RADIOPHARMACY: AN UPDATE

A TECHNOLOGIST'S GUIDE

TABLE OF CONTENTS

Foreword

Nuclear medicine, which encompasses the medical field of molecular imaging and radionuclide therapy, brings together many disciplines, and radiopharmacy requires that pharmacy, physics and medicine work together within a synergetic environment. It is undeniable that within nuclear medicine the baseline of any good procedure is the design and preparation of the radioactive pharmaceutical, referred to as the radiopharmaceutical.

After the introduction of a radiopharmaceutical into clinical practice, it is essential that the production and preparation of the radiopharmaceutical are done by highly qualified professionals, with a good theoretical background and highly developed practical skills. Within a multidisciplinary team, nuclear medicine technologists are professionals who are recognized for their competence in preparing radiopharmaceuticals and are also responsible for performing the control tests to determine the quality of the preparations.

The European Association of Nuclear Medicine (EANM) is a reference scientific body for the global nuclear medicine community. Taking this into account, in 2008 the EANM Technologist Committee

(EANM-TC) made a joint effort to produce a book entitled The Radiopharmacy. This publication was intended to help professionals from different locations and professions to optimise their practice in radiopharmacy. Due to the rapid evolution of nuclear medicine and its associated procedures and practices in radiopharmacy, the EANM-TC reached the decision that it is time to revisit the topic of radiopharmacy and to produce an update to the previous edition of this guide.

Radiopharmacy: an Update is the outcome of the work of the EANM-TC in drawing together the expertise of many authors in order to produce a guide that addresses a variety of radiopharmacy-related topics, from the history and basic principles of

radiopharmacy to the current generator- and cyclotron-produced radioisotopes, conventional nuclear medicine, PET and therapeutic radiopharmaceuticals. Additionally, good manufacturing practice (GMP), the translational approach to radiopharmaceutical production and radiation protection concerns in the design and workflow of a radiopharmacy are explored.

I would like to thank each of the authors of this book for lending their time to this project and for helping us to produce a guide that reflects their expertise. A special word of appreciation is due to the Translational Molecular Imaging and Therapy Committee for their contribution to this publication.

I would also like to express my gratitude

to our colleagues from the Society of Nuclear Medicine and Molecular Imaging– Technologist Section (SNMMI-TS) for their contribution to the book.

In addition, I am very grateful for the work of the EANM-TC editorial group and to Rick Mills for his editing, reviewing and support throughout the entire process. Lastly, the EANM Board and the Executive Office deserve my words of appreciation for their support in ensuring the continuity of this project. Radiopharmacy: an Update would not have been possible without the contribution of all the above mentioned. Thank you very much!

Andrea Santos
Chair of the Technologist Committee

Introduction

In 2008, the EANM Technologist Committee published a Technologist's Guide entitled The Radiopharmacy. It is available online through the EANM website and covers topics relevant to daily radiopharmacy practice, such as design, preparation, dispensing and documentation. Since then, many radiopharmaceutical practices have changed, especially with the introduction of new radiotracers.

This year's Technologist's Guide includes the basics, starting from history of radiopharmaceuticals, and proceeds to the high-end radiopharmaceuticals used in translational medicine. Illustrations and tables have been included to facilitate the understanding of certain principles. The most widely used radiopharmaceuticals in SPECT and PET have been dealt with separately because of the breadth of development since the previous publication in 2008. This year's Technologist's Guide also covers radiopharmaceuticals used in therapy. Authors from different backgrounds have contributed to the Guide, ensuring that it will be an important addition to the knowledge base required to perform radiopharmacy. It is an unmissable collection of information that will prove an essential aid in the clinical setting and will keep the technologist up to date with the latest radiopharmacy principles and practices.

MarieClaire Attard
Main-Editor on behalf of the editors

THE HISTORY OF
RADIOPHARMACY

by Marius Mada

1

EARLY PIONEERS

Two scientists paved the way for radiology and radiopharmacy at the end of the nineteenth century. Wilhelm Conrad Roentgen (1845–1923) discovered X-rays in 1895, which for the first time provided an insight into the living body. Only a year later, Henri Becquerel (1852–1908) found that certain naturally occurring substances emit radiation that is visible over distances and can penetrate black paper and blacken photographic plates. Marie and Pierre Curie discovered that uranium and polonium have this property and named this phenomenon radioactivity (Fig. 1). It soon became clear that these rays should find application in many different scientific fields, especially in medicine.

Following the initial application of X-rays in the treatment of skin diseases, radioactive substances were also used in this area. Scientific journals at the time reported successes in therapeutic hair removal and treatment for acne or copper rash associated with syphilis. Soon, the use of radioactive substances developed into a broad experimental field, with radium and thorium compounds being tried in almost all areas of medicine. A "radium frenzy" ensued, and radioactivity was used for the treatment of pulmonary tuberculosis, elephantiasis and epilepsy, to name just a few conditions.

Furthermore, tumours were treated with radioactive substances with promising results. In addition to external application, by 1905 internal application of radioactive materials (radium bromide or sulphate) was being performed using various methods of placing the substance. Metal capsules, needles, glass tubes and other suitable containers were used so that the radiation source could be removed from the body once the treatment had been completed. The radiation treatment of tumours continued following the First World War and progress was recorded particularly in measuring more precisely the dose applied. Already at this stage there were two approaches to treatment: one group of physicians considered a single, but sufficiently strong dose of radiation (intensive radiation) to be more efficient, while others saw advantages in using an interval therapy, i.e. multiple, regular but weaker radiation doses (fractionated radiation). For more than a decade, supporters in both camps tried to strengthen their position with numerous publications before the scientific dispute was settled in favour of fractionated radiation [1, 2].

DANGERS OF RADIOACTIVITY

With the increasing use of radionuclides, the need to protect healthcare professionals was soon recognised. Even today, Geiger counters and film dosimeters are standard equipment in radiology and nuclear medicine departments and are not dissimilar to the devices from the past (Fig. 2).

At the same time as the early devices to aid protection of healthcare professionals were being developed, attention was being drawn to the dangerous effects of radioactive substances on patients. It was observed that following prolonged exposure to radium plasters, inflammation or redness of the skin occurred, culminating in malignant inflammation. «[One] knows today that radium rays are able to cause carcinoma cells to fade and thus contribute to the healing of cancerous ulcers. However, it must be remembered that other tissues besides the carcinoma are strongly attacked by the rays.» In addition, it was observed that small mammals, such as mice, died within a few hours when exposed to intense radiation [3].

RADIATION THERAPY CULTURE

In the early twentieth century, the scientific world was fascinated by the therapeutic potential of radioactive compounds but was also aware of the dangers they can
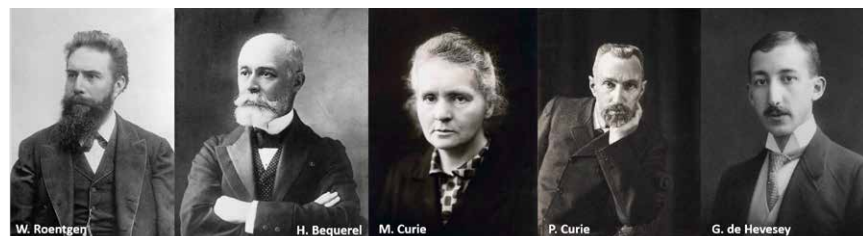

Figure 1: Pioneers in radiology and radiopharmacy

Figure 2: Left: The Hanford ring badge, used to determine exposures to the hands of workers manipulating radioactive sources. Centre: Film badge dosimeter from the Manhattan project. Right: End-window Geiger-Müller tube for measurement of radioactivity

pose. In a technical article presented at the British Pharmaceutical Conference in 1904, the pharmacist William Harrison Martindale (1874–1933) summarised the existing findings on radioactivity and its application in medicine, including the possible dangers, yet at the same time radioactivity was presented as a tried-and-tested therapeutic method against many diseases.

Backed by the scientific research and with a twist of entrepreneurialism, around the world radioactivity was praised as a miracle cure or panacea. The discovery of natural radioactive waters gave rise
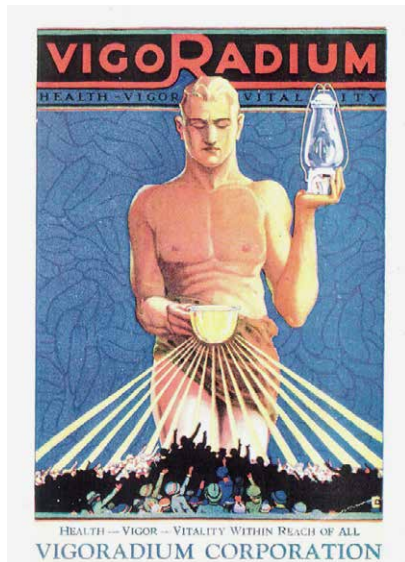

Figure 3: Advertisement for VigoRadium, water containing radium isotopes

to a considerable number of spas that promoted their use for therapeutic purposes. Especially in the United States, companies started commercialising radioactive drinking water, promising to rejuvenate the body, kill bacteria or purify the blood (Fig. 3). Radioactive water was also used in the food industry in recipes for biscuits or chocolate. The use of radioactive water at home, despite the absence of scientific evidence regarding the mechanism of cure, peaked in the early 1920s. However, the practice continued into the 1970s, with different brands using different radiation doses, some of them potentially dangerous to life.

Other radioactive preparations such as globules, suppositories, lozenges, tablets and even injections were also available. These were advertised to increase vitality, promote male strength or prevent aging. In addition, radioactive toothpaste, massage cream, cosmetics, soap, hair tonic and gelatin were offered for sale. For the treatment of rheumatic diseases or to strengthen the libido, radioactive preparations were integrated in the fabric of bedding, allowing longer exposure during sleep. By 1930, however, the American authorities found that 95% of the products they tested had no traces of radioactivity. It is fair to say that although such products were mis-sold, consumers were at least spared the hazards of radiation.

Up until the mid-1930s, the benefits of radiation for therapy were exploited by the salesmen trying to trade in the so-called universal panacea. Pharmacists then started to get more involved in the research into radioactivity and demanded that the preparation of these products be carried out by professionals. As a result, in 1938 the Federal Food, Drug and Cosmetic Act (FD&C) was passed in the United States, giving the Food and Drug Administration authority to oversee the safety of food, drugs and cosmetics. This act required that the safety of all new drugs must be proved to the FDA before they could be marketed to the public [4].

RADIATION AND DIAGNOSTICS

It is possible that the introduction of radioactivity into diagnostics was associated with an attempt to solve a culinary dilemma. The Hungarian chemist George de Hevesy (1885–1966) was working alongside Ernst Rutherford in Manchester, England, and was renting a full-board room. A Sunday speciality cooked by his landlady was meat pudding. de Hevesy suspected the lady was recycling the meat from the Sunday meal during the following week. To prove this, he spiked his leftovers with a small amount of a radioactive isotope that he was eventually able to trace a few days

later in the casserole. Faced with the facts, the landlady could only say, "This is magic!" He might have lost his room after this, but the principle used by George de Hevesy was later adopted in medicine and referred to as the "tracer principle". A radioactive substance is introduced into the human body in an amount sufficiently small as not to interfere with the molecular processes; its distribution then reflects the metabolic functions of the body, making this a great application for diagnosis and therapy.

The prerequisites for broad application of the tracer principle, however, were the discovery of artificial radioactivity and the large-scale production of radionuclides. The first cyclotron was developed in 1930 by Ernst Lawrence at the University of California in Berkeley. In December 1942, Enrico Fermi and co-workers achieved the first self-sustained nuclear chain reaction, which led to

Figure 3: 4: Ernst Lawrence developed the first cyclotron and Enrico Fermi was the first to create a nuclear reactor

the building of the nuclear reactor (Fig. 4). Compared with the cyclotron, the nuclear reactor could produce quantities of radionuclides millions of times greater. After the Second World War, Abbott Pharmaceuticals started selling the first radioisotopes [5]. By 1947, production facilities for medical and biological radionuclides had become available outside the United States, including at Harwell in the United Kingdom. These facilities mainly produced phosphorus-32, sodium-24 and iodine-131. Subsequently, more exotic isotopes were added to the list, such as strontium-89, yttrium-90, iodine-125, samarium-153, rhenium-186 and gold-198. In 1962, technetium-99m was proposed as a radionuclide agent for diagnostic purposes; it was to be extracted from molybdenum-99 in a generator, and this made possible the use of the radionuclide in many hospitals. In 1957 the prototype of the gamma camera was constructed by Hal Anger in United States using photomultipliers (Fig. 5). Within a decade, this instrument had become widely used across the globe. Following developments in computers and image reconstruction, in 1976 John Keyes developed the general-purpose single-photon emission computed tomo-graphy (SPECT) camera that we know today (Fig. 6).

GAMMA CAMERA

At the same time, the positron emission tomography (PET) scanner was being developed in the United States, and by the mid-1980s commercial cyclotrons had become available from CTI Corporation specifically for PET. Hybrid diagnostic imaging was just around the corner, with the first PET/CT scanner being developed by David Townsend and Ron Nutt in early 1990s (Fig. 7) and PET/MRI appearing towards the end of the decade [6]. By the time the first commercially available simultaneous PET/MRI scanner was installed in 2009, half a century after

Figure 5: Hal Anger, with his prototype gamma

Figure 6: John Keyes and the Humongotron SPECT camera

the gamma camera, the field of nuclear medicine and its radionuclides had been transformed beyond recognition.

Figure 7: Ron Nutt and David Townsend, who developed the first PET/CT scanner

RADIOPHARMACY

The development of the field of radiopharmacy or nuclear pharmacy (in the United States) as an independent pharmaceutical specialisation began after the Second World War, mainly in the United States. A reactor was built in 1943 in Oak Ridge (Tennessee), and the first radionuclides for civilian use and clinical application became available there after 1945. The 15th edition of U.S. Pharmacopeia was published in 1955 and contained for the first time several monographs on radiopharmaceutical preparations, for example sodium iodide (iodine-131) solution.

The task of radiopharmaceutical pharmacists at that time consisted mainly in the production of intravenous and oral formulations of artificial radionuclides and testing of radionuclide purity or pyrogens. Hospital pharmacists played a key role in the production and dispensing of such preparations, as the short-lived radionuclides required fresh preparation and immediate administration to the patient.

With the increasing use of radio-pharmaceuticals, interest in special training also grew. In-depth knowledge and skills were indispensable when handling radioactive and isotopically labelled drugs. Thus, the first tracer methodology courses were held at Purdue University (West Lafayette) in 1947. In the 1950s and 1960s, the range of courses in the United States grew steadily. In the mid-1970s, the Section on Nuclear Pharmacy of the American Pharmaceutical Association (APhA) was founded, and in 1978 the APhA published the Nuclear Pharmacy Practice Standards. These guidelines, which covered aspects including definition, scope, content, professional requirements, advanced knowledge and practical implementation, formed an important basis for the development of the area towards a special discipline. In the same year, the Board of Pharmaceutical Specialties (BPS) recognised Nuclear Pharmacy as its first

specialty. For acquisition of certification in Nuclear Pharmacy, proof of subject-related training or 4000 hours of professional experience was required [7].

Unlike in the United States, the development of radiopharmacy in Europe entailed the creation of additional further education regulations rather than new individual specialties. University-based advanced, additional and supplementary courses were the dominant form of specialisation. PhDs with a radiopharmaceutical focus have been offered in countries such as Greece, Spain, Hungary and the United Kingdom. At the ETH Zurich, the postgraduate course Radiopharmaceutical Chemistry/ Radiopharmacy was established in the mid-1990s in cooperation with the Universities of Frankfurt and Leipzig. This course is probably the most comprehensive of its kind to date. The course follows the guidelines of the European Association of Nuclear Medicine (EANM) and has been recognised by the latter.

The role of the nuclear medicine technologist (NMT) in radiopharmacy has mirrored the development of the field. During the past two decades, it has become a priority across the globe to ensure that NMTs have the necessary training and competencies, including skills that place NMTs alongside pharmacists, physicists and physicians [8].

SUMMARY

Radioactive compounds were being used against many diseases as long ago as the beginning of the twentieth century. Radiopharmaceutical products were tried and tested in the treatment of skin and cancer and were also considered to hold promise in offering a longer and healthier life. It was clear from the beginning, however, that the line between the benefits and the dangers of radioactivity is very thin. Conventional medicine tried to solve this balance using empirical methods. The pioneers of radiotherapy have to be credited with the fact that their work on the biological and pathochemical mechanisms of diseases and the effect of radioactivity provided, at an early stage, insights into the dangers of radioactivity while also creating the basis for modern nuclear medicine. After the Second World War, radiopharmacy established itself as an independent discipline of pharmacy, initially in the United States and subsequently across the world. Developments in nuclear medicine were driven in part by the success of radiopharmacy. In this context, the nuclear medicine technologist has acquired a recognised role in the delivery of safe and efficient radionuclide diagnosis and treatment.

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THEORETICAL BASICS OF RADIOPHARMACY

by Zéna Wimana

2

INTRODUCTION

Radiopharmacy is the art of preparing high-quality, radioactive, medicinal products for use in diagnosis and therapy. The production and handling of radiopharmaceuticals requires specific expertise. Different aspects need to be taken into consideration, including correct usage, storage of rapidly decaying diagnostic radionuclides, disposal of radioactive waste when using longer-lived therapeutic radionuclides and quality controls.

Unlike other medical imaging modalities, PET/SPECT cannot be performed without radiopharmaceuticals; similarly, unlike external beam radiotherapy, nuclear medicine therapy cannot be performed without them. Accordingly, radiopharmaceuticals prepared in the radiopharmacy are the cornerstone of nuclear medicine. In this chapter we will focus on the theoretical basics of radiopharmacy.

The starting point in the preparation of a radiopharmaceutical always depends on the goal, i.e. what we want to see or treat. This can be the function of an organ/system, a characteristic biomarker of a disease or a hallmark of cancer. Vector molecules have to be used against these targets, and have to demonstrate sufficient specificity to allow an appropriate contrast. The vector molecule is labelled with a radioactive flag, i.e. a radionuclide. This step in radiopharmaceutical production is commonly referred to as radiolabelling, and it starts with the choice of the most appropriate radionuclide. In this context it is to be noted, however, that a specific vector molecule is not always needed to allow imaging or treatment of a specific disease (e.g. when using iodine for thyroid pathologies).

CHOICE OF RADIONUCLIDE

The choice of radionuclide is predominantly based on three factors:

a. The purpose for which the radiopharmaceutical is to be used
b. The compatibility of the radionuclide with the vector molecule
c. The availability and price of the radionuclide

Purpose of using the radiopharmaceutical

Radionuclides are unstable atoms that attempt to attain a stable state through the release of ionising energy in the form of alpha (α), beta (β$^{-/+}$) particles or gamma (γ). The nature of each of these forms of emission and their characteristics determine the purpose (therapy or imaging) for which particular radionuclides are best suited. A radionuclide can need one or multiple steps to reach a stable atom, leading to a decay scheme containing different emissions.

Alpha particles are helium nuclei consisting of two protons and two neutrons. They are heavy particles and as a consequence of their large size they interact very strongly with matter, more so than other particles, and deposit their energy on a short path length [linear energy transfer (LET)]. Accordingly, they are much more potent for therapeutic purposes than other emission types. Furthermore, with regard to radioprotection they are stopped without difficulty (a simple piece of paper will suffice), making them more difficult to detect. Fortunately, the alpha-emitting radionuclides used in nuclear medicine also have gamma emission in their decay scheme, allowing detection. To date only one alpha radiopharmaceutical has been approved for clinical use (radium-223 or Xofigo), but several others are either in clinical trials or under development.

Beta particles are smaller charged particles and are either electrons (β^{-}) or positrons (β^{+}). The former are used for therapy and the latter for imaging. The smaller size of β^{-} compared with alpha particles means that they have a lower LET and are thus less potent in causing cell damage. However, this can also be an advantage in terms of toxicity. The use of β^{-} particles for therapy is now well established and still increasing. Their lower LET and longer range allow the treatment of larger tumours compared with alpha particles. To stop β^{-} particles, a denser material than paper is needed, such as Plexiglas or aluminium. In contrast to their negatively charged counterpart, β^{+} particles are used for imaging purposes. In fact, however, it is not the β^{+} particles themselves that are imaged, but secondary emitted gamma rays: when a β^{+} particle encounters an electron, a phenomenon termed annihilation takes place, whereby the mass of both particles is converted into energy and two gamma photons (511 keV) are emitted in diametrically opposed paths. These are the gamma photons that are detected by PET.

Gamma rays are electromagnetic emissions that are used for imaging. They have the least interaction with matter and thus can be detected outside the body, in contrast to the other types of emission. To stop gamma rays, even denser material is needed, such as lead.

An interesting and useful characteristic of radioactive decay, or radioactivity, is that it can be detected and quantified. The measured radioactivity is expressed

in the SI unit becquerel (Bq), with 1 Bq being equivalent to one disintegration per second (dps). However, for historical reasons (cf. *Chapter 1*), the unit Curie (Ci) is still commonly used in some countries, with 1 Ci being equivalent to the disintegration of one gram of radium. Of note, 1 Bq is equal to 2.7×10^{-11} Ci and 1 Ci is equal to 37 GBq.

The tools employed to detect and/ or quantify radioactivity depend on the nature of the emission and the range of radioactivity to be measured:

- A Geiger-Müller tube is used for the detection of ionising radiation (alpha, beta and gamma).
- A dose calibrator is used to measure gamma radiation in the higher range of radioactivity, such as is found in radioactive sources or generator eluate or during the dispensing of doses to patients.
- A gamma-counter is used to measure gamma radiation in the lower range of radioactivity, such as is found in biological samples (e.g. blood).
- A beta-counter is used to measure beta particles indirectly via a scintillation solution.
- Gamma cameras, SPECT and PET detect and quantify gamma ray emissions. While with SPECT the detected gamma radiation is emitted by the radionuclide directly, in the

case of PET, as mentioned above, it is emitted indirectly following annihilation of the β^+ particle with an electron.

Compatibility of the radionuclide with the vector molecule

Besides the type of emission, a radionuclide is characterised by its physical half-life ($t_{1/2}$), i.e. the time needed for half of the atoms to decay and reach the stable state. Although short-lived radionuclides can be considered advantageous in terms of radioprotection, the physical $t_{1/2}$ often turns out to be a limiting factor.

The physical $t_{1/2}$ of a radionuclide has to be sufficiently long to allow (1) the radiolabelling process, (2) the performance of quality control testing (excluding sterility), (3) administration to the patient and (4) adequate distribution of the radiopharmaceutical. The last-mentioned will be determined by the biological $t_{1/2}$ of the vector molecule. Consequently, to obtain satisfactory contrast and thereby allow correct diagnosis or successful therapy, the physical $t_{1/2}$ of the radionuclide and the biological $t_{1/2}$ of the radiotracer should be compatible. An example is the use of zirconium-89 (^{89}Zr, physical $t_{1/2}$=78 h) rather than short-lived gallium-68 (68Ga, physical $t_{1/2}$=68 min) to radiolabel large proteins such as antibodies (biological $t_{1/2}$=days).

Apart from the physical nature of the radionuclide, its chemical nature will also play a role in the choice (radiometals, halogens, lanthanides etc.).

Availability and price of the radionuclide

The availability and price of radionuclides depend on their mode of production. While there are several naturally occurring radionuclides, in the medical field radionuclides are synthetically produced by nuclear reactors, accelerators (cyclotrons) or generators (cf. *Chapters 4 and 5*). Of note, the successful application and wide availability of conventional nuclear medicine across the world have largely been based on the availability of the molydenum -99 / technetium - 99m (^{99}Mo/^{99m}Tc) generator.

RADIOLABELLING METHODS TO OBTAIN RADIOPHARMACEUTICALS

The radiolabelling method used to obtain a radiopharmaceutical is mainly dictated by the choice of radionuclide (see above). Some radionuclides will allow direct radiolabelling of the vector molecule whereas with others only indirect radiolabelling is possible.

Direct radiolabelling of the vector molecule

Direct radiolabelling can be achieved through either substitution or chelation

Substitution: An atom/group on the molecule is substituted by the radionuclide. This is achieved through an exchange between radioactive ions and non-radioactive groups in the molecule. The exchange can be with either an anion or a cation. The reaction itself is then called a nucleophile or an electrophile substitution, respectively. A well-known example of nucleophile substitution is the production of ^{18}F-fluorodeoxyglucose (FDG) with fluorine-18 (^{18}F).

Chelation: A molecule can have groups that can chelate cations. These groups contain electron donors that have a free electron pair that enable coordination binding (O, S and N). An example is the direct radiolabelling of molecules in kits with ^{99m}Tc.

It is to be noted that in order to enter such reactions the radioactive ion should first be in the correct oxidation state, through either reduction or oxidation.

Indirect radiolabelling of the vector molecule

When there is no favourable way to radiolabel the vector molecule directly, the molecule first has to be modified with a bifunctional chelator (and a spacer) to

allow radiolabelling through chelation.

The common traits required for a good bifunctional chelator are:

- Stable and kinetically inert: to avoid hydrolysis/trans-metalation/ chelation in vivo (e.g. binding of gallium-67 citrate to transferrin)
- Fast complexation: to allow the use of short-lived radionuclides
- Versatile conjugation chemistry
- Accessibility

Once the precursor has been modified, to facilitate this kind of reaction a trans-chelation occurs, whereby the radionuclide is first weakly chelated to a low-affinity chelator in the reaction medium and then trans-chelated to a high-affinity chelator in the desired molecule.

Many different chelators have been developed, including both linear (DTPA, HBED-CC, etc.) and macrocyclic (DOTA, NOTA, etc.).

METHODS FOR SYNTHESISING RADIOPHARMACEUTICALS

Indirect or direct radiolabelling methods can be executed by means of (a) manual, (b) automatic or (c) kit-based synthesis. It is to be noted that, independent of the method, the vast majority of radiopharmaceuticals are administered intravenously and aseptic techniques should therefore be

adopted, with sterile filtration as the final step in the radiosynthesis procedure if necessary. Furthermore, everything should be in place to protect the operator when preparing the radiopharmaceutical (cf. *Chapter 3*).

Manual synthesis

Manual synthesis is less and less common these days, but manual approaches are still employed at the start of the process of developing new radiopharmaceuticals. Although they have limitations in respect of radioprotection and can be quite cumbersome, they offer the flexibility to adapt and improve the synthesis through a trial and error approach. Nonetheless, manual synthesis requires specific expertise, dexterity and constancy, and if these are lacking then the reproducibility of the synthesis outcome will be poor.

Automated synthesis

Automated synthesis is based on the use of synthesis modules to allow the automation of the radiolabelling, purification and sterile filtration of the radiopharmaceutical.

Although semi-automated systems with stationary tubing were used initially, nowadays the systems used for automated synthesis are easily compatible with Good Manufacturing Practice (cf. Chapter 10). This has been achieved thanks to on-line

documentation of the manufacturing process and the use of disposable (sterile) cassettes, allowing full recording of the process and reducing the risk of cross-contamination. Furthermore, compared with manual synthesis, automated synthesis offers the adva*ntages of higher reproducibility and lower ra*diation burden to the operator. This approach also permits the fractionation and pre-concentration/purification of high amounts of incoming radioactivity. In general, the procedure for automated synthesis comprises the following key steps:

1. Preparation of the GMP cassettes: connect vials, perform self-tests and add precursor, reagents, buffer, etc.
2. Radionuclide transfer from cyclotron, generator or vial into the cassette
3. Radiolabelling: chemical reaction between the radionuclide and the vector molecule during an incubation at the desired temperature for a certain time
4. Purification of the reaction mixture: separation of the radiopharmaceutical of interest from the rest of the components in the reaction mixture (free radionuclide, buffer, etc.) by means of solid-phase extraction or high-performance liquid chromatography
5. Sterile filtration (0.22-µm filter)
6. Ready for patient injection

Kit-based synthesis

Kit-based synthesis consists in the reconstitution of proprietary single vials containing sterile, lyophilised precursor, buffer and scavenger. This represents an all-in-one easy-to-use approach that avoids the need for expensive equipment and lengthy procedures. Kit-based synthesis requires limited expertise, is very safe, is reproducible and is less cumbersome than the other radiolabelling approaches. It also results in important reductions in investment and production costs.

The availability of a wide range of such kits for different indications in combination with the generator is the reason for the success of ^{99m}Tc worldwide. Currently, kits are also being developed for ^{68}Ga, which may increase the accessibility of PET-based molecular imaging globally.

To this day, the lion's share of radio-pharmaceutical preparation in conventional nuclear medicine is by means of kit-based synthesis (cf. Chapter 6). Some important steps are as follows:

1. The generator is eluted in a vial (e.g. ^{99m}Tc) or activity is received in a vial [e.g. yttrium-90 (^{90}Y)].
2. The cap from the pharmaceutical vial is removed and subsequently swabbed with alcohol.
3. The appropriate activity is added.
4. Radiolabelling: chemical reaction between the radionuclide and the

vector molecule during an incubation at a desired temperature for a certain time, with or without shaking

5. Ready for patient injection

After any type of synthesis, the radiopharmaceutical undergoes quality controls (QCs). These can be quite limited, such as for kit-based synthesis when a leaflet describing the required QC is enclosed, or rather extensive, following the European Pharmacopoeia.

QUALITY OF RADIOPHARMACEUTICALS

Although the quality of the radiopharmaceutical after radiolabelling is often the main focus of concern, other important points have to be kept in mind to ensure the quality of the product. A short overview of the key components is provided below (more detail is provided in *Chapters 3, 5 and 6*). This discussion relates to hospital radiopharmacies; for descriptions of more extensive QC systems in GMP, please refer to *Chapter 10*.

Workplace

Within the workplace, the basic requirement is to ensure that the radiopharmaceuticals are prepared under the best conditions. In particular:

- The working space should be clean.
- Machines should be well maintained and calibrated.
- Precursors and other materials should be conserved appropriately.
- The laminar air flow should be maintained and tested regularly.

Reception

All incoming goods should pass a basic quality check at reception:

- Packaging should be inspected.
- Conformity assessment: Accuracy of the information on the documentation of incoming goods should be assessed (name, quantity, activity, certificate of analysis, leaflet, etc.).
- A swab/wipe test should be performed on delivered radionuclides or generators to ensure their integrity.

Radiopharmaceutical

Quality control of drugs is of extreme importance and is performed thoroughly by the pharma industry. In contrast to other drugs, most radiopharmaceuticals are prepared in hospital settings, outside of the pharma industry. Furthermore, radiopharmaceuticals are the foundation of nuclear medicine. Therefore, their quality and thus the implemented QC is of the utmost importance. In the QC, the physicochemical properties of the radiopharmaceutical are verified as well as the pharmacological ones.

Similarly to other drugs for intravenous injection, the appearance and the pH of the radiopharmaceutical have to be assessed. Appearance is assessed through visual inspection of the radiopharmaceutical, with attention to various features (e.g. clarity, presence of particles in the solution, colour, homogeneity). The pH is measured with pH indicator strips or a pH meter to ensure that the solution is within the injectable pH range. More specifically, for radiopharmaceuticals, different purities are evaluated, namely the radionuclide, chemical, radiochemical and bacteriological; the serological purity may also be assessed but this is seldom done. Table 1 provides an overview of these purities, showing their definition, the method of assessment and the consequences of impurities.

PRINCIPLES OF ACCUMULATION OF RADIOPHARMACEUTICALS

Radiopharmaceuticals are used in nuclear medicine to study specific targets and biological functions. Even though the number of radiopharmaceuticals could be limitless, the mechanisms underlying their accumulation in the human body are based on ten fundamental principles:

a. **Diffusion:** The radiopharmaceutical crosses the cell membrane passively. Diffusion is driven by the concentration difference between the two sides of the cell membrane. Example: Technegas accumulation in the lungs during a ventilation study.

b. **Ion exchange and transport:** The radiopharmaceutical accumulates in cells passively, via protein channels and carrier proteins present on the cell membrane. This phenomenon can also be referred to as facilitated diffusion. Example: Technetium pertechnetate (^{99m}TcO4-) accumulation during examination of the thyroid gland.

c. **Active transport:** In contrast to the passive use of protein channels and carrier proteins, active transport requires energy (ATP) to allow the radiopharmaceutical to cross the cell membrane. Example: Radioactive iodine accumulation in the thyroid gland for imaging or therapy.

d. **Compartmentalisation:** The radiopharmaceutical is restricted to the vascular compartment. Example: Use of radiolabelled red blood cells (RBCs) for determination of the blood pool or the investigation of occult bleeding.

	Definition	Method of assessment	Effects of impurities
Radionuclide purity	Ratio of the radioactivity of the desired radionuclide to the total radioactivity, expressed as a percentage.	Energy spectrum Decay assessment Differential measurements of radioactivity with lead shielding	Non-beneficial and undesired increase in radiation dose to the patient. Poor image quality.
Chemical purity	Ratio of the mass of the desired stated chemical form to the total mass, expressed as a percentage.	Colorimetric HPLC	Usually no direct impact Possible decrease in image quality
Radiochemical purity	Ratio of the radioactivity of the desired radiochemical form to the total radioactivity, expressed as a percentage.	TLC Radio-HPLC	Altered biodistribution Altered radiation dose Poor specificity Poor image quality
Bacteriological purity	Radiopharmaceutical is non-pyrogenic and without bacteria	LAL test Bacterial culture	Pyrogenic response Non-sterile solution can result in bacterial infection
Serological purity	Radiopharmaceutical is without virus	RT-PCR	Can result in viral infection

Table 1: *The different types of purity of a radiopharmaceutical HPLC, High-pressure liquid chromatography; TLC, thin-layer chromatography; LAL, Limulus amebocyte lysate; RT-PCR, reverse transcription polymerase chain reaction*

e. **Cell trapping:** The radiopharmaceutical is entrapped in the organ. Example: While passing the spleen, RBCs will endure a stress test to ensure their integrity; if they fail the test, they will remain in the spleen to die. Radiolabelled RBCs damaged by heat are sequestered into the spleen and thus can be used to image residual or ectopic spleen after splenectomy.

f. **Phagocytosis:** The radiopharmaceutical is phagocytised in the reticuloendothelial system (RES; liver, spleen and bone marrow). Example: Radio-labelled colloids for imaging of the bone marrow.

g. **Capillary blockage**: The radiopharmaceutical is entrapped in the capillaries owing to its size. Example: Macro-aggregated albumin radiolabelled with ^{99m}Tc (^{99m}Tc-MAA) for lung perfusion study.

h. **Metabolic trapping:** Accumulation of the radiopharmaceutical is based on the enhanced metabolism of the cell. While the radiopharmaceutical mimicking a building block/energy source of the cell enters the cell and undergoes the first step of metabolisation (e.g. phosphorylation), it is not completely metabolised and accumulates in the cell. The cell can demonstrate enhanced glucose, protein or lipid metabolism. Example: ^{18}F-FDG PET to investigate the enhanced glucose metabolism of cancer cells.

i. **Ligand binding:** The radiopharmaceutical is a ligand that will specifically bind to a determined receptor/enzyme. Example: ^{68}Ga/^{177}Lu-DOTATATE (somatostatin analogue) for imaging/treatment of neuroendocrine tumours.

j. **Ab-Ag complexes:** The radiopharmaceutical is an antibody or antibody fragment/derivative that will recognise and bind an antigen with high specificity. Examples: ImmunoPET with ^{89}Zr-trastuzumab against HER2 in a patient with HER2-positive breast cancer. Radioimmunotherapy with ^{90}Y-ibritumomab tiuxetan (Zevalin) against CD20 lymphocytes in lymphoma patients.

CONCLUSION

Although radiopharmaceuticals are medicinal products, the contained radionuclide makes them unlike any other pharmaceutical product. Radiopharmaceuticals are unique and are at the core of the diagnosis and the therapy performed in nuclear medicine.

This chapter has provided an overview of the theoretical basics of radiopharmacy, or the art of preparing high-quality, radioactive, medicinal products. Several topics have been discussed succinctly: the radionuclide, the radiolabelling and synthesis methods, the quality controls and the principles of accumulation of the radiopharmaceuticals. This discussion serves as an introduction to the following exhaustive chapters.

RADIOPHARMACY DESIGN AND RADIATION PROTECTION

by Lei Li Corrigan

INTRODUCTION

In the 1950s, hospital pharmacists started to establish facilities to handle radioactive materials [1]. These facilities later became radiopharmacies and were separated from central pharmacies as special equipment was needed for dispensing and radiation protection purposes.

Typically, a radiopharmacy is responsible for the dispensing of radiopharmaceuticals as active ingredients for targeted radiotherapy or as imaging agents for clinical single-photon emission computed tomography (SPECT) scans. Recent years have also witnessed the rapid development of fluorine-18, carbon-11 or gallium-68 labelled radiopharmaceuticals as imaging agents for position emission tomography (PET) [2]. Therefore, more and more radiopharmacies are establishing new facilities to produce tracers other than technetium-99m (^{99m}Tc). However, positron-emitting radioisotopes have higher energy and hence require additional radiation protection. This chapter focuses more on traditional radiopharmacy design for the dispensing of ^{99m}Tc tracers.

RADIOPHARMACY DESIGN

Technetium-99m has a half-life of 6 h. To maximise the number of doses that can be dispensed from one batch of product, the radiopharmacy is usually built immediately adjacent to the SPECT scanner, within the Department of Nuclear Medicine. ^{99m}Tc-labelled radiopharmaceuticals can also be shipped for use at different locations, and a big radiopharmacy may supply tracers to up to ten hospitals within a 1- to 2-h drive radius.

Most radiopharmaceuticals are manufactured and dispensed into sterile vials before being administered by intravenous injection. Directive 2003/94/EC, adopted by the European Commission, lays down the principles of and guidelines for good manufacturing practice (GMP) for medical products for human use. More detailed guidelines compliant with that Directive are used in assessing applications to manufacturing authorities and as a basis for inspection of manufacturers of medical products.

The EU GMP guideline Annex 1 defines the requirement for the clean controlled area where the manufacture of sterile products should be carried out. Clean rooms and clean air devices should be classified from A to D (Table 1) in accordance with EN ISO 14644-1:2015. EN ISO 14644-2 also provides information on testing to demonstrate continued compliance with assigned cleanliness classifications.

Grade	0.5 µm	5 µm
A	3520	20
B	3520	2900
C	352,000	2900
D	3,520,000	29,000

Table 1: *Maximum permitted number of particles per m3 equal to or greater than the tabulated size (at rest)*

Segregation of clean rooms is achieved by maintaining a pressure cascade within the facility between adjacent areas of different classification. Clean rooms must be fully sealed to maintain the pressure regimes and reduce ingress of particles or pathogens.

Floors, walls and ceilings in the suite should be finished in fully welded sheet vinyl, i.e. standard clean room material with coving at all floor-to-wall, wall-to-wall and wall-to-ceiling junctions. Walls, floors and ceilings (including the inlet side of air diffusers) in the suite should be designed and constructed in such a way that the surfaces are accessible for cleaning. All finishes in the facility should be compatible with the mechanical and chemical effects of the intended methods of cleaning and disinfection.

Due to the short half-life of ^{99m}Tc, radiopharmaceuticals are administered before completion of a sterility test. Hence, effectiveness of infection control is critical to product safety and sterility assurance. Products should be prepared aseptically in a grade A environment with continuous particle monitoring. Isolator or restricted access barrier system technologies, and the associated processes, should be designed so as to provide maximum protection of the grade A environment.

A typical radiopharmacy (Fig. 1) should include a grade D changing room, leading to a grade C production area where at least one isolator with a transport chamber as a grade B environment and a main chamber as a grade A environment are installed. Room layouts should ensure that personnel, material, products and waste flows do not compromise product integrity. A radiopharmacy should also include a grade D preparation or support room with a transfer hatch linked to the clean room. This is to ensure that materials' sanitization and transfer into the clean room occurs under a controlled environment and that the flow of personnel and materials is segregated. A typical entrance to a radiopharmacy clean room is illustrated in Figure 2.
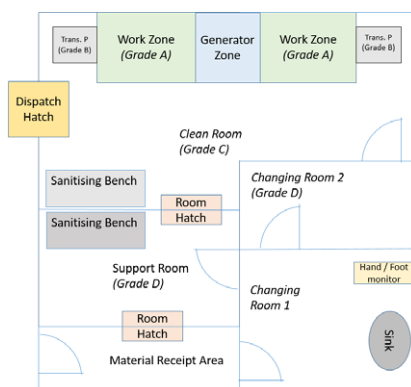
Figure 1: *Layout of radiopharmacy and room grading (illustrative)*

Some radiopharmacies have dedicated clean rooms for blood labelling procedures. In the design of the facility, adequate segregation of the tracer manufacturing process and the blood labelling process is very important to avoid cross-contamination. This entails segregation of the flow of personnel, materials and waste.

Ungraded rooms may also be included for storage of materials and quality control (QC) of products. The storage room, fridge and freezer require continuous temperature and humidity monitoring to ensure that materials are stored properly and are fit for purpose. The QC room should be segregated from the production area. Typically, the following equipment is installed in the QC room for QC testing: thin-layer chromatography (TLC) and/or high-pressure liquid chromatography

Figure 2: *Typical entrance to a radiopharmacy clean room: the clean room door is without a handle, a sticky mat is placed on the floor to remove dust from shoes, there is an interlock at the door, access to the room is restricted, and a sign on the door indicates that this is a radiation control area and shows contact details for the radiation protection supervisor*

(HPLC) to measure the radiochemicaland chemical purity, pH meter or pH paper to measure the pH value and a kit to test for the endotoxin level. Some radiopharmacies are also equipped with incubators to assist in the environmental monitoring of microorganisms.

RADIOPHARMACY PERSONNEL – RESPONSIBILITIES AND TRAINING

Key management personnel of a radiopharmacy include the Head of Production and the Head of Quality Control, who must be independent from each other and appointed by senior management. Senior technicians and technicians will be assigned in the production team or QC team for the performance of daily tasks. In a small unit, a technician can be trained to carry out production and QC protocols but cannot be involved in both production and QC for the same batch. Depending on the size of the unit, a separate Head of Quality Assurance and Head of Quality Unit may be appointed. The radiopharmacy must have an organisational chart that defines every position and the managerial hierarchy. Senior management must ensure that the duties of those in positions of responsibility are clearly defined in their job descriptions and that adequate training is provided to ensure competence in the execution of responsibilities.

A training plan should be defined for each role. Typically, the training in a standard operating procedure includes observation, performance of the procedure under close supervision and performance of the procedure independently during competency assessment. The competence of each staff member should be reviewed

regularly. Some training may be carried out in the form of group lectures: general GMP training, overview of the quality management system, policy of change management, how to handle deviations, etc.

RADIATION PROTECTION

The Euratom/European Union Basic Safety Standards Directive 2013 (BSSD) sets out updated safety requirements for the nuclear and radiological sector. Radiopharmacy should be compliant with each country's current ionising radiation regulations and health and safety legislation to guarantee the safety of the public.

Radiopharmacies should gain local Regulatory Body permits for radioactivity storage and emissions that cover bringing radioactivity into the lab, delivery of radioactive products to clients and management of radioactive waste.

Radiopharmaceuticals emit penetrating gamma radiation and high-energy electrons. They present an external hazard (hand and body exposure), a contamination hazard (primarily to the hands) and also an internal hazard if controls are not properly implemented.

In the United Kingdom, the role of the radiation protection supervisor (RPS) is defined in the Ionising Radiations Regulations 2017. The RPS is appointed

by the radiation employer to ensure compliance with the legislation in respect of work carried out in an area which is subject to local rules. The legal responsibility for supervision, however, remains with the radiation employer.

Radiation protection includes different aspects: the clean room should provide enough shielding to ensure safe handling and storage of the radioactivity; based on the work flow, there should be efficient radiation monitoring equipment in place when incidents occur; all staff should be trained in good practice in the handling of radioactive material (local rules); and radiation risk assessment should be performed before establishing a new protocol.

Radiation risk assessment should also be performed during the design of the radiopharmacy and a decision made on the level of shielding needed for safe handling and storage of the maximum amount of radioactivity permitted. A computerised clean room-compatible gamma radiation detector must be installed to provide continuous

monitoring. The location of the detectors should be determined during the design stage of the radiopharmacy.

All persons entering the radiation control area must wear the following personal protective equipment: lab coat, hairnet, overshoes, disposable gloves and safety eyewear. Staff are usually required to wear double gloves; the outer gloves are sacrificial and should be changed promptly if they become contaminated.

At the border of the radiation controlled area, the changing room or lobby should be equipped with the following for emergencies: a telephone for communication to the RPS when an incident occurs, a hand and foot monitor for contamination self-check (Fig. 3), a sink for quick decontamination and clean scrubs and shoes to change into before leaving the contaminated area.

Figure 3:
Left: mobile contamination monitoring equipment;

right: hand and foot monitor

The thermoluminescent dosimeter (TLD) is a type of passive dosimeter which is used to measure exposure from ionising radiation (Fig. 4). The TLD is normally worn on the trunk of the body but can also be worn on the extremities (e.g. for measuring doses to the fingers). TLDs measure the cumulative dose to the whole body and fingers over a period of time. The radiation dose received by each staff member is reviewed regularly by the RPS, and if the dose reaches the level warranting an investigation, a formal investigation will be carried out. This investigational level is determined based on the work load and the nature of each task and should be defined in the local rules.

Figure 4: *Examples of TLDs (1, 2) and APDs (3, 4)*

Active personal dosimeters (APDs) measure the dose and dose rate (Fig. 4). These measurements are displayed in real time so that the user has immediate feedback regarding current exposure. APDs are more expensive than TLDs and are usually worn by operators who have a higher chance of being exposed to large amounts of radioactivity.

Quality assurance (QA) is a broad concept covering every aspect that collectively or individually impacts the product quality. QA within a radiopharmacy entails the establishment and maintenance of a quality management system to ensure that radiopharmaceuticals are of the required quality for the intended use. QA is discussed in more detail in Chapter 10.

Quality control includes control and testing of raw materials, process control and testing of final products. The aim is to ensure that the product meets the specification before release for use.

The QC lab in a radiopharmacy is usually equipped with (1) a dose calibrator to ensure that the dose delivered is what is intended and (2) a radio-TLC scanner to determine the radiochemical purity of the final product. Some radiopharmacies are equipped with a pH meter for pH measurement and HPLC with a radio detector for quantitation of chemical and radiochemical impurities. Gas chromatography can be used for the quantitation of residual organic solvents

when applicable. A disposable kit has been licensed and marketed for rapid determination of the product endotoxin level by means of limulus amoebocyte lysate assay. This equipment can also be a valuable asset in the QC lab of a radiopharmacy.

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GENERATORS USED IN NUCLEAR MEDICINE

—

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4

INTRODUCTION

Radionuclide generators are a convenient way to produce and isolate medical isotopes used in nuclear medicine. All generators work on the same principle: a longer-lived isotope decays into a shorter-lived isotope, which can be isolated from the generator through a process called elution. The rate of ingrowth by the parent nuclide is approximately similar to the decay of the daughter nuclide. The eluted isotope (eluate) can be either administered directly into patients or labelled with a drug to create a radiopharmaceutical, each approach giving a desired distribution in the body. Common radionuclide generator systems used clinically are shown in Table 1.

A few key properties are required of generators in order for them to be successful. First, generators must use isotopes that have different chemical properties so that they may be separated chemically. For medical use, only the daughter isotope is desired, and eluting too much of the parent isotope can cause problems such as excess radiation dose to the patient or unsatisfactory radiopharmaceutical labelling. Generators must also be shielded properly and be portable so that they may be transported to nuclear pharmacies and medical centres that do not have a reactor or cyclotron nearby. Lastly, the generator must be prepared under sterile conditions and allow for an aseptic elution process so that the radionuclide is safe to administer to patients. The following sections will describe the properties of specific generator systems, including isotopes, structure, operation and quality control of the generators.

MOLYBDENUM-99/ TECHNETIUM-99M GENERATOR

The molybdenum-99/technetium 99m (99Mo/99mTc) generator is the most widely used generator in nuclear medicine and technetium-99m (99mTc) is used to produce many commonly used radiopharmaceuticals, as shown in Table 2. With a physical half-life of 66 h, the parent isotope, molybdenum-99 (99Mo), decays into the shorter-lived 99mTc, which has a physical half-life of 6 h.

Historically, three ^{99}Mo/^{99m}Tc generator systems have been developed: column chromatography, sublimation and solvent extraction generators. Of these three, the column generator system is the only type of ^{99}Mo/^{99m}Tc generator currently approved for clinical use [1]. The column is composed of aluminum oxide (alumina) onto which the ^{99}Mo is absorbed. Unlike ^{99}Mo, ^{99m}Tc does not have a high affinity for alumina; therefore, as the ^{99}Mo decays into ^{99m}Tc, the ^{99m}Tc can be washed off the column using saline (0.9%), generally with a volume of 5–20 mL. ^{99m}Tc, when eluted, is in the form ^{99m}Tc-pertechnetate (^{99m}TcO$_4^-$).

Column generators are designed as either liquid or solid column generators, of which the solid column generator is the design used clinically owing to ease of operation compared with the liquid column generator. Solid column generators exist in two styles: wet generators and dry generators. As the names suggest, either the generator column remains full of saline after elution (wet) or all the saline is removed from the column after elution (dry). Wet generators operate by leaving a large reservoir of saline connected to the in-port of the generator and applying an evacuated vial on the out-port. The vacuum on the vial pulls saline from the reservoir through the column, washing off any ^{99m}Tc, and the resulting eluate is collected in the vial on the out-port. The volume, and thus the concentration, depends on the size of the evacuated vial used. After elution, saline stays on the column, creating water radiolysis products (reducing agents) that cause a reduction of the ^{99m}Tc. As a result, there is less ^{99m}TcO$_4^-$ and lower yields during elution, making wet generators a less satisfactory choice compared with dry generators.

Dry generators tend to be more popular than wet generators and operate in a slightly different fashion. When one is ready to operate the generator, a saline vial of a specific volume is applied to the in-port and an evacuated vial of at least the volume of the saline vial is applied to the out-port. As long as the evacuated vial has enough vacuum, all of the saline in the vial will be pulled through the column and collected through the out-port. In the instance of a dry column system, the volume and concentration of the eluate are dependent on the volume of the saline charge applied to the system.

Yield refers to the amount of daughter isotope collected after elution of a generator. Using the ^{99}Mo/^{99m}Tc, the theoretical yield refers to the amount of ^{99m}Tc that could potentially be collected from the generator based on the amount of ^{99}Mo present and the efficiency of the generator. Many factors can potentially affect the yield of ^{99m}Tc from a generator. As previously stated, a reduction of the ^{99m}Tc can occur. The reduced ^{99m}Tc has a higher affinity for alumina, and thus will be more inclined to stick to the column. The time since the last elution also has an effect on yields, as the generator needs time to "recharge" and build up enough ^{99m}Tc. Generally speaking, ^{99}Mo/^{99m}Tc generators are most efficient when they are eluted every 24 h. Lastly, physical trauma or leaks in the generator system can reduce yields.

Two important quality control

tests, known as chemical purity and radionuclide purity, must be performed on the generator eluate. Chemical purity involves checking the elution for alumina which may have washed off the column. Alumina present in the eluate may affect the labelling of certain radiopharmaceuticals, including ^{99m}Tc-sulphur colloid and ^{99}Tc-labelled red blood cells (UltraTag). Alumina breakthrough testing is accomplished using commercially available colorimetric kits that have indicators to warn when the maximum levels of alumina in an elution have been reached. Any elution containing 10 µg of alumina per mL of ^{99m}Tc, or more, should be discarded.

Radionuclide purity testing of the elution, also called ^{99}Mo breakthrough testing, refers to checking for ^{99}Mo that may have been washed off the column and into the eluate. Injection of excess ^{99}Mo into patients is undesirable for many reasons, including the additional radiation dose to the patient due to the higher energy and longer physical half-life. The vial of eluate is placed in a dose calibrator and measured on the ^{99m}Tc channel to determine how much ^{99m}Tc is present. Next, the vial is placed in a lead shield and measured again in the dose calibrator, this time on the ^{99}Mo channel. Because of the difference in energy (Table 3), the lead will block out the low-level gamma rays from ^{99m}Tc and allow only the higher-energy ^{99}Mo to be measured. Once both measurements have been made, a ratio can be created.

The maximum allowable amount of ^{99}Mo breakthrough is 5.6 kBq of ^{99}Mo per 37 MBq of ^{99m}Tc at the time of administration. It is important to keep in mind that because ^{99}Mo has a longer half-life, the ratio of ^{99}Mo to ^{99m}Tc will increase over time as the ^{99m}Tc decays. This means that at the time of elution the eluate may be usable for patients, but as the ^{99m}Tc decays, the allowable ^{99}Mo limit may be passed. Most radiopharmacy software systems have programs that will automatically calculate the time of eluate expiration based on the measured values from the dose calibrator. ^{99}Mo breakthrough can occur for many reasons, including eluting with saline greater than a pH of 7, excessive elutions and channelling in the column, where only certain parts of the column are exposed to the saline.

STRONTIUM-82/RUBIDIUM-82 GENERATOR

Rubidium-82 (^{82}Rb), produced by the strontium-82/rubidium-82 (^{82}Sr/^{82}Rb) generator under the name Cardiogen-82® by Bracco, is a positron-emitting radiopharmaceutical used for myocardial perfusion imaging in positron emission tomography (PET). The parent isotope, strontium-82 (^{82}Sr), has long physical half-life of 25 days and decays into shorter-lived ^{82}Rb, which has a physical half-life of 75 s. The manufacturer-recommended dose is 1480 MBq per injection (1111–2220 MBq range), with the patient typically receiving an injection for both rest images and pharmacological stress images. The ^{82}Rb is infused at a rate of 50 mL/minute with a maximum of 100 mL volume administered [2].

The generator structure consists of a shielded column with an inlet and an outlet line. The column is an ion exchange column of tin oxide, for which ^{82}Rb, the parent isotope, has an affinity [3]. Due to the low binding affinity of the daughter isotope, ^{82}Rb, as ^{82}Sr decays into ^{82}Rb, the ^{82}Rb can be washed off the column by passing 0.9% NaCl through the column via the inlet line and collecting the eluate through the outlet line. The ^{82}Sr/^{82}Rb generator and infusion system are shown in Figure 1.

The short physical half-life of ^{82}Rb presents some challenges as far as elution and administration are concerned. Elution is performed with the patient's intravenous line connected directly to the generator infusion system and the patient lying on the imaging table. With the ^{82}Rb being infused directly into the patient, and imaging beginning immediately after injection, it is impossible to perform quality control after every elution. Quality control must therefore be performed at the start of the day, and not during each elution.

Quality control is performed on the generator daily before the generator is used for any patients. The first elution is discarded and the second elution is used to measure contamination levels. The second elution is placed in a dose calibrator and measured on the ^{82}Rb channel according to the manufacturer's recommendation. Following this initial measurement, the elution is set aside for one hour, allowing the ^{82}Rb to completely decay. After waiting one hour the elution should be measured in the dose calibrator again on the ^{82}Rb and/or ^{82}Sr channel. Ratio and correction factors are applied to determine the amount of ^{85}Sr present based on the amount of ^{82}Rb in the elution. Records of the total volume passed through the generator, including quality control elutions and patient infusions, must also be kept [2].

The alert and discontinuation trigger levels for ^{82}Sr and ^{85}Sr breakthrough testing are shown in Tables 4 and 5 respectively. When alert levels are reached, quality control testing should be performed again after every 750 mL that is passed through the generator. When discontinuation trigger levers are reached, the generator should no longer be used for patients.

Based on half-life and physical characteristics, the ^{82}Sr/^{82}Rb generator usually lasts for 4–8 weeks [3].

GERMANIUM-68/GALLIUM-68 GENERATOR

Gallium-68 (^{68}Ga) is a positron-emitting radionuclide that has gained popularity of late owing to its ability to be labelled to DOTATATE and prostate-specific membrane antigen (PSMA) targeting compounds. ^{68}Ga-DOTATATE is a somatostatin analogue indicated for localisation of somatostatin receptor-positive neuroendocrine tumours (NETs) using PET. ^{68}Ga-PSMA targeting agents are also used in PET and consist of ^{68}Ga bound to human PSMA ligands for the assessment of PSMA-expressing tumours, such as prostate cancer.

The generator from which ^{68}Ga is produced is appealing to investigators and clinicians because of the half-lives of both the parent and the daughter isotope. The parent isotope, germanium-68 (^{68}Ge), has a physical half-life of 270 days, allowing for a long shelf-life. The daughter isotope, gallium-68 (^{68}Ga), has a physical half-life of 68 min, allowing for patient imaging after delivery from a nearby nuclear pharmacy. The 68-min half-life allows sufficient time for imaging after injection while decay is sufficiently rapid to keep radiation exposure to the patient at a minimal level.

The general structure of the ^{68}Ge/^{68}Ga is similar to other generators in that it consists of a shielded column. Weighing about 10 kg, this generator features a glass column containing a titanium dioxide bed on which the ^{68}Ge is absorbed. The generator is eluted with 0.1 M HCl, which washes the daughter isotope, ^{68}Ga, off the column and into the collection vial at the end of the out-line [4]. The ^{68}Ge has a high affinity for titanium oxide and remains absorbed onto the column. The generator generally has an elution efficiency of at least 75% and can fully recharge every 7 h [4].

^{68}Ge breakthrough for this generator is measured as the activity of ^{68}Ge in the eluate compared with the activity of ^{68}Ge on the column. New generators generally have about 3×10^{-5} % breakthrough of ^{68}Ge. One situation where ^{68}Ge breakthrough may increase is when the generator goes unused for several days. In such a circumstance, it is recommended that the generator be eluted with 10 mL of 0.1 M HCl one day prior to resuming clinical use of the generator [4].

RUBIDIUM-81/KRYPTON-81M GENERATOR

Krypton-81m (^{81m}Kr), with a physical half-life of 13 s and an energy of 190 keV, is most commonly used for lung imaging.

The parent isotope, ^{81}Rb, has a physical half-life of just 4.7 h and is produced in a cyclotron. ^{81m}Kr is produced as a gas and is administered to the patient using a mask to assess ventilation to the lungs. Technical challenges exist with this generator, including the 20-h expiration time due to the short half-life of the parent isotope and the fact that medical centres must be near to a cyclotron able to produce the parent isotope.

The column is made of a strong cation-exchange resin and is eluted using either air or oxygen when performing lung ventilation imaging. ^{81m}Kr can also be used for lung, myocardial and cerebral perfusion studies. In these cases, the generator is eluted using an isotonic 5% dextrose solution in water [5].

STRONTIUM-90/YTTRIUM-90 GENERATOR

Yttrium-90 (^{90}Y) is a pure beta-emitting isotope with a physical half-life of 64 h, making it an ideal isotope for radionuclide therapy. Yttrium-90 is commercially available in many countries as a unit dose, without the need of a generator at the hospital or facility doing the procedure. It can be used to label an antibody or a peptide. ^{90}Y-ibritumomab tiuxetan (Zevalin) entered common use in the early 2000s as a radiotherapy

agent for the treatment of non-Hodgkin's lymphoma. At present, ^{90}Y is more often used with microspheres for selective internal radiation therapy (SIRT) for liver cancer. The ^{90}Y microspheres come in two varieties: resin (SIR-Spheres®) and glass (TheraSphere^{TM}). The generator is produced using a Dowex 50 cation exchange resin column onto which the parent isotope, ^{90}Sr (half-life of 28.6 years), is absorbed. The ^{90}Y can be eluted using 0.03 M EDTA with an efficiency of approximately 98% [6].

ZINC-62/COPPER-62 GENERATOR

Copper-62 (^{62}Cu) has a physical half-life of 9.7 min and is being used for a number of investigational research studies in PET imaging for the assessment of cardiac and brain blood flow. The parent isotope, zinc-62 (^{62}Zn), has a relatively short half-life of 9.3 h compared with other parent isotopes and is produced in a cyclotron. Because of the short half-life of the parent isotope, this generator must be replaced every 1–2 days

CONCLUSION

In summary, generators allow the delivery of short-lived medical isotopes to radiopharmacies and medical centres

that cannot afford or are too far away from cyclotrons and reactors. In order for generators to effectively deliver these isotopes, they must be portable, efficient, cost-effective and safe.

Despite the convenience of generators, the parent isotopes are still created from reactors or accelerators, which can limit availability. For example, while ^{99m}Tc is produced from a ^{99}Mo/^{99m}Tc generator, the ^{99}Mo is produced in nuclear reactors by fission of enriched uranium-235. The availability of generator-produced daughter isotopes is at the mercy of the production of the parent isotope. In order to keep a constant availability of generators, we must continue to investigate methods of production of the parent isotopes and also ensure that our medical reactors and cyclotrons are reliable sources of parent isotopes.

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Figure 1: *82Sr/82Rb generator*

Credit: PET Study Guide, 2d ed, by Paul E. Christian and Nancy M. Swanston. Reston, VA: SNMMI; 2015: p. 2.

TABLES

Parent	Half-life	Daughter	Half-life
^{99}Mo	2.75 days	^{99m}Tc	6 h
^{82}Sr	25 days	^{82}Rb	1.25 min
^{68}Ge	288 days	^{68}Ga	68 min
^{90}Sr	28.5 years	^{90}Y	2.67 days
^{227}Ac	22 years	^{223}Ra	11 days
^{81}Rb	4.7 h	^{81m}Kr	13 s

Table 1: *Common generators used in nuclear medicine*

Radiopharmaceutical	Use
99mTc-macro-aggregated albumin (MAA)	Lung perfusion
99mTc-mertiatide (MAG3)	Functional renal studies
99mTc-medronate (MDP)	Skeletal imaging
99mTc-sestamibi	Myocardial perfusion studies
99mTc-sulphur colloid	Gastric emptying and lymphoscintigraphy
99mTc-mebrofenin	Hepatobiliary imaging (HIDA scan)

Table 2: *Common ^{99m}Tc radiopharmaceuticals and uses*

Isotope	Energy (keV)	Physical half-life
^{99}Mo	740 and 780	66 h
^{99m}Tc	140	6 h

Table 3: *^{99}Mo and ^{99m}Tc properties*

Test	Limit
Cumulative eluate volume	14 L
^{82}Sr breakthrough	0.002 uCi/mCi ^{82}Rb
^{85}Sr breakthrough	0.02 uCi/mCi ^{82}Rb

Table 4: *Quality control alert levels for ^{82}Sr/^{82}Rb generators*

Test	Limit
Cumulative eluate volume	17 L
^{82}Sr breakthrough	0.01 uCi/mCi ^{82}Rb
^{85}Sr breakthrough	0.1 uCi/mCi ^{82}Rb

Table 5: *Quality control discontinue levels for ^{82}Sr/^{82}Rb generators*

CYCLOTRON- AND NUCLEAR REACTOR-PRODUCED RADIOISOTOPES

by Sergio do Carmo, Francisco Alves

INTRODUCTION

Nuclear medicine procedures, whether performed for diagnostic or for therapeutic purposes, rely on the decay of a radioisotope of adequate radiation characteristics. These unstable isotopes are not available in nature and therefore have to be artificially produced, through nuclear reactions. Three main direct or indirect nuclear processes leading to the production of the intended radioisotopes can be identified:

- Nuclear reactions performed in particle accelerators, namely cyclotrons
- Nuclear reactions performed in nuclear reactors
- Generators

CYCLOTRON RADIOISOTOPE PRODUCTION

Radioisotopes can be created through nuclide transmutation by bombarding stable target nuclei with charged particles (protons, deuterons or alpha particles). These charged particles need to be accelerated to energies of at least several MeV in order to overcome the target nucleus Coulomb barrier and lead to the nuclear reaction. As a result, a particle accelerator is required. Because of their practical characteristics and high current performance for the entire energy range of interest (10–100 MeV), cyclotrons have been almost exclusively chosen as the most convenient option for radioisotope production since the 1950s. Discussion of the working principles of cyclotrons falls beyond the scope of this work but a detailed description can be found in [1].

The choice and characterisation – and therefore determination of the feasibility and yield – of a nuclear reaction to be performed in a cyclotron require study and knowledge of physical quantities such as beam range, target material stopping power, cross section and thick target yield.

Stopping power

Radioisotope production in cyclotrons is based on charged projectile particles impinging on a medium containing the target material. This medium, which can be a gas, a liquid or a solid, slows down the particles as they penetrate. The average energy degradation of the beam per unit length of its path in the absorbing medium, dE/dx, termed as the stopping power and usually expressed in keV·µm-1, is given by Bethe's formula:

$$ -\frac{dE}{dx} = \frac{4\pi z^2 q_e^4}{m_e v^2} \; N \; Z \; ln\left(\frac{2m_e v^2}{I}\right) $$

Equation 1

where m_e and q_e are the mass and charge of the electron, z and v the atomic number and velocity of the particles of the beam, Z and N are the atomic number and number of atoms per volume of the target material crossed by the beam and I is the average excitation potential of an atom of this material.

Stopping power values are available in the literature for most elements and beam energy ranges, based on experimental measurements. Although precise theoretical calculations are complex, simpler semi-empirical expressions produce a good approximation. As can been seen in Figure 1, the stopping power decreases with the projectile energy and increases for heavier projectiles and with the medium density.

Figure 1: *Stopping powers in liquid water for several 100 MeV particles impinging in distinct absorbing media*

Range

The knowledge of stopping power variation with energy enables determination of the distance travelled by the beam through a medium until complete rest, as illustrated in Figures 2 and 3. This distance, denominated as range, R, corresponds to the integral of the inverse of the stopping power for the beam in this material, where the integration limits are the initial energy when entering the material, Ei , and 0:

$$ R = \int_0^{E_i} -\frac{dx}{dE} \; dE $$

Equation 2

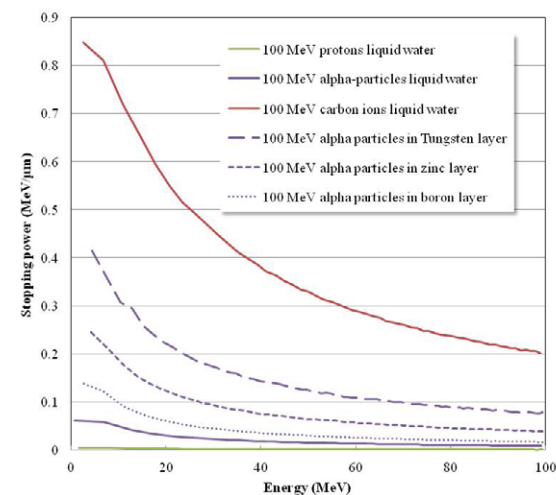Typically, for common energy ranges, targets for radioisotope production are:

- less that 1 cm thick when considering solid targets,
- a few centimetres long when impinging on boiling liquids,
- 10–20 cm long for gaseous targets.

Figures 2 and 3 show that the stopping power increases sharply within a very short distance when the beam energy slows down almost to rest, originating a small and well-defined volume where a

significant amount of energy is deposited. Known as the Bragg peak, this is the fundamental characteristic exploited in particle therapy.
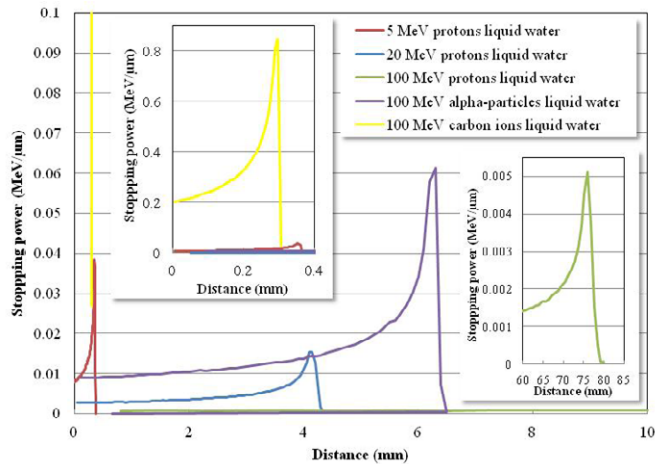
Figure 2:
Stopping powers in liquid water at atmospheric pressure for distinct energies and for different particles of equal energy
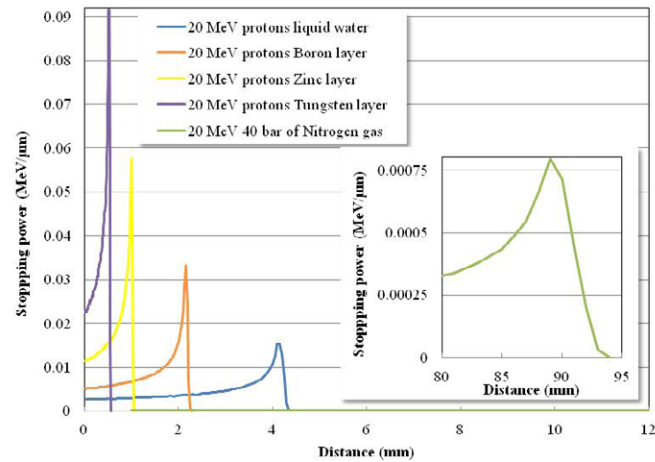
Figure 3:
Stopping powers for 20 MeV protons in different absorbing media

Cross section

The cross section is the metric that quantifies the probability that a given nuclear reaction will occur, per projectile and per target density unit. The cross section of a nuclear reaction is usually denominated σ and is defined by:

$$N_{NR} = N_p \, N_{target} \, \sigma$$

Equation 3

where N_{NR} is the number of nuclear reactions induced when a beam of N_p particles hits a surface with N_{target} nuclei per unit area.

Cross section is related to event probability and has area dimensions. It is usually expressed using the unit *barn, b*, defined as:

$$1 \text{ barn} = 10^{-28} \text{ m}^2$$

Equation 4

Cross sections are energy dependent, meaning that nuclear reaction probability depends on the energy of the incident projectile. The set of cross section values for a relevant particle energy range is denominated as the *excitation function* of the given nuclear reaction. The excitation function is usually depicted as shown in Figure 4 for the ^{68}Zn(p,n)^{68}Ga nuclear reaction.

Thick target yield

Since a projectile impinging on a target slows down as it penetrates the media, the amount of radioisotope produced by a particular nuclear reaction in a target can be estimated by integrating its excitation
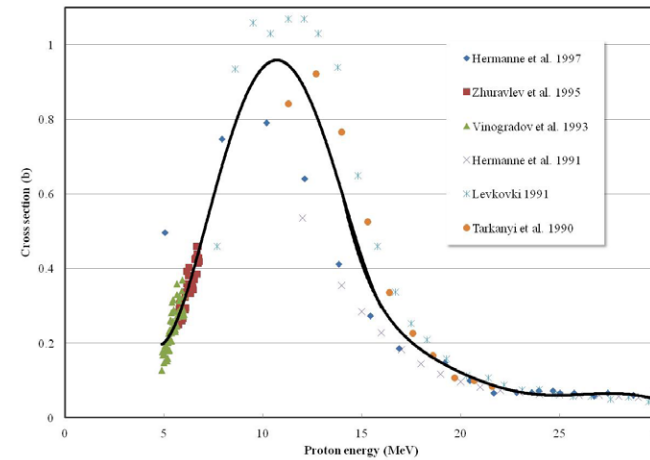
Figure 4:
Excitation function of the ^{68}Zn(p,n)^{68}Ga reaction, obtained from experimental measurements [2–7]. The line shown is a guide for the eye

function over the range of beam energies over the target material. The production route efficiency, termed as *thick target yield Y*, thus correlated to the beam stopping power and the reaction cross sections, is defined as:

$$Y = C \ \frac{N_A H}{M} \int_{E_f}^{E_i} \left(\frac{dE}{d(\rho x)}\right)^{-1} \sigma(E) \ dE$$

Equation 5

where N_A is the Avogadro number, ρ is the medium density M and H are respectively the atomic mass and the target material isotopic enrichment, and *C* is the concentration of the target nuclei in the target medium. The inverse of the stopping power multiplied by the excitation function $\sigma(E)$ is integrated along the beam energy values, from entering (E_i) to emerging from the target (E_f).

Although *Y* evaluates the absolute number of radioisotope nuclei produced per incident projectile, it is more conveniently (and therefore more commonly) defined as the activity production yield per unit of beam current and is thus expressed in terms of *MBq/µA*.

Since there is limited interest in considering the production yield without taking into account its decay during the irradiation duration, it is necessary to consider simultaneously the production rate and the decay during the irradiation to evaluate the activity produced. The temporal evolution of the number *N* of radioisotopes in the target during bombardment is given by:

$$\frac{dN(t)}{dt} = -\lambda \ N(t) + YI$$

Equation 6

where *I* is the beam current and λ the produced radioisotope decay constant. The product *YI* corresponds to the production rate.

As a result, the activity *A(t)* of the radioisotope produced (null at the beginning of the irradiation) after an irradiation duration *t* is:

Equation 7 shows that a saturation condition is reached for long irradiation

$$A(t) = \lambda N(t) = YI\left(1 - e^{-\lambda t}\right) = \frac{C \ N_A H}{M} \left(1 - e^{-\lambda t}\right) I \int_{E_f}^{E_i} \left(\frac{dE}{d(\rho x)}\right)^{-1} \sigma(E) \ dE$$

Equation 7

times and that the activity then tends to equalise, asymptotically, the rate of production (Figure 5), since a balance is reached between the number of nuclides being produced and the decay. In such conditions, a maximum producible activity is obtained, which equals the product *YI*.

For that reason, the *thick target yield* is also referred to as the expected activity, per unit of beam current, in *saturation* and is thus also expressed as $MB^q/µA_{sat}$. It has to be pointed out that a thick target yield must refer to a range of energies or only to an incident energy if the beam is completely stopped within the target.
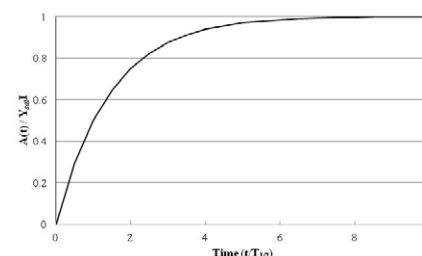

Figure 5: *Ratio between the activity A and the rate of production $Y_{sat}I$, as a function of time in units of half-life*

Cyclotron radioisotope production: research and quality control

When studying distinct possible routes for the production of a given radioisotope, there are several parameters that need to be taken into account simultaneously. As several different nuclear reactions can lead to production of the same nuclide, knowledge of their excitation functions is fundamental, keeping in mind the practical possibilities in terms of projectiles available and respective energy ranges. Then, in order to compare all the production possibilities and their optimisation, in terms of not only yield but also radionuclidic purity of the product, it is mandatory to evaluate fundamental parameters:

- The *thick target yield at saturation for the intended production channel,* as this represents the maximum yield that can be obtained for a target.
- *Thick target yields for nuclear reactions leading to radionuclidic impurities:* while radioisotopes from other elements can be removed chemically, radionuclidic impurities will remain in the final product and therefore have to be kept below acceptable levels.
- The evaluations provided from the thick target yields are used to determine carefully the most suitable *energy range* in order to minimise radioisotope impurities, especially if these have longer half-lives than the intended radioisotope as they will worsen the radionuclidic purity with time.
- Depending on the former results, it is also possible to establish the *target material purity* to be required, concerning both the *enrichment in the target nuclide* and also *minimisation of specific unwanted stable isotopes.*

- Such calculations must take into account the length of typical/ practical irradiations according to the half-life of the intended radioisotope.
- It is also fundamental to consider the *radionuclidic purity of the product after the irradiation,* so that it remains in agreement with the quality control (QC) requirements sufficiently long to enable its clinical use.

Knowledge and simultaneous optimisation of all these parameters are fundamental and equally important. Failure to maintain the quality levels definitively discards a production route regardless of its production yield.

Spallation

While nuclide transmutation through charged particle bombardment occurs with energies up to 100 MeV, a distinct nuclear process can become relevant for higher particle energies. Typically at energies higher than 100 MeV, the emission of nuclei from a heavy target nucleus in a process named *spallation* is likely to occur. The yield of the intended radioisotope and its purity are significantly influenced by the choice of the heavy target nucleus. For instance,

large quantities of ^{68}Ge are produced by spallation reactions on RbBr targets, simultaneously producing high specific activity ^{73}As as a by-product. However, although it is the preferred production route for some radioisotopes and for high radioisotope production efficiencies, this is not a widely used technique because facilities able to accelerate charged particles with such a high energy are scarce [8].

NUCLEAR REACTOR RADIOISOTOPE PRODUCTION

It is also possible to produce radioisotopes by bombarding target nuclei with neutrons from a nuclear reactor. Common nuclear reactors are known for their use for energy purposes – neutrons are used to generate heat that is further passed to a fluid – but some are alternatively used to produce radioisotopes for medical use. In either case, and despite such distinct purposes, nuclear reactors invariably consist of devices able to initiate and control the creation of neutrons through a self-sustained nuclear chain reaction. Although a detailed description of the working principle of a nuclear reactor is beyond the scope of this work, it is relevant to describe the fundamental nuclear reactions beyond radioisotope production.

Fission

A nuclear chain reaction is initiated by the *fission* of a large and unstable target nucleus created by collision of a neutron with a heavy stable isotope. Fission is a nuclear mechanism that consists in splitting an unstable nucleus into two fragments, usually of different mass number, denominated as fission pairs. Consecutive fission reactions are commonly observed, as the energetic fission products can subsequently originate new reactions, releasing more energy, gamma radiation and free neutrons. A portion of the neutrons produced may be absorbed by another starting stable nucleus and trigger further fission events, with the release of more and more neutrons. An adequate choice of the starting stable material guarantees a self-sustained nuclear chain reaction. Such a starting material is denominated as **fissile** *nucleus* (e.g. ^{235}U or ^{239}Pu).

As the resulting smaller nuclei can be chemically separated, this mechanism can be used to produce radioisotopes. For instance, the fission process generated from unstable ^{236}U, where ^{235}U is the fissile nucleus, enables the production of the medically relevant ^{99}Mo, used in the production of ^{99}Mo/^{99m}Tc generators, through the nuclear reaction:

$$^{235}U + n \rightarrow\ ^{236}U \rightarrow\ ^{99}Mo + ^{132}Sn + 4n$$
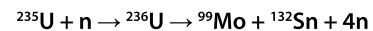
Equation 8

Neutron capture

The neutrons produced in the nuclear reaction chain can alternatively originate a nuclear process denominated as *neutron capture activation*. In that case, the high neutron flux is used to bombard a specific target nucleus. The nuclear reaction involved is usually a (n,γ) reaction, thus resulting in a different isotope of the target, but other elements can also be produced via (n,p) or (n,α) reactions. A relevant illustrative example refers to ^{99}Mo used in ^{99}Mo/^{99m}Tc generators since it can be obtained in nuclear reactors also through a neutron capture reaction:

$$n + ^{98}Mo\ \rightarrow\ ^{99}Mo + \gamma$$
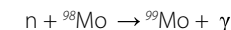
Equation 9

Analogously to what happens in charged particle reactions, the production of radioisotopes through neutron irradiation is influenced by the irradiation parameters, namely:

- Energy of the neutrons
- Neutron flux
- Target material
- Activation cross sections

As illustrated in Figure 6, activation cross sections vary significantly with the neutron energy, usually being much larger for low energies. As a result, *neutron moderators* such as water are used to

change the energy spectrum of the fast neutrons produced from the fission process, slowing them down into lower (usually named thermal) energies at which nuclear reaction probability is higher.
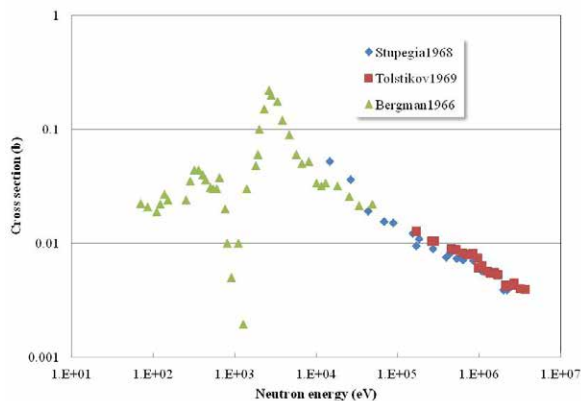
Figure 6: *Excitation function of the 89Y(n,γ)90Y reaction [9–11]*

The analogy with charged particle reactions remains when evaluating the production yields in activation reactions. In this latter case, the activation evolution with time is defined by:

$$\frac{dN(t)}{dt} = \phi\, \sigma_{act} N_T \; - \lambda\, N(t)$$

Equation 10

where $N(t)$ is the number of active atoms, Φ the neutron flux in neutrons/cm^2/s, σ act the neutron activation cross section (for the average neutron energy) in cm^2, N_T the number of target nuclei and λ the decay constant of the radioisotope produced.

The activity S, expressed in Bq/g, produced after an irradiation of length t is given by:

$$S = \frac{0.6\, \sigma_{act}\phi}{A}\left(1 - e^{-\lambda t}\right)$$

Equation 11

Equation 11, where A is the target atomic weight, shows that, for long irradiations, the activity is only limited by the neutron flux of the reactor.

Generators

A generator consists of a radioactive source providing a shorter half-life radioisotope in a chemical form suitable for consideration as a starting material for radiolabelling. Such systems enable the separation and extraction of the intended shortlived radioisotope (called the daughter) from a long-lived radioisotope (called the parent), the former being

obtained from decay of the latter, through a process of elution. The radioactive parent nuclide is previously produced though either reactors or charged particle nuclear processes (e.g. ^{99}Mo and ^{68}Ge respectively). Generators are widely used for several purposes, ranging from single-photon emission tomography (SPECT) and positron emission tomography (PET) diagnostic techniques (e.g. the ^{99}Mo/^{99m}Tc and ^{68}Ge/^{68}Ga systems respectively) to therapy (e.g. the ^{188}W/^{188}Re generator). Elution can be performed repeatedly as the decay of the parent radioisotope replenishes the amount of daughter nuclei, although the maximum activity of daughter radioisotope is determined and limited by the activity of the parent radioisotope.

It is possible to classify generators according to the difference between the half-lives of the parent and the daughter radioisotopes:

1. If the half-life of the parent radioisotope is much longer than that of the daughter radioisotope, so-called secular equilibrium is reached: the activity of the daughter reaches the activity of the parent as long as sufficient time is allowed between elutions (typically a few half-lives of the daughter), as depicted in Figure 7. This is, for instance, the case for the

^{68}Ge/^{68}Ga generator system (270.8 day half-life compared with 67.8 min). This sort of generator generally offers limited activity but is also characterised by a longer useful half-life of the device (due to the long half-life of the parent nuclide).

2. In contrast, if the parent half-life is not substantially longer than that of the daughter, transient equilibrium occurs. As the activity of the daughter increases due to the decay of the parent nuclide, the attenuation of the activity of the parent nuclide begins to be non-negligible (Figure 7). A maximum activity of the daughter radioisotope is reached after about 4 of its half-lives since the last elution [12]. The ^{99}Mo/^{99m}Tc is the most widespread example of such transient equilibrium: the 6-h half-life of ^{99m}Tc means that maximum activity is reached after approximately 24 h, making such a system very convenient in that it allows a single daily elution at a fixed time (Figure 8).

Gamma ray spectrometry

Regardless of the nuclear production process involved, it is fundamental to assess the quality of the radioactive product manufactured by identifying and
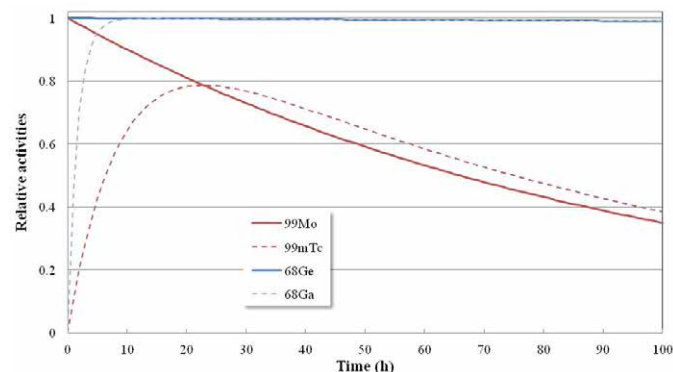
Figure 7: *Evolution of the activities of the parent and daughter radioisotopes for the ^{99m}Tc/^{99}Mo (red curves) and ^{68}Ga/^{68}Ge (blue curves) generators*
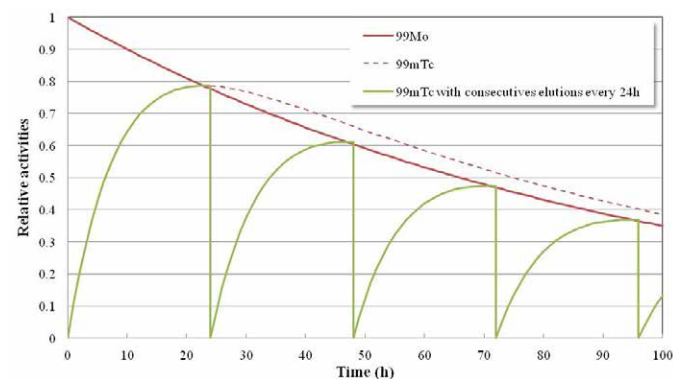
Figure 8: *^{99m}Tc activity profile with consecutive elutions every 24 h, i.e. once a day at a fixed time. Note that the maximum 99mTc activities are obtained 23 h after each elution*

quantifying all the radioisotope present in the final product. For this purpose, the radioisotopes produced are identified by their characteristic gamma rays, of specific energies and intensity branching ratio, emitted as they decay. Table 1 illustrates this intrinsic feature by presenting the characteristic gamma rays from the ^{66}Ga, ^{67}Ga and ^{68}Ga radioisotopes of gallium.
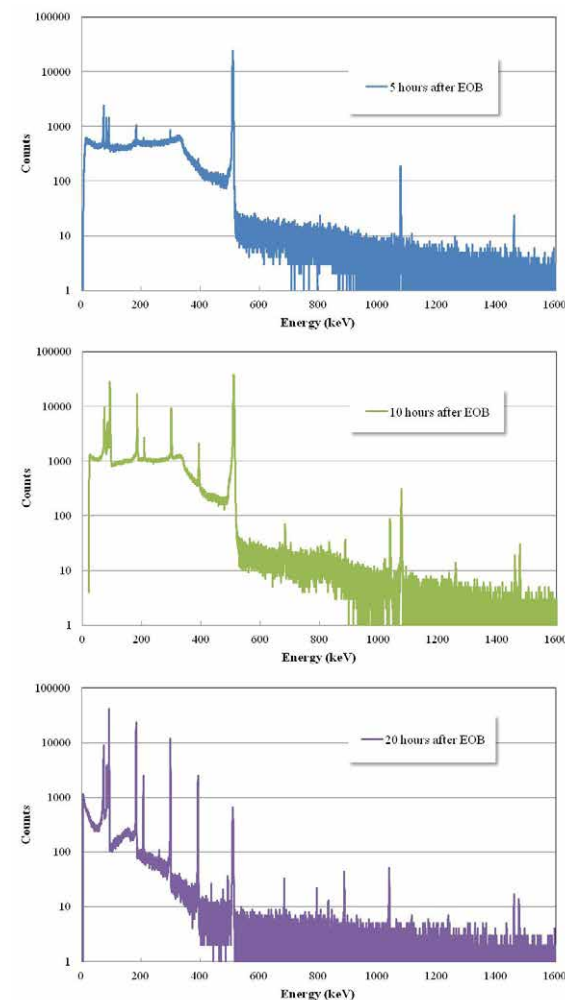
Figure 9: *Gamma ray spectra from cyclotron-produced ^{68}Ga at distinct cooling times*

The gamma ray emissions are detected, analysed and recorded with an analytical spectroscopy system based on a gamma radiation detector, usually a high-purity germanium detector (HPGe), that elaborates a gamma-ray energy spectrum (Figure 9). As several radioisotopes contribute to the spectra, with a wide range of activities and characteristic gamma ray intensities, it is necessary to acquire several spectra at distinct hours and/or days after EOB depending on the half-lives of the radioisotopes involved. Some short half-life and/or high-activity radioisotopes can initially completely mask other characteristic gamma rays, which in turn may be detectable or even predominant after an appropriate cooling time. Figure 9 illustrates such a phenomenon by presenting HPGe spectra of cyclotron-produced ^{68}Ga at distinct cooling times and showing that the radionuclidic impurities ^{66}Ga and ^{67}Ga are detectable only several hours after EOB, after almost total decay of ^{68}Ga.

THE ILLUSTRATIVE CASE OF ^{68}GA

For most radioisotopes, more than one production technique can be used. Each production route presents advantages and there is generally at least one suitable option for each particular radioisotope. For instance, ^{90}Y is almost exclusively produced via nuclear reactors while ^{18}F is commonly obtained worldwide using medical cyclotrons. The case of ^{68}Ga is particularly illustrative of such diversity as it is available through a ^{68}Ge/^{68}Ga generator (in this case the parent nuclide, ^{68}Ge, is usually obtained through a charged particle nuclear reaction) and is also produced directly with charged particle nuclear reactions (using either solid or liquid targets).

Concerning the ^{68}Ge/^{68}Ga generator, ^{68}Ga is conveniently obtained in 0.1 M HCl as ^{68}Ga(Cl$_3$) by elution of the generator matrix containing the ^{68}Ge. This technique presents undeniable advantages with respect to other ^{68}Ga production routes:

- It is a convenient source of ^{68}Ga for daily use, lasting for months.
- It enables independence from ^{68}Ga production centres.
- It can be used several (although limited) times per day.

However, the generator system also presents some non-negligible inconveniences:

- The relatively short half-life of the parent nuclide, ^{68}Ge, significantly limits the useful half-life of the device to about one year.
- There is limited activity per elution and decreasing eluted activity throughout the generator lifetime.
- The down-time between elutions (about 3–4 h) is non-negligible and limits use on a daily basis.
- There is a continuous possibility of breakthrough of the parent nuclide, ^{68}Ge.
- Metallic contaminants may be present.
- As the demand for ^{68}Ga is increasing vastly, the availability of such generator systems is becoming an issue and advance planning of purchase is mandatory to prevent a shortage.
- The price is high.

On the other hand, ^{68}Ga(Cl$_3$) is also available using accelerator-produced ^{68}Ga. While the production of the parent nuclide, ^{68}Ge, for ^{68}Ge/^{68}Ga generators requires mid-energy cyclotrons, ^{68}Ga can be directly obtained from low-energy biomedical cyclotrons, namely via the proton-induced ^{68}Zn(p,n)^{68}Ga nuclear reaction on ^{68}Zn. This process, involving the irradiation of a solid target containing ^{68}Zn, has long been established and available but it has not been a preferred production route owing to technical and radioprotection issues concerning solid target-based production techniques and the related low availability of facilities with such capability.

As a result of recent developments involving production through liquid targets, cyclotron-produced ^{68}Ga has become a viable alternative [13, 14] to generators and has been increasingly implemented in the worldwide installed "medical cyclotrons" dedicated to ^{18}F production.

Direct cyclotron production of ^{68}Ga presents several advantages over the ^{68}Ge/^{68}Ga generator system:

- Larger activities of ^{68}Ga are obtained.
- Consecutive productions are possible without downtime.
- Running costs are affordable.
- Production is decentralised, and potentially boosted by already existing biomedical cyclotron infrastructure.
- Independence from ^{68}Ge production centres avoids possible shortages.
- No long half-life contaminants are present in the eluate.

As the manufacturing technique for direct production of ^{68}Ga differs from the generator system, the production considerations and concerns are distinct. For instance, the radioimpurities present in the latter case correspond only to the radioisotopic impurities ^{66}Ga and ^{67}Ga. These are due to:

- residual contamination of the target material enriched ^{68}Zn with residual percentages of other radioisotopes of zinc, namely ^{67}Zn and ^{66}Zn,
- undesired nuclear reactions that take place simultaneously with the ^{68}Zn(p,n)^{68}Ga reactions.

Gallium-66 is produced through ^{66}Zn(p,n)^{66}Ga and ^{67}Zn(p,2n)^{66}Ga nuclear reactions, whereas ^{67}Ga is obtained from ^{67}Zn(p,n)^{67}Ga and ^{68}Zn(p,2n)^{67}Ga nuclear reactions. Since both ^{66}Ga and ^{67}Ga have a longer halflife than the desired ^{68}Ga, their presence will affect the radionuclidic purity of the accelerator-produced ^{68}Ga, which will also deteriorate with time since ^{68}Ga decays faster. However, although inevitable, the presence of the radioimpurities ^{66}Ga and ^{67}Ga can be minimised by carefully selecting fundamental production parameters:

- The production of ^{66}Ga via the ^{67}Zn(p,2n)^{66}Ga reaction can be minimised if the incident proton beam energy is kept below the 13.0 MeV threshold of this particular nuclear reaction (Figs. 10–12).
- The production of ^{67}Ga via the ^{68}Zn(p,2n)^{67}Ga reaction can be minimised if the incident proton beam energy is kept below the 12.0 MeV threshold of this particular

nuclear reaction (Figs. 10–12).

- The production of ^{66}Ga via the ^{66}Zn(p,n)^{66}Ga reaction can be minimised by minimising the amount of ^{66}Zn present in the enriched ^{68}Zn used (Figs. 11, 12).
- The production of ^{67}Ga via the ^{67}Zn(p,n)^{67}Ga reaction can be minimised by minimising the amount of ^{67}Zn present in the enriched ^{68}Zn used (Figs. 11, 12).
- Since ^{66}Ga and ^{67}Ga have longer half-lives than ^{68}Ga and the production yield of ^{68}Ga reaches saturation long before the yields of ^{66}Ga and ^{67}Ga, shorter irradiations are preferred.

Although some quality control (QC) parameters are naturally common to all manufacturing processes, such as half-life determination and pH, it is mandatory to establish some other tests to reflect the specificity of each production technique. Consequently, the monograph from the European Pharmacopoeia entitled *Gallium (68Ga) chloride solution for radiolabelling* [15], designed to cover the ^{68}Ga obtained from ^{68}Ge/^{68}Ga generators, is inadequate for accelerator-produced ^{68}Ga. Another document, *Gallium (68Ga) chloride (accelerator-produced) solution for radiolabelling* [16], was specifically published to address ^{68}Ga produced
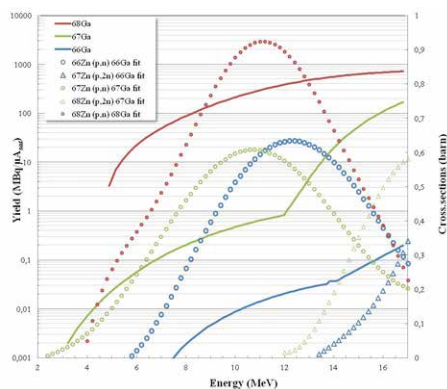
Figure 10: *Thick targets yields at saturation (solid curves) for the production of ^{66}Ga, ^{67}Ga and ^{68}Ga from a typical proton irradiation of a liquid target with ^{68}Zn and excitation functions (open symbols) of the different nuclear reactions involved (data from [14]).*

in liquid targets. Table 2 illustrates the differences by presenting the specifications relating to radionuclidic purity for both cases.
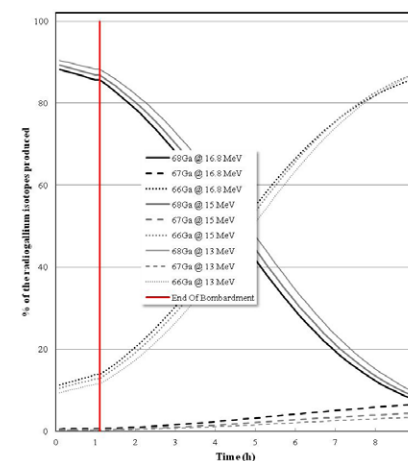
Figure 11: *Activity ratio of the radioisotopes of Ga produced during and after a typical irradiation of a liquid target containing ^{nat}Zn, for distinct impinging energies*
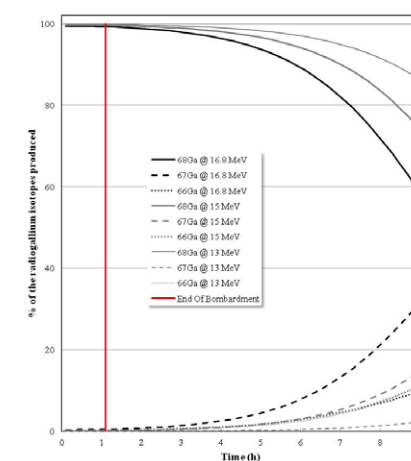
Figure 12: *Activity ratio of the radioisotopes of Ga produced during and after a typical irradiation of a liquid target containing enriched ^{68}Zn (made of 95% ^{68}Zn, 0.5% ^{67}Zn and 2% ^{66}Zn), for distinct impinging energies*

	Half-life	Main characteristic gamma rays	
		Energy (keV)	Branching ratio
^{66}Ga	9.49 h	511	114 %
		833	5.9%
		1039	37%
		2190	5.3%
		2751	22.7%
^{67}Ga	3.26 days	93.3	70.6%
		184.8	21.3%
		300.2	16.6%
		393.5	4.59%
^{68}Ga	67.8 min	511	178%
		1077	3.24%
		1883	0.142%

Table 1: *Characteristic gamma ray lines emitted by the ^{66}Ga, ^{67}Ga and ^{68}Ga radioisotopes of gallium*

	Radionuclidic testing specifications	
	From ^{68}Ge/^{68}Ga generators	Accelerator-produced ^{68}Ga
^{68}Ga activity	Minimum 99.9%	Minimum 98.0%
^{68}Ge activity	Maximum 0.001%	n.a.
^{66}Ga and ^{67}Ga	n.a.	Maximum 2.0%
Other radioimpurities	n.a.	Maximum 0.1%

Table 2: *European Pharmacopoeia's radionuclidic specifications for accelerator produced ^{68}Ga and for ^{68}Ga from ^{68}Ge/^{68}Ga generators [15, 16]*

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CONVENTIONAL NUCLEAR MEDICINE RADIOPHARMACEUTICALS

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Antonio Paulo

RADIOPHARMACEUTICALS

A radiopharmaceutical is a pharmaceutical preparation that has a radionuclide in its composition. Radiopharmaceuticals are used in nuclear medicine mainly for diagnosis but also for therapeutic purposes. They are almost invariably administered intravenously, although there are a few oral radiopharmaceuticals and even fewer gaseous preparations. Also, intradermal/subcutaneous injections are used when administering radiopharmaceuticals for sentinel node detection.

A radiopharmaceutical can be a simple chemical species such as the ions $^{131}I^-$, $^{201}Tl^+$ and $^{223}Ra^{2+}$, which are supplied as the salt forms $Na^{131}I$, $^{201}TlCl$ and $^{223}RaCl_2$, respectively. However, most radiopharmaceuticals have two basic components: a chemical moiety, carrying or not an active biomolecule, and a suitable radionuclide. The radionuclide provides the signal for detection outside the body, after administration, permitting assessment of the morphological structure or the physiological function of the target organ or system. When used for therapy, radiopharmaceuticals can deliver a radiation dose to target tissues [1–3].

In spite of the increased use of radiopharmaceuticals based on positron-emitting radionuclides for PET imaging, technetium-99m radiopharmaceuticals still have an important role in conventional nuclear medicine owing to the availability and affordability of the molydenum-99/technetium-99m ($^{99}Mo/^{99m}Tc$) generator and the wide variety of kits for preparation of the final radiopharmaceutical. In the early 1970s, the introduction of the lyophilised diethylenediamine penta-acetic acid (DTPA), pre-mixed with stannous tin to reduce pertechnetate, was a remarkable event that implemented the use of ^{99m}Tc compounds based on kits. Since then, due to the highly versatile chemistry of ^{99m}Tc, it has been possible to develop a variety of ^{99m}Tc compounds useful for many diagnostic procedures.

The first generation of ^{99m}Tc radiopharmaceuticals was developed by taking advantage of intrinsic properties (e.g. size, form, polarity) of the ^{99m}Tc compounds that determine their absorption, distribution, metabolism and excretion after administration. Most of these radiopharmaceuticals are still in use in nuclear medicine departments. They include the ^{99m}Tc-phosphonates, ^{99m}Tc-DTPA, ^{99m}Tc-colloids and ^{99m}Tc-macroaggregates of albumin (MAA) for imaging of bone, kidney, lymphatic system and lung perfusion, respectively.

The search for new and more efficacious ^{99m}Tc radiopharmaceuticals progressed with the design of new ligands that afforded ^{99m}Tc complexes with well-characterised molecular structures. Those studies led to the development of the so-called second generation of ^{99m}Tc radiopharmaceuticals, which includes brain and cardiac imaging agents such as ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO) and ^{99m}Tc-methoxyisobutylisonitrile (MIBI). The more recent ^{99m}Tc radiopharmaceuticals correspond to the generation of target-specific radiopharmaceuticals based on selected biomolecules for specific delivery of the radionuclides to biological targets such as antigens or receptors associated with disease. The design of these radiopharmaceuticals requires that the labelling procedure does not affect the biological activity of the biomolecule. The most common strategy comprises four components: a target-specific vector (e.g. monoclonal antibodies or their fragments, bioactive peptides), a bifunctional chelator, a linker and the radionuclide. The bifunctional chelator has the dual function of binding the ^{99m}Tc (or any other radiometal) and incorporating a chemically reactive functional group to attach the biomolecule responsible for the biological specificity of the final ^{99m}Tc compound.

PREPARATION OF RADIOPHARMACEUTICALS

Radiopharmaceutical preparation should be performed in high labelling yield and should provide a final product with high radiochemical, chemical, physical and microbiological stability, taking into consideration the following objectives: (1) *safety* for both the patient and the operator personnel; (2) *efficacy* to ensure relevant diagnostic information or optimal therapeutic effect; (3) *uptake at the site of action* to effectively target the required organ or system and remain there for sufficient time [2, 3].

The special characteristics of radiopharmaceuticals introduce some problems that are quite unusual when compared with non-radioactive pharmaceuticals. Most of these problems relate to the stability of the preparation and are due to the low concentration of the active compound. Furthermore, the expected radioactivity at the dispensing time may not be available owing to not only the radioactive decay but also adsorption or volatilisation of the radionuclide. The masses of radionuclides in a typical radiopharmaceutical are very small, so they are likely to adhere to vial surfaces. Moreover, high specific activities and high radioactive concentrations may be responsible for degradation by radiolysis. High dilution can also induce stability problems. Therefore, the concentration of the final

solution has to be optimised. Certain compounds protect against radiolysis as they act as scavengers for the free radicals produced by the internal radiation. Benzyl alcohol, a common preservative used to prevent microbial contamination, also acts as a scavenger.

Radiopharmaceutical preparations also include suspensions of aggregated particles. Particles should be prepared with non-toxic, non-antigenic and biodegradable materials, most frequently human serum albumin (HSA). The methodology for production of aggregates involves the denaturation of HSA solution chemically or by heating. The main disadvantage of the preparation is that particle size and shape are not well defined. The principal considerations to take into account with these preparations are the number of particles and the size of particles. Too few particles lead to unsatisfactory results, whereas too many may be hazardous to the patient. Larger particles may block some of the greater blood vessels and cause pulmonary hypertension, while smaller particles may be retained by the reticuloendothelial system.

Most radiopharmaceuticals are prepared using lyophilised kits since they considerably facilitate the radiopharmacy's practice. A kit is a prepacked set of sterile reagents of guaranteed pharmaceutical quality designed for the easy preparation of a specific radiopharma-

ceutical. The most common are those for labelling with ^{99m}Tc.

The widespread use of kits is related to their availability; their rapid and easy preparation; the closed procedure for kit radiolabelling; the stability, sterility and pyrogen-free quality of the main starting reagents; and the reproducibility, reliability and long shelf-life of the reagent mixture. Generally, the kit vials contain all the components for the formulation, either solutions or suspensions, with the exception of the radionuclide. Lyophilisation is performed to render the freeze-dried mixture ready for solubilisation in aqueous solution. Typically, radiolabelling is accomplished simply by addition of the radionuclide, e.g. ^{99m}TcO$_4^-$. Boiling may be required for some preparations.

The purpose and nature of the ^{99m}Tc kit components are as follows:

- *Ligand:* This is the most important constituent of a technetium cold kit as the radiopharmaceutical is the result of the radiolabelled ligand.
- *Reductant:* For practical reasons (water solubility, stability, low toxicity and effectiveness at room temperature), stannous salts (most commonly stannous chloride) are the preferred reductants in kit formulations. The reductant influences the oxidation state of ^{99m}Tc and, if a mixture of complexes is formed, the proportion of

each one. The amounts of Sn^{2+} and ligand in the kit are very important since too much tin induces hydrolysis of tin and consequently the production of hydrolysed-reduced ^{99m}Tc, which may compete with the formation of ^{99m}Tc complex. Too little tin, on the other hand, may lead to incomplete reduction of pertechnetate to the required oxidation state and consequently an unreliable yield of ^{99m}Tc complex.

- *Additives:* Additives or preservatives are added to kits to ensure their stability and efficacy. They must not react with any other ingredient of the radiopharmaceutical and should prevent its degradation. They include antioxidants, buffers, solubilising agents, fillers and transfer chelators. *Antioxidants* avoid degradation by oxidation since they compete with reductant to react with oxygen accidentally introduced into the preparation. They may also help in reductant conservation in the event of oxidant formation by radiolysis. Examples are ascorbic acid and gentisic acid. *Buffers* are also often used since radiochemical purity and biodistribution are pH dependent. *Solubilising agents* are used to increase the solubility in aqueous solutions of highly lipophilic ^{99m}Tc complexes such as ^{99m}Tc-MIBI. Surfactants may also be useful to prevent particle aggregation. Additives, such as gelatine,

act as stabilisers, forming a protective colloid on the primary particles. *Fillers* are used to improve the aspect of the lyophilised kit; sodium chloride and mannitol are the most common. *Transfer chelators* are additional ligands added to the kit formulation to maximise the yield of the desired complex formation through the process of ligand exchange. Ligands which form weak ^{99m}Tc complexes like gluconate, tartrate and citrate have been used. These ligands are used in cases of slow complex formation relative to the formation of hydrolysed-reduced ^{99m}Tc, as exemplified below for radiolabelling of ^{99m}Tc-mercaptoacetyltriglycine (MAG3) and ^{99m}Tc-tetrofosmin.

MOST COMMON DIAGNOSTIC RADIOPHARMACEUTICALS

Currently, most ^{99m}Tc radiopharmaceuticals are still of the first- and second-generation types [4]. The most commonly used in daily routine at nuclear medicine departments are discussed below, together with a few examples of the target-specific ^{99m}Tc radiopharmaceuticals in clinical use. A summary of their kit composition and labelling procedures is given in Table 1. Other radiopharmaceuticals, with isotopes such as indium-111 (^{111}In) and iodine-123 (^{123}I), are also briefly described [1–4].

Radiopharmaceutical	Clinical indication	Pharmaceutical ingredient	Other ingredientes / excipients	Labelling (Activity, volume, incubation)	Shelf life after labelling
^{99m}Tc-Colloids (e.g. Albumin nanocolloids)	Lymphoscintigraphy and Sentinel lymph node imaging , Bone marrow and inflammation imaging	Human albumin colloidal particles	Stannous chloride dehydrate, glucose anhydrous, poloxamer 238, sodium phosphate dibasic, anhydrous, sodium phytate, anhydrous	0.18 – 5.55 GBq Na ^{99m}TcO$_4$, 1-5 ml, Invert carefully, 5 - 10 min	6 h, 2 – 25 ºC Shake before withdrawing a dose
^{99m}Tc-Colloids (e.g. sulphur colloids)	Lymphoscintigraphy and bone marrow imaging; Gastroesophageal reflux scintigraphy	Sodium thiosulfate anhydrous	Vial:, Edetate disodium, Gelatine; Syringe A: 1.5 ml of 0.148 N hydrochloric acid solution; Syringe B: 1.5 mL aqueous solution of sodium biphosphate anhydrous and sodium hydroxide.	Up to 18.5 GBq Na^{99m}TcO$_4$, 1-3 ml, swirl; Add syringe A, boiling water, 5 min, cool 3 min; Add syringe B and swirl	6 h, 15 - 30 ºC
^{99m}Tc-DTPA	Dynamic renal scintigraphy; Measurement of glomerular filtration rate	Calcium trisodium diethylene-triamine pentaacetate	Stannous chloride dihydrate, gentisic acid and sodium chloride	Up to 11.1 GBq Na^{99m}TcO$_4$, 2-10 ml, 15 -30 min at 15 -25ºC	8 h, 25ºC
^{99m}Tc-DMSA	Functional and renal scintigraphy	Dimercaptosuccinic acid (Succimer)	Stannous chloride dihydrate inositol, ascorbic acid, sodium hydroxide	Up to 3.7 GBq Na^{99m}TcO$_4$, 5 ml (1 min shake),15 min	5 h, above 25ºC
^{99m}Tc-ECD	Cerebral perfusion Imaging;	N,N'-1,2-ethylenediy(bis-L-cysteine diethyl ester (Bicisate dihydrochloride)	Vial A: Stannous chloride dihydrate, disodium edetate, manitol, hydrochloric acid Vial B: disodium phosphate heptahydrate, sodium dihydrogen phosphate monohydrate,	3.7 GBq Na^{99m}TcO$_4$, 2 ml into Vial B + 1 ml Vial A, 30 min, r.t.	8 h, ≤25 ºC
^{99m}Tc-HMDP	Bone imaging	Hydroxymethylenediphosphonic acid (Oxidronic acid)	Stannous chloride dihydrate, ascorbic acid, sodium chloride	Up to 11.1 GBq Na^{99m}TcO$_4$, 2 – 10 ml (2 min swirl), 15 min	8 h, 2 – 8 ºC
^{99m}Tc-HMPAO	Cerebral perfusion Imaging; Radiolabeling of autologous leukocytes (preparation without stabilizer)	[(RR,SS)-4.8-diaza-3,6,6,9-tetra-methylundecane-2,10-dione bisoxime], (Exametazime)	Stannous chloride dihydrate, sodium chloride + Methylene blue sodium phosphates/sodium chloride (Stabilizer)	0.37 – 2.0 GBq Na^{99m}TcO$_4$, 5 ml, 10 sec Or Add 2 ml stabilizer within 2 min	30 min (without stabilizer) Or 4 h (with stabilizer)
^{99m}Tc-MAA	Pulmonary perfusion imaging	Macroaggregates of human albumin	Stannous chloride dihydrate, human albumin, sodium chloride	2,22 GBq Na^{99m}TcO$_4$ maximum, 5 – 10 ml, 15 min, r.t.	6 -8 h, 2 – 8 ºC

Table 1: *Information regarding the composition and preparation of most commonly used radiopharmaceuticals.*

Radiopharmaceutical	Clinical indication	Pharmaceutical ingredient	Other ingredientes / excipients	Labelling (Activity, volume, incubation)	Shelf life after labelling
^{99m}Tc-MAG3	Functional and renal scintigraphy	Mercaptoacetyl triglycine (Mertiatide)	Stannous chloride dihydrate, sodium (R,R)-tartrate dihydrate, sodium hydroxide, hydrochloric acid	Up to 2.5 GBq Na^{99m}TcO$_4$, 2 ml, 15 min (no heating)	6 h
^{99m}Tc-MDP	Bone imaging	Methylenediphosphonic acid (Medronic acid)	stannous chloride dihydrate, ascorbic acid	Up to 11.1 GBq Na^{99m}TcO$_4$, 2 – 8 ml (1 min swirl), 1-2 min	6 h, ≤25 ºC
^{99m}Tc-Mebrofenin	Hepatobiliary imaging agent	(2,2'-[[2-[(3-Bromo-2,4,6-trimethylphenyl)-amino]-2-oxoethyl] imino] bisacetic acid) (Mebrofenin)	Stannous fluoride dihydrate, methylparaben, propylparaben	Up to 3.7 GBq Na^{99m}TcO$_4$, 1 – 5 ml, 15 min	18 h, 20 – 25ºC
^{99m}Tc-MIBI	Myocardial perfusion imaging	2-methoxyisobutylisonitrile (MIBI)	Sodium citrate dihydrate, L-cysteine hydrochloride monohydrate, mannitol, stannous chloride dihydrate,	0.92 – 5.55 GBq Na^{99m}TcO$_4$, 1-3 ml, Vigorous shaking, 10 min in boiling water, cool 15 min r.t.	6 h, 15 – 25ºC
^{99m}Tc-Tektrotyd	Scintigraphy of primary and metastatic neuroendocrine tumors bearing somatostatin receptors.	HYNIC-[D-Phe^1, Tyr^3-Octreotide]	Vial I: Tricine, Stannous chloride dihydrate, manitol; Vial II: EDDA; disodium hydrogen phosphate dodecahydrate, sodium hydroxide	1 ml water into Vial I; Transfer 0.5 ml to Vial II + Up to 2.2 GBq Na^{99m}TcO$_4$ 1 ml maximum), 80ºC, 20 min, cool 30 min	6 h, ≤25 ºC
^{99m}Tc-Tetrofosmin	Myocardial perfusion imaging	1,2-bis[bis(2-ethoxyethyl)phosphino]ethane (Tetrofosmin)	Stannous chloride dihydrate, disodium sulphosalicylate, sodium D-gluconate, sodium hydrogen carbonate	Up to 8.8 GBq Na^{99m}TcO$_4$, 4 – 188 ml (mix 10 sec), 15 min	12 h, 2 -25ºC
^{111}In-Octreotide	Scintigraphy of primary and metastatic neuroendocrine tumors bearing somatostatin receptors.	Pentetreotide (DTPA conjugated Octreotide),	Gentisic acid, trisodium citrate, anhydrous, citric acid anhydrous, inositol	Transfer ^{111}In chloride into reaction vial.Gently swirl, 30 min, ≤25 ºC	6 h, ≤25 ºC

Table 1: *Information regarding the composition and preparation of most commonly used radiopharmaceuticals.*

99mTc-diphosphonates

^{99m}Tc-labelled diphosphonate complexes are routinely used for bone imaging, methylene diphosphonic acid (MDP; trivial name = medronic acid) and hydroxymethylene diphosphonic acid (HMDP, trivial name = oxidronic acid) being the most common ones.

^{99m}Tc-MDP and ^{99m}Tc-HMDP correspond to a mixture of technetium complexes whose composition depends strongly on the kit formulation and labelling conditions. The molecular structures of these complexes is not fully established but it is considered that they must consist of Tc(III) and Tc(IV) species. The bone uptake of ^{99m}Tc-diphosphonates is held to result from chemisorption processes at the bone surface. There is increased uptake in areas of greater surface area associated with increased bone metabolism (e.g. fracture, infection and tumour).

99mTc-mebrofenin

^{99m}Tc complexes with derivatives of iminodiacetic acid (IDA) are useful as hepatobiliary agents. Among the developed IDA derivatives, mebrofenin (2,2'-[[2-[(3-bromo-2,4,6-trimethylphenyl)-amino]-2-oxoethyl]imino] bisacetic acid) is the most commonly used in nuclear medicine. Most probably, ^{99m}Tc-mebrofenin corresponds to a mono-anionic and lipophilic ^{99m}Tc(III) complex

that contains two tridentate IDA ligands coordinated to the metal.

Like other IDA derivatives, ^{99m}Tc-mebrofenin is rapidly extracted from blood into bile by active transport via the anionic site on the hepatocyte membrane; this is the same site as is used for transport of bilirubin, and ^{99m}Tc-mebrofenin is therefore useful for the assessment of hepatobiliary function. ^{99m}Tc-mebrofenin is cleared through the hepatobiliary system but elevated serum bilirubin levels increase its renal excretion.

99mTc-DTPA

^{99m}Tc-DTPA was the first ^{99m}Tc radiopharmaceutical for renal imaging. The chemical structure of ^{99m}Tc-DTPA has not been fully determined but it is generally considered that it corresponds to a Tc(IV) complex with the metal coordinated by three nitrogen atoms and three oxygen atoms from the DTPA ligand.

^{99m}Tc-DTPA undergoes rapid blood clearance by glomerular filtration and is excreted unchanged into the urine. It is used to assess kidney function in a variety of conditions and to measure the glomerular filtration rate. A variable but low percentage of ^{99m}Tc-DTPA binds to the serum proteins, and the glomerular filtration rate is consequently lower than when using standard insulin clearance.

99mTc-DMSA

^{99m}Tc can form different complexes with DMSA (meso-2,3-dimercaptosuccinic acid) depending on the labelling conditions (e.g. pH, concentration of ^{99m}TcO$_4^-$, Sn(II)/Sn(IV) ratio, oxygen concentration and incubation time).

The renal imaging agent is obtained at low pH (pH 2–3) by labelling DMSA kits. It is a Tc(III) complex with a proposed structure (Tc(III)(DMSA)$_2$) that involves the coordination of two DMSA ligands to each Tc atom. After intravenous administration, ^{99m}Tc-DMSA (Tc(III)(DMSA)$_2$) accumulates slowly in the renal cortex, primarily in the cells of the proximal tubule. It is indicated for kidney imaging for the evaluation of renal parenchymal disorders. However, if the DMSA labelling is performed at higher pH, stereoisomeric ^{99m}Tc(V) complexes are formed (Tc(V)(DMSA)$_2$), having a Tc=O core coordinated by four thiolate groups of two DMSA ligands, which have been found to localise in medullary thyroid carcinoma.

99mTc-MAG3

^{99m}Tc-MAG3 is a hydrophilic and anionic ^{99m}Tc(V) oxocomplex stabilised by a tetradentate MAG3 ligand, coordinated through one sulphur atom and three nitrogen atoms. The original kit formulation for preparing ^{99m}Tc-MAG3 contains an S-benzoyl protected mercaptoacetyltriglycine ligand (betiatide).

Upon heating at 100°C, the benzoyl protecting group is released and the coordinating thiol (SH) group becomes available to coordinate the [Tc^V=O]$^{3+}$ core. More recently, a new formulation was introduced containing unprotected MAG3 that allows labelling at room temperature. For both formulations, the labelling involves a transchelation reaction between [^{99m}Tc^V-tartrate] and the incoming MAG3 ligand.

^{99m}Tc-MAG3 is highly bound to plasma proteins following intravenous injection. However, the protein binding is reversible and the radiotracer is rapidly excreted by the kidneys, in its intact form and primarily via active tubular secretion; it is therefore of value for the assessment of renal function.

99mTc-HMPAO

^{99m}Tc-HMPAO was the first approved ^{99m}Tc radiopharmaceutical for brain imaging. HMPAO ([4,8-diaza-3,6,6,9-tetramethylundecane-2,10-dione bisoxime; trivial name = exametazime) exists in two diastereomeric forms, D,L- and meso-, and acts as a tetradentate ligand that forms neutral and lipophilic Tc(V) oxocomplexes. The kit contains the D,L-racemate of HMPAO because its Tc(V) oxocomplex has a better brain retention compared with the congener complex obtained for the meso form of HMPAO. The Tc(V) com-

plex with D,L-HMPAO is unstable in aqueous solution due to the conversion of the primary lipophilic complex to a secondary hydrophilic complex, which is enhanced by the presence of reducing agents.

The shelf-life of the reconstituted HMPAO kit without addition of a stabiliser is 30 min, as the preparation shows a radiochemical purity lower than 80% after this time. After labelling, the addition of cobaltous chloride (CoCl$_2$) extends the shelf-life of ^{99m}Tc-HMPAO to 4 h. CoCl$_2$ stabilises the ^{99m}Tc-HMPAO preparation by oxidizing the excess of Sn(II) and by acting as a radical scavenger. ^{99m}Tc-HMPAO can also be applied in the labelling of autologous leukocytes. For this purpose, only the non-stabilised ^{99m}Tc-HMPAO can be used. It is generally considered that the brain uptake of ^{99m}Tc-HMPAO is due to the primary lipophilic complex and that brain retention is due to its intracellular conversion to the non-diffusible secondary hydrophilic complex.

^{99m}Tc-ECD

^{99m}Tc-ECD (ECD = *N,N′*-1,2-ethylenediyl(bis-L-cysteine diethyl ester) is a neutral and lipophilic Tc(V) oxocomplex suitable for brain perfusion imaging. The Tc=O^{3+} core is coordinated by two nitrogen atoms and two sulphur atoms from the tetradentate and trianionic ECD ligand, which confers a high stability to ^{99m}Tc-ECD

in aqueous medium. The ^{99m}Tc-coordination of the ECD backbone is quite stable. However, the presence of two ester functionalities makes it labile to enzymatic hydrolysis in vivo.

The ECD ligand exists as the L,L and D,D isomers. The ^{99m}Tc(V) complexes of both isomers show brain uptake but only the L,L isomer is retained in the brain. As a lipophilic complex, ^{99m}Tc-ECD localises in the brain by passive diffusion of its unionised form through the blood-brain barrier. Slow hydrolysis of the ester groups in blood and its rapid hydrolysis in brain tissue lead to the formation of more hydrophilic and polar metabolites (carboxylic acid derivatives), which explains the brain retention of ^{99m}Tc-ECD.

^{99m}Tc-MIBI

^{99m}Tc-MIBI (MIBI = 2-methoxyisobutylisonitrile) is a lipophilic and cationic ^{99m}Tc(I) complex, showing the Tc atom coordinated by six MIBI ligands. In the kit, MIBI is incorporated in the form of a Cu(I) complex to mitigate its toxicity and facilitate lyophilisation, since MIBI alone is a volatile liquid. The labelling is performed at 100°C to promote the reduction from Tc(VII) to Tc(I) and facilitate the release of MIBI from its Cu(II) complex to coordinate to Tc(I).

After intravenous injection, ^{99m}Tc-MIBI is taken up into the heart by passive diffusion through the myocyte membranes, in

proportion to myocardial blood flow and without involvement of the Na,K-ATPase membrane pump. Uptake is associated with intact cellular and mitochondrial membrane potentials, with a strong accumulation in myocyte mitochondria. The heart retention remains virtually unchanged after the time of injection. ^{99m}Tc-MIBI is used to assess myocardial perfusion in ischaemia and infarction conditions.

^{99m}Tc-tetrofosmin

^{99m}Tc-tetrofosmin (tetrofosmin = 1,2-bis[bis(2-ethoxyethyl)phosphino] ethane) is a lipophilic and cationic compound that corresponds to a Tc(V) dioxocomplex ([^{99m}Tc-(tetrofosmin)$_2$O$_2$]$^+$) containing two bidentate ether functionalised diphosphine ligands. Unlike ^{99m}Tc-MIBI, the preparation of ^{99m}Tc-tetrofosmin does not require heating and involves a transchelation reaction with gluconate as the transfer ligand, which avoids the formation of low-valent Tc complexes [e.g. Tc(III)] that could arise due to the reducing properties of the tetrofosmin ligand.

Similar to ^{99m}Tc-MIBI, ^{99m}Tc-tetrofosmin is taken up into the heart in proportion to myocardial blood flow without redistribution. ^{99m}Tc-tetrofosmin undergoes a faster hepatic clearance comparative to ^{99m}Tc-MIBI. Its uptake by the myocytes occurs by potential-driven diffusion across

the cellular and mitochondrial membranes. However, unlike ^{99m}Tc-MIBI, a significant part of ^{99m}Tc-tetrofosmin is bound in the intracellular cytosol of myocytes and is not located in the mitochondria.

^{99m}Tc-MAA

^{99m}Tc-MAA, macroaggregated albumin labelled with ^{99m}Tc, is primarily used for perfusion lung imaging. The MAA particles range from 10 to 90 μm in size and are formed by denaturation of the protein when stannous chloride and HSA are heated under controlled conditions. The nature of the ^{99m}Tc binding to MAA has not been fully elucidated in terms of the type of complexation and oxidation state of technetium. However, it is generally considered that the Sn(II) reduces disulphide bonds in the protein and the reduced technetium is coordinated by the formed SH groups.

The lung localisation of ^{99m}Tc-MAA involves the microembolisation (i.e. physical trapping) of the particles in capillaries and precapillary arterioles. The diameter of capillaries and precapillary arterioles is about 10 μm and 20–30 μm, respectively. ^{99m}Tc-MAA particles are slighter larger and, when injected intravenously, the first capillary beds encountered are the lungs. No particles larger than 150 μm should be present to avoid lung embolism.

99mTc-albumin colloid

This radiopharmaceutical corresponds to HSA microcolloid (also called nanocolloid) labelled with ^{99m}Tc. It contains very small particles: the mean size of the particles is 0.03 µm and almost 95% are smaller than 0.08 µm. The preparation of ^{99m}Tc-albumin colloid involves the initial reduction of Tc(VII) to a lower oxidation state with binding of the reduced Tc to albumin microcolloid, most probably through coordination by the protein SH groups, as mentioned above for ^{99m}Tc-MAA.

After intravenous injection, ^{99m}Tc-albumin colloid undergoes rapid blood clearance upon phagocytosis by reticuloendothelial cells in liver, spleen or bone marrow. After subcutaneous injection into connective tissue, 30%–40% of the ^{99m}Tc-albumin colloid particles are filtered into lymphatic capillaries, are then transported along the lymphatic vessels to regional lymph nodes and main lymphatic vessels, and are finally trapped in the reticular cells of functioning lymph nodes. ^{99m}Tc-albumin colloid is useful in lymphoscintigraphy to demonstrate the integrity of the lymphatic system and to differentiate venous from lymphatic obstruction, as well as for preoperative imaging and intraoperative detection of sentinel lymph nodes in several carcinomas.

99mTc-sulphur colloid

^{99m}Tc-sulphur colloid (^{99m}Tc-SC) is a colloidal dispersion of sulphur particles labelled with ^{99m}Tc. It is the only technetium radiopharmaceutical in clinical use in nuclear medicine that contains ^{99m}Tc in the non-reduced Tc(VII) oxidation state. Technetium appears in this oxidation state due to the formation of insoluble and quite stable technetium heptasulphide, Tc_2S_7. The formation of ^{99m}Tc-SC involves the following major steps: (1) upon boiling in acidic conditions, there is hydrolysis of thiosulphate with release of elemental sulphur, which aggregates, forming particles ranging in size from 0.1 to 1.0 µm; (2) ^{99m}Tc$_2$S$_7$ is formed during the reaction and becomes incorporated into the sulphur particles. The kit composition includes gelatine, which controls particle size and aggregation by forming a negatively charged protein coat on the particles, causing them to repel each other. EDTA is also present to chelate any Al^{3+} ion that may be present in the ^{99m}TcO$_4^-$ eluate and avoid its flocculation, which would lead to accumulation of excessively large particles in the lungs.

^{99m}Tc-sulphur colloid has different uses in nuclear medicine, such as liver/spleen imaging and bone marrow studies. Another common application is in lymphoscintigraphy to identify sentinel lymph nodes in melanoma patients.

99mTc-Tektrotyd

This target-specific radiopharmaceutical corresponds to the mixed-ligand complex ^{99m}Tc-EDDA/HYNIC-Tyr^3-octreotide, carrying a cyclic bioactive peptide (octreotide derivative) that recognises some of the somatostatin (sst) receptors, particularly subtype 2 and, to a lesser extent, subtypes 3 and 5.

^{99m}Tc-Tektrotyd contains [D-Phe^1,Tyr^3]-octreotide (TOC) as the sst-binding peptide and hydrazinonicotinic acid (HYNIC) as a ^{99m}Tc coordinating moiety. Furthermore, it is stabilised by EDDA (ethylenediamino-N,N'-diacetic acid), which acts as a co-ligand and coordinates to ^{99m}Tc following a tricine/EDDA exchange reaction. The molecular structure of ^{99m}Tc-EDDA/HYNIC-Tyr^3-octreotide has not been fully assessed. Nevertheless, it is generally accepted that it contains ^{99m}Tc(V) coordinated by one HYNIC moiety and two EDDA ligands.

^{99m}Tc-Tektrotyd is indicated for the detection of pathological lesions in which sst are overexpressed, namely neuroendocrine tumours (NETs). In NETs, sst receptor imaging is useful for primary diagnosis, staging of the disease, selection of patients for targeted therapy and monitoring of its efficacy.

111In-pentetreotide

^{111}In-pentetreotide ([^{111}In-DTPA^a]-oct-reotide) has been in clinical use since the 1990s and was the first radiopharmaceutical to be approved for peptide receptor imaging. ^{111}In-pentetreotide contains the biologically active ring of octreotide and a DTPA unit that is covalently bound to the D-phenylalanine group. ^{111}In-pentetreotide specifically binds to sst receptors, with particular affinity to subtypes 2 and 5.

^{111}In-pentetreotide, like ^{99m}Tc-Tektrotyd, is used for scintigraphic localisation of primary and metastatic NETs that bear sst receptors. In the past few years, however, its use has started to be replaced by gallium-68-labelled somatostatin analogues, which provide higher sensitivity and resolution using PET imaging.

123/131I-MIBG

Metaiodobenzylguanidine (MIBG) is a noradrenaline analogue that enters neuroendocrine cells by an active uptake mechanism via the adrenaline transporter and is stored in the neurosecretory granules, which results in specific accumulation in tissues of neuroendocrine origin. MIBG can be labelled with ^{123}I or ^{131}I. ^{123}I-MIBG is preferred for diagnostic procedures due to a more favourable dosimetry. However, ^{131}I-MIBG might be preferred to estimate tumour uptake and retention, within MIBG therapy planning and prior

to the administration of therapeutic doses of ^{131}I-MIBG.

$^{123/131}$I-MIBG can be obtained "ready for use" and with a radiochemical purity greater than 95% at expiry, as either "carrier-added" or "non-carrier-added" (n.c.a.) products. $^{123/131}$I-MIBG is used to detect and treat tumours of neuroendocrine origin, particularly those of neuroectodermal origin (phaeochromocytomas, paragangliomas and neuroblastomas). In addition, it can be useful for the study of disorders of sympathetic innervation, for example in ischaemic and non-ischaemic cardiomyopathy.

^{123}I-ioflupane

^{123}I-ioflupane (methyl (1R,2S,3S,5S)-8-(3-fluoropropyl)-3-[4-(^{123}I)iodophenyl]-8-azabicyclo[3.2.1]octane-2-carboxylate) is a cocaine analogue that binds to the dopamine transporter (DAT). Its structure contains a fluoropropyl group that enhances its selectivity towards the DAT over other neurotransmitter transporters.

^{123}I-ioflupane is commercially available as a high-specific n.c.a. product ready for use, and it is used for the differential diagnosis between essential tremor and degenerative parkinsonism. ^{123}I-ioflupane crosses the intact blood-brain barrier, distributes to the brain and preferentially binds to the DAT located predominantly on the nigrostriatal dopaminergic neurons. In healthy humans, the binding of ^{123}I-ioflupane to DAT allows the visualisation of the striata as two "half-moon"-shaped hot spots on SPECT images. However, loss of the nigrostriatal dopaminergic neurons results in loss of the DAT associated with those neurons and absence of those hot spots.

QUALITY CONTROL OF RADIOPHARMACEUTICALS

Quality control procedures are essential to ensure the quality of any radiopharmaceutical. Many of them are performed by manufacturers, but the final preparation of radiopharmaceuticals formulated as kits, the dispensing of individual doses and the radiolabelling of patient's autologous samples are accomplished at the radiopharmacy and the ultimate responsibility lies with the radiopharmacist. Thus, quick and discriminative tests should be applied to the dispensed radiopharmaceuticals before release [3, 5].

Quality control procedures include the assessment of radiochemical, radionuclidic and chemical purity as well as pharmaceutical tests that comprise physicochemical and biological testing [5].

RADIOCHEMICAL PURITY

Radiochemical purity (RCP) is defined as the proportion, expressed as a percentage, of the total radioactivity in the radiopharmaceutical that is associated with the desired radiolabelled chemical species. For most diagnostic radiopharmaceuticals, a RCP above 95% is desirable since the radiochemical impurities have a different biodistribution that will affect the quality of the image and consequently delay accurate diagnosis. However, for a few radiopharmaceuticals such a level of purity is not achievable (e.g. ^{99m}Tc-HMPAO: RCP >80%).

Radiochemical impurities may arise from an incomplete radiolabelling reaction or decomposition due to any factor (e.g. chemical impurities, temperature or pH, light, oxidizing or reducing agents).

As regards conventional ^{99m}Tc radiopharmaceuticals, the three types of radiochemical species that can be determined are: (1) ^{99m}Tc ligand of interest (desirable radiochemical form) and the most common radiochemical impurities, (2) free ^{99m}Tc pertechnetate (^{99m}TcO$_4^-$) and (3) hydrolysed-reduced ^{99m}Tc (insoluble ^{99m}Tc dioxide and/or ^{99m}Tc tin colloid). Nevertheless, for certain radiopharmaceuticals a fourth radiochemical impurity may also be present, i.e. ^{99m}Tc labelled to other secondary compounds (e.g. ^{99m}Tc-HMPAO may present radiolabelled hydrophilic species) or transfer ligands (e.g. ^{99m}Tc-glu-conate in ^{99m}Tc-tetrofosmin and ^{99m}Tc-tartrate in ^{99m}Tc-MAG3).

To assess the RCP, some form of physicochemical separation technique must be used to separate the various radiochemical species prior to the quantification of radioactivity and subsequent calculation of their proportion. Numerous methodologies can be employed, including thin-layer chromatography (ITLC), paper chromatography (PC), gel permeation chromatography, high-performance liquid chromatography (HPLC) and gel electrophoresis. The RCP analysis must be quick, accurate, based on relatively easy procedures and economical to gain the maximum amount of information in a minimum amount of time. Planar chromatography that includes PC and ITLC is the separation method universally employed in RCP determination, although solid phase extraction separation methods based on cartridges are coming into use.

Planar chromatography

In planar chromatography, the solvent (mobile phase) migrates up the strip, moving the radiopharmaceutical solution through the stationary medium (paper or ITLC) and separating the various radiochemical species. Intrinsic properties of the radiopharmaceuticals, such as particle size, molecular mass and charge,

determine the distance migrated. Solvent polarity also influences the solubility of each radiochemical species. Thus, solvent must be selected on the basis of its ability to separate the radiochemical forms on each stationary phase. The most commonly used solvents are: 0.9% saline, water, methyl ethyl ketone (MEK), acetone and methanol.

Miniaturised chromatography procedures are the most extensively used. The chromatographic strips or plates are cut into small sizes and the line of application (origin) and the expected solvent front are marked (Figure 1). A few microlitres of the radiopharmaceutical preparation is applied on the origin line. The chromatography strip is then developed in the appropriate solvent in a chromatography chamber, ensuring that the spotting point is not immersed. Following solvent migration along the strip to the solvent front, the strip is removed from the chamber and allowed to dry. Radiochemical species are separated and the strips can be either scanned with a radiochromatogram scanner or cut and counted for activity in a dose calibrator, gamma counter or any other adequate equipment.

Each radiochemical species migrates a characteristic distance that is represented as the relative front (R_f) value. The R_f is defined as the ratio of the distance

travelled along the stationary phase by each radiochemical species to the distance travelled by the solvent front. The fraction of activity detected at each R_f value allows determination of the RCP. For many conventional ^{99m}Tc radiopharmaceuticals (see typical R_f values in Table 2), the RCP analysis usually requires two chromatographic systems
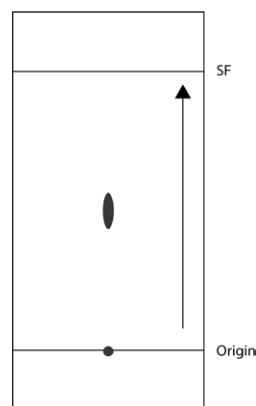

Figure 1: *Typical chromatography strip showing the position of the origin and solvent front (SF) lines*

to determine the percentage of each radiochemical impurity: ^{99m}TcO$_4^-$ and hydrolysed-reduced ^{99m}Tc. In the case of a particulate radiopharmaceutical such as a ^{99m}Tc-nanocolloid, neither the ^{99m}Tc colloids nor the hydrolysed-reduced ^{99m}Tc migrate, so both remain at the origin.

Radiopharma-ceutical	Chromatographic system (Solid phase / Mobile phase)	Radiopharmaceu-tical	Hydrolysed-re-duced ^{99m}Tc	^{99m}TcO$_4^-$	Other RC Impurities
^{99m}**Tc-Colloids** (e.g. Albumin nanocol-loids)	Whatman Nº1 or TLC-SA / metanol:water 85:15, v/v	0.0 (>95%)	-	0.7 – 1.0	-
^{99m}**Tc-Colloids** (e.g. sulphur colloids)	Whatman Nº1 or ITLC-SG / Acetone or saline	0.0	-	0.9 – 1.0	-
^{99m}**Tc-DTPA**	ITLC-SG/ Saline	0.9 - 1.0	0.0	0.9 - 1.0	-
	ITLC-SG / MEK	0.0	0.0	0.9 - 1.0	
^{99m}**Tc-DMSA**	Whatman Nº1 or ITLC-SG / MEK	0.0 (> 95%)	0.0	0.0 (≤ 2%)	-
^{99m}**Tc-ECD**	BaKer Silica gel / Ethyla-cetate	0.8 -1.0 (>94%)	0.0	0.0 – 0.2	-
^{99m}**Tc-HMDP**	Whatman 17 / Saline;	1.0	0.0	1.0	-
	Whatman 17 / meta-nol:water 85:15, v/v	0.0	0.0	0.4 – 1.0	
^{99m}**Tc-HMPAO**	ITLC-SA / MEK	0.8 – 1.0	0.0	0.8 – 1.0	0.0
	ITLC-SA / Saline	0.0	0.0	0.8 – 1.0	0.0
	Whatman Nº1/50% Acetonitrile	0.8 – 1.0	0.0	0.8 – 1.0	0.8 – 1.0
^{99m}**Tc-MAA**	Whatman Nº 1 / Acetone	0.0	-	0.8 – 1.0	-
^{99m}**Tc-MAG3**	ITLC-SA / MEK	0.0	0.0	0.8 – 1.0 (≤ 5%)	-
	ITLC-SA / H$_2$O	0.8 – 1.0	0.0 – 0.1 (≤ 2%)	0.8 – 1.0	
^{99m}**Tc-MDP**	Whatman Nº 1 or ITLC-SG/ Saline	0.9 - 1.0	0.0	0.9 - 1.0	-
	ITLC-SG / MEK	0.0	0.0	0.9 - 1.0	
^{99m}**Tc-Mebrofenin**	ITLC-SA / Saturated saline	0.0	0.0	0.9 – 1.0	-
	Whatman Nº1 / Ethylene glycol:water (1:1)	0.8 – 1.0	0.0	0.8 -1.0	
^{99m}**Tc-MIBI**	Baker Aluminium oxide /Ethanol	0.8 – 1.0	0.0	0.6 – 0.7	-
^{99m}**Tc-Tektrotyd**	ITLC-SG or ITLC-SA / MEK	0.0	0.0	0.8 – 1.0	-
	ITLC-SA / Water: Acetic acid (1:1)	0.8 -1.0	0.0	0.8 -1.0	
^{99m}**Tc-Tetrofosmin**	ITLC-SA / Aceton:Di-chloromethane 65:35 (v/v)	0.4 -0.5	0.0 – 0.1	1.0	-
^{111}**In-Octreotide**	ITLC-SG / 0.1 M Sodium citrate	0.0	-	1.0 (^{111}In-hy-drophilic impurities)	-

Table 2: *Recommended chromatographic systems for quality control of most commonly used radiopharmaceuticals*

Thus, the only radiochemical impurity that can be separated is free $^{99m}TcO_4^-$.

Table 2 presents some of the manufacturer-recommended chromatographic systems for quality control of conventional diagnostic radiopharmaceuticals. Sometimes, it is convenient to develop alternative RCP testing procedures. In such cases, the alternative procedure must be validated.

Solid phase extraction

Some manufacturers recommend the use of reverse-phase Sep-Pak® chromatography cartridges for the RCP determination (e.g. ^{99m}Tc-MAG3 and ^{111}In-octreotide). These Sep-Pak® cartridges work by solid-phase extraction, an extraction technique based on selective partitioning of one or more components between two phases, one of which is a solid sorbent and the other typically a liquid.

The Sep-Pak® C18 cartridge comprises a solid, non-polar sorbent that enables separation of the various radiochemical species that may either adsorb to the solid material or remain in the liquid phase. RCP analysis using these chromatography cartridges requires multiple steps:

1. **Preconditioning** of the sorbent with the organic solvent used to load the sample. The eluate is discarded. Thereafter, an equilibration step is performed with a low-strength solvent and the eluate

from this step is also discarded.
2. **Loading** the sample.
3. **Elution** of the fractions. The first elution step removes constituents that are less strongly retained on the sorbent. Then, the analytes are eluted using a solvent of higher eluting strength while more strongly adsorbed constituents are discarded with the sorbent. The elution of the fractions depends on the radiopharmaceuticals and should be carried out according to the manufacturer's instructions.

RADIONUCLIDIC PURITY

Radionuclidic purity is the ratio, expressed as a percentage, of the stated radionuclide activity to the total radioactivity. Radionuclidic impurities can have undesirable effects on the patient's overall radiation dose as well as on the image quality. Sometimes these impurities are radioisotopes of the same element as the desired radionuclide and cannot be removed, or they may be radioisotopes of an entirely different element. The most accurate method to determine radionuclidic impurities is the technique of gamma spectroscopy, which, however, is not commonly available at the radiopharmacy.

The radionuclidic purity test of particular interest in radiopharmacy relates to the most popular diagnostic radionuclide,

^{99m}Tc: the radionuclidic purity of ^{99m}Tc can be assessed by the ^{99}Mo *breakthrough test*, as ^{99}Mo contamination on the $^{99m}TcO_4^-$ can occur during a $^{99}Mo/^{99m}Tc$ generator elution. An approximate assay for ^{99}Mo breakthrough can be performed using a dose calibrator and a lead shield of around 6 mm thickness that attenuates the 140 keV gamma emission of ^{99m}Tc while allowing the penetration of a portion (around 50%) of the higher energy gamma photons of ^{99}Mo. Comparison of the attenuated ^{99}Mo with a standard allows rapid estimation of the contamination level. The activity of ^{99}Mo must not exceed 0.15 of the total ^{99m}Tc activity.

CHEMICAL PURITY

Chemical impurities are all non-radio-active materials that can either affect radiolabelling or directly produce adverse biological effects.

In $^{99}Mo/^{99m}Tc$ generators, alumina oxide (Al_2O_3) is used as the column material to which the ^{99}Mo radioactivity in the form of molybdate ion (MoO_4^{2-}) is bound. Alumina breakthrough results in the presence of significant quantities of aluminium ions (Al^{3+}) in ^{99m}Tc eluates, which can interfere in the radiolabelling reactions. This impurity can be measured by the *aluminium ion breakthrough test*, a colorimetric test of

the generator eluate. A drop of the eluate is placed on a test paper and compared with a drop of the same volume of a standard solution of Al^{3+}. If the colour of the eluate drop is less intense than that of the standard solution, the Al^{3+} concentration is acceptable.

OTHER ROUTINE QUALITY CONTROL TESTS

Appearance

The colour and clarity of each radio-pharmaceutical preparation should be checked. If a radiopharmaceutical is a true solution, no particulate matter should be present. ^{99m}Tc radiopharmaceutical preparations based on particles have varying degrees of turbidity in appearance, ranging from white to milky.

Particle size and number

To achieve the desired biodistribution, radiopharmaceuticals formulated as solutions or suspensions of particles must have the proper particle size and number for each clinical indication. The size of ^{99m}Tc-MAA particles can be determined using a light microscope and a haemocytometer. The total number of particles can be estimated from the number of particles in one millilitre, determined by counting the particles within the haemocytometer.

pH

To ensure stability and integrity, all radiopharmaceuticals must maintain the most appropriate pH. The pH range of most radiopharmaceuticals is 4–8.

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PET
RADIOPHARMACEUTICALS

by Lei Li Corrigan

7

INTRODUCTION

Positron emission tomography (PET) employs short half-life positron-emitting isotopes, such as carbon-11 (^{11}C; $t_{1/2}$ = 20.4 min) and fluorine-18 (^{18}F; $t_{1/2}$ = 109.7 min), for in vivo measurement of physiological processes. PET imaging has been widely used in both diagnostic medicine and clinical pharmaceutical research. The major clinical applications of PET are in oncology, cardiology and neurology. The use of positron-emitting isotopes in PET contrasts with single-photon emission computed tomography (SPECT), where labelling is performed using gamma-emitting isotopes such as technetium-99m (^{99m}Tc; $t_{1/2}$ = 6.01 h) and indium-111 (^{111}In; $t_{1/2}$ = 2.8 days).

Radionuclides used in PET imaging emit positrons upon decay. For ^{18}F, the decay results in the formation of an oxygen-18 atom and a positively charged beta particle (or positron) along with a neutrino. The positron subsequently annihilates with a nearby electron to produce two gamma quanta departing approximately 180° apart (Fig. 1). A cylindrical detector surrounding the imaging target collects the simultaneously emitted gamma quanta pair. Computer-aided image reconstruction of the data allows for the output of 3D images of the regions of interest. Because a pair of gamma quanta is detected in PET, compared with only one gamma quantum in SPECT, the spatial resolution of PET images is higher than that of SPECT images. However, the cost of PET is significantly higher than that of SPECT because of the cost of the cyclotron and the automatic radiolabelling process.
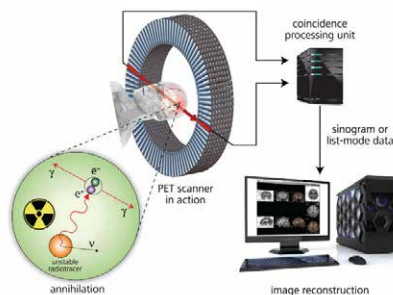

Figure 1: *Positron emission tomography*

The most widely used and studied PET tracer is [^{18}F]FDG. This tracer allows in vivo measurement of abnormal glucose metabolism, which is known to be a typical symptom in cancer patients. During the past 20 years, many automatic synthesisers and kits have been developed for the manufacture of [^{18}F]FDG and other ^{18}F-labelled tracers synthesised via nucleophilic fluorination of [^{18}F]fluoride. Other important ^{18}F-labelled tracers include [^{18}F]FMISO for the measurement of tissue hypoxia and [^{18}F]FLT for the measurement of cellular proliferation (Fig. 2), both of which are valuable diagnostic tools to measure response to cancer treatment.
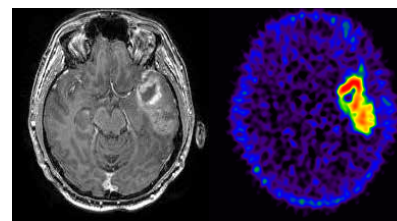

Figure 2: *PET study of a glioblastoma brain tumour using [^{18}F]FLT (from Price et al. [1])*

Carbon, oxygen and nitrogen are abundant in biologically active chemicals. Therefore, when these chemicals are labelled with carbon-11 (^{11}C), oxygen-15 (^{15}O) or nitrogen-13 (^{13}N), the radiopharmaceuticals metabolise in exactly the same way as their non-radioactive analogues. For example, ^{11}C-labelled amino acids such as L-[^{11}C]leucine and L-[^{11}C]methionine are used to measure protein synthesis rate, which is linked to brain function, and ^{15}O-labelled water or oxygen provides quick non-invasive measurement of oxygen metabolism, blood volume and cerebral perfusion in patients with acute brain injuries. Similar to ^{11}C-labelled amino acids, ^{13}N-labelled amino acids can be used for measurement of protein synthesis; however, the shorter half-life of ^{13}N ($t_{1/2}$ = 9 min) poses a big challenge to the production and availability of the radiotracers.

Small organic compounds with known biological activity as receptors to important proteins are labelled with ^{11}C for in vivo assessment of the protein activity. For example, (*R*)-etomidate and metomidate are licensed as short-acting intravenous agents for the induction of general anaesthesia and sedation in humans or animals. The primary pharmacological effect of this group of chemicals is to inhibit β-hydroxylase, a key enzyme in the biosynthesis of cortisol and aldosterone in the adrenal cortex. [^{11}C]Metomidate was developed as a PET ligand for the diagnosis of primary hyperaldosteronism and adrenocortical tumours.

[11C](*R*)-PK11195 has been used in PET scanning to visualise brain inflammation in patients with neuronal damage. Increases in [^{11}C](*R*)-PK11195 binding have been reported in patients with stroke, traumatic brain injury and chronic neurodegenerative conditions including Huntington's disease and Parkinson's disease.

Thioflavin T is the dye widely used to visualise and quantify the presence of misfold-

ed protein aggregates called amyloid, both in vitro and in vivo. Its radioactive analogue, [^{11}C]PIB, is used in PET to image beta-amyloid plaques in neuronal tissue and hence plays a critical role in investigational studies of Alzheimer's disease and other disorders (Fig. 3)
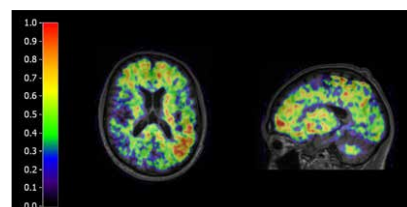
Figure 3: *Imaging beta-amyloid plaques with [^{11}C]PIB in Down syndrome (from Landt et al. [2])*

Due to the short half-life of ^{18}F, ^{11}C, ^{13}N and ^{15}O, a medical cyclotron is usually built next to the production facility. Radioisotopes generated from the cyclotron can then be immediately transferred to an automatic synthesiser for use in the labelling process (Fig. 4). Typically, when bombarding [^{18}O]H$_2$O with proton beams, the nuclear reaction in the cyclotron target produces [^{18}F] fluoride in ^{18}O-water. When bombarding nitrogen with oxygen with proton beams, the nuclear reaction in the gas target produces [^{11}C]CO$_2$ as the starting point of the radiosynthesis procedure.

Figure 4: *GE PETtrace 800 medical cyclotron*

[^{18}F]Fluoride or [^{11}C]CO$_2$ can subsequently be introduced in an organic compound via a single- or multi-step synthesis. This is the so-called radiolabelling process. For protection of the operator, the radiolabelling process usually occurs in an automatic synthesiser via a programmed time sequence (Figs. 5, 6). At the end of the radiolabelling process, the radiopharmaceutical will be purified, formulated, sterilised via membrane filtration and aseptically dispensed in a vial as final product.

Figure 5: *Above: GE FastLab2 synthesiser. Below: Synthra RNPlus synthesiser*

Figure 6: *Automatic dispenser for PET tracers*
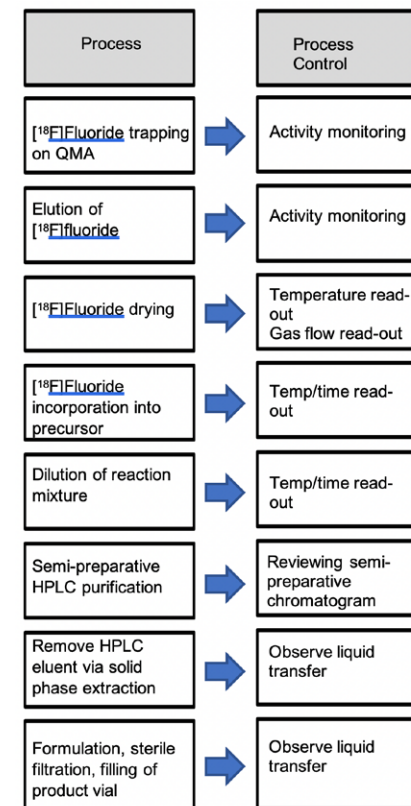
Figure 7: *Typical manufacturing process for PET tracers and process control*

Compared with ^{99m}Tc radiopharmaceuticals used in SPECT scans, the whole "radio-synthesis" process for PET tracers as shown in Figure 7 is very costly and time consuming. In addition, the availability of ^{18}F and

^{11}C radiochemistry limits the numbers of radiotracers that may be used in clinical studies. In the United Kingdom, most PET radiopharmaceuticals are unlicensed medicinal products and are supplied under a Medicines and Healthcare Products Regulatory Agency "Specials" licence. Only [^{18}F]FDG for injection is a licensed product.

As discussed previously, compared with SPECT tracers, ^{18}F- and ^{11}C-labelled PET tracers have the advantage of increased resolution but the disadvantages of being costly and dependent on a cyclotron. Recently, gallium-68 (^{68}Ga) has become an increasingly popular choice of radionuclide for PET studies owing to its practical and economic advantages. ^{68}Ga is a generator-eluted radionuclide with 89% decay by positron emission. The long half-life of its parent radionuclide, germanium-68 (270.8 days), allows use of the generator for up to one year. ^{68}Ga-labelled peptides, for example ^{68}Ga-DOTATOC, ^{68}Ga-DOTATATE and ^{68}Ga-DOTANOC, have been recognised as a new group of radiopharmaceuticals showing fast target localisation and blood clearance.

In recent years, antibodies have been extensively studied as attractive candidates for cancer therapeutics and drug delivery agents owing to their exquisite specificity and binding affinity. For similar reasons, resources have been invested in the de-velopment of suitable antibody-based radiopharmaceuticals with a radioisotope half-life matching the pharmacokinetic half-life of the labelled antibodies in vivo. Because antibodies have relatively slow pharmacokinetics, they often require a number of days to reach their optimal biodistribution within the body. The short half-life positron-emitting radioisotopes, e.g. ^{18}F, ^{11}C and ^{68}Ga, are consequently not a suitable choice in this context. Zirconium-89 (^{89}Zr) is gaining attention in PET clinical studies because its half-life is 78.4 h, which represents a good match to the pharmacokinetic of antibodies. Moreover, it irradiates a pair of relatively low-energy gammas (395.5 keV) after positron decay and is hence safer to handle ^{18}F and ^{11}C irradiate relatively high-energy gammas (512 keV)].

Radiolabelling of ^{68}Ga and ^{89}Zr is performed via chelators. For example, the most popular chelator for Zr^{4+} is desferrioxamine, which has three hydroxamate groups for binding the Zr^{4+} and a primary amine group. The primary amine group can be modified for conjunction to biomolecules, such as peptides, proteins or antibodies.

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RADIO-PHARMACEUTICALS USED IN THERAPY

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by Christelle Terwinghe,
Christophe M Deroose

8

INTRODUCTION

During the past decade, there has been significant growth in therapeutic nuclear medicine. Cancer cells can be killed by damaging their DNA, and other subcellular components, using radiation emitted by radionuclides accumulated inside or in the vicinity of the tumour cells. Targeted radionuclide therapy is typically a systemic treatment, with distribution of the radiopharmaceutical through the bloodstream and retention in tumour via specific uptake mechanisms. The radiopharmaceutical delivers a toxic level of radiation to the tumour cells, while healthy tissue is irradiated to a much lesser extent. For targeted radionuclide therapy, radionuclides are chosen that emit radiation with a short path length and with a high energy deposition along that path length; examples include beta particles, alpha particles and Auger electrons. In this chapter, the most commonly used therapeutic radiopharmaceuticals are described [1, 2].

From a logistic perspective, therapeutic radiopharmaceuticals can be categorised according to the complexity of the preparation to be performed at the clinical site where administration to the patient is to be performed:

1. Radiopharmaceuticals delivered in an individual dose that is entirely administered, e.g. sodium iodide-131 (Na^{131}I) capsules
2. Radiopharmaceuticals which are "ready to use" but which require dispensing of a specific amount of activity for administration, e.g. radium-223 dichloride (^{223}RaCl$_2$) vials
3. Radiopharmaceuticals that require a labelling procedure involving indirect radiolabelling of the vector molecule (for instance by use of a chelator), e.g. labelling of octreotate by lutetium-177 (^{177}Lu) through the use of a DOTA chelator

Depending on the complexity of the manipulations, a number of quality control tests should be performed:

- Upon receipt of the radiopharmaceutical, the package has to be controlled.
- The included documentation has to be checked, e.g. certificate of analysis and time of calibration.
- The surface of the container and the vial has to be swabbed to verify that the surface is not contaminated.
- The expiry date and time must be checked.

- The instrumentation has to be properly calibrated, with calibration factors for each specific radionuclide and in particular cases with calibration factors specific to the different materials used, e.g. container, vial and syringe. Instrumentation has to be maintained according to the manufacturer's instructions.
- The working space has to be clean and the procedure has to be performed in a sterile manner, e.g. swabbing surfaces with ethanol and working in a laminar airflow unit.
- The appearance of the product (colour, homogeneity etc.) has to be checked and should meet the manufacturer's description.
- The radionuclide purity has to be checked by recording the gamma-ray and X-ray spectrum if no chromatograms are available in the certificate of analysis.

Sodium iodide-131

In 1948, iodine-131 (^{131}I) became the first radiopharmaceutical to be used in humans for the treatment of benign conditions of the thyroid gland. ^{131}I is used to irradiate thyroid tissues in order to treat specific forms of hyperthyroidism (for this purpose a small amount of activity is employed, less than 1 GBq) and some forms of thyroid goitre. It is also used in the treatment of differentiated thyroid cancer, where the administered activity will ablate the post-surgical thyroid remnant (normal thyroid tissue) and accumulate within the malignant tumour cells, either in an adjuvant setting or for treatment of metastatic disease (activity varies from 1 to 6 GBq). Na^{131}I is typically administered orally. It can be provided in liquid form, in which case the prescribed activity has to be dispensed, but most commonly it is delivered in individual capsules. The activity has to be checked in an activity meter before administration to the patient.

In addition to the standard radiation protection measures, one has to take into account the fact that working with ^{131}I presents a potential radioactive contamination hazard because of the volatility of iodine. Manipulation of solutions poses the greatest danger and should therefore always be performed within a fume hood. Use of capsules is recommended to reduce the risk, but the capsules should still be preserved in well-closed containers in a fume hood or a well-ventilated room [3].

RADIOIMMUNOTHERAPY

Radioimmunotherapy (RIT) is a targeted radionuclide therapy using a radiolabelled monoclonal antibody with specificity for a tumour-associated antigen allowing direct delivery of therapeutic radionuclides to the tumour. RIT is currently used in the treatment of non-Hodgkin lymphomas (NHL).

The best-known RIT pharmaceutical is ^{90}Y-ibritumomab tiuxetan, a murine monoclonal antibody sold under the trade name Zevalin® (Spectrum Pharmaceuticals, Irvine, US). It is directed at the human CD20 antigen, which is expressed on B-cell NHL [4]. The labelling procedure to obtain the radiopharmaceutical is shown in Figure 1.

which binds to CD37, a glycoprotein expressed by B cells. Betalutin® is under study for patients with relapsed B-cell NHL [5]. It is delivered in a ready-to-use formulation, and the required activity has to be withdrawn from the vial and checked in an activity meter before administration.

1. NaAc buffer
2. ^{90}YCl$_3$
3. Ibritumab tiuxetan
4. Formulation buffer

Reaction Vial

1. Withdrawing of required activity
2. Activity measurement
3. Quality control
4. Administration within 4 hours.

Figure1: *Labelling procedure ^{90}Y-ibritumomab*

The quality control consists of a small drop deposition on an instant thin-layer chromatography silica gel (ITLC-SG) paper. The ITLC-SG paper is placed in a developing chamber, filled with saline to determine the radiochemical purity. The radiolabelled antibody is retained at the origin and non-bound yttrium-90 (^{90}Y) will be chelated by DPTA and migrate with the solvent front. The strip can be scanned by an autoradiography system or it can be cut and both halves counted in a scintillation crystal.

A promising new RIT radiopharmaceutical is ^{177}Lu-lilotomab satetraxetan (Betalutin®, Nordic Nanovector, Oslo, Norway),

Theranostics

Theranostics refers to a specific targeted therapy performed after a specific diagnostic scan using the same vector molecule radiolabelled with different radionuclides.

Peptide receptor radionuclide therapy

Peptide receptor radionuclide therapy (PPRT) involves the administration of radiopharmaceuticals that bind to the somatostatin receptor. A range of somatostatin analogues with bifunctional chelators, such as DOTATOC, DOTATATE, and DOTANOC (where DOTA refers to the che-

lator), labelled with various radionuclides, such as ^{177}Lu and ^{90}Y, are used in the treatment of neuroendocrine tumours. ^{177}Lu has a beta energy emission of 0.5 MeV and a maximum tissue penetration of less than 2 mm, which results in a high tumour dose and a steep gradient towards the surrounding healthy tissue. ^{90}Y has a maximum beta particle range of 12 mm and is therefore more suitable for larger tumours, with a higher probability of low-uptake areas that can still be irradiated by adjacent high-uptake areas (the so-called cross-fire effect). ^{177}Lu is both a beta- and a gamma-emitting isotope; the gamma emission allows the performance of imaging and dosimetry calculations [6].

Novel PRRT radiopharmaceuticals under development use bismuth-213 and actinium-225 (^{225}Ac), which are alpha emitters [7, 8]. Alpha emitters have the advantage of causing a much higher number of double-strand breaks in the tumour cell's DNA compared with beta emitters.

For the treatment of neuroendocrine tumours, which very frequently show high somatostatin receptor expression, ^{177}Lu-

DOTATATE is the most commonly used radiopharmaceutical. A gallium-68 (^{68}Ga) labelled DOTA-peptide (-TATA/-TOC/-NOC) PET scan (preferably), or if PET is not available, scintigraphy with indium-111 pentetreotide or a technetium-99m (^{99m}Tc) derivative has to be performed prior to treatment to confirm good uptake of the somatostatin analogue in the tumour lesions.

^{177}Lu-DOTATATE is commercialised under the name Lutathera® (Advanced Accelerator Applications, Saint-Genis-Pouilly, France) and is delivered ready-made. In some centres the radiopharmaceutical is prepared in house. This radiopharmaceutical is registered in Europe by the European Medicines Agency. A randomised controlled study, the Netter-1 trial [9], has demonstrated a profound effect on progression-free survival and quality of life [10] in patients with progressive midgut neuroendocrine tumours. A strong effect on overall survival has been suggested by an interim analysis, and the final analysis is awaited.
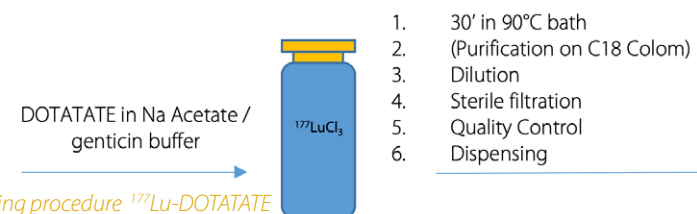

DOTATATE in Na Acetate / genticin buffer

^{177}LuCl$_3$

1. 30' in 90°C bath
2. (Purification on C18 Colom)
3. Dilution
4. Sterile filtration
5. Quality Control
6. Dispensing

Figure2: *Labelling procedure ^{177}Lu-DOTATATE*

The labelling can be performed semi-automatically or manually, depending on the availability of a synthesis module at the department. However, both methods require several steps, as shown in Figure 2. Fast dilution of the product after labelling is of great importance to avoid radiolysis owing to the small (<1 ml) volume of the labelled peptide.

Additionally, some quality control tests have to be performed:
- pH determination
- Radiochemical purity test by use of high pressure liquid chromatography or ITLC
- Radionuclide half-life determination
- Bubble point test of the used sterile filter
- Sterility test by use of bacterial culture media
- Pyrogenicity control by use of an endotoxin detection kit, e.g. limulus amoebocyte lysate (LAL) test

PSMA radioligand therapy (PRLT)

Prostate-specific membrane antigen (PSMA) is a protein overexpressed in the vast majority of prostate cancers. Several radiopharmaceuticals using ^{177}Lu attached to PSMA-binding vector molecules are used or are under investigation for the treatment of patients with prostate cancer after establishing high PSMA expression through a PET scan with a *gallium-68* or fluorine-18 (^{18}F) labelled PSMA ligand. ^{177}Lu-PSMA is one of the most promising radiopharmaceuticals for use in radionuclide therapy. The labelling and quality control are similar to those for ^{177}Lu-DOTATATE.

As with somatostatin analogues, radionuclides other than lutetium can be attached to the PSMA ligands. Very promising results have been shown with ^{225}Ac attached to PSMA, with complete responses in patients refractory to all standard therapies and ^{177}Lu-PSMA.

Other radionuclides that have been studied in the preclinical setting include terbium-161 and scandium-47 [11].

^{131}I-mIBG

Iodine-131 metaiodobenzylguanidine (^{131}I-mIBG) is used for the treatment of tumours arising from the neural crest, such as neuroblastomas (a tumour typically seen in children), paragangliomas and phaeochromocytomas. The targeting of the radiopharmaceutical is demonstrated by a diagnostic scan with ^{123}I-mIBG or a small amount of ^{131}I-mIBG.

^{131}I-mIBG is shipped and delivered on dry ice and has to be stored in the freezer. Before injection the radiopharmaceutical has to be defrosted and diluted. The required activity has to be withdrawn and checked before administration.

BONE-SEEKING RADIONUCLIDE THERAPY

Bone-seeking radionuclide therapy has been used for the palliation of pain caused by bone metastases for decades, mostly in patients with prostate cancer and to a lesser extent in those with breast cancer. A novel indication emerged after publication of the ALSYMPCA trial, where the alpha emitter ^{223}RaCl$_2$ was shown to prolong overall survival [12].

Patient selection is performed by diagnostic ^{99m}Tc-methylene diphosphonate scintigraphy or ^{18}F PET imaging examination shortly before planned treatment administration.

Strontium-89, samarium-153, rhenium-186, rhenium-188 and phosphorus-32 are radionuclides currently in clinical use in metastatic castration-resistant prostate cancer [13].

^{153}Sm-EDTMP

Samarium-153 ethylene diamine tetramethylene phosphonate (^{153}Sm-EDTMP) is used for palliation of bone pain in patients with multiple painful osteoblastic skeletal metastases and has demonstrated efficacy in patients with prostate, breast and other primary cancers. ^{153}Sm emits beta particles and gamma photons with a relatively short half-life (46 h) and a relatively high dose deposit. ^{153}Sm-EDT-MP is available under the trade name Quadramet® (Curium, Paris, France).

Quadramet® is supplied frozen in a 10-ml vial and has to be stored in the freezer. The vial contains 2–4 GBq in a concentration of 1.3 GBq/ml on the reference date. After defrosting, the required activity has to be dispensed (37 MBq/kg body weight). The activity in the syringe has to be checked in the activity meter.

^{223}RaCl$_2$

Radium-223 dichloride (^{223}RaCl$_2$) is an alpha emitter and has a natural affinity for newly formed osteoid, the organic precursor of bone tissue. It is the most recent addition to the radionuclide armamentarium for bone-seeking radionuclide therapy. The therapeutic regimen, validated by the randomised, controlled ALSYMPCA trial, consists of six intravenous injections (55 kBq/Kg body weight) every 4 weeks.

^{223}RaCl$_2$ is delivered ready to use under the tradename Xofigo® (Bayer Pharmaceuticals, Berlin, Germany). After calculation of the required activity, the volume of the solution to be withdrawn is determined. The activity in the syringe has to be checked by measuring the delivered vial before and after withdrawal of the solution in the activity meter.

SELECTIVE INTERNAL RADIATION THERAPY

Selective internal radiation therapy (SIRT), also known as radio-embolisation, is used in patients with inoperable liver tumours (primary tumours, hepatocellular carcinoma or cholangiocarcinoma) or liver metastases from other tumours (colorectal cancer, neuroendocrine tumours, breast cancer, ocular melanoma and others).

The therapy consists in injecting radioactive microspheres directly into the hepatic artery after catheterisation under X-ray guidance. The uptake in the tumour is typically higher than that in healthy liver tissue because of preferential perfusion of tumour by the hepatic artery and normal liver tissue by the portal vein.

For clinical use, three types of microsphere are available (all three are implantable medical devices from a regulatory point of view, and not radiopharmaceuticals): ^{90}Y-resin microspheres (SIR-Spheres®;

SIRTEX Medical, Lane Cove, Australia), ^{90}Y-glass microspheres (TheraSphere®, BTG, London, UK) and holmium-166 containing microspheres (QuiremSpheres®, Terumo, Leuven, Belgium). Some of them are delivered by patient-specific ordered activity and can be administered without manipulating the radiopharmaceutical. For SIR-Spheres, the required activity has to be dispensed from a bulk vial to the delivery V-vials. Prior to withdrawal, the required volume from the bulk vial has to be calculated and the spheres have to be mixed in suspension by slightly shaking them. The dispensed activity has to be determined by measuring the bulk vial before and after withdrawal. In both cases, with or without withdrawal, the activity has to be checked before administration [14, 15] (Table 1).

	SIR-Spheres®	TheraSphere®	QuiremSpheres®
Required manipulation	Dispensing	Measurement of the activity	Measurement of the activity
Radionuclide	Yttrium-90	Yttrium-90	Holmium-166
Material	Resin	Glass	Poly-L-lactic acid
Mean diameter (μm)	32	25	30

Table 1: *Overview of available microspheres for SIRT*

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BLOOD LABELLING FOR NUCLEAR IMAGING

by Naseer Khan, Giorgio Testanera

INTRODUCTION

Blood cells can be radiolabelled with various radionuclides, including predominantly indium-111 (^{111}In), technetium-99m (^{99m}Tc) and chromium-51 (^{51}Cr), for a variety of clinical applications. The radiolabelling process is carried out either in vitro, as in the case of radiolabelled leucocytes, or using an in vivo method, as in the case of radiolabelled red cells. In vitro methods involve the initial isolation of blood cells, radiolabelling with the suitable labelling agent and subsequent re-injection of the labelled cells into the patient [1, 2].

Red blood cells (erythrocytes), white blood cells (leucocytes) and platelets are all used in the labelling process. Red blood cells are the most abundant cellular elements. These cells are produced in the bone marrow. Haemoglobin, an important component of red blood cells, and other cellular proteins may contribute to the binding of radiolabels to the cells [1]. Leucocytes include granulocytes, monocytes and lymphocytes. Granulocytes migrate to the site of the infection and inflammation. The primary function of these cells is to phagocytose and destroy bacteria, and this function is exploited during the imaging process for the detection of the sites of infection. Platelets are derived from megakaryocytes in the bone marrow and are non-nucleated discs.

The ideal cell labelling agents will not damage the cells. The agent used for labelling must be neutral and sufficiently lipophilic that it can cross the cell membrane. Furthermore, the agent must have sufficient stability in vivo as elution of radioactivity during the course of the investigation could result in unnecessary irradiation of the other organs and in essence failure of the procedure.

REGULATORY REQUIREMENTS FOR BLOOD CELL LABELLING

The procedure should be carried out in a laminar flow hood or blood cell isolator as defined by the local regulations. General care should be taken to protect both blood components and the individual performing the procedure. As the labelled blood cells are re-injected into the patient, strict adherence to aseptic technique is essential. All reagents and glass/plasticware should be sterile and protective clothing (gloves, face mask and/or apron) must be worn during the manipulation of blood components. Simultaneous labelling of blood cells from more than one patient is discouraged, and if it is performed, special care needs to be exercised. One of the requirements is to have a clear physical barrier in place when handling different cells, and all syringes and test tubes must be labelled with the patient's name to avoid possible cross-contamination. The procedure is performed at room temperature unless otherwise specified in the package insert for the radiopharmaceutical. The volume of blood collected may vary and is generally between 30 and 50 ml but this needs to be adjusted in proportion to body weight in paediatric patients (Fig. 1). In the same way, the activity in paediatric patients needs to be in proportion to the body weight.

Figure 1: *Example of blood collection*

RADIOPHARMACEUTICALS USED FOR CELL LABELLING

The key radionuclides used in cell labelling are shown in Table 1. The choice of radionuclide depends upon the characteristics of the radionuclide as well as the length of the clinical study. The ligands labelled with either [99m]Tc-99m or ^{111}In form lipophilic complexes and are non-selective. Therefore, it is paramount to isolate the cells required prior to labelling. This part of the process is the most time consuming.

Radionuclide	Half-life ($t_{1/2}$)	Gamma energy (keV)
[99m]Tc	6 h	140
[111]In	67.9 h	171, 245
[51]Cr	27.7 days	320

Table 1: *Radionuclides used for blood cell labelling*

RADIOLABELLING OF WBCS AND PLATELETS

Non-selective, lipophilic ^{111}In and ^{99m}Tc complexes are used for radiolabelling of WBCs and platelets.

Clinical indications for labelled WBC scintigraphy

The main clinical indications for ^{99m}Tc-hexamethylpropylene amine oxime (HMPAO)-labelled WBC and ^{111}In-oxine-labelled WBC scintigraphy are localization of any occult site of infection and determination of the extent of the process [3–5]. Applicable disorders include:

- Osteomyelitis of the appendicular skeleton
- Infected joint and vascular prosthesis
- Diabetic foot
- Fever of unknown origin
- Postoperative abscesses
- Lung infections
- Endocarditis
- Neurological infections
- Infected central venous catheters or other devices
- Inflammatory bowel disease (^{111}In-oxine-labelled WBCs preferred)
- Intra-abdominal infection (^{111}In-oxine-labelled WBCs preferred)
- Kidney infections (^{111}In-oxine-labelled WBCs preferred)
- AIDS

^{111}In WBC labelling

Indium-111 is supplied in high specific activity with no carrier added, as the chloride in 0.04 mol/L hydrochloric acid. Oxine (8-hydroxyquinoline) is the ligand used for the ^{111}In labelling of leucocytes [4]. Oxine forms a 3:1 complex with indium that is neutral and highly lipophilic (see Fig. 4A below). This property of the ligand allows rapid diffusion of the complex through the cell membrane. Once inside the cell, the indium complex completely or partially dissociates, allowing indium binding to the intracellular proteins. ^{111}In-oxine will also label the transferrin present in the plasma; therefore cells need to be washed thoroughly to remove plasma during the labelling process (Figs. 2, 3). This complex gives a higher radiolabelling efficiency (80%–90%) as compared to the corresponding ^{99m}Tc complex. Therefore, ^{111}In-oxine-labelled WBCs are still widely used in clinical settings for infection imaging. The drawbacks of the complex are those associated with the physical properties of the radionuclide, i.e. the longer half-life and higher radiation energy [1, 4].

Figure 2: *Centrifugation of blood sample to remove plasma*

Figure 3: *Separation of blood components following centrifugation*

^{99m}Tc-HMPAO WBC labelling

Technetium-99m has been extensively used for radiolabelling of cells, and ^{99m}Tc-HMPAO has been developed as a cell labelling agent (Fig. 4B).
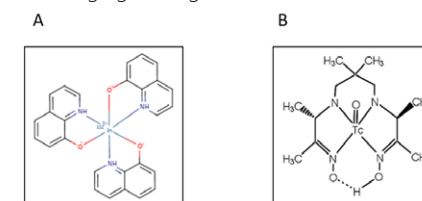
Figure 4: *A ^{111}In-oxine complex; B ^{99}Tc-HMPAO complex*

presence of a relatively small amount of stannous chloride. In addition, the age and radioactive concentration of the generator eluate can affect the formation of the primary complex. The extent of degradation is higher with generator eluate older than 2 h and with eluate from generator which has not been eluted within the last 24 h [1, 3, 6].

Clinical indications for ^{111}In-labelled platelet scintigraphy

The main clinical indication for ^{111}In-labelled platelet scintigraphy is idiopathic thrombocytopenic purpura (low platelet count). Patients with this condition may have therapeutic indications for splenectomy that need to be validated. The scan is then necessary to ensure

that the site of platelet destruction is the spleen. For performance of the scan, it is fundamental that the patient's platelet count is between 5000 and 90,000 per microlitre (5–90x10^9/L). A platelet count of less than 5000 may result in failure of the labelling procedure. If the platelet count is below 5000, the referrer may suggest medical therapy to bolster the platelet count [7].

Procedure for ^{111}In-labelled platelet scintigProcedure for ^{111}In-labelled platelet scintigraphy

This procedure has been described in detail by Mathias and Welch [7]. The general procedure for preparing platelets for radiolabelling is to carefully withdraw whole blood with a large diameter needle, ideally discarding the first millimetre since it will contain a large quantity of tissue thromboplastin fibrinogen. The whole blood should be drawn into a syringe containing either acid citrate dextrose or 3.8% trisodium citrate. After withdrawal, the whole blood should be gently rotated to uniformly mix the anticoagulant with the blood, and the blood is then transferred to a polypropylene

conical test tube by removing the syringe plunger and allowing the blood to flow slowly down the side of the tube.

To obtain the platelet-rich plasma (PRP), the blood is then centrifuged. A slow centrifugation speed for a longer time, i.e. 180 g for 15 min, or a fast speed with a shorter time, i.e. 1000 g for 2 min, is preferred [7]. The PRP should contain 60% population of platelets with very few contaminating cells present. Once they have been centrifuged, the cells are washed with a plasma-free medium once. The platelet pellet is resuspended gently into the buffer, forcing the solution over the pellet several times with a pipette. The cells are virtually lifted into the solution. The process is shown in Figure 5:
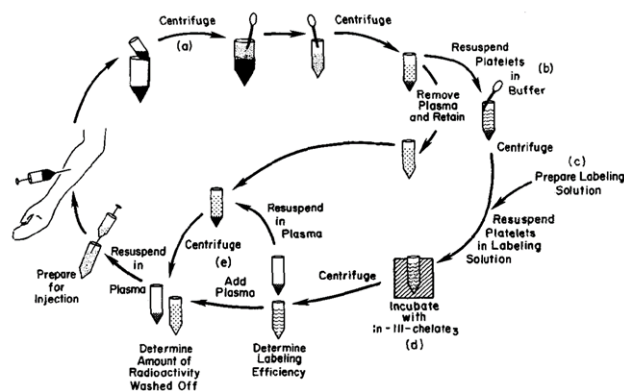

Figure 5: *Schematic of the radiolabelling of platelets (from [7], with permission)*

MEASUREMENT OF RED CELL MASS LABELLED WITH ^{51}CR

Polycythaemia is suspected when haemoglobin and haematocrit are raised. Routine laboratory screening does not differentiate true polycythaemia, i.e. elevated red cell mass (RCM: the total volume of red blood cells), from apparent, or pseudo-polycythaemia, i.e. reduced plasma volume. Radiolabelled cells can be used to measure RCM. Plasma volume can be calculated from the haematocrit or measured independently using radiolabelled human serum albumin [8–10]. Measurement of RCM is done using ^{51}Cr-sodium chromate, and measurement of plasma volume using radioiodine-labelled human serum albumin as a plasma label. There is evidence that the use of a protein with a molecular weight considerably greater than that of albumin will give a smaller estimate of the distribution space and probably a more accurate indication of the true plasma volume. However, in practice no suitable alternative preparations are available as yet.

Not only is measurement of RCM essential for the diagnosis of polycythaemia vera, but it is widely believed that measurement of RCM by the ^{51}Cr labelling method is very accurate and that the RCM is measured independently of the venous haematocrit. It is also widely believed that measurement of RCM must be done with ^{51}Cr [8, 11].

Clinical indications for red cell mass measurements

The main clinical indication for red cell labelling is to verify a clinical diagnosis of polycythaemia vera or thrombocytopenia [12].

Labelling procedure for red cell mass and plasma volume studies

In all cases prior to 1993, the method used was that recommended by the International Committee for Standardization in Hematology. This method has been widely published and therefore will not be described in detail. Briefly, it consists in labelling erythrocytes with ^{51}Cr to measure RCM and with radioiodine-labelled human serum albumin to measure plasma volume [13]. A 10-mL sample of venous blood is obtained and subsequently radiolabelled with ^{51}Cr Na$_2$CrO$_4$. Radiolabelled blood is re-injected and venous samples are obtained 15 and 30 min post injection. Radioactivity is counted in the blood samples and compared with known standards using a gamma counter to establish plasma and red cell volumes. The experimental or measured values are then compared with predicted values for height and weight of the patient.

QUALITY CONTROLS FOR CELL LABELLING

For routine clinical use, visual inspection of the final product and determination of the labelling efficiency are usually considered sufficient. It is also fundamental to visually assess the final product (Fig. 6) and to double check the production worksheet in the release project to ensure that all steps have been followed correctly and that no expired reagents have been used. In the implementation of the procedure, an accurate checklist needs to be provided and filled in by the operator. Microscopic inspection of clumping, the trypan blue exclusion viability test and the post-release sterility test may be used as additional quality controls [3].

Figure 6: *Visual assessment of the final product of 111In-WBC labelling*

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GOOD MANUFACTURING PRACTICE FOR RADIO-PHARMACEUTICALS WITHIN THE HOSPITAL ENVIRONMENT

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by Kishor Solanki

10

INTRODUCTION

Good manufacturing practice (GMP) is that part of quality assurance (QA) which ensures that radiopharmaceuticals (RP), which are medicinal products, are produced consistently and are fit for human administration. The patient must receive the correct radiopharmaceutical at the correct radioactive dose with a high radiochemical purity. Radiopharmaceuticals should be controlled to ensure compliance with quality standards, mostly set by the European Pharmacopoeia, and should be appropriate for the intended use.

Radiopharmaceuticals are radioactive; they therefore have a short shelf life and are prepared close to patient use in a hospital 'hotlab' or cyclotron. They are subject to European rules on radiation for medical applications [1]. The main points to highlight are the importance of ensuring the safety of personnel and the need for good aseptic techniques when preparing injectable radiopharmaceuticals. In general, radiopharmaceuticals are prepared for immediate use, i.e. within 24 h of preparation.

The commonly used radiopharmaceuticals (technetium-99m products) and generators (technetium/molybdenum) in hospitals should have European or national marketing authorisation and be prepared in accordance with the stated product specifications. Technetium-99m products start as sterile non-radioactive kits to which technetium-99m from the generator is added to prepare the final product. The marketing authorisation determines the radioactivity per radiopharmaceutical kit; therefore this amount should not be exceeded when this product is prepared in hospitals. The radioactivity burden and product expiry after addition of radioactivity are part of a manufacturer's marketing authorisation and the manufacturer has to provide related documented evidence and justification. Incorrect preparation could result in poor quality product that could compromise patient diagnosis.

This chapter will focus on the hospital environment, where the associated risks are lower in comparison with industrially manufactured pharmaceuticals. The industrial production of radiopharmaceuticals is required to follow volume 4 of the European EudraLex GMP guidelines [2] and related national regulations; EudraLex Annex 3 is specific to radiopharmaceuticals [3].

For nuclear medicine there are three EANM-associated guidelines:

- Guidance on current good radio-pharmacy practice for the small-scale preparation of radiopharmaceuticals using automated modules: a European perspective (2014) [4]

- Guidance on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals (2010) [5]

- Guidelines on current good radio-pharmacy practice (cGRPP) in the preparation of radiopharmaceuticals (2007) [6]

The time between preparation of radiopharmaceuticals, quality control (QC) and clinical use is very tight. At the international level, the Pharmaceutical Inspection Convention/Pharmaceutical Inspection Co-operation Scheme (PIC/S) guidelines aid in the harmonisation of practices [7] and place strong emphasis on release and recall procedures. Timing plays a very important role in the use of radiopharmaceuticals and hence there is strong need to have such procedures in place. The release procedure includes objective assessment of the prepared product. There is strong emphasis on ensuring that the radiopharmaceutical has been accurately prepared and on appropriate compliance with specifications. A recall procedure must be documented and reviewed at least once a year as an insurance policy in case the product is found not to meet specifications once all QC has been completed. The above GMP guidelines play an important role in clinical practice in terms of guaranteeing the safety of patients.

In essence, GMP is concerned with both production and robust QA/QC. Therefore, the key topics covered in this chapter will include:

- Control of preparation processes
- Quality assurance and quality control
- Key personnel
- Control and ongoing validation of premises and equipment
- Essential documentation
- Safe and secure storage and distribution of radiopharmaceuticals
- Good practices in relation to waste and expired and decayed products
- Need for overarching audits of quality management systems

CONTROL OF PREPARATION PROCESSES

Production operations must follow clearly defined and documented procedures to minimise the chance of errors. These procedures should ensure that the work area is clear and uncluttered. Work should be performed with only one radiopharmaceutical agent at a time and once the radiopharmaceutical has been prepared there must be appropriate 'line clearance' from the direct preparation area,

sometimes called the 'critical zone'. This 'critical zone' is the work area behind the lead glass shield of the laminar airflow cabinet or the central area inside an enclosed dispensing isolator. Long forceps or tongs should be used when lifting vials containing radioactive material. This will help reduce the exposure dose or the chance of contamination to the hands. Staff working in radiopharmacies should wear personal monitoring devices at all times in a radiation area. When these are not being worn to monitor occupational exposure, they should be stored in a low-radiation background area.

At every stage of processing, products and materials should be protected from microbial and particulate contamination. Sanitisation of all items should be undertaken in at least two stages (with a sporicide and 70% alcohol) prior to their use in a pharmaceutical laminar air flow cabinet or isolator. The sanitisation involves a spraying and wiping technique and each operation should be validated for these cleanroom practices. Care is required when using sporicides as they affect radiochemical purity. Aseptic, microbial-free conditions and techniques must be ensured when radiopharmaceuticals are reconstituted or drawn up for patient administration in order to prevent the introduction of

micro-organisms which cause infection. Vials should always be wiped with a fresh alcohol swab immediately before use. The needle tip must not be allowed to touch any other surface.

Radioactive, microbial and particulate cross-contamination should be avoided by appropriate technical or organisational measures. These measures should be detailed in documented work instructions and all staff should be trained in them. Each product should be prepared following line clearance and in an orderly fashion to permit batch segregation and stock rotation. Prevention of product crossover and misadministration is a key challenge in most radiopharmacies across the world. In this context, appropriate labelling plays an important role. Labels applied to containers, equipment or premises should be clear, unambiguous and in the previously agreed format. In addition to the wording on the labels, it is helpful to use colours to indicate status (e.g. bone scan). Checks on the radioactivity and product yields, and reconciliation of quantities, should be carried out and documented as necessary to ensure that there are no discrepancies outside acceptable limits.

In addition, the dose calibrator setting should be checked when assaying the

patient's dose. Dose calibrators should be cross-checked using a standard calibration source at least once a day. Results must be within an acceptance limit of 5%. The values from the dose calibration checks should be plotted on a graph (i.e. trended) to ensure stability of the equipment. Prepared products should be held 'in bond' until all evaluation of finished radiopharmaceutical products has been completed and documented, this being a necessary step before release of the product for patient administration. A clear set of standards and conditions must be established for this 'final release'.

The final product should be stored appropriately, in accordance with the instructions set out by the manufacturer of the radiopharmaceutical kit. In general, radiopharmaceuticals should be kept below 25°C and some should be stored in the refrigerator as this helps to maintain high radiochemical purity. The latter is important as radiopharmaceuticals are mainly dispensed from multidose vials and are used on a number of patients: it is important that the last patient also receives a high-quality product.

Deviations from instructions or procedure guidelines should be avoided as far as possible. Any deviation should be approved in writing by a competent person, with the involvement of the Quality Control Department. Rejected materials and products should be clearly

marked and stored separately in restricted areas until they have decayed before disposal.

Therefore, the basic requirements of radiopharmaceutical GMP are that:

- All manufacturing processes, and all critical steps within those processes, are clearly defined and documented, and all significant changes to the processes are validated.

- All necessary radiopharmacy facilities for GMP should be provided with:
  » Appropriately qualified and trained personnel
  » Adequate premises and space
  » Suitable equipment (e.g. L-lead screen, syringe shields, shielded waste bin, long forceps/tongs, stainless steel work trays, contamination monitor, dose calibrator) and environmental services
  » Correct materials (sealed calibrator reference sources)
  » Containers and labels
  » Approved procedures and instructions
  » Suitable storage and transport

- In the light of local experience there should be systematic review, and it should be demonstrated that radiopharmaceutical manu-

facturing processes are capable of consistently yielding products that are of the required quality and compliant with the specifications.

Starting materials should be purchased only from approved suppliers or licensed pharmaceutical wholesalers or, where possible, directly from the licensed manufacturer/producer. All radiopharmaceuticals and generators should have marketing authorisation and the purchasing team should have clear instructions and appropriate training.

For each delivery, the containers should be checked for integrity of the packaging and seal and for correspondence between the delivery note and the supplier's labels. QC tests should be undertaken with each new delivery of radiopharmaceutical generator and radiopharmaceutical kits. Each delivery or batch of material should be attributed a specific reference number or date, or given an identification mark such that it can be tracked from start to finish. This is important with regard to product liability, in the event that a patient reacts to the radiopharmaceutical.

All materials and products should be stored under the appropriate conditions. Refrigerated items must be maintained at 2°–8^oC and checked and controlled 24/7. Outdated or obsolete material should be destroyed, and this disposal should

be recorded specifically if the material is radioactive.

Risk assessment and validation studies should be undertaken before starting to use any new product in order to reinforce radiation safety and GMP. The defined procedures should be recorded together with the results and conclusions.

QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control (QA and QC) are that part of GMP which is concerned with sampling, specifications and testing.

Radiopharmacies should have approved standards for inspection and receipt of all radiopharmaceutical starting materials and finished products. The radiochemical purity testing methods, i.e. TLC and HPLC methods and sterility testing of radiopharmaceuticals, are set out by pharmacopoeias or professional guidelines.

The dose calibrator and other radiation monitoring devices should be cross-calibrated with radioisotopes in use. The dose calibrator should be checked daily using calibrated reference sources, e.g. caesium-137, and trended.

All records should be made in real time; the recording can be done manually and/or using recording instruments. It is

important to have completed documents before the prepared product is released for patient administration.

The finished products should contain active ingredients that are compliant with the qualitative and quantitative composition stipulated by the marketing authorisation, are of the purity required and are enclosed within their proper containers and correctly labelled. The patient must receive the correct radiopharmaceutical at the correct dose with a high radiochemical purity.

The majority of radiopharmaceuticals are administered by injections and therefore appropriate monitoring of environmental conditions is important for GMP purposes. This requires that accurate and sterile doses are prepared for patient administration. Aseptic techniques and sterile and pyrogen-free ingredients are used at all times to minimise bacterial and pyrogen contamination. Each operator should receive training in the aseptic technique, with validation using agar transfer plates or broth tubes.

Regular monitoring of the environment is essential. Agar plates exposed during preparation and found to contain pathogen species, especially Gram-negative bacteria and fungal spores, will require additional cleaning to ensure appropriate sanitary conditions for dispensing of radiopharmaceuticals. For radiopharmaceuticals, it is important to

undertake 'end of broth' testing as many radioactive samples cannot be sent to a microbiological testing laboratory for normal sterility testing.

Monitoring of radiation contamination on surfaces in the radiopharmacy should be done using suitable radiation monitors to prevent cross-contamination of other radiopharmaceutical products and spread of radioactivity to clinical areas.

No batch of product is released or supplied prior to certification by an authorised person that it is in accordance with the requirements of the relevant authorisations.

In order to cover all contingencies, a system should be designed to recall products, especially as radiopharmaceutical QC tests may require additional time. It is necessary to take action promptly if any QC parameters are unsatisfactory (out-of-specification) and if products are known or suspected to be defective. All instances of out-of-specification results and complaints relating to potentially defective products must be carefully reviewed according to written procedures. It is important to undertake root cause analysis.

Audits and self-inspections should be conducted in order to monitor the implementation of and compliance with GMP principles and to identify necessary corrective measures. Many countries have adopted the International Atomic Energy Agency (IAEA) guidance on quality

management audits in nuclear medicine practices [8]. The radiopharmacy checklist is based on the IAEA operational guidance on hospital radiopharmacy [9]; this guidance is risk based and categorises procedures in the field of hospital radiopharmacy according to three operational levels:

- Operation level 1a – Dispensing of radiopharmaceuticals purchased or supplied in their final form from recognised and/or authorised manufacturers or centralised radiopharmacies

- Operation level 1b – Dispensing of radioiodine and other ready-to-use radiopharmaceuticals for radionuclide therapy or palliation

- Operation level 2a – Preparation of radiopharmaceuticals from prepared and approved reagent kits, generators and radionuclides

- Operation level 2b – Radiolabelling of autologous blood cells, especially white blood cells

- Operation level 3a – Compounding of radiopharmaceuticals from ingredients and radionuclides for diagnostic application

- Operation level 3b – Compounding of radiopharmaceuticals from ingredients and radionuclides for thera-

peutic application

- Operation level 3c – Synthesis of positron emission tomography radiopharmaceuticals

- Staff training requirements in respect of each of these operational levels, including with regard to QC, are documented in [10].

KEY PERSONNEL

There must be sufficient qualified and trained personnel to carry out all the tasks which are required during the production of radiopharmaceuticals. There must be staff responsible for the production of the product and other staff who are responsible for the quality of the radiopharmaceutical.

Individual responsibilities should be clearly understood by the individuals and recorded. All personnel should be aware of the principles of GMP that are relevant to them. Competency-based training in radiopharmacy is essential to GMP [10,11] and all staff should receive initial and continuing training, including with regard to hygiene. Operators are trained to carry out procedures correctly, and this training should be documented and regularly updated. From a pharmaceutical point of view, there should be a minimum number of trained staff – capacity planning is

essential. In addition, for radioprotection there should be rotation of staff to share the radiation dose.

The head of production and the head of QC are key personnel, and each must be independent from the other. In small departments the person involved with preparation should not be responsible for the release of products.

The head of production and the head of QC have some shared responsibilities relating to GMP and quality. These may include, subject to any national regulations:

- Monitoring of compliance with the requirements of GMP

- Approval of written procedures and other documents, including amendments

- Monitoring and control of the manufacturing environment

- Ensuring cleaning of radiopharmacies and general hygiene

- Approval and monitoring of suppliers of materials

- Retention of records

- Inspection, investigation and taking of samples, in order to monitor factors which may affect product quality

- Staff training

CONTROL AND ONGOING VALIDATION OF PREMISES AND EQUIPMENT

Premises should be designed and equipped so as to afford maximum protection against the entry of unauthorised persons, insects or other animals. Steps should be taken in order to prevent the entry of unauthorised people. Security of radioactivity, sealed sources and radiopharmaceuticals is of paramount importance. Production, storage and QC areas should not be used as a right of way by personnel who do not work in them.

Lighting, temperature, humidity and ventilation should be appropriate and such that they do not, directly or indirectly, adversely affect the radiopharmaceutical products during their manufacture and storage. The layout and design must aim to minimise the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination and build-up of dust or dirt, which would adversely affect the quality of products.

Premises and equipment must be located, designed, constructed, adapted and maintained to suit the radiopharmaceutical operations to be carried out and provide optimal radiation protection. There are specific European and national rules for the design of radiopharmacies and there are different classes of the room and biosafety cabinets [discussion of this topic is beyond the scope of this chapter; how-

ever, for sterile preparation of radiophar-maceuticals an EU Grade A environment (bacteria and particle free) is required]. These specifications are in place to ensure sterile preparation of radiopharmaceu-ticals; in addition, they should prevent cross-contamination of radiopharmaceu-ticals.

Premises should be carefully main-tained, ensuring that repair and main-tenance operations do not present any hazard to the quality of products. They should be cleaned and, where applicable, disinfected according to detailed written procedures. The radiopharmacy cleaning staff should also be trained and should use dedicated cleaning mops etc. that are specific to radiopharmacy and not used elsewhere (mainly to prevent spread of radioactive, microbial and particulate con-tamination). Cleaning materials should be kept under control at all times. Any chang-es should be documented and systemati-cally followed up.

ESSENTIAL DOCUMENTATION

Good documentation constitutes an es-sential part of the QA system and is key to operating in compliance with GMP requirements. The various types of doc-ument and media used should be fully controlled and defined in the Quality Management System. The main objective

of the system of documentation utilised must be to establish, control, monitor and record all activities which directly or indi-rectly impact on all aspects of the quality of radiopharmaceuticals.

The instructions and procedures [standard operating procedures (SOP)] should be written in an instructional form in clear, stepwise and unambiguous national language, specifically applicable to the facilities provided. There should be detailed written procedures for cleaning, sanitisation and disinfection SOP according to local practice.

The documentation types required in typical radiopharmacies are:

- Quality manual
- Site plan together with organisational charts describing key positions and operational links
- Work instructions, with SOP, covering: manufacturing formulae, sanitisation, processing, labelling and dose calibrator checking, testing of radiochemical purity and final release.
- Protocols and associated forms that provide instructions for performing and recording certain critical and discrete operations.
- Specifications that describe in detail the requirements with which the

generator and radiopharmaceutical must conform and thereby serve as a basis for quality evaluation.

- Records that provide evidence of various actions taken to demonstrate compliance with instructions, e.g. activities, events, investigations and, in the case of manufactured batches, a history of each radiopharmaceutical batch of product, including its use in patients.
- Records that include all data on which quality management decisions are based.
- Documentation and release procedures which ensure that the necessary and relevant tests are actually carried out and that their quality has been judged to be satisfactory.
- Certificates of analysis that provide a summary of the generator and kit testing results on samples of products together with evaluation of compliance with stated specifications.

In addition, records and reports of any risk assessment, environmental microbiology, validation, exercises, projects or investigations should be retained. Each of these reports should be structured and should contain the following details: objective, results, discussion, conclusions

and recommendations. Records of any significant deviations should be retained and they should provide evidence of the investigation undertaken and the reasons for GMP non-compliance.

A complete history of a generator and radiopharmaceutical batch to be traced to patients is to be retained in a comprehensible and accessible form.

SAFE AND SECURE STORAGE AND DISTRIBUTION OF RADIOPHARMACEUTICALS

The storage and distribution of all radioactive products should be controlled to minimise any risks with respect to their quality. It is important to meet legal requirements.

GOOD PRACTICES IN RELATION TO WASTE AND EXPIRED AND DECAYED PRODUCTS

Radioactive waste should be fully documented and controlled at each stage. This should be done in real time. The level of radioactive waste is generally authorised in the site license, which covers both intake and disposal of solid and liquid waste. The radiopharmacy staff have an important role and they should be trained to ensure regulatory compliance.

Only designated, labelled and properly

shielded containers should be used for disposal of radioactive waste. All used syringe needles should be disposed of in a separate container made of a strong material to reduce the chance of injury from a radioactive or contaminated needle.

NEED FOR OVERARCHING AUDITS OF QUALITY MANAGEMENT SYSTEMS

Any significant deviations from instructions and guidelines must be fully recorded and investigated. In addition, any complaints about radiopharmaceuticals should be properly examined, the causes of quality defects investigated and appropriate measures taken in respect of the defective products to prevent recurrence.

A programme of self-inspections should be conducted in order to monitor compliance with GMP principles and to serve as the basis for proposal of corrective measures. The International Atomic Energy Agency (IAEA) guidance on quality management audits in nuclear medicine practices [8], already discussed above, is hospital specific and can be used for self-assessment and external peer-reviewed audits. Reports should contain all the observations made during the inspections and, where applicable, proposals for corrective measures. Statements on the actions subsequently taken should also be recorded.

The quality risk management system should ensure that:
• Evaluation of the risk to quality is based on scientific knowledge and experience with the process and is ultimately linked to protection of the patient.
• The level of effort and the documentation of the quality risk management process are commensurate with the level of risk.

SUMMARY

This chapter has described essential GMP rules and practices for radiopharmaceuticals prepared in hospitals for use in nuclear medicine procedures and has outlined the necessary QC of radionuclide generators and radiopharmaceuticals. There is a need for well-managed and well-controlled documentation systems that ensure appropriate control of processes, premises and equipment at all times. Quality management systems, risk assessment, self-inspection and external audits are an integral part of radiopharmacy practices. High standards of practice are important for all radiopharmaceuticals prepared for use in patients.

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TRANSLATIONAL APPROACH TO RADIO- PHARMACEUTICAL DEVELOPMENT

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INTRODUCTION

The translational process for radiopharmaceuticals (RPs), like that for conventional drugs, is a long and strenuous process. It starts with several steps of in vitro and in vivo preclinical testing research, which must be completed before clinical research can begin on an RP candidate. These evaluation and control steps aim to provide the necessary information for distribution and safety assessment permitting characterisation of potential adverse effects in humans.

Such preclinical experiments aim to demonstrate that the radiotracer:

- marks the targets and/or the mechanisms which it is designed to measure (specificity)
- shows a cell-killing effect in the case of a therapeutic RP
- shows suitable kinetics and metabolic stability
- does not display toxicity in healthy tissues and organs
- uptake is modulated by changes of target expression (sensitivity)

EVALUATION STEPS NEEDED FOR CLINICAL TRANSLATION

In vitro evaluation

Molecular targeting

One of the first aspects to take into account when developing a new imaging or therapeutic RP is the high uptake or tropism for the potential target site, and the need to prove that the RP specifically marks what it is designed to measure.

For radioligands (RPs which bind to receptors), the in vitro binding assay can be performed by incubating a fixed target concentration with increasing concentrations of the radiolabelled compounds. Bound radiolabelled tracer and free tracer are then separated by filtration and counted for radioactivity. Binding data can be analysed with curve-fitting software to calculate K_d (the ligand concentration that binds to half the receptor sites at equilibrium) and B_{max} (the maximum number of binding sites) to determine affinity.

Binding potential (BP) is the ratio of B_{max} (receptor density) to K_D (radioligand equilibrium dissociation constant), as defined by Mintun et al. [1]:

$$BP = \frac{B_{max}}{K_D} = \text{receptor density} \times \text{affinity}$$

where B_{max} is the total density (concentration) of receptors in a sample of tissue, K_D is the (radioligand) equilibrium dissociation constant and the affinity of ligand binding is the inverse of K_D.

Targeting potential can also be assessed by cell uptake studies, performed by incubating the radiolabelled agent (e.g. antibody) with the cell expressing target (e.g. antigen).

Plasma protein binding

Like conventional drugs, RPs bind to plasma proteins to variable degrees. The estimation of plasma protein binding is one major determinant of the RP distribution in the body. This can be assessed by incubating the RP with fresh human plasma and performing measurements using several techniques such as dialysis, ultrafiltration and trichloroacetic acid (TCA) precipitation.

Metabolic stability

Another aspect to be considered in the preclinical development of an RP candidate is the metabolism. Metabolic stability can be assessed in vitro using human liver microsomes containing cytochrome P450 (CYP, a dominant group of metabolising enzymes) or other subcellular hepatic fractions which contain non-CYP enzymes (such as acetyl transferase or glucuronyl transferase) involved in drug metabolism. Hepatocytes and liver slices can also be used and are physiologically more relevant for measurement of the hepatic metabolism of RP.

Although in vitro and ex vivo experimental models can never accurately mimic the complexity of a whole organism, their simplicity allows procurement of initial information regarding the metabolism of the compound.

In vivo evaluation

Biodistribution studies

Although in vitro and cell uptake studies are extremely useful, in vivo animal studies are still required before RP candidates can progress from the stage of in vitro testing to the stage of toxicity assessment and, finally, first-in-human (FIH) studies.

In tissue biodistribution studies, the RP is injected into disease-bearing animals such as mice, rats or rabbits. All animal experiments have to be conducted in accordance with the European guidelines (2010/63/UE) [2] and have to be approved by the national or local animal use ethics committee.

This preclinical evaluation process often starts with imaging studies (PET or SPECT imaging) which can be coupled with autoradiography on tissue sections or ex vivo biodistribution studies, in which organs are harvested, weighed and counted for radioactivity following the injection of a radiotracer. All these methods are aimed at confirming that the RP interacts (in vivo or ex vivo) with the intended target (affinity) and shows minimal uptake in healthy tissues.

RP specificity for the target can also be

evaluated in vivo by imaging techniques using animal models expressing a modulation of target levels through under- or overexpression (such as tumour, overexpressed receptor). Note that, interestingly, RP uptake can be compared with that observed in "knock-out" mice which do not express the target of the RP.

Imaging is usually performed in the static or dynamic acquisition mode:

- **Static acquisition mode:** This is the basic acquisition mode, where the radiotracer distribution is assumed to be static throughout the acquisition. In this mode, a single image is generated representing the radiotracer distribution over the complete acquisition time. Acquired images are then processed by dedicated software for radioactive signal quantification. Usually, a semi-quantitative analysis is performed and the results are expressed in terms of the standardised uptake value (SUV), which is the ratio of the image-derived radioactivity concentration in a region of interest to the whole body concentration of the injected radioactivity and body weight, or the percentage of the injected radioactivity per gram of organ or tissue (%ID/g).

- **Dynamic acquisition mode:** In this acquisition mode, images are acquired according to a predefined temporal framing scheme (one acquisition interval). This mode is used when it is necessary to follow the uptake and clearance of an RP over an extended period. It provides more information on the kinetics of the RP by using absolute PET quantification and modelling. Indeed, pharmacokinetics (PK) parameters (clearance, volume of distribution, etc.) of the radiotracer can be evaluated from these biodistribution studies. The elimination half-life can be measured by collecting serial samples of blood at different time intervals after radiotracer administration and measuring the plasma radioactivity. Urinary and faecal excretion can also be determined quantitatively by collecting urine and faeces at defined intervals after RP administration and measuring radioactivity in the samples.

Even if PK evaluation in animals is still mandatory in the preclinical data package, estimation of PK in humans can also benefit from in vitro studies (e.g. regarding solubility, plasma stability) and bioanalytical methods and pharmacokinetic/pharmacodynamic (PK/PD) modelling.

Animal toxicity studies

Before an investigational RP can be administered to humans as part of an FIH trial, it must undergo safety testing in non-clinical studies [3]. RPs are a special class of drugs comprising two parts, one "cold" or non-radioactive (e.g. antibody, peptide) and one radioactive (e.g. fluorine-18, copper-64, gallium-68, iodine-131, actinium-225). Thus, the toxicity of RPs may be driven by the non-radioactive as well as the radioactive component. Considering the first version of the new guideline on non-clinical requirements for RPs [4], three schemes are possible:

- If the non-radioactive part of the RP is a known compound and if preclinical studies are available, then there is no need for additional toxicity studies if there is available information or data demonstrating that the radioactive atom does not change the pharmacology of the compound.

- If minimal modification of the structure of the non-radioactive compound has been performed (this is sometimes necessary for radiochemistry), then the possible risk related to that modification has to be considered.

- If the non-radioactive compound is unknown and no preclinical toxicity data are available, then full toxicity studies have to be performed and conducted under Good Laboratory Practice (GLP) regulations.

In general, it is recommended that toxicity tests are carried out in two different mammalian species (one rodent and one non-rodent) and that the risk of RP overdose is evaluated. This has in the past been achieved by injecting an acute dose of RP and monitoring animals for clinical signs and changes in parameters such as body weight and food intake, with subsequent post-mortem examination. Today, however, this approach has largely been replaced by so-called extended single-dose toxicity studies entailing assessment in only one mammalian species – usually a rodent with evaluation at day 1 and 14 days post dose administration to assess acute and delayed toxicity and/or recovery. Recently, a new approach (summarised in Table 1) has been proposed, based on the definition of three distinct toxicological limits [5]: toxicological limit 1 <1.5 µg, toxicological limit 2 <100 µg and toxicological limit 3 >100 µg.

Therapeutic RPs are regarded in every respect in the same way as any other medicinal product and therefore clinical

trials must comply with regulations regarding investigational medicinal products (IMPs). The mutagenic and carcinogenic potential of the non-radioactive component may be evaluated and studies should be designed to assess the radiation exposure of tissues due to the radioactive part, in order to predict the radioactive exposure in humans and to mitigate radiation-induced toxicity. Note that the radiation is a major contributor to cancer induction, so dosimetric considerations can cover the carcinogenic potential of the RP. Note also that radiation-induced clinical toxicity is covered by Directive 2013/59/Euratom [8].

Microdosing studies

The concept of microdosing assumes that key PK parameters of a new chemical entity (NCE) that is developed as a drug can be evaluated using very small doses (microdoses) of the investigational compound. Since such low doses are likely to be too small to have any pharmacodynamic effects or cause any major side effects after a single dose, it should be possible to undertake such studies in humans without having to perform the classical toxicology studies at therapeutically effective doses that are mandated prior to regular phase 1 trials.

This new approach, developed almost two decades ago [9], has been proposed as a powerful complementary tool to the existing approaches, and is often achievable with PET agents [10, 11]. The approach is known as human "microdosing studies" according to the European Medicines Agency (EMA) or as "exploratory clinical trials" according to the Food and Drug Administration (FDA). Both the EMA in 2004 [12] and the US FDA in 2006 [13] recognised the concept and its legitimacy with respect to the conduct of such studies. It is important to note that microdosing clinical studies are not meant to replace traditional phase 1 clinical trials.

The use of microdosing studies can be considered in the development process for RPs. In this case, very low single doses of the tested RP are administered to very few human subjects (healthy volunteers or patients) to investigate target receptor binding or tissue distribution in a PET study and/or to obtain basic PK parameters (such as volume of distribution, clearance and $t_{1/2}$) without the introduction of pharmacological effects.

According to the EMA, a microdose is defined as less than $1/100^{th}$ of the dose calculated to yield a pharmacological effect of the test substance. This calculation is based on primary PD data obtained by in vitro and in vivo studies. Typically, the maximum dose must be less than 100 μg or 30 nMol [14]. The use of such a low amount of the RP means that human body exposure is limited, so no therapeutic, toxic or radiotoxic effects are expected.

To sum up, taking a RP from the bed to the bedside involves several steps in development, evaluation and control, entailing the participation of different disciplines, as illustrated in Figure 1.

GOOD MANUFACTURING PRACTICE (GMP) FOR RPS

Beside their efficacy for specific indications, RPs intended for use in humans should be sterile, pyrogen-free and safe. This implies that RP production should be transferred from the conventional laboratory to a GMP facility with a controlled environment, where manufacturing and quality control can be undertaken in a way compliant with the legal framework and regulatory procedures and in accordance with the principles of GMP for medicinal products [15].

GMP requires dedicated clean room facilities with the highest classification to ensure aseptic preparation of RPs. Preparation and quality control of RPs should be conducted only by competent and appropriately qualified personnel. The person responsible for preparation should be clearly identified and ideally should not be the same person as is responsible for quality control.

Production

In the clinic, RP synthesis requires much higher levels of radioactive materials, fast reaction times and reproducible results. Hence, it is mandatory to develop a straightforward GMP-compliant radiosynthesis using fully automated radiosynthesisers and including suitable procedures for quality control.

Quality control

All quality control procedures that are applied to non-radioactive drugs are applicable to RPs. Furthermore, since most RPs are short-lived products, the methods used for quality control should be fast and effective. Usually, tests must be completed before release of the drug product; however, some RPs with very short half-lives may have to be released and used after assessment of batch documentation even if all quality control tests have not been completed. The necessary steps in quality control are summarised in Table 2.

Additionally, GMP and the Clinical Trials Regulation impose the need for authorisation and require that a qualified person, according to Directive 2001/83, is responsible for the release of RPs.

Quality assurance

Furthermore, GMP implies the need for a highly sophisticated quality management framework where all the operations concerning production and manufacture

as well as quality control have to be documented and accurately recorded. Prior to clinical trials, standard operating procedures (SOP) for the preparation, quality control and quality assurance of RPs, as well as specifications for starting materials, should be in place. This ensures harmonisation of practice, traceability and maintenance of standards.

FIRST-IN-HUMAN IMAGING STUDIES

Before its use in FIH clinical trials, the RP has to be classified as an "investigational medicinal product" (IMP) and several mandatory documents, including an Investigator's Brochure (IB) and an Investigational Medicinal Product Dossier (IMPD), have to be prepared and submitted to the competent authorities and the ethics committee to obtain written approval. The IMPD should include all the useful information relating to the chemical and the RP quality of the compound, as well as non-clinical data relating to pharmacology, pharmacokinetics, toxicology and dosimetry [16].

Guidance on the preparation of IMPDs for RPs was published by the Radiopharmacy Committee of the EANM in 2014 [17]. Furthermore, a detailed study protocol has to be prepared in which every step of the protocol is documented.

Acquisition of informed consent from the healthy volunteer or patient is also mandatory.

As RP imaging agents are usually employed at an extremely small mass dose (in the nanogram to microgram range) with no pharmacological effects, a very low incidence of adverse events and a short half-life, FIH studies aim to provide information on feasibility, target specificity, stability, safety biodistribution, pharmacokinetics and metabolism. Radiation dosimetry information regarding use of the RP in humans is also obtained to uncover any side effects.

The following are key aspects in the design of an FIH study:

- Study population: healthy volunteers and/or patients can be enrolled in one or multiple cohorts
- Demographic information from each subject (weight, body surface, age, gender etc.)
- Test RP and reference radiotracers (e.g. test compared with ^{18}F-FDG), or a standard of reference (histology or radiological imaging)
- Administered dose: usually, a single dose is administered via the intravenous route
- Choice of imaging parameters (scan duration, acquisition mode, number of scans per subject) for biodistribution and dosimetry

- Blood/urine sampling intervals for pharmacokinetic study
- Safety profile

FIH study design for diagnostic radiotracers is quite straightforward compared with interventional drug FIH clinical trials, where, for instance, healthy volunteers or patients receive a single dose of the investigational drug or a placebo, starting with a very low dose for the first cohort. Thereafter, the dose is escalated in the following cohorts (or stopped depending on the tolerability and safety). Single ascending dose studies are usually followed by multiple ascending dose studies in a very similar design, where the subjects receive multiple doses of the drug (or placebo).

FIH trials should be designed in a way that permits optimal results from the study, without exposing excessive numbers of subjects and while ensuring their safety. Thus, the EMA advises that it is usually appropriate to design the administration of the first dose so that a single subject receives a single dose of the active IMP, with justification of the period of observation before the next subject receives a dose. This is in order to mitigate the risks associated with exposing all subjects in the same cohort simultaneously [18].

In contrast to imaging studies, the FIH design of interventional drug studies takes into consideration the pharmacological effect of the drug, so the starting dose, maximum dose and exposure and maximum duration of treatment are carefully considered. Also, beside the route and frequency of administrations, the half-time and washout time of the IMP are determined, as are the sequence and interval between dosing of subjects within the same cohort. In the case of dose escalation increments, decisions on transition to the next dose increment cohort or next study part (if the FIH study includes several parts) must take into account tolerability and safety; moreover, stopping rules and the safety parameters that require monitoring have to be clearly established. Note that even if FIH clinical trials are primarily designed to assess the safety and tolerability of an interventional drug, the PK and, when appropriate and feasible, the PD are often included in order to facilitate the link with the non-clinical data and support dose escalation decisions. For both radiotracers and interventional drugs, the study design should take into consideration all the acquired preclinical knowledge, incorporating all available toxicology and pharmacology information on the compound candidate to ensure the safety of the subjects.

Compounds under <1.5 µg	Compounds under <100 µg		Compounds above 100 µg
One can consider that there is no risk (no genotoxic impurity related risk if <2 mg [5, 6]).	The microdosing approach can be considered [7]. In this case, two different approaches are possible:		Potential chemical toxicity studies have to be performed, including extended single dose toxicity studies [18] in rodent and non-rodent species in addition to genotoxicity assessment (Ames).
	Approach 1: Would involve not more than a total dose of 100 µg, more than 1/100th of the non-observed adverse effect level (NOAEL) or more than 1/100th of the pharmacologically active dose. Toxicology studies consist in extended single-dose toxicity studies in one species, usually a rodent, with evaluation 14 days post dose to assess delayed toxicity and/or recovery. Genotoxicity studies are not recommended. For highly radioactive compounds such as PET probes, appropriate PK and dosimetry should be performed.	**Approach 2:** Consists in a maximum of 5 administrations with washout periods (>6 half-lives), with a total cumulative dose of <500 µg and with each administration <1/100th of the NOAEL and <1/100th of the pharmacologically active dose. Toxicology studies consist in a 7-day repeated dose toxicity study in one species, usually a rodent, and genotoxicity studies are not recommended. For highly radioactive compounds such as PET probes, appropriate PK and dosimetry should be performed.	Given that RPs are not usually administered to pregnant women, there is no need for teratogenicity studies. As RPs are given as low doses (exposure is limited to a single dose or a few doses), there is no need for either genotoxicity or carcinogenicity studies. Chronic toxicity studies are also usually not necessary.

Table 1: *Possible approaches for toxicity evaluation depending on the mass of the compound*

Parameter	Evaluation methods/ objectives
Appearance	Evaluated by visual inspection: the solution must be clear and free from visible particles.
pH	pH is verified using paper strips. Initially, the pH paper should be validated against standard buffers and should be in the physiologically acceptable range (5.5–8).
Chemical purity	The purity of the precursor is determined by proton NMR and elemental analysis. This test is done once per batch of precursor.
Radiochemical purity/ yield	Radiochemical purity is assessed by HPLC and radio-TLC.
Radiochemical stability	Radiochemical stability is often tested in serum and saline using HPLC with a radioactivity detector or TLC and a radioactivity scanner (radio-TLC).
Radionuclide purity	Radionuclide purity can be determined by gamma spectrometry or by determination of the half-life.
Residual solvents (e.g. acetonitrile and dehydrated alcohol)	Presence of residual solvents is evaluated by gas chromatography.
Microbiology	Bacterial endotoxins: the limulus amoebocyte lysate test is the most popular.
Sterility	Sterility testing must be initiated after several hours of preparation. To assure sterility, each batch of product has to be tested using culture vials with aerobic and anaerobic materials and incubated with culture vials for at least 4 days at 37°C. Sterility is assayed by visualising the cloudiness of the solution.

Table 2: *The necessary steps in quality control for PRs used in the clinic*
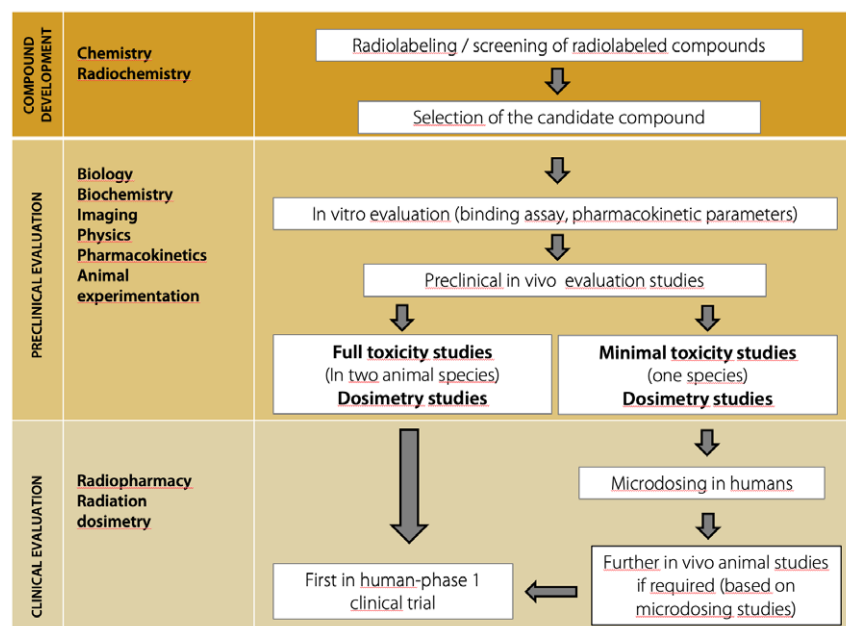
Figure 1: *Schematic of the RP evaluation steps needed for clinical translation*

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