Integrity of $^{111}$In-radiolabeled superparamagnetic iron oxide nanoparticles in the mouse☆

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A B S T R A C T
Introduction: Iron-oxide nanoparticles can act as contrast agents in magnetic resonance imaging (MRI), while radiolabeling the same platform with nuclear medicine isotopes allows imaging with positron emission tomography (PET) or single-photon emission computed tomography (SPECT), modalities that offer better quantification. For successful translation of these multifunctional imaging platforms to clinical use, it is imperative to evaluate the degree to which the association between radioactive label and iron oxide core remains intact in vivo.

Methods: We prepared iron oxide nanoparticles stabilized by oleic acid and phospholipids which were further radiolabeled with $^{59}$Fe, $^{14}$C-oleic acid, and $^{111}$In.

Results: Mouse biodistributions showed $^{111}$In preferentially localized in reticuloendothelial organs, liver, spleen and bone. However, there were greater levels of $^{59}$Fe than $^{111}$In in liver and spleen, but lower levels of $^{14}$C.

Conclusions: While there is some degree of dissociation between the $^{111}$In labeled component of the nanoparticle and the iron oxide core, there is extensive dissociation of the oleic acid component.

1. Introduction

Superparamagnetic iron oxide (SPIO) nanoparticles, magnetite ($\text{Fe}_3\text{O}_4$) and maghemite ($\gamma$-$\text{Fe}_2\text{O}_3$) with a particle size of 5–25 nm and appropriate surface coatings have potential as drug delivery nanoparticles because they can be targeted to a tumor or diseased organ by using an external magnet [1–3]. They also have high capabilities as magnetic resonance imaging (MRI) contrast enhancers [4,5]. The essential requirement for the use of SPIO nanoparticles in these applications is a narrow distribution of particle sizes smaller than 100 nm with a high magnetization value that results in the characteristic superparamagnetism of the nanoparticles [1]. SPIO nanoparticles are good contrast agents for use in MRI, but this modality does not readily provide quantitative distribution data. SPIO nanoparticles have been labeled with $^{59}$Fe to allow quantitative biodistribution studies based on dissection of animal tissues [6]. However, the decay properties of $^{59}$Fe are not favorable for nuclear medical imaging [7]. A radionuclide commonly used in clinical nuclear medicine is $^{111}$In. It emits gamma-rays of energies 0.17 and 0.25 MeV, which combine the features of good ability to penetrate the human body, with efficient detection using nuclear medicine imaging instruments [7]. Also, the half-life of $^{111}$In is 2.8 days, which offers a reasonable “shelf-life” in the radiopharmacy with acceptably rapid clearance from the patient by physical decay after imaging studies have been completed. Furthermore, the electron-capture mode of decay avoids the higher radiation doses to tissues associated with nuclei that emit beta particles.

Nanoparticles have shown a tremendous potential in various biomedical applications. The ease of combining different imaging agents in a single platform, by means of using nanoparticles, paves the way for multimodal imaging using a single agent. Each imaging component can provide complimentary information based on which, the diseased sites can be diagnosed and detected efficiently with greater accuracy, both qualitatively and quantitatively. However, it is imperative to study the stability and integrity of these imaging agents conjugated to nanoparticles via different interactions (ionic interactions, covalent conjugations, etc.). The co-localization of different imaging agents in similar organs or diseased site can provide limited information on stability of these multimodal imaging platforms. Thus, it is very critical to assess the stability of these interactions once nanoparticles are administered in systemic circulation.

The objective of this manuscript is to study the in vivo integrity of ligand stabilized SPIO in systemic circulation. We adapted the synthetic approach of Jain et al. [8] to prepare oleic acid-stabilized hydrophobic iron oxide nanoparticles, which were further stabilized with different phospholipids to impart aqueous dispersibility for in vivo administration in mice. We incorporated different radioisotopes: (a) $^{111}$In using DMPE-DTPA on SPIOs platform for multimodal imaging; (b) $^{59}$Fe to label the iron oxide core and, (c) $^{14}$C to label the oleic acid. All three

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radiolabels were studied to quantify the distribution of each component and correlate the integrity of SPIOs in circulation. We expected that after intravenous administration in mice, the labeled nanoparticles would be accumulated by reticuloendothelial cells in the liver and spleen, and therefore that if the $^{111}\text{In}$ remained associated with the nanoparticles, the radioactivity should be found predominantly in these organs. Furthermore, if the nanoparticles remain intact in the blood systemic circulation after administration, then all the components (iron oxide core, intermediate oleic acid layer and phospholipid-DTPA-$^{111}\text{In}$) on the surface should be delivered to various organs to the same degree. We believe that since the core of nanoparticles is labeled with a radioisotope of iron ($^{59}\text{Fe}$), and the oleic acid is labeled with a radioisotope of carbon ($^{14}\text{C}$), then the organ distributions of these nuclides after intravenous administration should be the same as that seen with $^{111}\text{In}$-labeled SPIO nanoparticles. Furthermore, the biodistribution of $^{111}\text{In}$ after administration of $^{111}\text{In}$-labeled nanoparticles should be distinct from the biodistribution patterns of other $^{111}\text{In}$ species ($^{111}\text{In}$-citrate, DTPA-$^{111}\text{In}$, and DMPE-DTPA-$^{111}\text{In}$). While our studies were in progress, Freund et al. [6] reported that $^{14}\text{C}$-oleic acid label may be rapidly removed from the iron core after intravenous injection of similarly labeled SPIO nanoparticles, leading them to suggest that $^{14}\text{C}$-oleic acid-stabilized nanoparticles require careful assessment before using them for in vivo studies [6]. This study provides further insight into the stability of these ligand-stabilized nanoparticles and how these self-assembled nanoformulations could be engineered in the future for efficient in vivo applications.

2. Materials and methods

2.1. Animals

Male Swiss Webster mice (Charles River Laboratories, Cambridge, MA) weighing 25–30 g were used for all in vivo studies. Mice were maintained at the animal facility of Division of Laboratory Animal Medicine (DLAM) on 12 h alternating light and dark periods, with access to food and water ad libitum. Mice were treated humanely in compliance with NIH guidelines for the use of laboratory animals, and according to a protocol approved by Northeastern University Institutional Animal Care and Use Committee (IACUC).

2.2. Materials

Iron (II) chloride tetrahydrate (99+%, Acros Organics) (FeCl$_2$·4H$_2$O), iron (III) chloride hexahydrate (99+%, Acros Organics) (FeCl$_3$·6H$_2$O) and ethanol (200 proof, USP/NF) were purchased from Fisher Scientific (NJ). Sodium chloride (ACS Reagent, 99+%), oleic acid (99%), ammonium hydroxide solution (ACS reagent, 28.0–30.0% NH$_3$ basis), chloroform (ACS Reagent, 99.8+%), and HPLC grade water were purchased from Sigma-Aldrich (Saint Louis, MO). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-mPEG2000, CAS 474922-77-5) and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N,N-diethyltrimethylammoniumpropionate (DMPE-DTPA, CAS 384832-89-7) were purchased from Avanti Polar Lipids (Alabaster, AL). $^{59}\text{Fe}$ radionuclide ferric citrate, $^{59}\text{Fe}$ ferric citrate chloride, $^{14}\text{C}$ oleic acid, Solvable™ and Ultima Gold™ XR were purchased from Perkin Elmer (Waltham, MA).

2.3. Preparation of SPIO nanoparticles

The nanoparticles were prepared from ferric and ferrous chloride by co-precipitation and stabilization with oleic acid with slight modifications from the published procedure [8,9]. Briefly, 15 mL of 0.1 M FeCl$_2$·4H$_2$O with 30 mL of 0.1 M FeCl$_3$·6H$_2$O was mixed in a round bottom flask equipped with a thermometer. The molar ratio of Fe$_2^{3+}$ and Fe$_3^{3+}$ was kept at 1:2.2. The solution was bubbled with argon and stirred for 20 min in the chemical hood before heating. When the temperature reached 80 °C, 3 mL of 5 M NaOH was added drop-wise, the clear pale yellow–green solution immediately turned dark brown–black indicating the formation of iron oxide nanoparticles. At that point, 100 mg of oleic acid was added to the mixture. The heating continued for another 30 min while the temperature was maintained at 80 °C. The sample was then allowed to cool to room temperature (RT). The resulting SPIO nanoparticles were separated from the solution using a strong magnet and the particles were washed with ethanol twice, dried under argon, and re-dispersed in chloroform. Using a 10 mL syringe (Fisher Scientific, NJ), the re-dispersed SPIO nanoparticles were filtered through PVDF 0.45 μm filters (Millipore™ Millex™ Sterile Syringe Filters, Fisher Scientific, NJ) to remove large aggregates, dried under argon, carefully weighed, and re-dispersed in chloroform to produce a 20 mg/mL suspension of SPIO nanoparticles.

PEGylated SPIO nanoparticles were prepared by the rehydration method [8,9]. In brief, 10 mg of DSPE-mPEG2000 and 2.5 mg of SPIO nanoparticles were mixed in chloroform. The organic solvent was removed by using a rotary evaporator to complete dryness. The film was then warmed in 80 °C water for 1 min and rehydrated using 1 mL of HPLC grade water. The film was placed in a sonicking bath for 20 min to obtain a suspension of PEGylated SPIO nanoparticles. Uncoated nanoparticles were removed by applying an external magnet to the bottom of the tube, and the supernatant was removed to a fresh vial.

2.4. Characterization of nanoparticles

The particle size distribution and surface charge (zeta potential) of the PEGylated SPIO nanoparticles were determined by dynamic light scattering (DLS) measurement with Brookhaven Instrument's 90 Plus particle size analyzer (Holtsville, NY) [12,13]. The particle size was determined at a 90° angle and at 25 °C, and the average count rate was adjusted in the range of 100–500 kcps by proper dilution (10 μL of the PEGylated SPIO nanoparticles solution was diluted in 2 mL deionized water). The mean number and diameter of the nanoparticles were obtained on MSD distribution mode. The average zeta potential values were determined based on the electrophoretic mobility using the Smoluchowski–Helmholtz equation. Each sample was analyzed five times with 10 cycles each time, and an average value was obtained from the five measurements. The PEGylated SPIO nanoparticles were stored at 4 °C and their stability was assessed on day 2, day 3, day 4, day 5, day 6 and day 7.

A JEOL 100-X transmission electron microscope (Peabody, MA) accelerating at 80 KV was used for analyzing the structure of the iron core of the PEGylated SPIO nanoparticles [14]. 10 μL of the PEGylated SPIO nanoparticles solution was diluted in HPLC grade water, placed on the carbon-coated copper grids, and allowed to air-dry at RT.

2.5. Radiolabeling PEGylated SPIO nanoparticles with $^{111}\text{In}$

To radiolabel PEGylated SPIO nanoparticles with $^{111}\text{In}$, the nanoparticles were synthesized as described above along with addition of 0.5 mg/mL of DMPE-DTPA to the lipid film [10,11]. 1 mL of DMPE-DTPA containing SPIO nanoparticles was incubated for 1 h with 10 μL of $^{111}\text{In}$ citrate solution (diluted to approximately 8–10 μCi by using 0.5 M HCl) at RT, to allow for the transchelation of $^{111}\text{In}$ from a weak citrate complex into a strong DTPA complex. The radioactivity was measured in an ionization chamber (CAL/RAD MARK IV, Fluke Biomedical, Cleveland, OH) with a setting of 676. The unbound $^{111}\text{In}$ was removed by a 30 kilodalton cutoff microcentrifuge filter (Amicon Ultra 0.5 mL centrifugal filters, Sigma-Aldrich, St. Louis, MO) at 14,000 rpm for 15 min at 4 °C for 3 times. The radiolabeled PEGylated SPIO nanoparticles were recovered from the filter using 1.5 mL of 0.9% NaCl solution.
2.6. Preparation of $^{111}$In radiolabeled DTPA-$^{111}$In solution.

0.02 mg of DTPA was dissolved in 1.25 mL of saline. The solution was incubated for 1 h with 5 μL of $^{111}$In citrate solution (about 4–5 μCi) at RT, to allow for the transchelation of $^{111}$In.

2.7. Preparation of $^{111}$In radiolabeled DMPE-DTPA-$^{111}$In solution

0.02 mg of DMPE-DTPA was dissolved in 1.25 mL of ethanol-emulphor-saline (1:1:18). The solution was incubated for 1 h with 5 μL of $^{111}$In citrate solution (about 4–5 μCi) at RT, to allow for the transchelation of $^{111}$In.

2.8. Preparation of $^{59}$Fe or $^{14}$C-oleic acid radiolabeled SPIO nanoparticles

Nanoparticles radiolabeled with $^{59}$Fe in the SPIO core were prepared by adding a small volume (15 μL, 15 μCi) of radioactive $^{59}$Fe$^{3+}$ chloride solution to the mixture of ferrous and ferric chloride solutions before addition of ammonia (see procedure for preparation of unlabeled nanoparticles, earlier in this section). Similarly, nanoparticles radiolabeled with $^{14}$C-oleic acid were prepared by adding a small volume (0.25 mL, 25 μCi) of $^{14}$C-oleic acid solution to the oleic acid that was added after addition of ammonia. In both cases, neither the additional volume nor the extra mass of material should have any effect on the formation and coating of the nanoparticles, since this is being done in a volume of 45 mL using approximately 250 mg of iron and 100 mg of oleic acid. The radioactivity in the preparation of $^{59}$Fe-labeled SPIO nanoparticles was estimated using the ionization chamber. The radioactivity in the preparation of $^{14}$C-oleic acid-labeled SPIO nanoparticles, tissues were measured using a liquid scintillation counter (LS6500, Beckman Coulter, Brea, CA). After the formulation of $^{59}$Fe- and $^{14}$C-oleic acid-labeled SPIO nanoparticles, the PEGylated nanoparticles were prepared as described above, except that rehydration was done using 1.5 mL saline instead of HPLC grade water.

2.9. Biodistribution studies

Radioactive compounds were administered to conscious mice via a tail vein in 0.2 mL of vehicle. At indicated times, mice were euthanized by cervical dislocation, and samples of blood, urine and solid organs (brain, heart, liver lung, spleen, kidney, testis, fat, bone, muscle and skin) were collected. Each sample was carefully weighed in a tared tube. $^{111}$In and $^{59}$Fe were assayed in a gamma-counter (COBRA) using windows of 100–300 keV and 800–1600 keV, respectively. Counting standards were also assayed, and data were expressed as percent injected radioactivity per gram wet weight. For mice administered with $^{14}$C oleic acid-labeled SPIO nanoparticles, tissues were completely dissolved in Solvable™ after weighing, and then bleached with 30% hydrogen peroxide before addition of UltimaGold™ XR liquid scintillation fluid. The samples were then assayed for $^{14}$C using the liquid scintillation counter. Biodistribution data were expressed as percent injected radioactivity per gram tissue wet weight (%IA/g).

3. Results

3.1. Characterization of PEGylated SPIO nanoparticles

Fig. 1 is a schematic of the phospholipid stabilized SPIO nanoparticles showing the possible hydrophobic–hydrophobic interaction between the oleic acid and lipid part of the phospholipid. The PEGylated nanoparticles were stable and did not show signs of aggregation after 1 week at 4 °C (Figure S1). The hydration of oleic acid stabilized SPIONs using phospholipids increased the overall hydrodynamic size of nanoparticles from 5–25 nm to 40 ± 7 nm. The surface zeta potential of phospholipid stabilized SPIONs was −37 ± 7 mV. These results are in agreement with previous studies [12,13].

3.2. Efficiency of $^{111}$In labeling of PEGylated SPIO nanoparticles

Incubation of DTPA-substituted nanoparticles with $^{111}$In-citrate gave initial labeling yields of >70%. After 3 filtration steps >98% of radioactivity was associated with the particles (Table S1).

3.3. Biodistribution of $^{111}$In-radiolabeled SPIO nanoparticles

The tissue disposition of $^{111}$In after intravenous injection of labeled particles is shown in Table 1. Liver exhibited the highest radioactivity concentrations at all times (10 min, 30 min, 1 h, 2 h, and 24 h) followed by spleen. Radioactivity in the blood decreased with time after administration, as to a lesser degree did radioactivity in lung and heart, probably reflecting the large amounts of blood in these organs. In contrast, radioactivity in liver increased with time. This rise was significant (p < 0.05) between 10 and 30 min. Our interpretation is that $^{111}$In remained bound to nanoparticles in blood during the period of our experiments, and that the nanoparticles

![Fig. 1. Schematic of a nanoparticle consisting of a superparamagnetic iron oxide (SPIO) core of diameter 15 nm that was stabilized by addition of oleic acid, and then modified by addition of distearoylphosphatidylethanolamine-PEG2000 and dimyristoylphosphatidylethanolamine-DTPA to provide a hydrophilic coating and immobilized chelating groups to bind $^{111}$In$^{3+}$. Inset shows the SPIO formulation synthesized with $^{59}$Fe and $^{14}$C-oleic acid.](image-url)
continued to be accumulated by liver reticuloendothelial cells, and remained trapped in these cells. Similarly to the case with liver, the concentration of radioactivity in spleen and bone did not decrease over the course of our experiments, again consistent with trapping by reticuloendothelial cells in these organs [15,16]. Radioactivity concentrations decreased in the order skin > muscle > testes > fat > brain, and also decreased between earlier and later time-points in these tissues. It is likely that all radioactivity measured in the brain was actually in the brain vasculature, since it was very low at all times, and its concentration was 3–5% of that in the blood. Kidney had the third highest concentrations of radio-indium at all times, after liver and spleen which could reflect trapping of smaller sized nanoparticles in the glomerulus [17,18], though this is speculative. Since $^{111}\text{In}$ was recovered in urine at all time-points, some loss of $^{111}\text{In}^{3+}$ or other $^{111}\text{In}$ labeled species from the nanoparticles must occur.

### 3.4. Biodistribution of other $^{111}\text{In}$-labeled species

The dispositions of $^{111}\text{In}$ from the citrate, DTPA and DMPE-DTPA chelates were all distinct from each other and from that seen with the $^{111}\text{In}$-nanoparticles (Table 2). As expected from its use as a kidney function radiopharmaceutical, $^{111}\text{In}$-DTPA was very rapidly cleared into the urine [19]. The distribution of label from DMPE-DTPA-$^{111}\text{In}$ was most similar to that observed with label from $^{111}\text{In}$-labeled particles, but liver, spleen and bone levels were lower for DMPE-DTPA-$^{111}\text{In}$ than for the labeled particles, while the blood, heart and lung concentrations were higher.

### 3.5. Biodistribution of $^{59}\text{Fe}$ and $^{14}\text{C}$-oleic acid-radiolabeled PEGylated SPIONs

When the iron core of the SPIO nanoparticles was labeled with $^{59}\text{Fe}$, the distribution pattern at each time-point was marked by higher fractions of injected radioactivity in liver and spleen than for $^{111}\text{In}$ (Table S2), and lower concentrations elsewhere. However, bone, kidney, blood and urine contained more radioactivity than other organs (except liver and spleen). The fraction of injected $^{59}\text{Fe}$ in liver decreased from 55% to 45% between 10 min and 24 h ($p < 0.05$).

When nanoparticles labeled with $^{14}\text{C}$ oleic acid were injected into mice, a different pattern of distribution (Table S3) was observed than when the nanoparticles were labeled with $^{59}\text{Fe}$. Although the liver contained the highest concentration of $^{14}\text{C}$ at 10 and 30 min post injection, this halved (18% IA/g to 9% IA/g) between the two early time-points. Spleen, blood, kidney, heart and lung all contained approximately equal concentrations of $^{14}\text{C}$ (about 3% IA/g) at both 10 and 30 min, while the concentrations in testis and brain were much lower (about 1% IA/g). $^{14}\text{C}$ levels in urine were higher than those in non-hepatic tissues at 10 and 30 min. By 24 h after administration of labeled nanoparticles, radioactivity concentrations in all tissues, including liver, were low (<2% IA/g). Fig. 2 summarizes the dispositions of $^{111}\text{In}$, $^{59}\text{Fe}$ and $^{14}\text{C}$ from labeled nanoparticles at 10 and 30 min, and 24 h.

### 4. Discussion

Our major purpose was to obtain information about the extent to which $^{111}\text{In}$, a radionuclide with good properties for in vivo imaging in humans as well as in animal models, remains associated in vivo with the iron oxide core of labeled nanoparticles. To that end, we radiolabeled particles with $^{59}\text{Fe}$, in the core, and also with $^{14}\text{C}$-oleic acid, the surfactant, as well as with $^{111}\text{In}$, and compared the biodistributions of the labels. As seen in Fig. 2, the distributions of $^{111}\text{In}$ and $^{59}\text{Fe}$ are broadly similar, in that the organs with most active reticuloendothelial systems, liver and spleen, have the highest uptake levels of these two nuclides, especially at 24 h. The organ with the greatest discrepancy between $^{111}\text{In}$ and $^{59}\text{Fe}$ is the kidney, which has an 8-fold higher concentration of $^{111}\text{In}$ than $^{59}\text{Fe}$ at 24 h. In contrast to the two metallic radionuclides, $^{14}\text{C}$ exhibited much lower concentrations in liver and spleen, and our data thus confirm the recent conclusions of Freud et al. [6] that oleic acid used as a surfactant during preparation of SPIO nanoparticles does not remain firmly attached to the iron oxide core in vivo, or at least that not all the oleic acid remains bound.
The differences in distribution of either $^{111}\text{In}$ citrate or of DTPA-$^{111}\text{In}$ from those obtained with $^{111}\text{In}$-labeled nanoparticles (Tables 1 and 2) support the view that a substantial fraction of the $^{111}\text{In}$ is not lost from the particles in either of these chemical forms. However, the somewhat similar distribution of DMPE-DTPA-$^{111}\text{In}$ to that of $^{111}\text{In}$-labeled SPIO nanoparticles, in terms of high uptake in organs containing reticuloendothelial cells, raises the question of whether some of the uptake seen with labeled nanoparticles in liver and spleen is due to recirculation of dissociated DMPE-DTPA-$^{111}\text{In}$ to these organs. In other words, if this labeled phospholipid itself preferentially distributes to liver and spleen, which would be possible if, for example, it forms a micelle taken up by reticuloendothelial cells [20], then the fact that $^{111}\text{In}$ from the labeled SPIO nanoparticles is found in liver and spleen does not absolutely prove that the labeled nanoparticles remain intact in vivo. The higher uptake of iron-labeled than indium-labeled nanoparticles at 10 min (Fig. 2) is consistent with the notion that during the distribution phase some of the radioactivity dissociates from the nanoparticles in the form of DMPE-DTPA-$^{111}\text{In}$. As demonstrated in the experiment where DMPE-DTPA-$^{111}\text{In}$ itself was administered, in this case much of the dissociated radioactivity would be subsequently taken up and retained by the liver and spleen. Thus, at 10 min post injection the radioiron/radioindium ratio in liver is 2:1, but due to recirculation of DMPE-DTPA-$^{111}\text{In}$ at 24 h it has decreased to 1.25. An alternative explanation for differences between $^{111}\text{In}$ and $^{59}\text{Fe}$ is that during the labeling with $^{111}\text{In}$, this radioactive metal becomes associated preferentially with a subset of the particles, which exhibit a different distribution pattern than the majority of the particles. The particles are heterogeneous in size, and smaller particles, which have a higher ratio of surface to volume, must have a higher ratio of surface lipid to iron oxide core. Therefore they are expected to have more bound DTPA moieties per iron atom, and presumably a higher ratio of $^{111}\text{In}$ to $^{59}\text{Fe}$. Differences in the dispositions of $^{111}\text{In}$ and $^{59}\text{Fe}$ labeled particles could therefore reflect differences in tissue distribution patterns of particles of different sizes (or other characteristics) and not necessarily rapid dissociation of the $^{59}\text{Fe}$ and $^{111}\text{In}$ labeled components.

The potential advantages of radiolabeled SPIO nanoparticles as dual modality (PET/MRI or SPECT/MRI) imaging agents are well recognized, and this field recently been reviewed [4]. SPIONs have been labeled with the PET nuclides $^{11}\text{C}$ [21], $^{64}\text{Cu}$ [22–25], and $^{68}\text{Ga}$ [26,27], and with the SPECT nuclide $^{99m}\text{Tc}$ [28,29], but we are not aware of published reports of labeling with $^{111}\text{In}$, except for a PhD dissertation that does not include experiments with animals [11]. None of these reports describes experiments to evaluate particle integrity in vivo. Two evaluated integrity in vitro, in human blood plasma [26,29]. The most directly comparable study to ours, involving mouse experiments with oleate-stabilized particles with a DSPE-PEG coating, but labeling with $^{64}\text{Cu}$, is that of Glaus et al. [22]. Their biodistribution data showed similar high uptake in the liver and spleen at 30 plus min, but a larger increase between 10 and 30 min than ours. Bone and kidney dispositions were similar to those we saw for $^{111}\text{In}$, but other tissues (blood, lungs, heart, kidney, muscle, fat) had higher concentrations of $^{64}\text{Cu}$ at all times. The Glaus et al. data [22], while broadly similar to our $^{111}\text{In}$ data (Table 1) in that they show high uptake in organs with active reticuloendothelial uptake systems, suggest that the $^{64}\text{Cu}$ labeled particles, if they do not
decompose after injection into the mouse, clear from the circulation more slowly than our $^{111}$In-labeled particles. Combining the two studies, the rapidity of clearance appears to be $^{59}$Fe $>$ $^{111}$In $>$ $^{64}$Cu. Differences between the present study and that of Glaus et al. are that they: used BALB/c mice rather than Swiss–Webster; used mice weighing 20 g rather than 25 g; injected labeled particles under anesthesia rather than to conscious animals, and attached a metal chelating group to the end of the PEG chain, rather than using a separate lipid-anchoring lipid incorporating the chelating group. It is unlikely that any of these factors would greatly affect the distribution of labeled particles.

5. Conclusions
To our knowledge there have been no previously published studies that specifically evaluated the question of in vivo integrity of radiolabeled SPIONs intended for use with nuclear medical imaging technologies. The recent paper by Freund et al. [6], however, demonstrated that $^{14}$C-oleic acid does not remain with the $^{59}$Fe labeled iron oxide core, and the present study confirms their report. In view of the great potential advantages of radiolabeled SPIONs as dual modality imaging agents, critical studies of their in vivo stability, including radiochromatographic analysis of tissues, are needed.

Appendix A. Supplementary data
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nucmedbio.2014.08.014.

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