Detection of endogenous magnetic nanoparticles with a tunnelling magneto resistance sensor

BY A. IONESCU1,*, N. J. DARTON2, K. VYAS1 AND J. LLANDRO1

1 Cavendish Laboratory, University of Cambridge, Cambridge CB3 0HE, UK
2 Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge CB2 3RA, UK

The magnetotactic bacterium Magnetospirillum sp. has been cultured and the properties of its endogenous magnetic nanoparticles characterized. Electron-microscopic analyses indicate that the endogenous magnetite nanoparticles in Magnetospirillum sp. are coated with a 3–4 nm thick transparent shell, forming a magnetosome. These magnetite nanoparticles had diameters of 50.9 ± 13.3 nm, in good agreement with the diameter of 40.6 ± 1.2 nm extracted from magnetometry. Each Magnetospirillum sp. bacterium contained chains of 5–25 magnetosomes. Superconducting quantum interference device magnetometry results indicate that the extrinsic superparamagnetic response of the bacterial solution at room temperature can be attributed to the reversal of the magnetization by physical rotation of the nanoparticles. The intrinsic blocking temperature of a sample of freeze-dried bacteria was estimated to be 282 ± 13 K. A tunnelling magneto resistance sensor was used to detect the stray fields of endogenous magnetic nanoparticles in static and quasi-dynamic modes. Based on the tunnelling magneto resistance sensor results, the magnetic moment per bacterium was estimated to be approximately 2.6 × 10⁻¹³ emu. The feasibility of this detection method either as a mass-coverage device or as part of an integrated microfluidic circuit for detection and sorting of magnetosome-containing cells was demonstrated.

Keywords: endogenous nanoparticles; magneto-resistance sensing; Magnetospirillum sp.; blocking temperature

1. Introduction

The emerging multi-disciplinary field of biomagnetism and magnetic-based biosensors has attracted a considerable amount of interest in the last few years. This interest has been spurred on by the successful integration of magnetic nanotechnology into biomedicine and life sciences, for example, in magnetic directed drug delivery by magnetic resonance imaging (Martel et al. 2007; Darton et al. 2008), in hyperthermia treatment, magnetic actuation of cells (Pankhurst et al. 2009) and in bioassays using magnetic labels (Megens & Prins 2005). These techniques are based on magnetic nanoparticles that are normally chemically synthesized (Roca et al. 2009) and subsequently coated for biocompatibility and ligand attachment (Berry 2009). However, one of the

*Author for correspondence (ai222@cam.ac.uk).

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drawbacks of using chemically synthesized particles is their inhomogeneous size and moment distribution (Micklem et al. 2008 and references therein). Particularly in magnetic nanoparticle-based bioassays, these inhomogeneities lead to a poor magnetic quantification by current sensor technologies such as tunnelling magneto resistance (TMR) and giant magneto resistance (GMR) effect-based sensors (Llandro et al. in press). The question arises whether or not magnetic nanoparticles endogenously produced in magnetotactic bacteria (Blakemore 1975) could offer a more mono-disperse and versatile alternative for use in biomagnetic technologies.

In 1991, a helically shaped magnetotactic bacterium, Magnetospirillum sp. AMB-1, was isolated from freshwater sediment in Kewanee, Tokyo (Matsunaga et al. 1991). The magnetic response of this microaerobic bacterium is due to its ability to synthesize magnetite ($\text{Fe}_3\text{O}_4$) nanoparticles in intracellular structures called magnetosomes. These comprise a magnetite mineral crystal surrounded by a lipid bilayer membrane about 3–4 nm thick. Most magnetotactic bacteria in the Northern Hemisphere move towards the magnetic north, so are ‘north-seeking’, whereas those isolated in the Southern Hemisphere are ‘south-seeking’. It is thought that they have this magnetic-pole-seeking capability to enable them to move downwards to the less-aerated sediments, low in oxygen, in their aqueous habitat (Bazylinski & Frankel 2004).

There is currently an absence of reported studies on the magnetic response of endogenous magnetic nanoparticles in a bacterial solution. However, there have been a number of studies on dry frozen samples of magnetotactic bacteria containing chains of magnetosomes and of released magnetosomes (Denham et al. 1980; Moskowitz et al. 1988; Pan et al. 2005). While the entire magnetosome chain in a bacterium responds as a single-domain magnetic dipole, the released magnetosomes have a slightly higher saturation magnetization, smaller coercive fields and a lower remanent/saturation magnetization ratio. This can be explained by the fact that released magnetosomes are closer together and not separated by cell membranes and cytoplasm. In previous studies, these endogeneous magnetite nanoparticles, measured to have physical diameters of 40–50 nm, are observed to have a single magnetic domain, supported by a variety of measurement techniques. However, there is evidence that some of the larger endogenous particles contain multiple magnetic domains (Moskowitz et al. 1988). This was further supported by measurements of mineral magnetite nanoparticle deposits, in which the limit for single-domain magnetite nanoparticles was estimated to be $48 \pm 5$ nm. The critical size for the onset of superparamagnetism at room temperature (RT) was estimated to be $33 \pm 8$ nm (Dunlop 1973). For the purposes of the study presented here, endogenous nanoparticles will be treated as containing a single magnetic domain.

In this work, the morphology and magnetic properties of magnetosome-containing Magnetospirillum sp. were studied with a view to designing a sensor to detect cells based on endogenous magnetic nanoparticle content. The TMR sensor illustrated in figure 1 was used to interrogate each cell for its magnetic characteristics. Here, we present static and quasi-dynamic measurements demonstrating the feasibility of using a TMR sensor for single nanoparticle detection and quantification in an approach combining biochemistry, magnetics and spintronics, ‘biomagnetronics’.

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TMR sensing of endogenous nanoparticles

magnetotactic bacterium

Figure 1. Schematic of the TMR sensor over which a bacterium containing endogenous magnetic nanoparticles passes. The TMR sensor detects the chain of endogenous superparamagnetic nanoparticles. As the bacteria are transported by capillary flow, a perpendicular applied magnetic field $H$ magnetizes the nanoparticle chain and generates a stray field, $B$. The in-plane components of $B$ are detected alternately by the TMR sensor as each bacterium moves over the sensor.

2. Material and methods

*Magnetospirillum* sp. was purchased from ATCC, Middlesex, UK (catalogue no. 700264). The 1653 Revised Magnetic Spirillum Growth Medium (MSGM) was used to culture the *Magnetospirillum* sp. This was made up in 11 MilliQ water with 10 ml Wolfe’s Vitamin solution (ATCC, Middlesex, UK), 5 ml Wolfe’s Mineral solution (ATCC, Middlesex, UK), 2 ml ferric quinate (for 0.01 M solution: 0.27 g FeCl$_3$, 0.19 g quinic acid, 100 ml MilliQ water), 0.45 ml resazurin (0.1%), 0.68 g KH$_2$PO$_4$, 0.12 g NaNO$_3$, 0.035 g ascorbic acid, 0.37 g tartaric acid, 0.37 g succinic acid, 0.05 g sodium acetate, adjusted to pH 6.75 with NaOH (Sigma, UK).

*Magnetospirillum* sp. were cultured in microaerobic conditions in MSGM media sealed in 50 ml sterile Falcon tubes at 28°C for three to four weeks until a black sediment of bacteria collected at the base of the tubes. The magnetic
response of the bacteria was tested with a 0.5 T NdFeB magnet (e-magnets, Sheffield, UK) placed against the Falcon tube. All cells were fixed in 10 × BD cellfix for transmission electron microscope (TEM) analysis (Philips CM100), scanning electron microscopy (SEM) (FEI FEG XL30), superconducting quantum interference device (SQUID) measurements (Quantum Design) and for use with the TMR sensor. For the SQUID measurements of dried bacteria, samples of the 10 × BD cellfix suspended bacterial solutions were freeze dried.

The TMR effect sensing device was constructed out of a 2 × 6 μm² magnetic tunnel junction (MTJ; Micro Magnetics Inc.), with a maximum sensitivity of 0.2 × 10⁻⁵ T, on a silicon chip that was placed onto a polydimethylsiloxane sheet. Sensor calibration curves were taken using a Helmholtz coil attached to a LakeShore Model 450 gaussmeter. The sensor response was monitored using PICOSSCOPE software. The sensor was operated in an AC Wheatstone-bridge configuration and the signal was extracted by using a lock-in technique. An external electromagnet (Magnet-Schultz Type GMH) was used to supply a perpendicular magnetic field of 80 Oe at the sensor position. The electromagnet was driven by a square wave from a TTI TG315 function generator between 0 and 11 V at a frequency of 1 Hz. A residual current device was included to prevent circuit damage from spikes in the power supply.

3. Endogenous nanoparticle size measurements

The cultured microaerobic Magnetospirillum sp. demonstrated a rapid magnetic response to a 0.5 T NdFeB magnet (figure 2). The black magnetotactic bacteria migrated to the side of the tube closest to the magnet within 5 s.

TEM studies confirmed that the magnetotactic bacteria cultured were approximately 2 μm long and 0.5 μm in diameter with a helical morphology (figure 3a,b). Calculation of the approximate volume of these ellipsoid-shaped bacterium \((4πa^2b/3)\) gave a value of \(2.62 \times 10^{-19} \text{ m}^3\) per bacterium. It was observed that each Magnetospirillum sp. had at least one chain of between 5 and 25 magnetite nanoparticles. Each magnetite nanoparticle appeared to be surrounded in a transparent coat with a thickness of around 3–4 nm (figure 3c) thought to correspond to the magnetosome lipid bilayer. These magnetosome nanoparticle crystals appear slightly cuboid in geometry (inset, figure 3b; Bazylinski & Frankel 2004).

From 237 measurements of magnetite nanoparticles, the particle diameters can be seen to fit a Gaussian distribution with a mean of 50.9 ± 13.3 nm (figure 4).

To explore whether the endogenous magnetic nanoparticles respond to the applied magnetic-field gradient as individual particles or as a chain, SQUID measurements of the magnetic properties of the nanoparticles were undertaken. 50 μl of the bacterial solution was pipetted into a plastic sample holder specially designed for liquid and low-temperature measurements (De Los Santos et al. 2009). Figure 5 is a plot of the superparamagnetic response curve observed at RT following removal of the small net-paramagnetic contribution of the combined diamagnetic water and paramagnetic sample holder, \(χ_p\). The Langevin equation (3.1) was fitted to the normalized \(M–H\) data to deduce an approximate size of the magnetic element responding to the applied magnetic

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Figure 2. A series of images showing: (a) magnetotactic bacteria in nutrient media in a graded sterile Falcon tube (the gradations on the tube indicate volume in millilitres) and (b) placement of a magnet resulting in (c) collection of black-coloured magnetotactic bacteria adjacent to the magnet within 5 s.

Figure 3. TEM images of: (a,b) *Magnetospirillum* sp. showing their helical morphology and chains of magnetite nanoparticles. (c) Enlargement of the chain of magnetosomes seen in (b) in which a 3–4 nm thick magnetosome lipid bilayer, transparent in appearance, envelopes the magnetite crystals. Scale bars, (a,b) 500 nm and (c) 100 nm.

Figure 4. Histogram of *Magnetospirillum* sp. magnetite nanoparticle diameter distribution from 237 measurements of the particle diameter. They can be seen to fit a Gaussian distribution curve (solid line) with a mean particle diameter of 50.9 ± 13.3 nm.
Figure 5. $M$–$H$ measurement at RT of *Magnetospirillum* sp. showing the field dependence of the normalized magnetic moment (open squares) with the corresponding Langevin fit (solid line).

Field in the SQUID (Connolly *et al.* 2005; Darton *et al.* 2008). The equation is given by

$$\sigma = Nm\mu_B \left( \coth \left( \frac{m\mu_B H}{k_B T} \right) - \frac{k_B T}{m\mu_B H} \right) + \chi_p H,$$

where $\sigma$ is the mass magnetization, $N$ is the number of particles per gram of magnetite, $m$ is the number of Bohr magnetons per particle, the constant $\mu_B$ is the Bohr magneton, $k_B$ is the Boltzmann constant, $T$ is temperature (293 K) and $H$ is the applied magnetic field. We normalized the equation by removing first the paramagnetic contribution and dividing the mass magnetization through the coefficient $Nm\mu_B$. This was necessary as the samples of bacteria analysed contained some free magnetic particles from lysed dead cells making an accurate estimate of the mass magnetization difficult. The resulting Langevin fit to the experimental data is also shown in figure 5. By fitting $m$ to the normalized magnetization curve to the Langevin equation, the number of $\mu_B$ per nanoparticle is yielded. As the number of $\mu_B$ (the magnetic moment per unit cell) and density of bulk Fe$_3$O$_4$ is known, assuming spherical particles, the diameter of the elements responding to the magnetic field can be approximated as $40.6 \pm 1.2\text{nm}$. This calculated diameter indicates that the magnetic response of the material is due to individual nanoparticles comparable in size to those observed in TEM (figure 4). The deviation of the Langevin fit observed at low magnetic fields may be owing to the particle-size distribution or the ‘extrinsic’ superparamagnetic response.

4. Measurement and calculation of the blocking temperature

For ‘intrinsic’ superparamagnetic particles, the blocking temperature, $T_B$, represents the temperature at which the hysteretic response is lost for a particular experimental time frame. This means that below the blocking temperature,
a hysteretic response is observed, because the thermal activation energy is not large enough to allow the alignment of the particle moment with a sufficiently small external field. The exchange interaction between the individual particles is dominant in small fields and at low temperatures. Therefore, the spins of the nanoparticles couple preferentially by a ferromagnetic (in the case of Fe₃O₄, ferrimagnetic) interaction rather than a dipole–dipole interaction, which forms the basis of the Langevin model, equation (3.1).

Assuming spherical particles, the rotational energy barrier to alignment is given by the magneto-crystalline anisotropy energy per unit volume of Fe₃O₄, \( K_1 \) \((1.12 \times 10^5 \text{erg cm}^{-3})\). This holds true by neglecting other anisotropy contributions that could arise from the shape anisotropy (deviation from a spherical symmetry) and surface anisotropy, which would orient the magnetization into the plane of the surface and thereby create a disordered surface region with a reduced magnetization (spin–glass behaviour). Therefore, for an \( M–H \) measurement taken over a certain experimental time frame, the blocking temperature should satisfy the relationship (McHenry et al. 1994)

\[
T_B = \frac{K_1 V}{k_B \ln \frac{\omega_0}{\omega}},
\]

where \( V \) represents the volume of the particle \((6.91 \times 10^{-17} \text{cm}^3\) for a 25.45nm diameter particle), \( k_B \) is the Boltzman constant, \( \omega \) is the inverse of the experimental time (approx. \(3.47 \times 10^{-5} \text{Hz}\) for an approx. 8h SQUID measurement) and \( \omega_0 \) is the inverse of the magnetic relaxation time. However, for ferrofluids, such as the bacterial solution, the relaxation time, \( \tau \), is composed of the Brownian relaxation time, \( \tau_B \), (Rosensweig 2002) and the Néel relaxation time, \( \tau_N \) (Néel 1949, 1955),

\[
\omega_0 = \frac{1}{\tau} = \frac{1}{\tau_B} + \frac{1}{\tau_N}.
\]

In the bacterial solution, there are two possibilities for Brownian relaxation; owing to the rotation of the individual particles inside the cytoplasm and rotation of the bacterium in the 10 × BD cellfix solution. The Brownian relaxation is given by

\[
\tau_B = \frac{3\eta V}{k_B T},
\]

where \( \eta \) is the viscosity (for a particle in cytoplasm, \(2.5 \times 10^{-3} \text{Pa s}\) (Mastro et al. 1984), and for a bacterium in H₂O, \(8.9 \times 10^{-4} \text{Pa s}, \text{at RT}\)). This yields a Brownian relaxation time of \( \tau_B^P = 1.28 \times 10^{-4} \text{s} \) \((\omega_B^P = 7811.4 \text{Hz})\) for the particle and \( \tau_B^B = 0.17 \text{s} \) \((\omega_B^B = 5.8 \text{Hz})\) for the bacterium.

To calculate the magnetic relaxation time, \( \tau_N \), owing to the magneto-crystalline anisotropy, Néel gives the following approximation:

\[
\tau_N = \frac{m_e}{eH_c} \frac{1}{3G \lambda + DM_S^2} \sqrt{\frac{\pi G k_B T}{2V}} \exp \left( \frac{H_c M_S V}{2k_B T} \right),
\]

where \( m_e \) is the mass of an electron, \( e \) is the electron charge, \( H_C \) is the coercive field (extrapolated from figure 6 at 0K as 709 Oe), \( G \) is the modulus of rigidity \((38 \text{GPa for Fe₃O₄}; \text{Kashiwagura et al. 2002})\), \( \lambda \) is the
mean magnetostriction constant of magnetite approximated by averaging three crystallographic axes, \(4 \times 10^7\) (Bickford et al. 1955), \(D\) is a numerical coefficient that takes into account the shape of the particles (for a sphere \(4\pi/5\)), \(M_s\) is the saturation magnetization, 473.8 emu cm\(^{-3}\). This yields a very large value for \(\tau_N\) tending towards infinity owing to the exponential term in equation (4.4) and therefore, \(\omega_N = \sim 0\) Hz. As such, we can conclude that the 50 nm nanoparticles inside the bacterium should be ferrimagnetic with a relaxation time dominated by Brownian rotation of the nanoparticles inside the cytoplasm. This gives rise to 'extrinsic' superparamagnetism at RT in the bacterial solution. Therefore, the approximation of the blocking temperature by equation (4.1) is not possible.

It should be noted that the resulting value calculated for the Néel relaxation of our 50 nm particles from equation (4.4) above differs significantly from the value of 1 GHz (McHenry et al. 1994) often generically cited in literature. Indeed, Néel’s original paper on superparamagnetic nanoparticles states this frequency, \(\omega_N\), is far from being a constant, changing by 9 to 10 orders of magnitude upon doubling the particle’s volume (Néel 1949, 1955).

In an attempt to measure the intrinsic magnetic blocking temperature, a 50 µl sample of the bacterial solution was freeze dried and analysed in temperature-dependent SQUID measurements. Figure 6 shows the temperature dependency of the coercivity of the nanoparticles. For low temperatures, this can be fitted by (McHenry et al. 1994)

\[
H_C = H_{CI} \left(1 - \frac{\sqrt{T}}{T_B}\right),
\]

\[\text{(4.5)}\]
where $H_{CI}$ is the coercive field at 0K. Therefore, the intrinsic blocking temperature can be estimated from a linear plot of the coercivity against $T^{1/2}$ (figure 6). This yields an experimental blocking temperature, $T_B$, as $282 \pm 13$ K and $H_{CI}$ as approximately $709 \pm 23$ Oe. This indicates that the majority of nanoparticles could be ferrimagnetic at RT and that the superparamagnetic response is extrinsic. The particle-size distribution also leads to an average coercivity of approximately $220 \pm 30$ Oe at RT in the dried frozen bacterial sample in agreement with other published values (Proksch et al. 1995).

The size distribution of the dry frozen endogenous nanoparticles, measured by TEM, leads to a range of blocking temperatures in ‘intrinsic’ superparamagnetism. However, in the bacterial solution, Brownian relaxation time plays the dominant role, giving rise to ‘extrinsic’ superparamagnetism (Rosensweig 1997). Depending on the viscosity of the sample of magnetosomes in bacteria and magnetosomes free in solution, this can lead to the observation of hysteresis loops at RT with varying coercive fields (data not shown). Separation and analysis of the free magnetosomes and the bacteria is currently being undertaken.

5. Static tunnelling magneto resistance sensor measurement

The TMR sensors, sourced from MicroMagnetics, were run at 8 kHz using a lock-in amplifier with a time constant of 5 ms. These sensors are only sensitive to stray fields in the plane and along the small axis of the device. In order to detect the extrinsic superparamagnetic particles, we applied an out-of-plane field, with a magnitude of 80 Oe, which was switched on and off with a frequency of 1 Hz in order to distinguish an induced magnetic signal from signal drift. A small in-plane component of the field was detected by the sensor at all times owing to field imperfections of the electromagnet. No drift in field strength was found in this magnet while being in this mode of operation over the time scale (less than 2 h) of the experiment. Therefore, any changes to the field detected, i.e. a change in the amplitude of the 1 Hz square-wave signal, would indicate the presence of a superparamagnetic particle.

The TMR sensor was first tested with deionized water to provide a negative control. To establish whether the TMR sensor was sensitive enough to detect the endogenous magnetic nanoparticles, it was tested by pipetting a solution of *Magnetospirillum* sp. (concentration approx. $1 \times 10^7$ cells ml$^{-1}$) onto the sensor surface. A glass cover slip was used to flatten the solution over the TMR sensor and reduce the rate of evaporation. In addition to the 1 Hz square wave, an offset in the voltage was detected for both solutions, as shown in figure 7. We associate the offset in voltage to a capacitive coupling between the electrodes of the sensor and the media. The magnitude of the offset was found to be reproducible and specific to the media used.

Figure 8 represents a series of images taken before and during the experiment. Figure 8a shows the sensor and its environment before the introduction of the liquid samples, and figure 8b was taken after addition of the 1 ml deionized water control. Some imperfections in the surface of the protective layer above the sensor circuit in the form of dark spots can be seen in these micrographs. Figure 8c was taken after the first addition of *Magnetospirillum* sp. solution and figure 8d taken...
Figure 7. TMR sensor response to the presence of deionized water (trace a) and *Magnetospirillum* sp. solution (trace b). The dotted trace represents the externally applied AC magnetic field. Black line, H2O experiment; grey line, magnetic bacteria experiment; dotted line, voltage supplied to the magnet.

Figure 8. Microscope images showing the TMR sensor (labelled with white arrows): (a) before the introduction of samples, (b) following addition of the deionized water control sample, (c) after addition of *Magnetospirillum* sp. at a concentration of approximately $1 \times 10^7$ cells ml$^{-1}$ and (d) after addition of a solution of *Magnetospirillum* sp. at a concentration of approximately $1 \times 10^9$ cells ml$^{-1}$. Bacteria are indicated by black arrows. Scale bars, 20 µm.
Figure 9. TMR sensor output amplitudes corresponding to measurements of deionized water control and solutions of *Magnetospirillum* sp. at a concentration of approximately $1 \times 10^7$ cells ml$^{-1}$ (‘bacterial sol’$^n$) and dried concentrated *Magnetospirillum* sp. cells (‘dried conc. bac.’).

after two days with a repeated addition of *Magnetospirillum* sp. solution. In both these figures, lumps of dark translucent bacteria can be seen in the vicinity of the TMR sensor, which can be associated with changes in amplitude of the signal voltage.

In order to derive magnetic information about the samples, the amplitude of the 1 Hz square wave was measured and plotted during the experiment, as seen in figure 7. Each point in figures 9 and 10 represents several measurements of the amplitude. The error arises owing to the magnetic noise of the environment. Both figures show clear detection of superparamagnetic particles in the vicinity of the sensor. A large change in signal was detected when the bacterial solution was concentrated close to the sensor with help of a permanent magnet and allowed to dry (figures 9 and 10). After these experiments, the sensor was imaged by SEM (figure 11), and a chain of four nanoparticles can be seen to have settled onto the sensing area. It is probable that these particles are the remnants of a bacterium following desiccation, resulting in the large increase in signal and the subsequent shift in baseline in the measurements of day 3.

As expected from a static measurement of a mass-coverage device, the addition of the magnetic solutions led to a change in the detected amplitude. The size of this detected amplitude depended on the position of the nanoparticles with respect to the sensing area. The amplitude change could either be seen as an increase or a decrease of the detected stray field from the electromagnet.
Figure 10. TMR sensor output voltages corresponding to measurements of dried concentrated *Magnetospirillum* sp. cells ('dried conc. bac. day 1' and 'new baseline day 3') and repeated measurements of a solution of *Magnetospirillum* sp. at a concentration of approximately $1 \times 10^9$ cells ml$^{-1}$ ('conc. bac. sol$^n$').

Figure 11. SEM image of a chain of four nanoparticles (inset) near the centre of the elliptical-shaped TMR sensor. Scale bars, 1000 nm; inset, 100 nm.
6. Quasi-dynamic tunnelling magneto resistance sensor measurements

Detection sensitivity of our TMR sensors can vary between 0.2–1.3 Oe. For the quasi-dynamic measurements, a TMR sensor able to detect stray fields down to 0.2 Oe was used. The calibration curve for this sensor is shown in figure 12.

For these measurements, a 1 µl droplet of *Magnetospirillum* sp. (concentration $4 \times 10^8$ cells ml$^{-1}$) on the sensor was stirred by an additional rotating magnet. The bacterial solution was then allowed to settle on the sensor, resulting in several recorded singular events indicated by a change in the voltage amplitude of the sensor response (figure 13). The response observed would be expected from the stray field of a magnetic particle passing in the vicinity of the TMR sensor (Shen *et al.* 2005). The events recorded had a voltage peak height of approximately 77 µV, superimposed on the signal voltage. These events were only recorded in the presence of the *Magnetospirillum* sp. solution and were absent in measurements of the deionized water control, ruling out the effects of random noise.

In order to support these observations, the theoretical magnetic stray field of a fully magnetized 50 nm diameter Fe$_3$O$_4$ nanoparticle was calculated at different distances to the TMR sensor (figure 14). The TMR sensors used in these experiments were covered with a 200 nm thick contact and a 200 nm thick passivation layer, giving the minimum separation between the sensor and the sample as 400 nm. The stray field $H$ of a magnetic dipole of length $l$ placed perpendicular on top of the sensing area of the TMR sensor at the distance $d$ away is approximated for the case that $d \gg l$ in cgs units by (Bozorth 1951)

$$H = \frac{m}{d^3} \times \left(1 - \frac{3l^2}{4d^2}\right),$$  \hspace{1cm} (6.1)

with $m$ being the magnetic moment of a single Fe$_3$O$_4$ nanoparticle and $l = 50$ nm, its diameter. The plot of the relation can be seen in figure 14. From this figure, it is clear that the theoretical active distance of the 0.2 Oe sensitive sensor lies between 400 and 540 nm above the sensor. In figure 14, it can also be seen that the event voltage of 77 µV observed in figure 13 can, in principle, be produced by a 50 nm magnetic nanoparticle passing the sensor at a height of approximately 420 nm.

7. Estimation of the magnetic moment per bacterium

The presence of four nanoparticles on the TMR sensing area, seen in figure 11, led to an amplitude change in the detected signal. This amplitude change can be approximately quantified using the sensor calibration curve presented in figure 12 and equation (6.1). The sensor calibration curve yields a stray field arising from the electromagnet at the position of the sensor of approximately 4 Oe, from inspection of the amplitude height of the square signal in figure 13. This holds true, regardless of the different sensor sensitivities, as the position of the sensors with respect to the electromagnet remains constant. Therefore, the amplitude height of the baseline (approx. 10 µV) from figure 9 represents the same stray field (4 Oe) arising from the electromagnet.

The amplitude change of approximately 5 µV, seen in figure 9, upon detection of a bacterium (four nanoparticle chain) settling on top of the sensor area thus represents a change of approximately 2 Oe. The most stable direction
Figure 12. Calibration curve of a TMR sensor versus an externally applied magnetic field yielding a response of $0.175 \text{ mV Oe}^{-1}$. Black square, sensor signal; solid line, linear fit: $Y = 0.355 - 0.175X$.

Figure 13. TMR sensor response in the presence of the *Magnetospirillum* sp. solution (solid trace) with a singular event at 2.4s evidenced by a peak of approximately 77 $\mu$V superimposed on the signal voltage. The dotted trace represents the externally applied AC magnetic field. Solid line, signal with bacteria solution; dotted line, voltage supplied to the magnet.

(ground state) of the magnetization vector without an applied field must lie along the chain direction. Even though the out-of-plane applied field of 80 Oe from the electromagnet might not be able to fully reverse the magnetization and overcome the magneto-crystalline anisotropy, it will induce an oscillation in the
Figure 14. Theoretical stray field of a fully magnetized 50nm diameter Fe₃O₄ nanoparticle versus the distance to the TMR sensor. The bottom solid line represents the sensitivity of our best sensors. Dotted line, single 50nm nanoparticle; solid line, best TMR sensitivity.

magnetization vector of the particles. The TMR sensor is only sensitive to the in-plane direction changes in the vector component of the perturbed magnetization. The out-of-plane component of the perturbed magnetization vector will therefore not be detected. However, the changes to the in-plane component from the ground state can be detected (approx. 2Oe). We are using equation (6.1) to estimate the magnetic moment generated by the chain of nanoparticles \( l = 200 \text{nm} \). The distance, \( d \), between the nanoparticle chain and the sensing area of the TMR sensor is approximatively 425nm. They are tilted approximately 45° to the in-plane detection direction, and therefore the real stray field of the chain corresponds to \( H_{\text{chain}} = 2 \text{Oe}/\cos(45°) = 2.8 \text{Oe} \). Equation (6.1) yields a value of approximately \( 2.6 \times 10^{-13} \text{emu} \) for the chain of four nanoparticles. This is only an approximation of the true moment, as the field is not uniform over the sensor area owing to the proximity and small size of the chain, yet this is in good agreement with literature values for magnetic moments of a whole magnetotactic bacterium that range from \( 1–1.6 \times 10^{-13} \text{emu} \) (Rosenblatt et al. 1982; Proksch et al. 1995).

8. Conclusions

In this work, the strain of magnetotactic bacteria, *Magnetospirillum* sp. was cultured and characterized by electron microscopy and magnetometry. From the electron microscopy, the lipid bilayer surrounding the nanoparticles was observed as a 3–4nm thick transparent envelope around each particle. TEM measurement of particle sizes yields a diameter value of \( 50.9 \pm 13.3 \text{nm} \), which is in good agreement with the diameter of \( 40.6 \pm 1.2 \text{nm} \) extracted from the Langevin fit to the normalized magnetization. Although the magnetosomes were
seen to form chains of up to 25 particles in the bacteria, they responded
magnetically as single 40 nm superparamagnetic magnetite particles in solution
at RT. The resulting extrinsic superparamagnetic response at RT of the bacterial
solution can be attributed to the reversal of the magnetization by physical
rotation of the nanoparticles. A freeze-dried bacterial sample yielded an intrinsic
blocking temperature of $282 \pm 13 K$, allowing the particles to potentially exhibit
ferrimagnetism at RT when rotationally constrained.

Successful detection of stray fields arising from endogenous magnetic
nanoparticles in static and quasi-dynamic modes was achieved with a TMR
sensor. In the static mode, a change in the measured amplitude upon detection
of the *Magnetospirillum* sp. solution when compared with the negative control
supports the possibility of using this sensor as a mass-coverage magnetic-
detection device for superparamagnetic nanoparticles. In the quasi-dynamic
mode, singular events were recorded for the bacterial solution (in contrast to
the negative control) which can be attributed to the detection of stray fields
arising from the motion of *Magnetospirillum* sp. across the sensor. This result
demonstrates the feasibility of integrating a TMR sensor into a microfluidic
circuit to enable sorting of magnetosome-containing cells. Finally, based on the
detection of a four nanoparticle chain on top of the TMR sensing area, a rough
estimate of the magnetic moment per bacterium was calculated as approximately
$2.6 \times 10^{-13}$ emu, which is in good agreement with literature values. To our
knowledge, this is the smallest magnetic nanoparticle moment detected by a
TMR-based device to date.

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