Methods for Radiolabelling Nanoparticles

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The use of radiolabelled nanoparticles (NPs) is a promising nuclear medicine tool for diagnostic and therapeutic purposes. Thanks to the heterogeneity of their material (organic or inorganic) and their unique physical and chemical characteristics, they are highly versatile for their use in several medical applications. In particular, they have shown interesting results as radiolabelled probes for positron emission tomography (PET) imaging. The high variability of NP types and the possibility to use several isotopes in the radiolabelling process implies different radiolabelling methods that have been applied.

nanoparticles Imaging 68Ga

1. Radiolabelling with Copper-64

The use of ^{64}Cu for the radiolabelling of NPs is raising interest in both the preclinical and the clinical field. Its long relative half-life allows one to study the biodistribution and tumor targeting of the radiolabelled NPs for up to 48 h [1]. The chemical properties of this radiometal allows the use of different chelators that can be conjugated to different molecules. However, the conjugation of them with the chelator could influence the properties of the NPs and reduce the capability of the specific targeting technique.

1.1. Direct Radiolabelling

The direct labelling of the NPs with ^{64}Cu can be obtained with those nanomaterials that are defined as electron donors that have a high affinity with those radioisotopes that are defined as electron acceptors.

$^{64}Cu^{2+}$ ions ($3d^9$) require an electron to have a stable electronic configuration, and for this reason, it is easy to label it with the donor nanomaterials. Shi et al. employed graphene nanomaterials as electron donors for ^{64}Cu, thereby performing a stable direct labelling procedure without the use of BFCs. They showed that the labelling procedure is influenced by the temperature of the reaction and the concentration of the NPs. The highest labelling efficiency (LE), 75.5 ± 1.7%, was obtained with a concentration of 0.5 mg/mL^{-1} at 75 °C after 60 min of incubation [2].

The same method was applied to radiolabel silica NPs (SNPs), which were synthetized with the incorporation of oxygen atoms that were arranged in symmetry to be the electron donors for ^{64}Cu. The radiolabelling occurred by simply incubating the free radioisotope at 70 °C for 60 min, and there was a final radiochemical yield (RCY) of 99% after the centrifugation of it. The RCY improves with increasing temperatures (from 4 to 70 °C), but no correlation has been shown when one is varying the pH (5.7–8.8) [3].

Other silica NPs cannot bind ^{64}Cu stably; they dissociate rapidly under the physiological [4].

Several other metal nanomaterials can be labelled with metallic radioisotope by following the same principle of chemical affinity.

Single-well carbon nanotubes (SWCNTs) were directly radiolabelled with ^{64}Cu using a one-step procedure by incubating the isotope and the NPs under a sonication condition for 1 h. However, the stability of the radiopharmaceutical decreased up to 50% in the serum, thereby confirming the poor stability of this radiolabelling approach for SWCNTs [5].

1.2. Radiolabelling with Bifunctional Chelators (BFCs)

DOTA is the most frequently used BFC for ^{64}Cu labelling since after the complexation with Cu^{2+}-ions, it forms a stable complex, thereby leaving two carboxylic functions that are free to conjugate with the NPs and other molecules. The most frequently used method radiolabelling of NPs with ^{64}Cu is a post-synthesis process: the NPs are synthetized, coupled with the BFC, and the isotope is added at the end [6].

2. Radiolabelling with Gallium-68

^{68}Ga is a generator-produced isotope with a relatively low cost when it is compared to the cyclotron-produced isotopes. Despite it achieving non-excellent spatial resolution imaging in PET due to the high energy of positrons on it and its very short half-life (68 min), ^{68}Ga is a promising isotope for NP radiolabelling. Like ^{64}Cu, ^{68}Ga can be radiolabelled either directly or indirectly with a chelating agent, such as DOTA, NOTA, NODAGA, or other BFCs that create a very stable complex with gallium (III)-cation [7]. The widely used purification methods for ^{68}Ga-NPs are based on solid-phase extraction (SPE) or size-exclusion chromatography (SEC). However, other methods such as ultracentrifugation have also been applied [8].

2.1. Direct Radiolabelling

The QDs with ZnS cores and a PEG-OCH_3 coating (QD-OCH_3) were radiolabelled with ^{68}Ga through a cation exchange at nearly room temperature in an aqueous solution, thereby obtaining a very high LE. The QDs were doped with ^{68}Ga by incubating $^{68}GaCl_3$ in a sodium acetate buffer for 15 min at 37 °C. The NPs can subsequently be functionalized with peptides to improve their specificity [9].

Magnetite NPs (Fe_3O_4 MNPs) were radiolabelled without a chelator by adding a solution of sodium citrate and $^{68}GaCl_3$ and incubating them at 90 °C for 40 min. Before purification, the RCY was ~70%, as determined by radio-ITLC analysis, but after the purification, the sample showed a radiochemical purity >91% [10]. Another strategy for radiolabelling without the use of BFCs, is the core-doping of the NPs with a radioisotope using microwave-assisted heating. This method has several advantages, such as a reduced reaction time in comparison to the traditional methods, a high reproducibility, and a high LE and yield [11].

Pellico et al. radiolabelled the IONPs used this method by combining $FeCl_3$ and dextran (to ensure a colloidal stability) with the generator eluate $^{68}GaCl_3$ and heating the mixture to 100 °C (in 54 **s**) with microwave irradiation at 240 W for 10 min. This method turned out to be very efficient and reproducible with a high RCY, and after the purification, this was of 93.4 ± 1.8 [12].

Ligand anchoring group-mediated radiolabeling (LAGMERAL) has been demonstrated to be an efficient strategy for labeling Fe_3O_4 NPs. These were initially labelled with ^{99m}Tc as proof of concept, and then, they were labelled with ^{68}Ga. This method is based on the interaction between the metal radioisotope and the diphosphonate anchoring groups of the PEG-coated NPs [13][14].

2.2. Radiolabelling with Bifunctional Chelators

PEG-modified nano-graphene sheets were conjugated with NOTA and functionalized with a TRC105 antibody for the in vivo targeting of the early stages of many tumors. NOTA was firstly attached to the NPs by binding them to PEG molecules, and this step was followed by the addition of ^{66}Ga and its incubation for 30 min at 37 °C under a constant stirring condition [15]. ^{66}Ga is an equivalent of ^{68}Ga for PET use, but it has a physical half-life of 9 h, which makes more suitable for the pre-clinical kinetic studies.

Cobalt ferrite magnetic NPs that are functionalized with an aptamer-targeting under-glycosylated mucin-1 (uMUC-1) were firstly conjugated with NOTA in an $NaHCO_3$ buffer solution while it was vortexed and mildly stirred at 4 °C, and then, radiolabelled with the ^{68}Ga. The reaction mixture was incubated for 1 h after it was briefly vortexed for up to 24 h, and it had a high stability [16].

The IONPs were also radiolabelled with NOTA. NOTA was added into the IONPs solution and mixed for 2 h. The reaction mixture was then washed, and finally, it was purified using a PD-10 column [17].

The BFC DOTA was used for the labelling of polyamide dendrimers (PAMAM) that were conjugated with αυβ3 receptors for the detection of tumor angiogenesis in mouse models with Ehrlich's ascites tumors (EAT). The conjugation occurred with the addition of a DOTA-NHS ester to the dendrimer's solution. The mixture was stirred at room temperature for 48 h, and subsequently, ^{68}Ga was added in the solution. The reaction mixture was stirred and incubated at 90–100 °C for 15–30 min [18].

Hajiramezanali et al. conjugated SPIONs with N,N,N-trimethyl chitosan (TMC)-coated magnetic nanoparticles (MNPs). The conjugation with DOTA was performed using the amine groups of TMC on the surface of the NPs. It was possible to purify the final solution by centrifugating it because the functionalized NPs were precipitated. The radiolabelling procedure with ^{68}Ga was allowed by adding a $^{68}GaCl_3$ solution that had been previously eluted with 0.2 M HCl. The mixture was vortexed for 10 **s** and heated at 90 °C for 5 min. This method was very efficient, and it showed a radiochemical purity that was higher than 98% and a stability, in vitro in the human serum, of 92% after 120 min and of 86% after 180 min [19].

The radiolabelling of porous zirconia (ZrO_2) NPs was performed using DOTA as BFC, which was successfully adsorbed on the surface of the NPs. ^{68}Ga-radiolabelling was performed by mixing the DOTA-ZrO_2 solution with ^{68}Ga that had been previously preconditioned using AG 1-X8 resin columns at 95 °C and at a pH 4 for 20 min [20].

NODAGA is another chelator that can be used for the labelling of NPs with ^{68}Ga. AGuIX NPs are ultrasmall rigid NPs (5 nm) that are made of polysiloxane and surrounded by gadolinium chelates. Due to their size, they are sufficiently small to escape hepatic clearance. They were functionalized with NODAGA for the following radiolabelling process with ^{68}Ga to be performed. The labelling between the NPs and the BFC occurred by dissolving the NODAGA in DMSO, and then, it was gradually added to the AGuIX solution under a stirring condition for 5 h at room temperature. The in vivo studies showed that these NPs remain unmetabolized up to at least 60 min post-injection, thereby making them an excellent imaging agent with there being passive accumulation in the diseased area [21].

The NODAGA was used also by Lahooti et al. for the radiolabelling of ultra-small superparamagnetic iron-oxide nanoparticles (USPION) [22] and by Körhegyi et al. for the labelling of chitosan and poly-glycolic acid (PGA) NPs. In particular, the NODAGA-NHS solution, which had been previously prepared, was added in a dropwise manner to a chitosan solution, and the reaction mixture was stirred at room temperature for 24 h. The chitosan–NODAGA conjugate (CHI-NODAGA) was purified by a dialysis procedure and after the synthesis of folate-labelled PGA, the stable self-assembling NPs were produced via an ionotropic gelation process between PGA-PEG-FA and the CHI-NODAGA conjugate under a continuous stirring condition at room temperature to give an aqueous solution of the conjugated NPs. The radiolabelling was then performed by adding ^{68}Ga into the solution and incubating it at room temperature for 15 min [23].

Hydrophilic superparamagnetic maghemite NPs, which were coated with a lipophilic organic ligand and entrapped into polymeric NPs that are made of biodegradable poly(lactic-co-glycolic acid) (PLGA) which is linked to PEG were conjugated on their surface with NODAGA through a classic peptide bond. The purification was carried out by filtering the solution. After the conjugation with NODAGA was achieved, the ^{68}Ga eluate was added to the vial, and it was heated at 60 °C for 30 min [8].

3. Radiolabelling with Zirconium-89

Metallic radionuclides are excellent candidates for PET applications. ^{89}Zr, thanks to its half-life of 3.3 days, has been successfully used with many biomolecules that have long circulation times, such as the antibodies for immuno-PET applications. Similarly, the NPs that have a log-plasmatic half-life may benefit from being labelled with this radioisotope.

3.1. Direct Radiolabelling

The direct labelling with ^{89}Zr can be performed by using the chemical affinity between the isotope and the NP. In the literature, among the most significant results, the silica based-nanomaterials are often easily radiolabelled with

several isotopes due to the affinity of the silanol groups with the oxophilic cations [4]. Indeed, the radiolabelling of the silica NPs with ^{89}Zr is possible thanks to the strong interaction between the hard Lewis base (deprotonated silanol groups) and the hard Lewis acid ($^{89}Zr^{4+}$). Chen et al. used the favorable characteristics of the radiolabeled ultrasmall cRGDY-conjugated fluorescent silica NPs (C' dots) to radiolabel them with ^{89}Zr. As it is underlined as in this approach, is important to consider the pH and the temperature of the reaction, which should be between pH 8–9 and 50–75 °C, respectively. Indeed, a decrease in the pH (2–3) leads to a protonation of the silanol groups that cannot bind the positively charged ^{89}Zr.

3.2. Radiolabelling with Bifunctional Chelators

DFO is a cyclic hexadentate chelator that is widely used to chelate ^{89}Zr. Compared to DTPA, DFO shows a greater stability in vivo, without affecting the in vivo biodistribution of the NPs, and allowing a radiolabelling process to be performed at mild temperatures and with a neutral pH [24][25][26].

The radiolabelling via the ^{89}Zr-DFO coupling method usually provides a first step, whereby the NPs are conjugated to DFO, and this is followed by the addition of the isotope.

The DFO can also be used to stably label the isotope in the core of the NPs. For example, Li et al. radiolabelled liposomal NPs with the ligand-exchange method. The authors labelled the 8-HQ (oxine) to the isotope, thereby allowing the delivering of ^{89}Zr into the liposomal cavity where it was previously encapsulated in the DFO. Briefly, the authors added the DFO into the NPs solution, and this was followed by 30 min of incubation at 35 °C and 5 min of sonication, thereby allowing the encapsulation of DFO into the liposomal cavity. Then, the radioisotope was chelated with 8-HQ (oxine). The final mixture was kept at room temperature for 30 min before the addition of the DFO-liposome solution, which was followed by another 60 min of incubation. The volume ratio of the final solution of ^{89}Zr:8-HQ:DFO-liposome was 2:1:3. The RCY was 98%, but after its storage for 48 h at 4 °C, this was reduced to 83% [27].

Ferumoxytol (superparamagnetic iron oxide NPs that are coated with polyglucose sorbitol carboxymethylether) was labelled with ^{89}Zr-DFO, which was used as a PET/MRI contrast agent. For the success of the radiolabelling process, a modification of the surface chemistry of the drug was needed and, in particular, an amination of the particles to bind the DFO to Ferumoxytol was carried out.

After the radiolabelling process, which consisted of adding ^{89}Zr in the modified ferumoxytol and mixing them at 37 °C for 1 h, they analyzed the LE before its purification (>90%) and the radiochemical purity (99%, and this remained stable for over 24 h at 37 °C in mouse serum) [28].

High-density lipoprotein (HDL) has been radiolabelled with a high efficiency in several studies, and it is usually applied to image tumor-associated macrophages (TAMs) or activated macrophages in atherosclerosis. The ^{89}Zr physical half-life matches the biologic half-life of HDL, thus making ^{89}Zr-HDL a perfect radiopharmaceutical. For these studies, the labelling process required a previous modification of HDL with a DFO. The conjugation was

obtained via a reaction between the DFO and the lysine amino group of ApoA-1. This method showed a high radiochemical purity [29][30][31][32][33][34][35].

Dextran nanoparticles were studied as a nuclear probe for the detection of inflammatory leukocytes in atherosclerotic plaque. Before the radiolabelling was performed, the NPs were modified with epichlorohydrin through a cross-link reaction, and then, they were aminated with an ethylene diamine, thereby obtaining amino-dextran NPs (DNP-NH2). Finally, they were conjugated with p-isothiocyanatobenzyl desferoxamine (SCN-Bz-Df) and radiolabelled with ^{89}Zr, and then, they were added to the final mixture at room temperature [36].

AuNPs were radiolabelled with ^{89}Zr and conjugated with a monoclonal antibody (cetuximab) for to test their quantitative imaging performance in a PET application. The monoclonal antibody was first radiolabelled with ^{89}Zr via desferal moiety, and then, it was conjugated with AuNPs using carbodiimide chemistry. The radiochemical purity after the purification was >95%. The immuno-PET showed a higher tumor-to-background ratio of ^{89}Zr-cetuximab-AuNPs than ^{89}Zr-cetuximab did alone, without there being significant differences in the biodistribution, thereby proving that it is a promising tool for a future theragnostic approach. In another study that was conducted by the same group, AuNPs were conjugated with the anti-CD105 antibody which had been previously radiolabelled with ^{89}Zr using the same strategy. These NPs were used to perform a quantitative PET imaging of mice bearing tumors. The results confirmed its high specificity in vivo [37][38].

4. Radiolabelling with Iodine-124

Among the positron-emitting radionuclides, iodine-124 (^{124}I) has the longest half-life ($T_{1/2}$ = 4.2 days). This characteristic, when it is combined with its chemical properties, contribute to its wide diffusion in the study of NPs pharmacokinetic [39].

There are few data that are available in the literature regarding direct labelling, such as the remote loading method or the use of Iodo-beads and Iodogen, or via Chloramine-T oxidation. On the contrary, for indirect labelling, various techniques have been proposed, including the use of Bolton–Hunter reagent as BFC. Some of these techniques reach the best performing at high temperatures, which can be a limit of them.

4.1. Direct Radiolabelling

For the iodine radiolabelling of liposomal NPs, the direct labelling method demonstrated to have a higher efficiency than the indirect method using the Bolton–Hunter reagent did [40][41]. For this reason, a direct method to encapsulate ^{124}I in the liposomal NPs has been used. Here, isotopes are conjugated with compounds that allow the passive crossing of them through the membrane of the NPs. The most frequently used compound for the remote loading of ^{124}I in the liposomal NPs is the amino diatrizoic acid (ADA), a iodinated contrast agent that is usually applied in Computed Tomography (CT). The compound is first conjugated to the isotope, and then, thanks to solutions that are based on citrate or ammonium sulphate that create a transmembrane pH gradient, the

compound is able to cross the lipid membrane. The non-protonated compound, once it is inside the liposomal NPs, is protonated and cannot be released from the inner core [42].

A novel class of NPs, which are defined as “upconversion NPs (UCNPs)”, are composed by fluorescent metal-based materials such as $NaYF_4$, $NaGdF_4$, $NaLaF_4$, LaF_3, GdF_3, CeO_2, $LiNaF_4$, etc. They are characterized by an emission in the near-infrared (NIR) spectrum, thus resulting in a high degree of the penetration of the light through the biological tissues [43][44]. Lee et al. combined the optical properties of Er^{3+}/Yb^{3+} which was co-doped $NaGdF_4$ NPs using PET/MRI property imaging, thereby developing a multimodal tool for tumor angiogenesis imaging. The UCNPs were radiolabelled with ^{124}I using Iodo-Beads. The NPs that were functionalized with the arginine-glycine-aspartic acid (RGD) motifs had a surface-exposed tyrosine residue that allowed the direct conjugation of them with ^{124}I using the polystyrene beads. The resulting radiolabelling yield was approximately 19%, and the in vivo tumor uptake of ^{124}I-c(RGDyk)2-UCNPs was ~2%ID/g at 4 h, thus confirming that there was radiolabelling instability due to the de-iodination of radioiodine from the NPs. Further studies are needed to improve the stability of radiolabelling [45].

The same method was applied for polymeric NPs that were synthetized by poly(4-vinylphenol) (PVPh) polymers. The large number of phenolic groups on their polymeric backbone allowed an easy radio-iodination to occur, thus resulting in a high radiolabelling yield (~90%). The PVPh-NPs were incubated with iodination beads (Iodo-beads) including the ^{124}I isotope. When the beads were removed, the reaction was stopped. The NPs were then conjugated with three different mAbs: anti-adhesion molecule of platelet-1 endothelial cells (PECAM-1), anti-thrombomodulin (TM), and anti-PV1. The results showed that the NPs targeting PECAM-1 enabled a high-quality PET image to be obtained of the pulmonary vascularity in the murine models [46]. A similar approach was used with Iodination vials (Iodogen), where the iodine nuclides are blocked in the reaction vials. The isotope covalently labels the tyrosine motifs on the NPs’ surface [47].

By contrast, the Chloramine-T method has been used to radiolabel Gold NPs. Iodination was performed by adding the Chloramine-T reagent to the solution containing the isotope and the NPs. The free isotope was then removed by ultracentrifugation. The ^{124}I-AuNPs were used for in vivo tumor imaging through a micro-PET in a breast cancer mice model and to track the trafficking of the dendritic cells to evaluate the efficacy of the DC-based immunotherapy [48][49].

It has been reported that iodine isotopes have a high affinity for gold nanomaterials, thus resulting in them having a direct and strong bond with them [50]. ^{124}I-labeled gold nanostar probes (^{124}I-GNS) that are used for brain tumor imaging are selectively brain-tumor-targeting thanks to the EPR effect, thus making the ^{124}I-GNS nanoprobe promising for its future clinical applications to diagnose brain tumors [51].

4.2. Radiolabelling with Bifunctional Chelators

The Bolton–Hunter method has been successfully used to radiolabel silica NPs with ^{124}I. The NPs with an average diameter of 20–25 nm and surface-free amino groups were efficiently conjugated with a covalent linkage to the

NHS ester group that had been previously radiolabelled with ^{124}I (^{124}I-NHS) for the PET imaging to be performed in vivo [52].

5. Radiolabelling with Fluorine-18

Fluorine-18 that is labelled with a deoxyglucose molecule ([^{18}F]-FDG) is the main radiopharmaceutical that is used in clinical PET imaging. The main drawback of this radionuclide is its short half-life ($T_{1/2}$ = 109.7 min), which restricts its use to studies of small molecules with a fast biodistribution. The NPs generally have a longer pharmacokinetic that does not match with the half-life of this isotope, thus limiting its use in nanomedicine.

5.1. Direct Radiolabelling

One strategy for directly radiolabelling the NPs with ^{18}F is based on bombarding the nanomaterials with a neutron or proton, whereby an atom of the NP undergoes a nuclear reaction, thereby providing a radionuclide in situ. This strategy was applied for the radiolabelling of ^{18}O-enriched tin oxide (Al_2O_3) NPs by their direct irradiation with 16 MeV protons. The nuclear reaction allowed the transmutation of ^{18}O in ^{18}F. This method provided the precise control of the isotope position, thus achieving a high radiochemical stability. However, its application is limited to inorganic nanomaterials since the organic NPs can be affected and modified in their structure by the nuclear reaction. Furthermore, this method requires specific instrumentation with a high management costs [53]. Unlike the metal radionuclides that prefer to undergo labelling via chelators, the halogen radionuclides, such as ^{18}F, are usually labelled directly with a chemical group (chemical adsorption) or with a prosthetic group (indirect labelling) on the surface of the NPs. Chemical adsorption usually occurs with the reaction between the soft acids and the soft bases or between the hard acids and the hard bases, thereby creating strong coordination bonds between the isotope and chemical groups on nanomaterials. Several studies have been reported in the literature, showed the strong affinity between ^{18}F and the rare earth NPs, such as $KGdF_4$, $NaYF_4$:Yb, Gd-$NaYF_4$:Yb, $NaYF_4$:Yb, and $NaYF_4$:GdYb. The chemical adsorption of fluorine on the NPs' surface is a simple and fast method, whereby only the incubation of the isotope with the NP leads to a chemical stability of the compound with a RCY that is higher than 90% and a high radiochemical stability in vivo. The main limitation of this approach is the high temperatures that are required to achieve the conjugation [54][55][56].

Rare-earth fluoride NPs, such as yttrium trifluoride (YF_3) nanoparticles could be radiolabelled by mixing [^{18}F] the potassium fluoride solution with an aqueous solutions of NPs at room temperature, which would be followed by a 5 to 10 min incubation procedure. The free ^{18}F can then be removed by centrifugation. Excellent radiolabelling yields were reported, which were in the range of 80–95% [57]. This strategy could be also used with magnetic nanoparticles, such as $MnFe_2O_4$ and Fe_3O_4, where the radiolabelling process consists of adding a [^{18}F] sodium fluoride solution in a solution of NPs and incubating them while they are continuously shaken at room temperature for 10 min [58]. Indeed, UCNPs that are composed of lanthanide nanocrystals (Gd^{3+}/Yb^{3+}/Er^{3+}) with co-doped $NaYF_4$ were efficiently and directly radiolabelled with ^{18}F through a simple incubation. The strong binding between Y^{3+} and F^- allowed for a high LE. In vivo, the low bone uptake demonstrated the stability of this radiopharmaceutical.

The advantage of lanthanide materials is that they are characterized by their luminescent and magnetic properties, which provide a high spatial resolution and a high sensitivity when they are used in MRIs and fluorescent imaging, while the positron-emitting radionuclide provides functional information in PET imaging. Indeed, with a single nano-radiopharmaceutical, it is possible to obtain multimodal imaging at the molecular level with high sensitivity [59].

5.2. Radiolabelling with Bifunctional Chelators

For the indirect surface labelling of ^{18}F with prosthetic groups, it is typical that the copper-catalyzed azide–alkyne cycloaddition click chemistry is applied [60]. With this method, the prosthetic groups of the but-3-yn-1-amine modified USPIONPs, maleimide-AuNPs, and aminated IONPs were efficiently conjugated with ^{18}F under mild conditions and with high yields [61][62][63].

Nanodiamonds (DNPs) are sp3-carbon NPs, which are a promising biomaterial due to their good biocompatibility, possibility to be functionalized for drug delivery and ability to cross the cell membrane. The radiolabelling of these NPs was made possible by covalently attaching the ω-aminopropyl groups to the surface of the DNP, a reaction that occurs under mild conditions with high yields and is a well-established methodology for functionalizing various solid materials, including silicas and metal oxides. The resulting amino-DNPs were treated with ^{18}F-SFB (N-Succinimidyl 4-[^{18}F] Fluorobenzoate), thereby obtaining ^{18}F-radiolabelled NPs. In the biodistribution studies, it was observed that these NPs accumulate in the lung, spleen, and liver and are excreted into the urinary tract [64].

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