Nanoparticulated magnetic drug delivery systems: Preparation and magnetic characterization

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Abstract. This paper describes how magnetic resonance can be successfully used as a tool to help customize and quantify nanosized magnetic particles while labeling cells and administered in animals for targeting different biological sites. Customization of magnetic nanoparticles is addressed here in terms of production of complex magnetic drug delivery systems whereas quantification of magnetic nanoparticle in different biological compartments emerges as a key experimental information to assess time-dependent magnetic nanoparticle biodistribution profiles. Examples of using magnetic resonance in unfolding information regarding the pharmacokinetics of intravenously-injected surface-functionalized magnetic nanoparticles in animals are included in the paper.

1. Introduction
The successful development of a nanosized magnetic drug delivery system (MDDS) requires full assess to different information regarding the material’s properties and both in vitro and in vivo tests. Ultimately, the pre-clinical tests and clinical trials of a new MDSS is nowadays expected to be supported by the very knowledge of the computer-simulation of the interaction between the drug itself and the template material that carries, site-target, and delivers the drug. In between these two ends we found the synthesis and the physical characterization of the drug loaded and drug unloaded MDSS (l-MDDS and u-MDDS). Most of the currently developing MDSS based on the platform offered by precipitated and colloidal-suspended magnetic nanoparticles include iron-oxides, particularly cubic ferrites. Within this scenario magnetic resonance (MR) may play a key role in help in characterizing and understanding the very properties of the l-MDDS and u-MDDS. In addition to the use of magnetic resonance (MR) for the characterization of magnetic nanoparticulate-based materials the MR technique can also be successfully employed as an analytical tool to track these materials while in contact with biological systems, including time-dependent biodistribution of magnetic nanoparticulate-based materials incubated with cells or intravenously-injected in animals. This paper is focused on the description of the use of MR to assess important information regarding magnetic nanoparticulated materials while used to carry on in vitro and in vivo tests. The use of magnetic colloids as a material platform to produce l-MDDS and u-MDDS will be briefly described as well. Indeed, besides MR [1-3] other techniques have been used to characterize magnetic colloids, magnetic nanocomposites and MDSS, providing assess to morphological/crystalline aspects [4-9], magnetic properties [10, 11], and magneto-optical responses to DC/AC magnetic fields [12-15]. Applications of the l-MDDS discussed in this paper include cell-labeling [16-18], photodynamic therapy (PDT) [19-22], and magnetohyperthermia (MHT) of cancer cells and tissues [23-25].
2. The material platform provided by nanosized magnetic particles

Nanosized magnetic particles can be conveniently suspended as a very special colloid in which the suspended nanoparticle possesses a permanent magnetic moment. This special colloid, usually named magnetic fluid (MF), uses aqueous or organic carrier fluids, therefore providing an exceptional material platform to encapsulate the previously-synthesized magnetic nanoparticle in any hosting template in a very controllable way. Stable MF samples containing as much as 15% in particle volume fraction have been produced and shelved for long periods of time. Besides the Brownian motion electrostatic and steric repulsions are the main mechanisms supporting the MF colloidal stability. Electrostatic interaction is the dominant mechanism in ionic MFs whereas steric repulsion is the dominant mechanism supporting the MF colloidal stability in organic-based MFs. In more complex systems, such as in the biocompatible magnetic fluids (BMF), both electrostatic and steric repulsion mechanisms take place simultaneously, providing very stable magnetic colloids even at physiological conditions. Nevertheless, biocompatibility of magnetic fluids is assigned only after performing and evaluating in vitro and in vivo tests while is often considered a dose dependent issue.

The most extensively used synthesis route for MF samples described in the literature starts with the co-precipitation of the metal ions from their aqueous salt solution using weak or strong base addition under controlled chemical species (hydroxyl and metal-ions) concentration, stirring speed, and temperature. Peptization of the precipitated magnetic nanoparticle as ionic MF can be accomplished in low-pH as well as in high-pH values [26, 27]. Fresh precipitate containing magnetic nanoparticle can be suspended in both non-polar [28, 29] and polar-based media [30, 31]. Starting with MF samples magnetic nanosized particles can be encapsulated as anionic or cationic magnetoliposome (ML), trapped in magnetic nanoemulsion (MNE) or hosted in magnetic nanocapsule (MNC). The ex-situ incorporation of magnetic nanosized particles using the MF technological platform provides full control for nanoparticle concentration with excellent uniformity. MDDS consisting of biocompatible magnetic fluids (BMFs), magnetoliposomes (MLs), magnetic nanoemulsions (MNEs) and magnetic nanocapsules (MNCs) are presently under production and under biological test worldwide [19-23, 32-39].

Interesting aspects regarding MNE formulations are the enhanced drug solubilization, good thermodynamic stability and easily-achieved scaling up for mass production [40]. Whereas surface passivation of the suspended nanoparticle needs to be properly addressed to prevent chemical degradation and aging effect PDT and MHT may promote phase changes of the magnetic component in MDDS. In order to investigate the phase stability of the material and identify the thermally- and optically-induced production of new magnetic phases different spectroscopic techniques have been employed. Magnetic resonance, for instance, provides very useful information regarding magnetic nanoparticle core and surface engineering and particle-particle interaction. In addition, due to the strong MR signal provided by nanosized magnetic particles the technique can be used as an excellent spectroscopic tool to probe biodistribution of MDDS while introduced in animals or used to label cells.

Figure 1 presents a typical particle size (core diameter) histogram plot obtained from the analysis of transmission electron microscopy (TEM) micrographs. The vertical bars in Fig. 1 represent the experimental data whereas the solid line represents the best curve-fitting using the log-normal distribution function. Average particle diameter \(D\) and diameter dispersion \(\sigma\) obtained from the data shown in Fig. 1 was 5.6 nm and 0.22, respectively. The sample whose data are presented in Fig. 1 consists of spherical nanosized magnetite surface-coated with DMSA (dimercaptosuccinic acid). While suspended in physiological medium the DMSA-coated magnetite nanoparticle can be used to target cells (\textit{in vitro} tests) and organs (\textit{in vivo} tests). The next section will describe how MR can assist the \textit{in vivo} and \textit{in vitro} tests using surface-coated oxide-based magnetic nanoparticles suspended in physiological medium.
Figure 1. Typical particle size distribution of magnetite nanoparticles surface coated with DMSA. The inset is a representative TEM micrograph of the sample.

3. Magnetic resonance in cell labeling and organ targeting

Evaluation of the magnetic nanoparticle content in a given biological site is easily obtained via MR measurements as long as a calibration curve is provided. Figure 2 shows typical MR spectra recorded from two BMF samples, each one presenting distinct nanoparticle contents ($2.5 \times 10^{13}$ particle/mL and $5.0 \times 10^{16}$ particle/mL). Resonance spectra recorded in a given range of nanoparticle concentration are used to build the calibration curve, as shown in Fig. 3. The vertical axis in Fig. 3 represents the area under the MR spectrum (absorption curve) whereas the horizontal axis represents the magnetic nanoparticle concentration (particle/mL). The samples whose MR spectra are shown in Fig. 2 are based on magnetite nanoparticles (9.4 nm in average diameter) surface-coated with Dextran and DMSA. Biocompatible magnetic fluid samples containing magnetite nanoparticle surface-coated with Dextran and DMSA were labeled DEX-MAG and DMSA-MAG, respectively. The room temperature MR data, as shown in Fig. 2, were recorded using a commercial spectrometer (Bruker ESP-300) tuned around 9.42 GHz.

Figure 2. Typical magnetic resonance spectra of nanosized magnetite particles coated with Dextran and DMSA and suspended as biocompatible magnetic fluid samples (DEX-MAG and DMSA-MAG), at different particle concentration.

Figure 3. Typical magnetic resonance calibration curve. The sample used was the biocompatible DMSA-MAG, whose spectra were presented in Fig. 2.
Magnetic resonance was used to investigate the effects of incubating mouse Raw cells with DEX-MAG, with emphasis in finding the efficiency of magnetic nanoparticle (MNP) internalization by the cells. Symbols in Fig. 4 represent the time \((t)\) dependence of the DEX-MAG concentration \((C)\) in Raw cells [18]. The DEX-MAG concentration in the Raw cells versus time is described by 
\[
C(t) = C_o [1 - \exp(-k_c t)],
\]
where \(C_o = 53 \times 10^{14}\) particle/mL and \(k_c = 0.23\) min\(^{-1}\). The solid straight line in Fig. 4 (semi-log plot) represents the best curve-fitting of the experimental data (solid triangles) according to the previously described exponential behaviour. Above 12 hours we found the experimental data falling well below the straight solid line in Fig. 4, indicating a systematic reduction of the DEX-MAG concentration, probably due to cell metabolization, as suggested by independent analysis using light microscopy analysis. Indeed, the half-life associated to the MNP internalization by the cells is given by 
\[
t_{1/2} = 0.69/k_c = 3\ s.
\]
Likewise, MR was used to investigate the time dependence of the MNP concentration in the blood circulation and in several organs following an intravenous bolus dose administration of sample DEX-MAG to female Swiss mice. Animals between 8 and 10 weeks of age, were used in this study. In order to perform the MR experiments 100 \(\mu\)L of the DEX-MAG sample containing about \(10^{17}\) particle/mL were used. After injection the animals were sacrificed and the samples collected. Before the MR measurements were carried out the animals were submitted to a full perfusion procedure. One hour after the injection MNP were found mainly in liver and spleen, while small amounts of magnetic material were observed elsewhere. Blood samples from the control were mixed with the DEX-MAG sample in order to obtain the calibration curve. Open squares in Fig. 5 represent the time-decay (disposition) of the dextran-coated magnetite nanoparticle concentration in blood [41]. The data indicate that the bolus dose reaches the systemic circulation where, in a short period of time, it is homogenously distributed. The solid straight line going through the open squares in Fig. 5 represents the best fit of the MR data using one-exponential decay, thus signalling the presence of a single biological compartment [42]. Therefore, the DEX-MAG concentration in the blood versus time is described by 
\[
C(t) = C_o^B \exp(-k_B t),
\]
where \(C_o^B\) and \(k_B\) are the MNP concentration at \(t = 0\) and the rate constant for elimination of the MNP from blood, respectively. The best fit of the MR data recorded from the blood samples using the equation presented above provides \(C_o^B = 110 \times 10^{14}\) particle/mL and \(k_B = 0.10\) min\(^{-1}\). The half-life associated to the MNP disposition from the compartment is given by 
\[
t_{1/2} = 0.69/k_c = 6.9\ min.
\]
In addition, data in Fig. 5 allow determination of mouse blood clearance as 
\[
C\ = \frac{\text{dose}}{\text{dose}} \int C(t)dt = \text{dose} \times k_B \times C_o^B = 90\ \mu\text{L/min} \ [43].
\]
Finally, the volume of distribution \((V)\) in the mouse systemic circulation was 
\[
V = CL/k_B = 900\ \mu\text{L}.
\]
Figure 5 describes how fast MNP is transferred from the bloodstream compartment to the liver and spleen (LS) compartment. Open circles in Fig. 5 represent the time-dependence (absorption) of the dextran-coated magnetite nanoparticle concentration in liver and spleen. The time dependence of the MNP concentration in the organs (liver and spleen) is described by 
\[
C(t) = C_o^{LS} [1 - \exp(-k_{LS} t)],
\]
where \(C_o^{LS} = 35 \times 10^{14}\) particle/mL and \(k_{LS} = 0.05\) min\(^{-1}\). This finding allows us to estimate the effective half-life associated to the MNP absorption by the two compartments (liver plus spleen) as 
\[
t_{1/2} = 0.69/k_c = 13.8\ min.
\]
Mass-balance involving the two compartments (B and LS) indicates that about \(8 \times 10^{15}\) nanoparticles were found in liver and spleen 60 min after injection, while in the same time window about \(16 \times 10^{15}\) particles left the bloodstream. Blood, liver, and spleen volumes were quoted as 1.5, 2.3, and 0.2 mL, respectively. We argue that about \(8 \times 10^{15}\) particles were captured by the blood mononuclear phagocyte cells (neutrophils and monocytes) and retained in the liver and spleen. The blood mononuclear phagocyte cells containing nanoparticles is certainly removed out by the perfusion procedure before the MR data were recorded using the liver and spleen samples. Based on the mass-balance the dashed line in Fig. 5 (with \(k_{LS} = 0.10\) min\(^{-1}\)) would be the expected curve describing the time dependence of the MNP concentration in liver plus spleen if the organs were not submitted to the perfusion procedure. Consequently, due to the perfusion procedure, the open circles and the associated solid straight line (see Fig. 5), both representing the time-dependence of the dextran-coated magnetite nanoparticle concentration in liver and spleen, lie just below the dashed line. Therefore, semi-log plots as presented in Fig. 5 allow direct
observation of the mass-balance scenario. Note in addition that all straight lines in Fig. 5 extrapolate to the unit as the time tends to zero.

Figure 4. Time dependence of the magnetic nanoparticle concentration. The data were obtained from the MR spectra recorded from mouse Raw cells after incubation with DEX-MAG [18]. Note the natural logarithm vertical scale.

Figure 5. Time dependence of the magnetic nanoparticle concentration. The data were obtained from the MR spectra recorded from blood (open squares) and liver plus spleen (open circles) after intravenous injection of DEX-MAG as a bolus dose in female Swiss mice [41].

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