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A New Quantitative Method to Determine the Uptake of SPIONs in Animal Tissue and Its Application to Determine the Quantity of Nanoparticles in the Liver and Lung of Balb-*c* Mice Exposed to the SPIONs

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We propose a new method for determining the quantity of superparamagnetic iron oxide nanoparticles (Fe_3O_4, SPIONs) embedded in animal tissue using magnetization measurements. With this method, the smallest detectable quantity of magnetite nanoparticles in a tissue sample is ~1 μg. We showed that this method has proved being efficient. In this study, we focused in determining the quantity of SPION confined in lung and liver tissue of mice injected with ~13 nm magnetite superparamagnetic nanoparticles. Furthermore, the method allowed us to detect the magnetite nanoparticles present in animal tissues without letting the natural iron ions present in the tissue or blood interfere with the measurements.

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A comprehensive understanding of the bio-distribution of SPION throughout the body is of great importance due to the wide variety of applications that SPION currently enjoy in medicine, ranging from NMR diagnostics to drug delivery systems and hyperthermia for cancer treatment. Different approaches based on quantitative chemical and physical methods employed to evaluate the presence of SPIONs in animal tissues have been studied before. For this purpose there are available the ferrozine assay, Prussian Blue, Atomic Absorption Spectroscopy and Plasma coupled Spectroscopy methods,[1–4] but they are qualitative in nature and/or can not distinguish between the endogenous iron found in proteins from the iron present in SPIONs. Alternative methods including NMR[5] and ferromagnetic resonance (FMR)[6] are more desirable since they are quantitative and provide a magnetic signal that is characteristic of SPION. However, they involve expensive equipments and the sample preparation in these cases results painstaking.

We developed a quantitative method for measuring the quantity of SPIONs in an animal tissue based on the superparamagnetic properties of these particles. SPIONs' magnetization curve as a function of magnetic field and temperature $M(H, T)$ is given by the Langevin function: $M(H, T) = M_S[\coth(x) - (1/x)]$, where $x = \mu H/k_B T$, k_B is the Boltzmann constant, μ is the magnetic moment of the nanoparticle and M_S is the saturation magnetization, which is usually given normalized by mass (emu/g) or volume (emu/cm^3). The measurement of the M_S value of a sample gives the amount of magnetic phase in the whole sample in a very precisely. $M(H)$ curve of organic materials usually shows diamagnetic behaviour (or occasionally a paramagnetic one), which corresponds to a linear response with negative (or positive) slope when a moderate magnetic field is applied. The clear differences between the $M(H)$ curves corresponding to the SPIONs and organic tissue allows us to separate these contributions easily. By comparing the M_S value of the sample with the normalized M_S value of the particles it is easy to determine the exact quantity of SPIONs present in the sample.

We performed a study based on the quantification of SPIONs in different animal tissues to test one potential use of this method. SPIONs coated with oleic acid

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were prepared following a chemical route as described elsewhere.[7,8] To improve the biocompatibility of these nanoparticles, the oleic acid coating was replaced by a layer of DEXTRAN (MW $\sim$ 5000), following a similar procedure as described in Ref. [9]. SPIONs were morphologically characterized by means of Transmission Electron Microscopy (TEM) images, which were obtained in a Philips Electron Microscope operating at 200 kV. TEM images (bottom panel of Fig. 1(a)) show that spherical-like shape nanoparticles have a narrow size distribution with mean diameter $\langle d \rangle = 13$ nm and dispersion of $\sigma = 0.15$, as obtained from the log-normal fitting of the diameter histogram. Magnetization versus magnetic field measurements (-10 kOe–10 kOe) were performed in a commercial Vibrating Sample Magnetometer (VSM) at room temperature. These measurements show a characteristic curve of a superparamagnetic system (Fig. 1(a)) with $M_S = 32.8$ emu/g (DEXTRAN coating and other factors such as size reduction, decrease the value of M_S per gram of nanoparticle in comparison to the bulk value of 90–92 emu/g).

In order to prove the plausibility of the use of magnetization measurements as a quantification method of the magnetic nanoparticles present in animal tissue, we prepared three samples of bovine muscular tissue by injecting an aqueous suspension of magnetite nanoparticles to the tissue. Samples $M1$ and $M2$ were prepared by adding 22 ± 1 μl (2.6×10^{-4} $g_{magnetite}$/ml) and 80 ± 1 μl (3.6×10^{-4} $g_{magnetite}$/ml) of a nanoparticle suspension (equivalent to 5.8 ± 0.6 μg and 28.9 ± 0.4 μg of nanoparticles) injected in 0.1486 ± 0.0002 g and 0.1247 ± 0.0002 g of muscular tissue, respectively. A third sample without nanoparticles was also measured ($M0$). In the $M(H)$ curve of $M0$, we observed a characteristic diamagnetic curve, while for samples $M1$ and $M2$, the overlapping of the diamagnetic and superparamagnetic contributions is evident (Figs. 1(b) and (c)). The extrapolation of the high magnetic fields data to zero field determines M_S for each sample. As a result, for $M1$ and $M2$ the saturation magnetization was determined giving 5.9 ± 0.1 μg and 31.7 ± 0.1 μg respectively. This was found taking into account the M_S of our magnetite nanoparticles. These obtained values are close to the nominal ones given by the design of the samples.

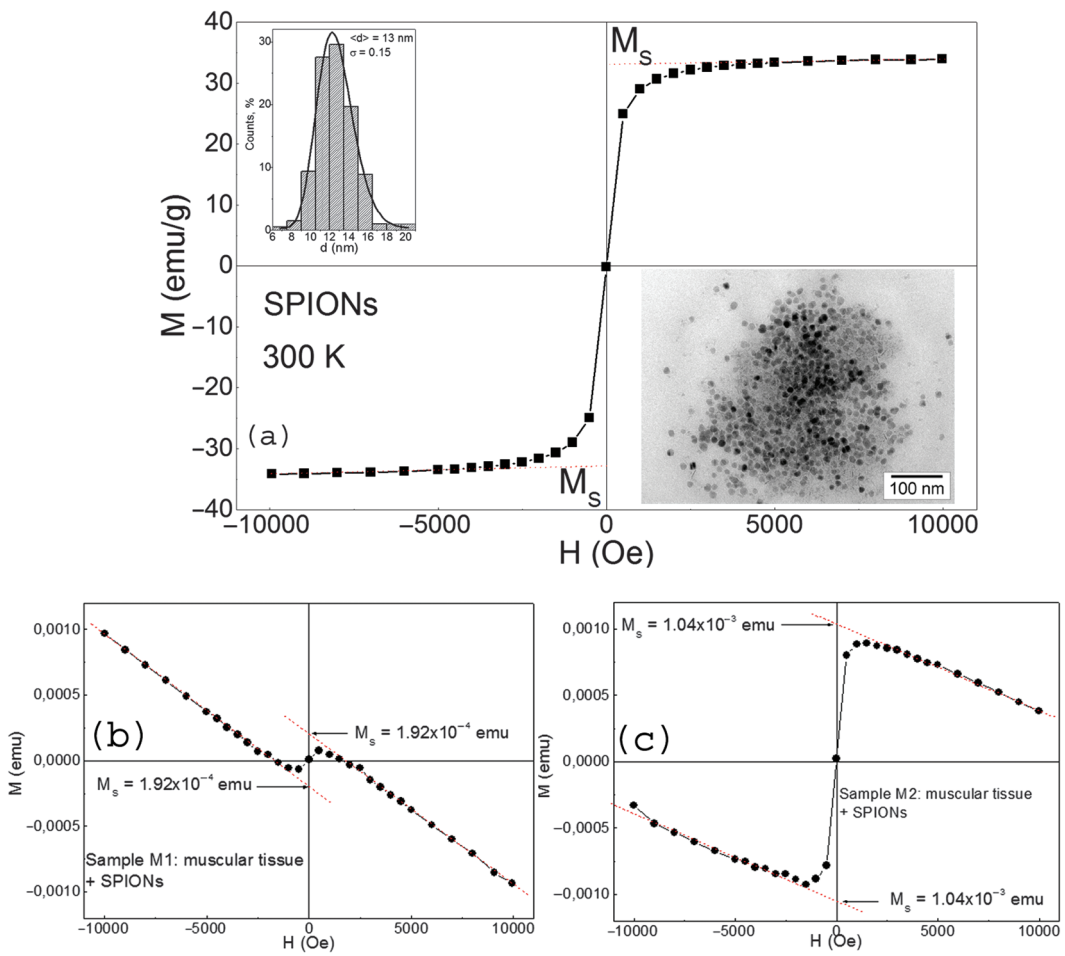

Fig. 1. (a)–(c): $M(H)$ curves of the SPIONs, sample $M1$ and $M2$, respectively measured at 300 K (bottom panel: TEM image of 13 nm SPIONs; top panel: diameter histogram fitted with a log-normal distribution).

Six *ex-vivo* bovine muscle tissue samples for each concentration of nanoparticles were measured. In all cases the results were quite similar with dispersion attributable to the size distribution of the drop volume of the suspension which was added to the tissue.

We used this method to determine the quantity of SPIONs present in liver and lung tissues of mice previously injected with an aqueous suspension of nanoparticles. Eight Balb-*c* mice aged 7 weeks were injected with 200 μl of an aqueous suspension containing DEXTRAN coated SPIONs (0.5% m/v) (2 control mice without injecting were also studied). The injection was applied into the tail vein with an overall mass of 1 mg of Fe_3O_4 per injection. 24 hs after the injection, the animals were sacrificed. All animal procedures were performed following NIH guidelines for animal care. Liver and lung tissues were extracted and fixed in formalin solution for 48 hs. Then these tissues were passed through a series of ethanol solutions (70, 96, and 100% v/v respectively). The liver was chosen because its high content of endogenous iron is known to interfere with most of the current techniques used to detect iron in animal tissues. Meanwhile, the lung was selected because the presence of magnetic particles in lung vessels might increase the risk of thromboembolism if these particles agglomerate. In all the samples the concentration of nanoparticles in different tissues was found to be within an expected distribution for the population of mice studied. The data which was previously reported corresponds to a mouse which absorbed a quantity of nanoparticles that is closest to the average value of the distribution. We focused our presentation of the results on the sensitivity and the accuracy of the proposed method.

Figures 2(A) and (B) show the magnetization measurements of liver and lung tissues where the superparamagnetic magnetization contribution is visible. In the same figure, the magnetization measurements of the same tissues from a non-injected mouse (control specimen) are plotted for comparison displaying only the diamagnetic behaviour (Figs. 2(C) and (D)). In each case the concentration of magnetite nanoparticles was clearly determined from the M_S values (see extrapolation of Figs. 2(A) and (B)): 29.1 ± 0.1 μg of magnetite in 114.2 ± 0.1 mg of liver tissue and 92.4 ± 0.3 μg of magnetite in 39.1 ± 0.1 mg of lung tissue. Table I summarizes the quantity of magnetic nanoparticles for all samples.

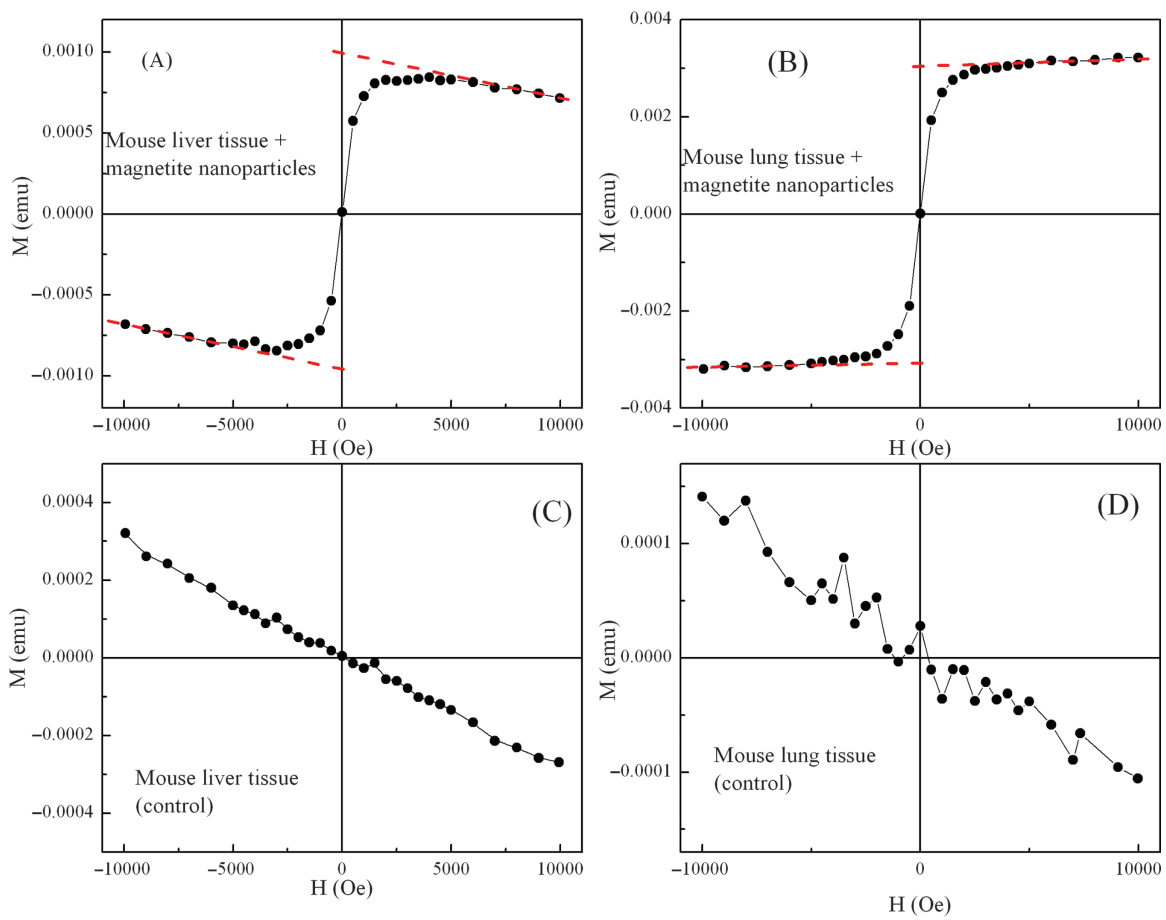

Fig. 2. (A) and (B) present the $M(H)$ curves of the liver and lung samples of a mice injected with a suspension of SPIONs, respectively. (C) and (D) present the respectively $M(H)$ measurements from a non-injected mouse. The solid lines are only a guide for the eye and the dotted lines are the extrapolation of the high field magnetization to zero field, indicating the saturation magnetization.

Table I. Determination of the quantity of SPIONs present in different animal tissues.

Sample	Sample mass (g)	M_S (emu/g)	SPIONs mass from M_S (μg)	SPIONs mass added (μg)
$M1$	0.1486 ± 0.0001	$1.92 \pm 0.02 \times 10^{-4}$	5.9 ± 0.1	5.8 ± 0.6
$M2$	0.1247 ± 0.0001	$1.04 \pm 0.02 \times 10^{-3}$	31.7 ± 0.1	28.9 ± 0.4
Liver	0.1142 ± 0.0001	$9.53 \pm 0.02 \times 10^{-4}$	29.1 ± 0.1	–
Lung	0.0391 ± 0.0001	$3.05 \pm 0.02 \times 10^{-3}$	92.4 ± 0.3	–

In conclusion, we presented a simple method for determining the quantity of SPIONs embedded in animal tissue by using magnetization measurements, avoiding any interference caused by the natural iron ions. Since the magnetometer sensitivity is $\sim 1 \times 10^{-5}$ emu, it is then possible to discriminate a signal of ~2–3 emu from the diamagnetic signal of the tissue. The minimum detectable quantity of nanoparticles using our method is ~1 μg of SPIONs in the sample. By applying this method, we determined that the quantity of SPIONs embedded in liver and lung of mice is 0.25 μg and 2.4 μg per mg of tissue, respectively. This supports the assumption that lung tissue absorbs a larger quantity of SPIONs than the liver tissue. Further studies must be done to extend the understanding of this issue.

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