_S or $A_S(CF) = N_A \lambda / M$ in this situation only (modern IUPAC

terminology).

Roughly speaking the radiochemical processing methods can be distinguished in: *dry methods* and *wet methods*.

4.2 Targetry

The technology of *high beam intensity power targets* P (more than 100 μA protons, or some tens μA alphas) and *high beam power density* P_D targets has been strongly boosted in the last decades. Roughly speaking a "some kW target" is considered high power, even if the significant quantity is the power density into the target. Today the more powerful targets in the world (LANL-IPF and INR-Troitsk) are able to manage up to 150 - 250 μA at 100-150 MeV proton beams on solid or melted metal and alloys targets with good thermal conductivity, meaning a power density of some 10 $kW \cdot g^{-1}$. Beam powers like 70 MeV protons x 750 μA = 52.5 kW or higher are considered out of limits of present technology and would require a strong technological effort. Indeed, the medical radionuclides target technology must not be compared to that of the high power targets (MW) for radwaste transmutation in spallation neutron sources. In fact medical radionuclides require high purity and specific activity (see section on QC) that cannot be achieved in ADS technology.

A relevant part of this technology deals with heat dissipation, radiation damage and mechanical stress of thin metal windows used to contain the gas and liquid targets. A wide range of metals and alloys has been studied depending on target material, radionuclide produced and other items. Target and window cooling is provided by highly engineerized water and gas streams of proper pressure, temperature and thermo-hydraulic specifications. In several practical cases (i.e. short-lived positron emitters) an effective *in-target chemistry* is achievable (*hot-atom chemistry*, *recoil-labelling*), gaining many simple radioactive precursors (i.e. $^{11}CO_2$, ^{11}CO, $^{11}CH_4$, $^{13}NH_3$, $^{13}N_2$, $H_2{}^{15}O$, $^{15}O_2$, $^{18}F^-$, $^{18}F_2$, many others) already suitable for further labelling steps of more complex labelling intermediates, to be used for the final labelling procedures of biomolecules and drugs.

4.3 Gas Target

Gas is a very suitable target material (when applicable), because of the easy pipeline transfer of irradiated gaseous material from the target and the cyclotron vault to the *hot*

radiochemistry laboratory. Of course the beam power dissipated in the target normally does produce a *density reduction* of the gas material and a consequent decrease of the theoretical yield is expected, if no well designed feedback systems on the beam intensity are installed. Moreover, the shape of the gas target must be optimized to minimize these effects. The operations are carried out by *remote controlled* fluidic equipment like: electrovalves, pressurized vessels, flow meter controls, on-line activity detectors, on-line purification and quality control systems. Targetry, purification and QC procedures are easily remotized under PC control. Typical target materials are nitrogen-14, enriched nitrogen-15, enriched oxygen-18, neon-20, enriched Kr and Xe isotopes, but in principle any volatile element or compound can be considered to this purpose, even if in case of compounds a substantial *radiation radiolysis* must be expected. The recovery yield of the radioactive product from the gaseous target must be faced and optimized. In fact there might easily be loss of high specific activity radionuclides due to *adsorption* on target holder materials, transfer tubing and valve systems. The method of either *flowing* or *recirculating* gas targets was investigated too, in order to achieve an *on-line separation* of very short-lived radionuclides. In case of oxygen-15 this method proved very effective and is used routinely in the clinical practice.

4.4 Liquid Target

the technology of *liquid water* irradiation is very well developed since the discovery in the ‘80s of the efficacy of $^{18}O(p,n)^{18}F$ or $^{16}O(^{3}He,p)^{18}F$ and $^{16}O(p,n)^{13}N$ reaction routes on either natural or enriched liquid water. In this case, as in the case of gas targets, a density reduction and even bubbling of liquid target is expected, even if with an improved Targetry technology it is possible to irradiate routinely 1 mL of pressurised water with several tens μA of 17 MeV proton beams, without significant losses (i.e. 17 W / g μA). Liquid or melted metals have been irradiated too (enriched Hg isotopes, Rb and Cs metal). The radiation induced *radiolysis* of liquid materials must be taken into account too. Moreover, the *corrosion* of metallic target holders by the liquid - and water in particular - is a hard technological problem, in particular when highly reactive products are present. The method of either flowing or recirculating liquid targets was investigated in some details, even if the target volume would became significantly higher, with somewhat non-tolerable decrease of specific activity.

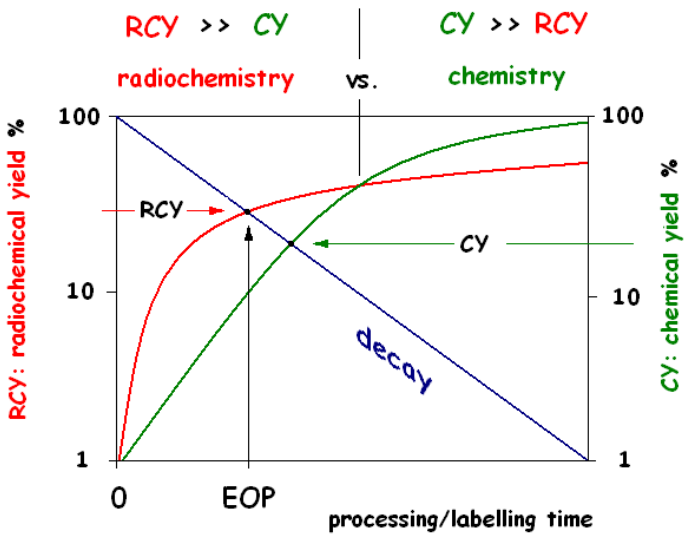
4.5 Solid Target

in this case the best target does consist of either a high melting point or/and a high thermal conductivity material, but in many practical cases the radioactive product could be volatile and can be lost during the irradiation (^{211}At, ^{123}I, ^{124}I). On-line separation methods of radioactive products are envisaged and implemented in such cases.

In case of low melting point target materials, the technology of irradiating compounds or alloys was already adopted successfully (i.e. Na^{127}I instead of $^{127}I_2$, $Cu_3{}^{75}As_2$ instead of ^{75}As, melted Rb instead of solid Rb, $^{124}TeO_2$ instead of ^{124}Te element, many others). The solid target must be driven to the hot cell facilities in *hot radiochemistry laboratories* by using either pneumatic or remote controlled rail systems. The solid targets are either dissolved in acidic media and subsequently separated, or brought to dry distillation equipment for separation of volatile species (i.e. ^{211}At, ^{73}Se or ^{124}I).

As a rule, few steps and fast separation methods are preferred in spite of the lower overall chemical yield CY%. In fact one has rather to optimize the Radiochemical Yield (RCY%), due to the short half-life of many radionuclides (RCY% = CY% exp(-λ t)), in order to maximize the amount of labelled species at the End Of radiochemical Processing, EOP. In practice a fast and simple chemical method is envisaged in comparison to more classical chemical methods with low kinetics and complex chemical procedures (as in figure).

Of course, in all cases the transfer systems must be accurately sealed and radiation shielded to be driven to the hot laboratories.



5. RADIOPHARMACEUTICALS

5.1 General Remarks

We discuss here the radionuclide binding to chemical substances, the radio-pharmaceuticals (i.e. radiotracer according to von Hevesy principle), which allow biological pathways once injected in vivo. From the chemistry point of view the radionuclides are divided in two principal groups according to whether they are metal or non metal. In fact the labeling methods of radiopharmaceuticals use reactions which are completely different for the two groups. All the radionuclides previously proposed are metals, but iodine. For this reason we mainly report here the state of the art of metal labeled radiopharmaceuticals. These latter have brought a great development in nuclear medicine, since technetium has had a wide spread use in clinical diagnosis [1]. The use of a radiometal requires handling *coordination complexes* to keep the radionuclide permanently bound to the bio-active molecule, and coordination chemistry studies oriented to ligands with backbones, which provide useful biological interactions.

In designing radiometal-based radiopharmaceuticals, important factors to consider include the radiometal half-life, the mode of decay, and the cost and availability of the isotope. For diagnostic imaging, the half-life must be long enough to chemically synthesize the radiopharmaceutical and perform the diagnostic analysis, but short enough to limit the dose to the patient. Radiometals for coordination complex-based radiopharmaceuticals used in gamma scintigraphy and PET range in half-life from about 10 minutes (^{62}Cu) to several days (^{67}Ga). The desired half-life is dependent upon the time required for the radiopharmaceutical to localize in the target tissue. For instance, heart or brain perfusion agents require short half-lives, since they reach the target quickly, whereas tumor-targeted radiopharmaceuticals based on monoclonal antibodies (Mabs) need long half-lives.

In table 1 some radiometals still used or which are going to be used in nuclear medicine are reported with the production method, half-life, type of radiation, and relative energy.

Table 1. Radiometals used for labeling radiopharmaceuticals

Radionuclides	Method of production	$T_{1/2}$	Radiaz. (E. in MeV)
^{60}Cu	$^{60}Ni(p,n)^{60}Cu$	24 m	β^+ (3.9-3.0)
^{61}Cu	$^{61}Ni(p,n)^{61}Cu$	3.3 h	β^+ (1.20)
^{62}Cu	$^{62}Zn/^{62}Cu$ gen.	9.8 m	β^+(0.51)
^{67}Cu	$^{67}Zn(n,p)^{67}Cu$	2.58 d	β^-(0.54), γ(0.185)
^{67}Ga	$^{66}Zn(d,n)^{67}Ga$	78,3 h	γ(0.09), γ(0.18), γ(0.3)
^{68}Ga	$^{68}Ge/^{68}Ga$ gen.	68.3 m	β^+(0.51)
^{90}Y	$^{90}Sr/^{90}Y$ gen.	2.67 d	β^-(2.28)
^{111}In	$^{111}Cd(p,n)^{111}In$	2.8 d	γ(0.17), γ(0.34)
^{153}Sm	$^{152}Sm(n,\gamma)^{153}Sm$	1.95 d	β^-(0.8), γ(0.103)
^{177}Lu	$^{176}Lu(n,\gamma)^{177}Lu$	6.71 d	β^-(0.50), γ(0.21, 011)
^{186}Re	$^{185}Re(n,\gamma)^{186}Re$	3.77 d	β^-(1.08), γ(0.131)
^{188}Re	$^{188}W/^{188}Re$ gen.	16.95 h	β^-(2.13), γ(0155)
^{201}Tl	$^{203}Tl(p,3n)^{201}Pb(EC)^{201}Tl$	73 h	γ(0.13), γ(0.17)
^{212}Bi	$^{224}Ra/^{212}Pb/^{212}Bi$ gen	1.0 h	α(7.8) γ(0.72)

Many of the metal labeled radiopharmaceuticals are also used for metabolic radiotherapy, which is bound to become more and more a valid support in the remission and regression of solid tumors. The success of Radiotherapy is related to the capability of radiation particles to reach tumor cells. Radiopharmaceuticals have the possibility to enter in inmost contact (at molecular level) with cancer cells and sometimes go inside them, and therefore the destructive action of radiation can be very efficacious. On the other hand, ionization radiations create damage also in normal cells and radiopharmaceuticals selectivity for cancer, with respect to healthy tissues, is necessary. Selectivity is dictated by the bio-specificity of the labeled molecule for a tumor site (receptor, membrane, blood irroration, etc.). In few words, radiotherapy needs are similar to those of radiodiagnosis, although more restricted and dramatic.

Table 2.

Radioisotopes for Radiotherapy

Isotope	Half-life (days)	β^-_{max} (MeV)	Range in soft tissue (mm)	Eγ (KeV)
^{165}Dy	0.1	1.29 (83%) 1.19(15%)	5.7	95 (4%)
^{156}Sm	0.4	0.7 (51%) 0.4 (44%)		None
^{188}Re	0.7	2.12 (72%) 1.96 (25%)	11.0	155 (15%)
^{166}Ho	1.2	1..85 (51%) 1.77 (48%)	8.5	81 (6%)
^{105}Rh	1.5	0.57 (75%) 0.25 (20%)		319 (19%)
^{153}Sm	1.9	0.67 (78%) 0.81 (21%)	2.5	103 (28%)
^{198}Au	2.7	0.96 (99%)	3.6	411 (96%)
^{90}Y	2.7	2.28 (100%)	11.0	None
^{186}Re	3.7	1.07 (74%) 0.93 (21%)	3.6	137 (10%)
^{175}Yb	4.2	0.47 (87%)		396 (7%)
^{177}Lu	4.2	0.48 (78%)	1.7	208 (11%)
^{32}P	14	1.71 (100%)	7.9	None

Parameters such as *physical half-life of β⁻-radiation*, *energy of β⁻-radiation* and its *percentage*, *tissue penetration range*, and *energy* and *percentage of γ-radiation* determine the efficacy of the radio-therapy application and suggest the irradiation protocols (Table 2). The γ-radiation, if emitted, is useful for imaging the drug uptake and bio-distribution during therapy. The radionuclide is chosen on the basis of the optimization of all the above parameters for the specific clinical application, and the imaging capability. The feasibility of clinical application depends on the availability of a labeled molecule which fixes the radionuclide upon the target tumor for the time necessary for a therapy protocol.

5.2 Properties of Metal Complexes

Designing metal complexes for imaging and radiotherapy requires correlating aspects of the coordination chemistry with *in vivo* behavior. Factors to consider include redox properties, stability, stereochemistry, charge and lipophilicity of the metal complex. The target organ or tissue will dictate the desired characteristics of the metal complex. For example, it is known that negatively charged compounds tend to clear thought the kidneys, many positively charged ions accumulate in the heart, and an overall neutral complex is required for crossing

the blood-brain barrier. Lipophilic complexes will generally have more uptake in the liver or in fatty tissues. Stereochemistry is important when targeting complexes to specific receptors. Another important factor is complex stability; while thermodynamic stability of non-radioactive metal complexes can help predict *in vivo* behavior, it is often not indicative of *in vivo* stability. Sometimes inertness (kinetic stability) was seen to be most important in keeping the complex unaltered during clinical application. There are few absolute rules, and it is a continuous learning process to correlate the characteristics of the metal complex to the *in vivo* behavior.

In receptor based radiopharmaceuticals the labeling metal compound determines the *in vivo* behavior in dependence of size, lipophilicity and activity of the biomolecule. The role is then correlated to how much the modification of the native biomolecule reduces the specific activity. From the up to date results, we can say that *the relationship between the maintenance of bio-activity and the modification of structure of the bio-molecule* is variable and it depends, first of all, on the dimension of the bio-active molecule but also on the type of affinity mechanism. The dimension factor is easily understandable, when we consider that the modification is completely supported by a big molecule of which, usually, only a small part participates in the specific uptake. The smaller the molecule, the higher is the influence of the ^{99m}Tc-complex in the modification of the biological behavior.

The affinity mechanism depends on the specific bio-molecule and is related to the particular functional groups, the spatial distribution, and the biochemical interactions of a precise part of the bio-molecule. On the other hand, the modification affects not only the affinity property but even the complete *in vivo* behavior: i.e. *uptake in non-target organs, membrane perfusion, plasma retention, etc*.. In other words, the percentage of fixation to the receptor is also dependent on the capability of the radiopharmaceutical to reach the site of uptake (transport capability).

5.3 Metal Based Radiopharmaceuticals

We consider here the radiopharmaceuticals that may be labeled with some of the radionuclides obtainable with the proposed Cyclotron. Although ^{124}I-radiopharmaceuticals are important tracers that may alone justify the Cyclotron installation, mainly for the applications in the pharmaceutical research, they are not dealt here since we restricted ourselves to metal tracers. Also ^{82m}Rb and ^{103}Pd as far as $^{212/213}$Bi, ^{225}Ac and ^{228}Pa labeled

molecules were not yet investigated since at the moment the radionuclides are still used as simple salts coming from the production targets.

5.4 Technetium and Rhenium

^{94m}Tc is a positron emitter that allows the already known Tc-radiopharmaceuticals to be imaged with PET. The limit, right now, is due to the impossibility to produce enough radioisotope quantity to meet the hospital needs.

Rhenium is the group 7 congener of technetium and the chemical similarity between the two elements stems from the lanthanide contraction observed for second and third row transition metals [2]. The coordination compounds of the two elements are similar in terms of size, geometries, dipole moments, lipophilicity, etc.. As a consequence, non-radioactive rhenium has often been used as an alternative to ^{99}Tc in preliminary investigations [3]. The isotopes of rhenium are primarily used as therapeutic agents, and as such have lead to the development of therapeutic ^{186}Re ($T_{1/2}$ = 3.78 d E_{β}- = 1.07, 0.93 MeV) and ^{188}Re ($T_{1/2}$ = 16.9 h, E_{β}- = 2.1 MeV). The γ-emission following β^- decay in ^{186}Re (Eγ = 137 keV) and ^{188}Re (Eγ = 155 keV) allows imaging which is useful when considering the ultimate fate and dosimetry of the radiopharmaceutical used for a therapeutic application.

The above parameters show that rhenium is a promising element for radiotherapy. Moreover rhenium has two additional advantages: firstly, there are two isotopes (186g and 188), with good but different therapeutic and diagnostic properties. Secondly, owing to its chemical similarities with technetium, we can exploit all the chemical and biological results already obtained for this latter. It is on the basis of their similarities that "matched pairs" of diagnostic ^{99m}Tc and therapeutic $^{186(or188)}$Re radiopharmaceuticals are being developed. Even “matched pair" generators (^{99}Mo/^{99m}Tc and ^{188}W/^{188}Re) have been produced. However, Tc and Re analogues are not the same. They have different stability and some different chemical properties. A major difference between analogous Tc and Re complexes is that their redox potentials can differ significantly, with technetium complexes being more easily reduced. This has practical consequences for nuclear medicine since reduced rhenium radiopharmaceuticals have a greater tendency to re-oxide back to perrhenate (ReO_4^-) than the analogous technetium complexes to pertechnetate (TcO_4^-), or tetraoxidotechnetato(1-) [3]. A further difference is that rhenium complexes are *more inert to substitution* than their technetium analogues. The magnitude of such chemical differences depends on the

compound, and their quantitative delineation provides new opportunities in radiopharmaceuticals development. Chemical differences between Technetium and Rhenium can be very useful when they are exploited to satisfy the different needs of diagnosis and therapy. In practice Rhenium-labeled molecules are employed only when a therapeutic use is possible and required. The major use of rhenium as radiotherapeutic agent is in the treatment of bone metastases. ^{186}Re has been complexed to hydroxyethylidene bisphosphonate (HEDP) [4], which localizes in bone by bridging hydroxyapatite. ^{186}Re-HEDP is an effective palliative treatment of metastatic bone pain [5,6].

Reference [7], a book about technetium, rhenium and other metals, has a large bibliography on bio-molecules labeled with Rhenium for tumor therapy, although, for the moment, no Rhenium based radiopharmaceutical is on the market. The major problem is the quality assurance of its production and labeling procedures. The product, ready for injection, must be prepared with a kit procedure, in the hospital, just before use. This means that an authorized radiopharmacy with authorized operators must be active in every hospital. The radiopharmaceuticals already labeled with Rhenium reported in literature are ^{188}Re-Somatostatin and its analogues [8-10]. The BFC (bi-functional chelator) has been studied with good results, although it is mainly retained in the liver owing to its high lipophilicity. P829 is a radiopharmaceutical, FDA approved as diagnostic for tumor when labeled with ^{99m}Tc [11].

5.5 Gallium and Indium

The coordination chemistry of gallium is well known [12-16]. The most prevalent oxidation state of gallium in aqueous solution is +3, and this is the oxidation state most relevant to radiopharmaceutical chemistry. The complexation of Ga(III) is dominated by ligands containing oxygen, nitrogen and sulphur donor atoms. Gallium has well established coordination numbers of 3, 4, 5, and 6 depending on the ligand. Generally the most stable complexes in vivo are six-coordinated and gallium is in +3 oxidation state. The ionization potential, ionic radii and coordination number of Ga(III) are very similar to those of Fe(III): In fact Fe(III) has half-filled 3d orbitals, similar to Ga(III) which has a filled 3d orbital.

Three radioisotopes of gallium have decay characteristics suitable for gamma scintigraphy or PET imaging. ^{67}Ga ($T_{1/2}$ = 78h) is cyclotron produced, decays by γ-emission and is used in gamma scintigraphy. ^{67}Ga has been employed in humans since 1953 [17]. ^{68}Ga

(T1/2 = 68 min) comes from the $^{68}Ge/^{68}Ga$ generator [18], decays by 89% β^+-emission, and is used in PET imaging. The long half-life of the parent isotope ^{68}Ge ($T_{1/2}$ = 280 days) provides the generator a self-life of about 2 years, allowing PET imaging at facilities without a cyclotron. Also ^{66}Ga ($T_{1/2}$ = 9.4h) is a cyclotron produced β^+-isotope, and begins to be studied as tracer of slow clearing bio-molecules [19, 20].

Gallium complexes may become good radiopharmaceuticals if they are: 1) stable to hydrolysis (formation of hydroxido compounds), and 2) more stable than Ga(III)-transferrin. In aqueous solution, hydrated Ga(III) ion is stable only under acidic conditions, and $Ga(OH)_3$, the insoluble species, is forming just as pH increases. Between pH 3 and pH 9.5, insoluble $Ga(OH)_3$ is the prominent species, whereas above pH 9.6, the soluble tetrahydroxidogallate anion $Ga(OH)_4^-$ forms. The preparation of Ga(III) coordination complexes is usually performed by ligand exchange reaction, since the precipitation of $Ga(OH)_3$ occurs more rapidly than complexation with ligands that bind Ga(III) at a slower rate. For instance, $GaCl_3$ is generally previously treated with weakly coordinating ligand such as acetate or citrate, and then this Ga(III) species is used to prepare coordination complexes of higher stability.

Gallium complexes, once injected in vivo, must also be resistant to exchange with the plasma protein transferrin. The large stability constant of Ga(III)-transferrin (log Ki = 20.3) [21] and the high plasma concentration of this protein (0.25g/100mL) thermodynamically favour the *in vivo* exchange of many Ga(III) complexes with transferrin. Most of radio-gallium complexes used as radiopharmaceuticals have very high thermodynamic stability or are kinetically stable to exchange with transferrin. Ligands that form highly stable complexes are generally multi-dentate and contain carboxyl, amino or thiol groups. The first radiopharmaceutical labeled with ^{67}Ga was ^{67}Ga-citrate, used in tumor imaging almost 30 years ago [22]. Few years later researchers determined that the ^{67}Ga was actually binding transferrin in vivo. Today, ^{67}Ga-citrate/transferrin remains a widely used radiopharmaceutical for the clinical diagnosis of certain types of tumors, such as Hodgkin's disease, lung cancer, non-Hodgkin's lymphoma, malignant melanoma and leukemia. The mechanism of ^{67}Ga-citrate/transferrin uptake into cancer cells has long been studied. The current theory is that the ^{67}Ga-transferrin compound binds to the transferrin receptor present on tumor cells, and is often incorporated into the cell by receptor-mediated endocytosis.

^{68}Ga citrate/transferrin has also been used in diagnostic imaging with PET, but, owing to the shorter half-life of ^{68}Ga, the diagnostic procedures are different. For instance, ^{68}Ga-

transferrin has been used to quantify pulmonary vascular permeability using PET, where ^{68}Ga-transferrin is taken up in the lungs immediately after injection. The PET has quantification capabilities that ^{67}Ga gamma scintigraphy has not. Because of the convenient half-life of ^{68}Ga as a PET radiotracer, and the easy availability from generators, considerable interest has been devoted to the development of ^{68}Ga-labelled molecules, as either myocardial and cerebral agents or tumor targeting agents. During the last 10 years, there have been significant advances in the development of ^{68}Ga-labeled myocardial imaging agents. Uncharged, lipophilic Ga(III) complexes of 1,1,1-tris(5-methoxy-salycilal-dimino-methyl)ethane [5-MeO(sal)$_3$tame] were investigated as ^{68}Ga myocardial imaging agents with limited success [23]. In fact their increased lipophilicity brought high accumulation in the liver. Also ^{68}Ga-[(4,6-MeO$_2$sal)$_2$BAPEN]$^+$ exhibits significant myocardial uptake and retention over the neutral salicylandimine ligands [24].

A series of lipophilic Ga(III) complexes of the type 1-aryl-3hydroxy-2-methyl-4-pyridinones have been found to exhibit high heart uptake in rabbit and dog models [25]. Although these complexes were only stable for a short time *in vivo*, the complexes were stable long enough for a first pass extraction by heart, and, for one of the complexes, the brain. Other ligands of the N_2S_2 type (BAT-TECH) [26] showed myocardial imaging. However the heart activity was washed out over time while the blood activity remained constant after 30 minutes. A further complex of ^{68}Ga: THM$_2$BED [27] was evaluated as a heart agent. It was taken up in the heart and slightly in the brain, but had a high accumulation in the blood, while quickly washed out of heart and brain. Some complexes have shown a higher uptake in the brain and have been evaluated as brain imaging agents. Anyway, it is difficult to find radiogallium complexes that accumulate in normal brain.

As already mentioned ^{68}Ga-labeled pyrrolidone derivatives showed uptake in rabbit brain that appeared to accumulate over several hours [28], while ^{68}Ga-THM2BED showed slight uptake in the brain at very early times post-injection, but rapid wash out [29]. It has been shown that the small, neutral and lipophilic complex of ^{68}Ga labeled with *tris*(2-mercaptobenzyl)amine (S_3N) ligand crosses the blood brain barrier [30]. The ^{68}Ga-S_3N complex does not exhibit “first-pass” uptake into the brain, but a rather slower uptake in the brain followed by slow washout, with a brain/blood ratio of 3.5 by 15 minutes post-injection and increasing to 5.2 by 60 minutes. This agent shows to be the most promising as for brain imaging of any ^{68}Ga complex evaluated to date.

Many other compounds have been synthesized and studied with gallium, but, since the

chemistries of gallium and indium are very similar, we will consider their complexes together.

	[ML]/[L][M]		pM	
Ligand	**Ga(III)**	**In(III)**	**Ga(III)**	**In(III)**
transferrin	19.8	18.3	19.7	18.3
EDTA	21.0	24.9	20.0	22.1
DTPA	24.3	29.0	20.2	24.9
PLED	32.3	26.5	24.7	19.0
HBPLED	31.0	29.0	21.8	19.7
DMPLED	27.3	21.5	25.5	19.7
Me_4HBPLED	31.9	33.0	24.6	20.6
HBED	38.5	28	28.7	20.0
TIIMBED	34.2	30.7	21.2	17.8
t-butyl-HBED	36.3	31.3	23.3	18.3
Me_4HBED	34.2	30.7	22.2	18.8
SIHBED	37.4	29.4	27.2	19.2
HBMA	30.5	26.3	19.1	14.9
rac-TMPHPG	32.5	26.0	20.7	13.8
meso-TMPHPG	34.0	26.6	20.7	13.3
rac-EHPG	33.9	26.7	23.3	16.1
meso-EHPG	32.4	25.3	22.0	14.9
NOTA	30.1	26.2	26.4	21.6
DOTA	21.3	23.9	15.2	17.8
TETA	19.7	21.9	14.1	16.2
TACN-HB	40.5	33.3	23.4	16.6
TACN-TX	44.2	34.0	25.2	15.0
TACN-HP	42.0	25.08	32.1	15.2
TACN-mcHP	45.6	28.02	34.9	17.4
TACN-TM	34.2	36.1	23.9	23.6
4SS	24.7	27.4	22.6	21.7
5SS	27.4	30.9	22.1	23.7
6SS	41.0	39.8	31.6	30.9
EDDASS	35.6	37.0	29.0	30.4
EC	31.5	33.0	NR	NR

Table 3 – Stability constants of Ga(III) and In(III) Complexes

In table 3 the stability constants of Ga(III) and In(III) polyaminopolycarboxylate, hydroxyaromatic, macrocyclic and amine-thiol complexes have been reported. It can be noted that stability constant values of indium and gallium homologues are similar. The polyaminopolycarboxylate ligands EDTA and DTPA form strong complexes with Ga(III) and In(III), having six-coordinate octahedral configuration. Pyridoxylethylenediamine derivative, such as N,N'-dipyridoxylethylene-diamine-N,N'-diacetic acid (PLEN) [32], form Ga(III) and In(III) complexes with a single negative charge. The Ga-PLEN complex is more thermodynamically stable than either Ga-EDTA or Ga-DTPA; however the In-PLED complex shows an intermediate stability that is larger than In-EDTA and smaller than In-DTPA.

Another type of hydroxyaromatic ligand for Ga(III) and In(III) , the N,N'-bis(2-hydroxy-3,5-dimethylbenzyl)ethylenediamine-N,N'-diacetic acid (HBED), formed a complex that was 10 orders of magnitude less stable than either Ga-EDTA or Da-DTPA, while the In-HBED complex was 10 orders less stable than the Ga compound [33], and about one order less stable than In-DTPA. The stability of Ga(III) complexes decreases derivatising HBED with various substituents on the phenyl ring.

The addition of alkyl substituents ($TNMe_4HBED$, t-butyl HBED) significantly increased the amount of initial uptake of ^{68}Ga and ^{111}In-labeled compounds in the liver in rats [34].

The most interesting class of ligands studied with Ga(III) and In(III) have been macrocyclic chelators. They form very stable complexes and they allow the conjugation of the radiometals to peptides.

Three carboxylic acid derivatised macrocyclic chelators evaluated with Ga(III) and In(III) are NOTA, DOTA, and TETA. The crystal structure of Ga-NOTA is already known. The stability constants of the In and Ga complexes possess the same trend for both the metals: NOTA > DOTA > TETA [35]. The lower stability of In-NOTA in respect to Ga-NOTA could be due to the larger radium of the In(III) cation (94 pm) vs. the Ga(III) cation (76 pm) and the smaller cavity size of NOTA. The higher selectivity of DOTA and TETA for In(III) is more likely due to steric factors. A large number of human tumors are somatostatine receptor positive, and chelating systems like DOTA, NOTA and TETA were used to modify octreotide derivatives and deliver Ga-68 or In-111 to the tumor cells[36].

Today there is a great interest in the investigation and clinical usage of somatostatine analogues (octreotide) labeled with Ga-68 through DOTA or NOTA chelating agents. These compounds may be particularly employed in the study of neuro-endocrine tumors (NETs).

Other Gallium and Indium complexes are under investigation, but they are out of the interest of this report.

5.6 Copper

Copper offers several radioisotopes for either imaging (^{60}Cu, ^{61}Cu, ^{62}Cu and ^{64}Cu) or therapy (^{64}Cu and ^{67}Cu). The positron-emitting diagnostic isotopes have a wide range of half-lives (10 min to 12.7h) and are cyclotron or generator produced. High purity and high specific activity ^{60}Cu, ^{61}Cu, and ^{64}Cu will be soon obtainable by biomedical cyclotrons [37]. The well known chemistry of copper, an element ubiquitous in nature, is restricted to two principal oxidation states (I and II). Copper is an oligo-element present in the human body in low

amount, and its biochemistry and metabolism are well known. Kinetically inert copper complexes for long term targeting and trapping (e.g. radiolabeled antibodies) have been developed. Other complexes may be selectively trapped in tissues by redox-catalysed ligand exchange mechanisms (e.g. blood flow tracers).

Only few papers report the metabolism studies of copper chelates. A recent study [38] deals with two macrocyclic chelates such as cyclam (1,4,8,11-tetraazacyclotetradecane) and 15aneN5 (1,4,7,10,13-pentaazacyclopenta-decane). The study demonstrated that the choice of chelate can dramatically affect the bio-kinetics, distribution and metabolism of the radiopharmaceutical that will ultimately determine the clinical usefulness of the drug. A recent study on four copper chelates has shown that their charge and lipophilicity play a role in kidney retention of copper radiolabeled antibodies and transchelation of the copper appears to be a significant factor for accumulation in the liver.

Another series of copper complexes have been studied as hypoxia imaging agents. The bis(thiosemicarbazone) complex, Cu(II)diacetyl-bis (N4-methylthio-semicarbazone) (^{62}Cu-ATSM) is selectively trapped in hypoxic tissue. This neutral, square-planar complex exhibits high membrane permeability and low redox potential. The analogous complex, Cu(II)-pyruvaldehide-bis (N4-methylthiosemicarbazone) (Cu-PTSM), is a proven blood flow tracer that becomes trapped in most major tissues (e.g. brain, heart, liver, kidney), and even tumors.

By a small modification through an addition of a methyl group to PTSM (pyruvaldehyde to diacetyl) the redox potential of the complex are altered. Cu(ATSM) has a lower redox potential (-297 mV) compared to that of Cu(PTSM) (-208 mV). This difference in redox values has been related to the selective trapping of Cu(ATSM) in highly reductive hypoxic tissue, but not in less reducing normal tissue. Further modifications in thiosemicarbazones affect the redox properties of the complexes and, as a consequence, good radiopharmaceuticals are found as cerebral, myocardial and hypoxia imaging agents.

In the fields of bio-molecules copper has been used for labelling octreotide. Octreotide has been conjugated to two bifunctional chelates, VPTA and TETA for labeling with ^{64}Cu [39]. Because of the lability of copper, macrocyclic chelates are necessary to form complexes that are stable in vivo. CPTA, a derivative of cyclam, forms Cu(II) complexes having a +1 charge, whereas the Cu-TETA complex has a –1 charge. ^{64}Cu-CPTA-octreotide and ^{64}Cu-TETA-octreotide have high affinity for the SSR both in vitro and in vivo, but the biological clearance is very different between the two conjugates. The ^{64}Cu-CPTA conjugate clears very low almost exclusively through the liver, while ^{64}Cu-TETA-octreotide primarily clears

through the kidneys, with very low liver accumulation. These results demonstrate that the bifunctional chelating complex (BFC) has a major impact on the biological behavior of radiometal-BFC-biomolecule conjugates.

^{64}Cu-TETA-octreotide is currently being evaluated as a PET imaging agent for neuroendocrine tumours [40]. Preliminary results showed that ^{64}-TETA-octreotide was able to detect even more SSR positive lesions than the currently used agent, ^{111}In-DTPA-octreotide and gamma scintigraphy.

6. HEALTH PHYSICS ASPECTS IN RADIONUCLIDE PRODUCTION AND PROCESSING

In all cases – due to the manipulation of un-sealed sources of large activity – the radiochemical separation must be carried out in radiochemistry laboratory of suitable class II in accordance to ISO and UNICEN 7815 as modified by 10491:1995, and classified Controlled Area. On the other hand operations involving radioactive material must be designed, equipped, and conducted to protect personnel as much as it is practical against the hazards of ionizing radiation (ALARA criterion). The protective measures must take into account the nature of the operation, the radionuclides involved with particular attention to the quantities that will be used, their radiotoxicity, and their chemical and physical form.

This kind of laboratory may have features that depend on the level of the hazard of the operations, according to some common criteria. In particular for high level of hazard the laboratory must be separated from other working areas and the minimum requirements for this area include:

1. The atmosphere in the laboratory is maintained at negative pressure with respect to other parts of the building. The negative-pressure ventilation must have a minimum exhaust velocity and a minimum number of room ventilation changing per hour. The air conditioning system must be independent on that of the main building and the air must be completely expelled each time.
2. Operations are carried out in glove boxes equipped with negative-pressure ventilation and a high-efficiency filtration (HEPA) system. Other protective devices (shielding, remote handling devices, air locks, bag-out ports, etc.) may be included according to the operation degree of hazard.
3. The walls and floors are smooth and protected with impermeable coverings that are ease to be decontaminated.
4. The coverings of work surfaces are either disposable or selected for ease of contamination cleanup.
5. Access to the workplace is limited to those persons actually needed to perform the operation.
6. Protective clothing, such as lab coats, and gloves and protective equipment, such as respirators, are used as specified by the health physicist.
7. Radioactive materials are stored in glove boxes, source pits, water pools, or other

devices, commensurate with the degree of hazard and the nature of the material.

8. A monitoring program is maintained to detect atmospheric contamination, external radiation, and surface contamination. Alarm devices should be installed to warn personnel of external radiation or airborne contamination exceeding permissible levels.

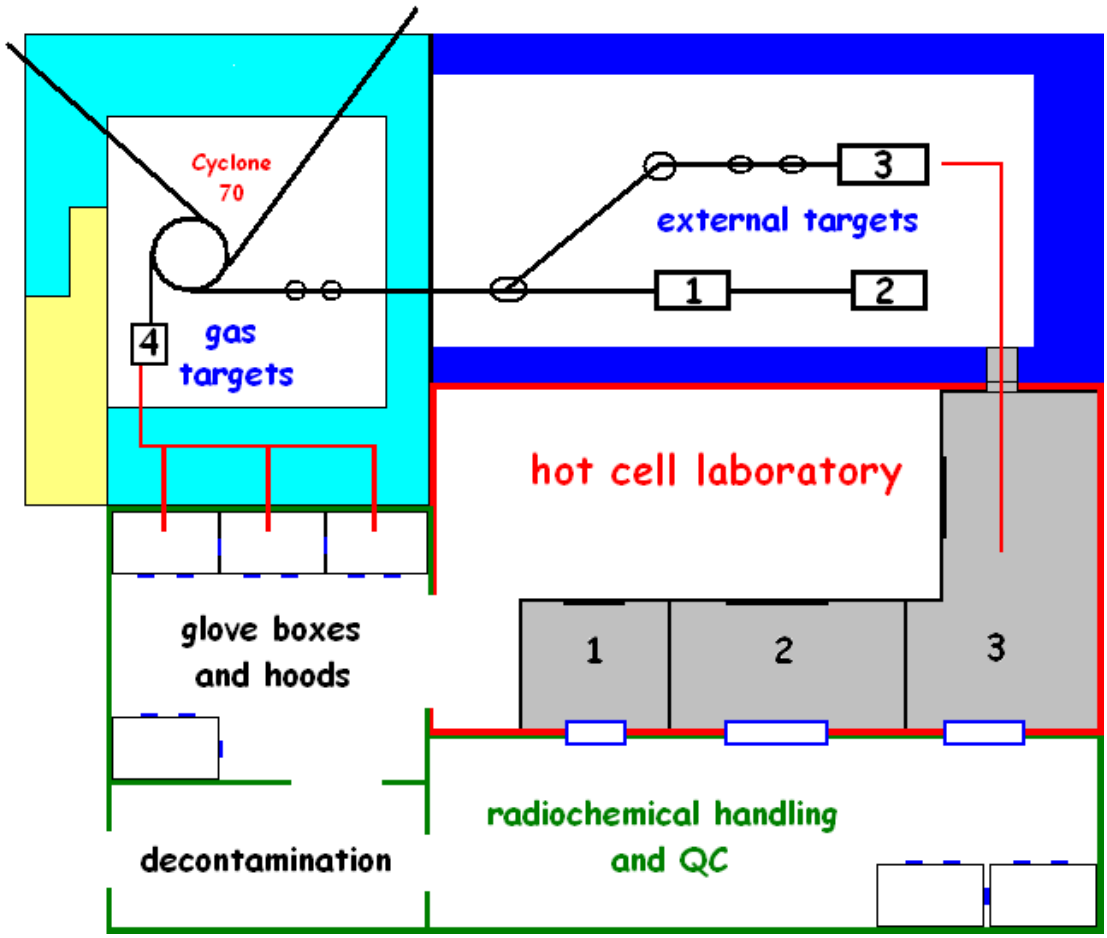
9. Special receptacles are provided for separate collection of solid and liquid residues generated during operations.

To this purpose the personnel involved must be trained to “high activity” radiochemical procedures in order to:

- Acquire the criteria for the adequate application of the radioprotection philosophy, starting from the basic principles of radiological protection: justification, optimization of practices and dose limitation.
- Plan of professional practices with an adequate training in order to keep the doses as low as it is reasonably possible (ALARA principle),
- Adequate the procedures taking into account: elements to be utilized, techniques, time required for the practice, how to work minimizing radiological risks for the worker and the other workers, the necessity to successfully manage all the situations and in particular emergency conditions. The personnel has to be familiar with the use of radiometric, radioanalytical and analytical equipment and with dosimetric concepts as for exposure and internal contamination.

The use of hoods and glove-boxes equipped with lead-glass is effective to facilitate the eye control of the procedures. However, the use of TV cameras and PC control systems must be taken into account.

A schematic (not to scale) example of a small radionuclide production facility is drawn in the next Figure, containing: a small cyclotron, on-line gas target assembly (4), other external targets (1-3), hot cell laboratory (two side access), glove-boxes and hood laboratory, quality control laboratory. The hot-cells are of 5-15 cm Pb equivalent depending on the activity and energy of gamma photons, and lead-glass windows are required. The gas (and liquid) targets are connected by pipelines to glove-boxes and hot cells. The solid targets are transferred to the hot cells by pneumatic or rail transportation.


Cyclone
70
3
external targets
1
2
4
gas
targets
hot cell laboratory
glove boxes
and hoods
1
2
3
radiochemical handling
and QC
decontamination

7. QUALITY CONTROL / QUALITY ASSURANCE OF RADIONUCLIDES AND LABELLED COMPOUNDS

As *Quality Control* or *Quality Assurance* (QC / QA) of either a radionuclide or a labelled compound (i.e. radiotracer, radiopharmaceutical) the international community means the experimental determination of the *Typical Range* of quantities like:

Radionuclidic Purity	RNP% (t)	95-99 %
Radiochemical Purity	RCP% (t)	95-99 %
Chemical Purity	CP(t)	
Specific Activity	A_S (t)	GBq / μg
Isotope Dilution Factor	IDF (t)	dimensionless
Activity Concentration	C_A (t)	MBq / g
Biological Purity	pH, sterility, apirogenicity, osmolarity, isotonicity	

and, moreover, the experimental determination of :*Stability (with time)* of all previous parameters (both in-vial and in-vivo).

Radionuclidic purity does refer to the presence of radioactive species accompanying the radionuclide of interest in the radioactive specimen (irradiated target, radiochemically processed target, labelled compound, radiopharmaceutical). This definition does not take into account the chemical form of the different radionuclides present in the radioactive specimen. Any kind of emitter (gamma, X, beta, alfa) is considered a radionuclidic impurity and its percentage must be experimentally determined by the proper radiometric equipment (gamma-X spectrometry, beta and alpha spectrometry by liquid scintillation counting, high resolution alpha spectrometry by semiconductor detectors, others).

The non-radioisotopic impurities can be – in principle – separated by the radionuclide of interest by radiochemical methods. The isotopic impurities can be minimized by a proper choice of irradiation conditions followed by suitable cooling times during the various steps of radiochemical separation and after the EOP as well, based on the different half-lives of different radionuclides. In case of decay chains the radionuclidic decay can drive to the production of non-radioisotopic species.

The radionuclidic purity is normally expressed as a percentage and varies with time depending on the half-lives of different radionuclides.

The accurate knowledge of radionuclidic purity is fundamental in order to calculate the dose to both the patient and the personnel involved. Moreover, the waste of radioactive specimen containing long half-lived and highly radiotoxic radionuclides can improve the dose to the general population leading to environmental concerns. At last the high gamma energy radionuclidic impurities can decrease the quality of the radiological images.

Radiochemical purity does refer to the chemical form of the different radioactive species present in the radioactive preparation. In this case, if the radionuclide of interest is 100% pure for the radionuclidic point of view, it refers to the different chemical forms of the main radionuclide and it is reported as a percentage too. Due to the chemical instability on many chemical compounds due to different chemical and physical agents, the radiochemical purity varies with time and must be assessed by any kind of analytical and radioanalytical method. Moreover, in the present case, the high ionizing radiation fields involved can improve strongly the radiolytic decomposition of the labelled compounds (radiolysis and auto-radiolysis).

Radiochemical purity has a much larger relevance than radionuclidic purity as for both diagnostics and therapy, because the presence of unexpected radioactive species may provide an undesired uptake of activity in an unpredicted target and lead to an undesired dose to healthy organs in case of radiotherapy.

Of course, the radiochemical stability must be investigated both in vial, before the administration to the patient and in-vivo after the administration. In this last case it is possible to assess the in-vivo stability by imaging (gamma-camera, SPET, PET) or by analysing patient fluids and excreta (in practice blood, serum and urine).

Chemical purity does refer to the presence of non-radioactive chemical species in the radioactive preparation. These species can be toxic to the patient or can compete with the chemistry of the radiotracer under investigation. It must be taken into account that high specific activity radionuclides and labelled species are constituted by a very small massic amount of radioactive chemicals. As a consequence, very small amounts of chemicals - and metals in particular – can strongly interfere and modify the metabolism of the radiopharmaceutical compound. Trace or ultra-trace concentrations of chemical and metals (ppm, ppb or ppt), that are of negligible significance in case of normal pharmaceutical chemistry can somewhat create large concerns in case of radiopharmaceutical chemistry.

Additives, sterilizing media, physiological media that are intentionally added to the radioactive preparation, are not considered indeed chemical impurities, but must be chemically controlled before use for the radioactive preparation. Any kind of analytical and radio-analytical technique is suitable for the determination of chemical purity of labelled species. In practice, there is a number of specific chemical species that must be controlled because it is known their effectiveness in interfering with the radiodiagnostics and radiotherapeutic performance of labelled radiotracers.

Specific Activity (massic and molar) is defined as the ratio between the activity of labelled species (considered of 100% radionuclidic purity) and the mass or molar amount of labelled species. The NCA A_S is somewhat close the theoretical CF value, but the experimental determination of its real value is mandatory for most practical applications of radiopharmaceuticals due to a series of items: 1) chemical toxicity of non radioactive carrier, 2) low solubility of low specific activity compounds in body fluids and compartments, 3) non specificity of radiopharmaceutical compounds, designed for specific receptor binding investigations on low concentration receptor in neurology, oncology.

Any kind of analytical and radioanalytical technique is suitable for the determination of the amount of stable isotopic and molecular carrier in the labelled species. The Isotope Dilution Factor is defined as the ratio between the A_S(CF) and the real NCA one and it is a quantitative parameter suitable to understand the degree of dilution of the radiopharmaceutical by the inactive carrier.

Activity concentration (massic or volumic) is simply the ratio between the activity of the radiopharmaceutical and the mass or volume of the radioactive solution or material.

Biological purity does refer like for any kind of pharmaceutical compound to be administered to living organisms (cells, animals, humans) in order to guarantee its biocompatibility. In practice it is necessary to perform a series ot tests and procedure to guarantee the sterility, the apirogenicity, the osmolarity, the isotonicity and the pH of the sample to be administered.

8. CONCLUSION

A new Cyclotron Isotope Production Center should help to cope with the growing needs of Nuclear Medicine. Research on new radionuclides requires not only a powerful beam line, but also extended structures to prepare targets, extract radionuclides, study radiopharmaceuticals, and host animal wards for in vivo experimentation. Should this Center be realized, the INFN Laboratories of Legnaro might become the leading institution for the Italian Nuclear Medicine isotope research and the hub where scientists gather to employ state of the art equipment and share experience and knowledge.

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