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Magnetorelaxometry of magnetic nanoparticles with fluxgate magnetometers for the analysis of biological targets

Frank Ludwig[a,*], Erik Heim[a], Sascha Mäuselein[a], Dietmar Eberbeck[b], Meinhard Schilling[a]

[a]*TU Braunschweig, Institut für Elektrische Messtechnik und Grundlagen der Elektrotechnik, Hans-Sommer-Str. 66, D-38106 Braunschweig, Germany*
[b]*Physikalisch-Technische Bundesanstalt, Section 8.21, Abbestr. 2-12, D-10587 Berlin, Germany*

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Abstract

A magnetorelaxometry system based on sensitive fluxgate magnetometers for the analysis of the relaxation behavior of magnetic nanoparticles is presented. The system is tested with a dilution series of magnetite. The results are directly compared with data obtained with a SQUID magnetorelaxometry system measured on the same samples. Advantages of using fluxgates rather than SQUIDs for magnetorelaxometry are discussed.

Keywords: Nanoparticles; Relaxometry; Relaxation; Fluxgate magnetometers; SQUID; Assay; Magnetite

1. Introduction

Magnetic nanoparticles are used in a wide range of biological and medical applications. Recently, the MAgnetic Relaxation ImmunoAssay (MARIA) was proposed and developed [1–4]. In an immunoassay, the goal is to detect and quantify specific biological targets. Using magnetic nanoparticles as labels has the advantage that they are stable, nontoxic and can be used in opaque media as well. In MARIA the relaxation behavior of the magnetic nanoparticles is measured after switching off a magnetizing field. The key point is that magnetic nanoparticles bound to specific targets can be distinguished from unbound ones by their different relaxation times. If the molecules labeled with magnetic nanoparticles bind to a molecular structure that is fixed to a solid phase (the so-called solid-phase magnetic relaxation immunoassay), the nanoparticles are immobilized and the Brownian relaxation is suppressed. Thus, MARIA provides a quantitative measure of the amount of bound molecules even in the presence of unbound magnetic markers, i.e., without washing them out.

Very recently, magnetorelaxometry focused on the detection of magnetically tagged molecules in

*Corresponding author. Tel.: +49 531 3913863; fax: +49 531 3915768.
E-mail address: f.ludwig@tu-bs.de (F. Ludwig).

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solution, since in real biological system binding of the target molecules to a solid-phase immunoassay often is not applicable and requires long incubation times. As a sample system, Eberbeck et al. [5] studied magnetic nanoparticles bound to modified latex spheres and studied the binding kinetics. Grossman et al. [6] demonstrated the detection of *Listeria monocytogene* bacteria in suspension.

So far, all magnetorelaxometry experiments were performed with superconducting quantum interference devices (SQUIDs), fabricated either from the conventional superconductor Nb (e.g. Refs. [1,2,5]) or from the high-T_c superconductor $YBa_2Cu_3O_{7-x}$ (YBCO) [3,4,6]. SQUIDs are known to be the most sensitive magnetic field sensors and have magnetic field noise values down to below 1 fT/Hz$^{1/2}$ for Nb [7] and down to a few fT/Hz$^{1/2}$ for YBCO [8,9]. Obviously, it would be highly desirable to have room temperature sensors since SQUIDs require cryogenic temperatures. Among the various room temperature magnetic field sensors that are capable of measuring AC and DC magnetic fields, fluxgates exhibit the lowest magnetic field noise values. Commercial fluxgate magnetometers have white-noise values in the range of a few pT/Hz$^{1/2}$ [10], our own wire-wound fluxgate magnetometers have noise values down to about 350 fT/Hz$^{1/2}$ at 1 kHz, the lowest value reported so far for a fluxgate magnetometer [11]. In a fluxgate magnetometer, a ferromagnetic core is periodically driven by a primary coil into saturation and generally the second harmonic of the signal induced in a secondary coil is measured in a feedback circuit.

The aim of the present work is to investigate whether magnetorelaxometry can be performed with fluxgate magnetometers and to determine the detection limits.

A fluxgate differs from a thin-film SQUID magnetometer not just by the higher noise and operation at room temperature without cooling but by the following points:

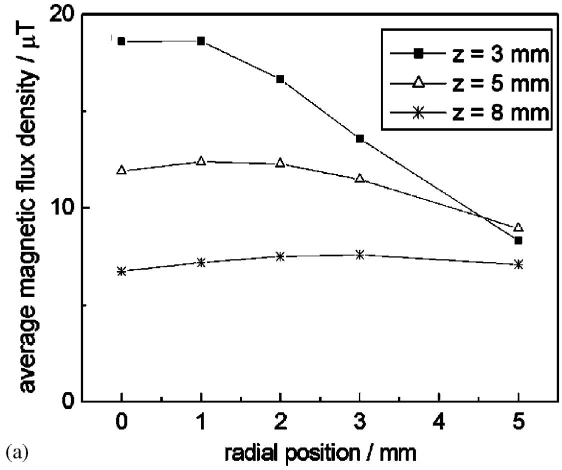
1. For a SQUID, the signal is proportional to the average magnetic flux penetrating the pickup structure. In a fluxgate magnetometer, the signal is proportional to the magnetic flux density averaged over the volume of the core.
2. A thin-film SQUID is a vector magnetometer, i.e., only magnetic field components normal to the pickup and SQUID structure couple flux to it. The core of high-sensitive (non-thin-film) fluxgate magnetometers is a three-dimensional object and the sensitivity in the various directions depends via the demagnetization factor on its shape. For rod-shaped cores, as used in this work, the sensitivity along the long axis of the core dominates, i.e., only magnetic fields parallel to it couple effectively to the sensor.
3. Since the fluxgate core consists of ferromagnetic material which is periodically driven into saturation, the fluxgate itself might affect the magnetic behavior of the magnetic nanoparticles.
4. While a SQUID only detects magnetic flux/magnetic field changes, the output signal of a fluxgate magnetometer is a measure for the absolute value of the magnetic field in its sensitive axis. This allows one to measure with a fluxgate both the magnetic signal when the magnetizing field is on and the relaxation behavior after switching off the field. In all SQUID magnetorelaxometry experiments reported so far, due to slew rate limitations, the flux-locked loop of the readout electronics could not be closed until a few 100 μs after switching off the field.

2. Experimental setup

In our magnetorelaxometry system a commercial fluxgate magnetometer from Bartington Instruments, Ltd. [10] is used. It has a white noise level of 3 pT/Hz$^{1/2}$ with a $1/f$ corner at a few Hz. The fluxgate was driven with an excitation frequency of 15 kHz. For the magnetization of the magnetic nanoparticles a Helmholtz coil with a diameter of 13.8 cm was used. The homogeneity of the magnetic field across a volume of 5 cm × 5 cm × 5 cm is better than 0.2%. Magnetic fields up to about 1 mT, representing a typical value in magnetorelaxometry [1–6], can be applied. The use of a Helmholtz coil principally has the potential to align the (vector) magnetic field sensor so that it sees only field distortions from the magnetic sample but no direct field components from the coil.

To determine the optimal arrangement between fluxgate, sample and magnetic field finite element (FEM) simulations were performed. Here a magnetic dipole was placed in the center of the coordinate system with its moment in z direction (as a result of the magnetizing field in z direction). For the core of the fluxgate a length of 20 mm and a diameter of 1 mm was assumed. A permeability $\mu_r = 80,000$ was used in the simulations. For a constant moment of the magnetic dipole, the magnetic flux density in the core direction averaged over the core volume was determined. It was found that—for the same distance between core end and magnetic dipole—the signal is about a factor of two larger when the core is oriented perpendicular to the z-axis. One important point is that the flux lines are concentrated in the ferromagnetic core. This flux concentration effect increases the signal by about one order of magnitude. The basic results showing the dependencies on core position are depicted in Fig. 1(a). For comparison, the dependence of the signal on sensor position for a SQUID loop with a diameter of 3 mm and its sensitive axis in z direction is plotted in Fig. 1(b). As reported by Matz et al. [12], for this SQUID arrangement the sensor should not be too close to the magnetic sample, otherwise spatially distributed magnetic dipoles inhomogeneously contribute to the total signal. For a fluxgate, due to the averaging effect, the signal depends much less on the exact radial position with respect to each other even for relatively small overall distances. As expected, the signal is at a maximum if the magnetic dipole is located at the end of the core.

All fluxgate experiments reported in this paper were performed with the fluxgate oriented with its sensitive axis perpendicular to the z-axis. The center of the sample holder was separated by about 5 mm from the fluxgate core. The measurements were performed in a magnetically shielded room. Without any sample, the fluxgate was manually positioned in the Helmholtz coil to minimize the signal. Residual field values of about 100 nT could be achieved, indicating an alignment to about 10^{-4}.



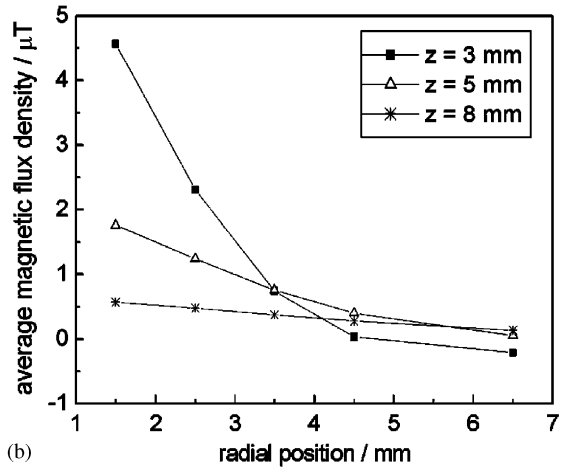


Fig. 1. (a) Dependence of the average magnetic flux density in the fluxgate core on radial position for different distances between magnetic dipole and core. The magnetic dipole moment is oriented in z direction, the fluxgate core is oriented perpendicularly to z. (b) Dependence of the average magnetic flux density through a SQUID loop with 3 mm diameter on radial position for different distances between magnetic dipole and SQUID. The SQUID loop normal is oriented in z direction. A value for the dipole moment of 2×10^{-12} Wb m was used in the FEM simulations.

To be able to observe very fast relaxation processes, the magnetic field should be switched off as fast as possible. Generally, the switchoff process follows an exponential function. A special drive electronics was developed which provides a linear decay of the magnetic field from typically 1 mT down to zero within about 300 µs.

3. Results

To test the performance of our prototype system a series of diluted magnetite samples was prepared. The initial concentration was 421 mol (Fe)/m^3, the sample volume was estimated to be about 150 μl. Dilutions were carried out with mannit solution. All data were 50 times averaged to improve the signal-to-noise ratio. A disadvantage of the used Bartington fluxgate magnetometer is that the excitation frequency amounts to 15 kHz, requiring low-pass filtering which does not distort the signal for frequencies up to about 3 kHz. To obtain the individual traces, the difference between the averaged and filtered measurement curves with and without the sample was calculated.

Typical time traces, recorded for a magnetizing field of 0.72 mT, are depicted in Fig. 2. To analyze the individual curves they were fitted with an exponential function

$$f(t) = B_{\mathrm{off}} + B_0 \exp\left(-\frac{t}{\tau}\right)$$

as expected for the Brownian relaxation of monodisperse magnetic nanoparticles, and alternatively with a stretched exponential function [5,13]

$$f(t) = B_{\mathrm{off}} + B_0 \exp\left(-\left(\frac{t}{\tau}\right)^{\beta}\right).$$

Although the stretched exponential function is rather phenomenological—to our knowledge, there is no microscopic model deriving it from a superposition of nanoparticles with a certain size distribution—it is a consequence of a distribution of hydrodynamic volumes of magnetic nanoparticles and aggregates. B_{off} is an offset field, B_0 the amplitude which is expected to be proportional to the concentration of magnetic nanoparticles, τ is the time constant and β is a number reflecting the size distribution. It was found that the stretched exponential function describes the experimental data by far better than the simple exponential function, indicating that the magnetite dilutions indeed contain a certain distribution of hydrodynamic sizes. The fitting parameters are depicted in Fig. 3 versus Fe content in mol. All parameters are normalized to their values at the highest magnetite concentration. The amplitude B_0 nicely scales with concentration down to about 1 μmol Fe and then there seems to be a saturation. The time constant τ and the exponent β are within the error margins independent of concentration as expected since the dilution experiment should not influence the nanoparticle size and concentration but only their concentration. Values of about $\tau = 5\,\mu s$ and $\beta = 0.22$ were found, the latter being a typical value known from literature [5]. Since—as stated above—there is no microscopic model for the stretched exponential function, it is hard to interpret these two fitting parameters quantitatively. Introducing a phenomenological time constant τ_{eff} which is the time at which the magnetic signal decayed to $1/e$ from its field-on value after switching off the field, one obtains a value of about 150 μs.

Also shown in Fig. 3 are the corrected fluxgate signals when the magnetic field is on. As can be seen, deviations from the linear relationship between signal and concentration level off at higher concentrations, indicating that this measure is more susceptible to mechanical or magnetic distortions. In any case, the fact, that—with our

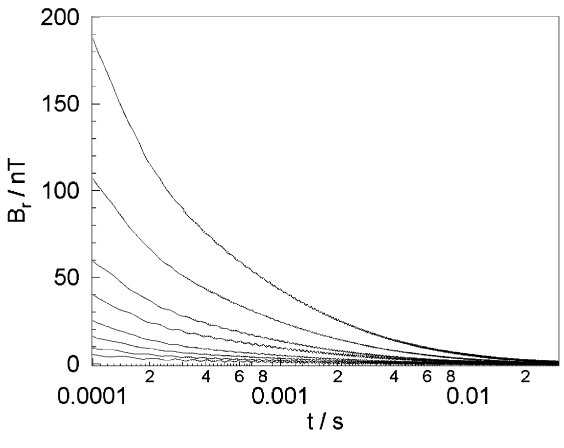


Fig. 2. Magnetorelaxation curves measured for a series of diluted magnetite samples. All curves were 50 times averaged. The magnetite concentration steadily decreases from top to bottom. The slight oscillations are residues of the 15 kHz fluxgate excitation. Note that the magnetizing field of the Helmholtz coil reaches zero at $t_0 = 300\,\mu s$ so that an analysis of the relaxation behavior of the MNPs can be performed only after this time.

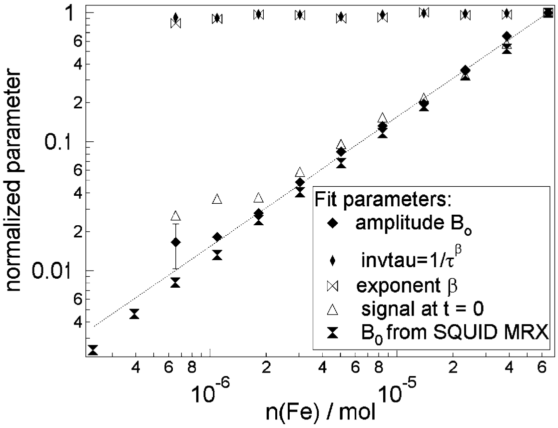


Fig. 3. Normalized fitting parameters obtained from fitting the relaxation curves by a stretched exponential function versus Fe content. The dotted line shows the theoretical dependence for a parameter that linearly scales with the Fe concentration. For the amplitude B_0 obtained for a Fe content of 0.65 μmol the error bar is shown. The error bars continuously decrease with increasing Fe concentration; at the maximum concentration the error amounts to about $\pm 0.1\%$.

fluxgate system—one obtains both the signal when the field is on and the relaxation amplitude, provides an additional degree of freedom for the analysis. The signal when the field is on is caused by the field of all magnetic nanoparticles of the sample proposed that the fluxgate is properly aligned perpendicularly to the magnetic field direction. In contrast, the relaxation behavior depends on the time constants of the magnetic nanoparticles and the fact whether Brownian or Néel relaxation dominates.

To get a direct comparison with the well-established SQUID magnetorelaxometry, the same dilution series was measured with the SQUID setup at the PTB Berlin. Details of the SQUID system are published elsewhere [12]. In this system, the Nb SQUID is arranged with its sensitive axis in z direction, i.e., in the direction of the magnetizing magnetic field. Since the signal of the sample with the highest magnetite concentration was rather high for the SQUID sensor, the distance between SQUID and sample holder was increased to about 27 mm. As for the fluxgate measurements, the best fit to the relaxation curves was obtained for a stretched exponential function. To get a direct comparison, the normalized amplitudes B_0 are plotted in Fig. 3. As can be seen the signal decreases with decreasing Fe concentration slightly faster than linearly. The other fit parameters are comparable to those derived from the fluxgate measurements.

4. Discussion and summary

It was demonstrated that high-sensitive fluxgate magnetometers have the potential to be used for magnetorelaxometry. A direct comparison with SQUID magnetorelaxometry shows that nanoparticle concentrations can be determined from the relaxation behavior at least down to concentrations of 1 μmol Fe in 150 μl aqueous solution. So far we do not have a clear explanation for the saturation of the fluxgate signal B_0 for Fe contents below 1 μmol. Taking the magnetic field noise as the ultimate limit, the detection limit of the used fluxgate should be about three orders of magnitude higher than that of the SQUIDs, provided that the number of averages is the same. The error in the fluxgate measurements was of the order of about 1 nT which is considerably higher than the about 100 pT estimated from the fluxgate noise, the chosen bandwidth and the number of averages. A possible reason might be insufficient mechanical stability of the fluxgate sensor with respect to the Helmholtz coil, especially when exchanging samples.

There are several ways to further improve the performance of fluxgate magnetorelaxometry. First, the mechanical construction has to be improved to detect even smaller nanoparticle concentrations. Second, one could use fluxgate magnetometers with lower noise. As already mentioned, our best fluxgate magnetometers have a white noise level that is almost one order of magnitude lower than that of the used one. Third, more work is needed to understand the influence of sample volume and position with respect to the sensor, the magnitude of the magnetizing field and influence of the ferromagnetic core on the sample magnetization.

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