Positrons as Imaging Agents and Probes in Nanotechnology

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Abstract. Positron emission tomography (PET) tracks a positron emitting radiopharmaceutical injected into the body and generates a 3-dimensional image of its location. Introduced in the early 70s, it has now developed into a powerful medical diagnostic tool for routine clinical use as well as in drug development. Unrivalled as a highly sensitive, specific and non-invasive imaging tool, PET unfortunately lacks the resolution of Computer Tomography (CT) and Magnetic Resonance Imaging (MRI). As the resolution of PET depends significantly on the energy of the positron incorporated in the radiopharmaceutical and its interaction with its surrounding tissue, there is growing interest in expanding our understanding of how positrons interact at the atomic and molecular level. A better understanding of these interactions will contribute to improving the resolution of PET and assist in the design of better imaging agents. Positrons are also used in Positron Annihilation Lifetime Spectroscopy (PALS) to determine electron density and or presence and incidence of micro- and mesopores (0.1 to 10 nm) in materials. The control of porosity in engineered materials is crucial for applications such as controlled release or air and water resistant films. Equally important to the design of nano and microtechnologies, is our understanding of the microenvironments within these pores and on surfaces. Hence as radiopharmaceuticals are designed to track disease, nuclear probes (radioactive molecules) are synthesized to investigate the chemical properties within these pores. This article will give a brief overview of the present role of positrons in imaging as well as explore its potential to contribute in the engineering of new materials to the marketplace.

1. Positron Emission Tomography (PET) and Drug Development

Positron emission tomography (PET) was developed some 30 years ago, however it was not until the 1990s that it was deployed as a tool for imaging the physiology and pathology of the brain, and later the heart. It is now widely used, in many countries for the detection and staging of a variety of malignant diseases. PET differs from other nuclear medicine procedures such as single photon emission tomography (SPECT), by its ability to detect coincidence signals, which allows the quantification of regional tissue radioactivity.[1-5] This is achieved by injecting a PET imaging agent or radiopharmaceutical incorporating a positron-emitting radioisotope such as $^{11}$C, $^{13}$N, $^{15}$O or $^{18}$F. These radioisotopes decay to emit a positron that loses sufficient energy to form positronium, and undergo annihilation to emit two 511 keV gamma rays (see figure 1). Those positrons that do not form positronium will undergo an alternate process called free electron annihilation. The probability of the former process is generally higher. The ability to detect these gamma rays in coincidence in two detectors at 180 degree is the key to locating the position of the radiopharmaceutical in the body.

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Figure 1 The positron annihilation process generates two gamma rays that are detected in coincidence by the PET camera.

Figure 2 A comparison of spatial resolution (relative size of symbols) sensitivity and potential toxicity of non-invasive imaging modalities.

2. Emerging Positron Radioisotopes

Many drug development companies have already implemented PET technology (using $^{11}$C and $^{18}$F radioisotopes) to fast track their research programmes. The short half lives and the requirement for specialised skills to incorporate these isotopes have limited the application of PET to small molecules (i.e. < 500 mol wt) with fast biological clearance rates. For larger molecules, (such as peptides, antibodies, DNA and nanoparticles) that have slower clearance rates, a PET isotope with longer half life is more appropriate. Copper 64, ($^{64}$Cu) is an emerging PET isotope that has a positron energy similar to $^{18}$F, but a longer half life (7 fold) which makes it ideal for attaching to larger target agents (i.e. > 1000s mol wt). It can be produced in a reactor (using natural copper or enriched zinc targets) and in a cyclotron (using enriched nickel or zinc targets). [7-18] The first routine production for $^{64}$Cu, as a by-product of the $^{68}$Ga production (using enriched $^{66}$Zn) using a 30 MeV was developed in
Australia by 1996.[11,19] In more recent times, it has been produced using low energy (15-12 MeV) cyclotrons and $^{64}$Ni targets.[13]

Unlike $^{11}$C and $^{18}$F, $^{64}$Cu can not be covalently attached or incorporated into the molecule and therefore needs to be attached through a bi-functional ligand. That is a ligand incorporating two parts; one for linking to the target agent or drug, and the other to complex the $^{64}$Cu. An array of polyazamacrocycles, polyazacarboxylate macrocycles and hexaaaza cage ligands have been designed for radiolabelling with $^{64}$Cu. However, only the hexazacage, sarar\(^2\) can complex $^{64}$Cu quantitatively over a wide pH range (4-9) at room temperature which makes it ideal for easy production and use in “kit formulations” (e.g. mixing of two vials of reagent at clinical site).[19] Furthermore, the three-dimensional encapsulation of $^{64}$Cu yields a kinetically inert complex that is ideal for the risk assessment of target agents. It ensures the PET signal is always associated with the target agent and therefore more accurate information regarding the biological pathway of the target agents can be obtained.

| Isotope  | Half-life | Positron Energy (average keV) | Gamma energy and probability (keV ; %) |
|----------|-----------|-------------------------------|---------------------------------------|
| $^{82}$Rb | 72 sec    | 1535                          | 776.5 ; 13                             |
| $^{15}$O  | 122.24 sec| 735                           | Nil                                   |
| $^{11}$C  | 20.39 min | 385                           | Nil                                   |
| $^{18}$F  | 109.77 min| 249                           | Nil                                   |
| $^{68}$Ga | 67.63 min | 836                           | 1077 ; 3                              |
| $^{64}$Cu | 12.7 hours| 278.2                         | 1345 ; 1.27                           |
| $^{124}$I | 4.176 days| 1540                         (max) | 603 ; 62.9 1691;10.88                   |

While the range of positron radioisotopes (see Table 1) generates plenty of opportunity for developing radioactive mimics of drugs, the relatively poor resolution of PET hinders its wider deployment in patient management. The resolution of PET is highly dependent on the energy of the positron, as the annihilation process requires the positron to slow to energies below ~100 eV and form positronium or annihilate with free electrons. A comparison of Monte Carlo simulations of the path length of different positrons in various tissues illustrated in figure 3, shows how the resolution of the PET image can depend not only on the energy of the positron but also the type of tissue from which it annihilates.[20]

For human PET cameras the resolution is of the order of 3 – 5 mm (for whole body) with $^{18}$F radiopharmaceuticals. The potential resolution achievable for animal PET cameras is estimated to be 0.50 to 0.75 mm. However this will only be achievable with improvements in the following areas: sensitivity of detectors, count rate capability, statistical reconstructions, the development of high specific activity radiopharmaceuticals and the use of appropriate positron emitting radioisotopes. Understanding how positrons interact with molecules and materials is fundamental to developing strategies to improve the performance of PET and therefore its applications.

See abbreviations for definitions
3. Positrons and Materials

Nature has developed exquisite biological systems that have the ability to sense, react, regulate, grow, regenerate and heal. Nature does this by designing systems (such as the cell) that are porous in nature and contain multiple microenvironments (e.g. nucleus, ribosome, and lysozomes) that can capture, conduct chemical reactions, release and adapt to their specific surroundings. The ability to design and synthesize materials (hard and soft matter) that can perform these types of operations and furthermore, progress their development into the marketplace is a key challenge for contemporary materials science. One fundamental property that appears to govern the design and performance of many new materials is their micro (＜2 nm) and mesoporosity (2 – 50 nm). Understanding the role of porosity in novel materials has implications in the design of catalysts, adsorbents, filtration and controlled release materials as well as anti-corrosive and water repellent films.

The sizes of the holes in materials can be readily determined using a number of routine techniques, such as transmission electron microscopy (TEM), Brunauer, Emmett and Teller (BET) surface analysis, and positron annihilation lifetime spectroscopy (PALS). TEM can give information at the atomic level however it does involve extensive sample preparation and many materials of interest are unstable under electron beam irradiation and high vacuum conditions. BET technique uses inert gases to probe the porosity and surface area of materials to give information on pore sizes from 3 – 100 nanometers. BET also requires high vacuum conditions and can give information about pore connectivity provided it is connected to surface pores. PALS can provide information on holes as small as an atomic vacancy (≈0.2 nm) to those up to 10 nm in bulk materials as well as in films. The advantage of PALS is the minimal sample preparation, making it ideal for comparing changes in the properties of materials under going various processes.

PALS works by releasing a positron (from a $^{22}$Na source; 10μCi) into desired material (~1 cm$^3$ position on either side of the source) that eventually annihilates with an electron in the pore or defect to emit gamma rays.[21] Hence the outgoing gamma rays carry information about the electronic density and pore architecture at the site of annihilation (see figure 4). Not only can the pore size and distribution be resolved, but information about the connectedness of pores can also be obtained. In figure 4 the extended decay profile for quartz indicates the presence of lower electron density or larger pores compared to the annealed nickel.

These lifetime spectra are fitted to the continuous decay form:

$$S_{LT}(t) = \int \lambda \alpha(\lambda) \exp(\lambda t) d\lambda$$

(1)

where $\lambda$ is an inverse of the positron lifetime (=1/$\tau$) and $\alpha(\lambda)$ is the probability density function (PDF) of the annihilation rate.[22]

The most common application of PALS has been in the semi-conductor industry; used to determine the presence and concentration of defects in these materials. More recent PALS has been used to study porosity in polymeric, ceramic, alloys and functionally gradient materials. Recently we have reported the use of PALS to study nanoporosity in complex biological materials such as wool and silk powders.[23]

4. Nanoporosity and Nuclear Probes

While understanding how the engineering of a material changes with the presence of micro and mesoporosity, it is equally important to understand the chemistry within these microenvironments or
nanopores. Particularly, when these materials are expected to react and respond to their changing environment by capturing a molecule from a solution for later release in another solvent system. Thus as one would design a drug for imaging disease in the body, nuclear probes are designed with different chemical characteristics (i.e. charge, size and hydrophobicity) to probe the physical and chemical properties of these nanopores.

Nuclear probes (radioactive target molecules) are highly sensitive (up to $10^{-5}$ ppb) and because they emit gammas, they can be used to study the interaction of materials with liquids (including high concentrations of electrolytes) and gases with negligible processing. Furthermore, studying of static or dynamic processes under experimentally relevant conditions can be adapted for high-throughput analysis. For example, three silk powders (Eri silk: Eri 1, Eri 2; Mulberry silk: Mulb; see figure 5), milled under various conditions, to different sizes ($9 - 4.3 \mu m$) and morphology; showed no change in microporosity ($0.48 \text{ nm}$) or surface area when analysed by PALS or BET, respectively.[24] Exposing the silk powder to metal ions (i.e. $^{64}$natCu$^{2+}$, $^{57}$natCo$^{2+}$ and $^{109}$natCd$^{2+}$) at various pH showed changes in rate and therefore selectivity for these metal ions. Figure 5 shows a typical plot for $^{109}$natCd$^{2+}$ absorption by these powders. These data suggest molecular structure of powders did not significantly alter compared to fibres on processing. However the processing the fibres into powders did increase the rate of diffusion of metal ions to binding sites.

Figure 5 Absorption of $^{109}$natCd$^{2+}$ to silk powder at various pH at 15minutes and 24 hours.

In addition, a range of nuclear probes (A – D; see figure 6) with overall positive and negative charges that are similar in size, show selective uptake by hollow silica shells over a range of pH. Negative (i.e. $D=\text{[Co-dota]}^{2-}$) and positively (A=\text{[Co-diamsar]}^{2+}; B=\text{[Co-sarar]}^{2+}; C=\text{[Co-sara-OH]}^{2+}$) charged nuclear probes have optimum absorption into hollow silica particles at pH 3 and 7, respectively. This indicates that there is an overall positive charge within the micropores of the silica particles that on incubation in buffer pH 7 could be made neutral or negative and therefore become responsive to the uptake of positively charge molecules as seen in figure 6. More interestingly, the subtle changes in molecular structures showed significant changes in uptake of these probes.

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See abbreviation for definitions
5. Conclusion

A review of the strategies used to advance the development of pharmaceuticals to the marketplace offers valuable lessons for the commercialisation of nanotechnologies. In particular, the design of relevant structure/activity tests that can act as gateways or in the risk assessment of material early in the R and D program, are valuable long term strategies for the cost-effective development of materials (scheme 1). Positrons and highly sensitive and selective nuclear probes are essential tools for investigating the size, concentration and distribution of pores and defects in materials as well as their chemical properties. They can be readily applied within materials assessment programs and are adaptable to high-throughout analysis. Using nuclear probes, processes can be monitored within seconds, over days and up to months. Together with current materials characterisation tools they can increase our confidence in our ability to predict and accurately measure performance of materials. Nuclear probes can also be used to develop methods for the cost-effective scale up of synthesis and to assess or to forecast potential risk of these materials.
Scheme 1. Demonstrates the application of positrons and nuclear probes to the commercialisation path of new materials.

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Abbreviations

Because the IUPAC names for the ligands are long and complicated, those ligands described in this paper have been abbreviated as follows:
sarar = 1-N-(4-aminobenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine
dota = (1,4,7,10-tetraazacyclododecane-N,N,N,N,-tetraacetic acid)
diamsar = 3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine
hydroxybenzyldiamsar = 1-N-(4-hydroxybenzyl)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine.
Complexes of these ligands will be denoted as M[(ligand)]n+, e.g. [Cu(sar)]2+.

CT Computer Tomography
MRI Magnetic Resonance Imaging
US Ultrasound
SPECT Single Photon Emission Computer Tomography
PET Positron Emission Tomography
TOF-PET – Time of Flight Positron Emission Tomography