A magnetic resonance disruption (MaRDi) technique for the detection of surface immobilised magnetic nanoparticles

S.T. Parslow, T. Hughes-Riley, M.I. Newton and R.H. Morris*

*corresponding author rob.morris@ntu.ac.uk

Telephone +44 115 8483315

School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, United Kingdom

Abstract

There are numerous assays that result in a surface with bound magnetic nanoparticles (MNP) whose number is proportional to the concentration of the analyte of interest. The techniques used to explore such assays are typically complex and costly. Since the presence of such MNP disrupts the pulsed magnetic resonance signal that would normally be detected from a fluid covering the surface, we present a measurement technique to quantify such assays. In this work we identify and characterise a suitable fluid for such measurements, namely 10cSt viscosity PDMS oil of thickness 250μm. We demonstrate that the T_2^{eff} relaxation time from the PDMS reduces as the proportion of the surface area covered with MNP increases. Most significant however, is a linear decrease in the signal amplitude from the PDMS as a function of MNP coverage. This is observed both for the integral over 4096 echoes and also in the Hahn echo promising simplified console electronics for rapid measurements.

Keywords: Magnetic Nanoparticles; MNP; Spin-Spin Relaxation Time; T_2^{eff}; Magnetic Resonance; MaRDi.

1. Introduction

An increasing number of assays result in a surface where the number of bound magnetic nanoparticles (MNP) is proportional to the concentration of analyte being measured[1-5]. These include colorimetric, refractive index change and optical reflectance methods for quantifying the number of bound MNP. Despite the fact that such MNP locally disrupt the magnetic field and hence the resulting magnetic resonance (MR) signal that would normally be detected[6], magnetic resonance would normally be considered too complicated and expensive to offer a viable characterisation technique. MR spectrometers, used in pulsed MR experiments, are usually expensive because they are designed to operate over a wide range of frequencies and use different pulse sequences to give a range of measurement parameters. If a single liquid were to be used and a single parameter measured, this would significantly reduce the cost and complexity of the hardware required. In this work we sought to identify a suitable liquid, determine a suitable thickness of that liquid which would provide the maximum disruption and ease of measurement caused by the surface bound MNP and to identify an appropriate magnetic resonance parameter to measure.

2. Experimental methods

Acrylic test sticks were laser cut with wells etched into the sticks to hold the test liquid approximately 6mm x 4mm x 2mm in length, width and height respectively giving a volume of approximately 50 µL. A probe was constructed using a set permanent magnets arranged in a Halbach geometry[7] which provided a static magnetic field of 507 mT (described in detail in the supplementary material). A CPMG sequence[8], described and shown schematically in supplementary material (S5 and S6 respectively), using the following parameters was applied to the coil using a commercial spectrometer (KEA[2], Magritek, NZ) running their proprietary software (Prospa, Magritek, NZ): Echo Time=400 µs; Number of echoes=4096; Number of averages=128; Pulse length=11.7 µs, Repetition time=2 s. The appropriate detection liquid was determined by collecting data from a number of different candidates including various cooking oils, water and silicone oils. The collected echoes were transformed using the Lawson and Hanson non-negative least squares (NNLS) analysis function built into Prospa, producing a relaxation spectrum which provides the relative weighting of each relaxation component. For example, a mono- exponential T_2^{eff} decay will produce a single peak centred at its T_2^{eff} value, whilst a bi-exponential will have two peaks one centred on each of the components' T_2^{eff} values with the heights representing their relative magnitudes. A standard biotin-avidin method was used to immobilize MNPs on to a polystyrene surface that was cut to fit in the acrylic wells (details of immobilization procedure are also included in the supplementary information). Prior to immobilization, some of the surfaces were laser engraved to remove different areas of the biotin. The MNP used were BNF-starch-redF 100nm (Micromod Partikeltechnologie GmbH) and the presence of immobilised MNP were confirmed using bright field images from a confocal microscope an example of which is shown in supplementary information figure S4. The surface area covered by MNP was determined to be 26%±2% for the non-engraved biotin surfaces and less than 3% for the engraved surfaces due to non-specific binding.

3. Results and Discussion

Both water and PDMS oil demonstrated a dominant (near single) relaxation component whereas cooking oils exhibited multiple relaxation components (see for example sunflower oil in Figure 1) which is not desirable as the effect of the MNPs will be different for each peak, complicating the analysis. As PDMS was available in a number of different molecular weights (MW), a series of different values were assessed to determine the relationship between the samples T_2^{eff} relaxation value and the oil's MW, expressed in this case as viscosity with increasing MW providing increasing viscosity. It was found that the T_2^{eff} relaxation value increased as viscosity decreased (Figure 2). As the T_2^{eff} relaxation time is expected to reduce with the presence of the MNP[6], the 10 cSt viscosity oil provided a sufficiently long starting T_2^{eff} of 700 ms and was of sufficiently low viscosity to flow easily filling the test stick, so was selected as the test liquid.

Different thicknesses of the 10 cSt PDMS oil layer were used to investigate the thinnest layer that would provide a sufficiently reproducible T_2^{eff} measurement, as

this would maximise the effect of any surface bound MNP (Figure 3). Values below 125 µm, corresponding to a volume of 3 µL of PDMS, showed significant increase in scatter of the data due to the limited volume fraction of the coil filled with MR liquid sample (known as the fill factor). Surface roughness of the sample holder required a slightly larger volume than this to provide reliable measurements of signal degradation due to the presence of MNP so a thickness of twice the minimum value was used giving a total fluid volume of 6 µL. The sample sticks containing different fractions of MNP coverage were measured to determine the value of T_2^{eff} and also the signal intensity using the integral of the echoes. In Figure 4 we show the non-linear reduction in T_2^{eff} as a function of the increase in the proportion of the surface area covered with MNP. From this we can see that there is little effect on T_2^{eff} for coverage of less than 7% but above this T_2^{eff} reduces. In Figure 5, a linear decrease in the signal amplitude, taken as the integral of 4096 echoes, as a function of the percentage of the surface area covered in MNP is shown with a gradient of 5 au/cm^2. This same trend was also apparent for the integral first echo alone (often incorrectly referred to as the Hahn echo) also shown in Figure 5 although it is less sensitive with a gradient of 0.01 au/cm^2.

4. Conclusion

We have demonstrated that the number of surface bound MNP is directly proportional to the intensity (integral) of one or more spin echoes. A three 'well' system that provides one reference well containing only oil, one reference well with a known MNP surface coverage and one well with the unknown coverage, most usefully as a function of analyte binding, would allow self-consistent determination of the amount bound. The wells with the oil alone and the known coverage give the possibility of straight line calibration allowing the unknown coverage to be determined independently of environmental parameters for example, this would remove problems associated with long term temperature drift in the permanent magnets which may affect the signal. The ability to determine the binding fraction from the integral of one or more echoes, with the increasing publication of simplified spectrometer designs[9,10], makes magnetic resonance a viable technique for determining the quantity of surface bound MNP.

Acknowledgements

STP gratefully acknowledges Nottingham Trent University for funding under the Vice Chancellors Bursary Award

1. C. Esseghaier, G.A.R.Y. Suaifan, A. Ng, M. Zourob. *J. Biomed. Nanotechnol.* 2014, **10**, 1123.

2. X. Hun, Y. Xu, X. Luo. *Microchim. Acta.*, 2015, **182**, 1669.

3. G.A.R.Y. Suaifan, C Esseghaier, A. Ng, M. Zourob. *Analyst*, 2013, **138**, 3735.

4. C. Esseghaier, A. Ng, M Zourob. *Biosens. Bioelectron.* 2013, **41**, 335.

5. S. Wignarajah, G.A.R.Y. Suaifan, S. Bizzarro, F.J. Bikker, W.E. Kaman, M. Zourob, *Anal. Chem.*, 2015, **87**, 12161.

6. A. Hall, V.J. Mundell, C. Blanco-Andujar, M. Bencsik, G. McHale, G. M.I. Newton, G.W.V. Cave. *Chem. Commun.*, 2010, **46**, 2420.

7. K. Halbach. *Nucl. Instrum. Methods.*, 1980, **169**, 1.

8. S. Meiboom, D. Gill. *Rev. Sci. Instrum.*, 1958, **29**, 688.

9. N. Sun, Y. Liu, L. Qin, H. Lee, R. Weissleder, D. Ham. *Solid-State Electron.,* 2013, **84**,13.

10. H-Y. Chen, Y. Kim, P. Nath, C. Hilty. *J. Mag. Res.*, 2015, **255**, 100.

Figures

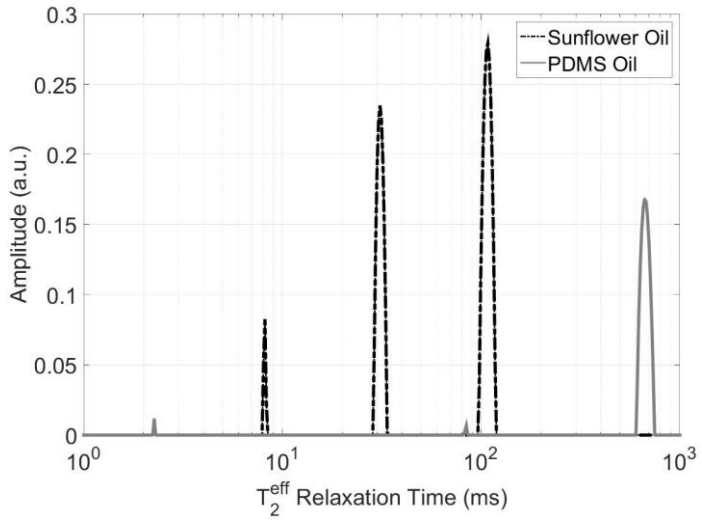

Figure 1. A relaxation spectrum for 10cSt PDMS oil and sunflower oil showing the different T_2^{eff} components. The PDMS oil shows one dominant and two negligible peaks, whereas the sunflower oil has three significant relaxation components.

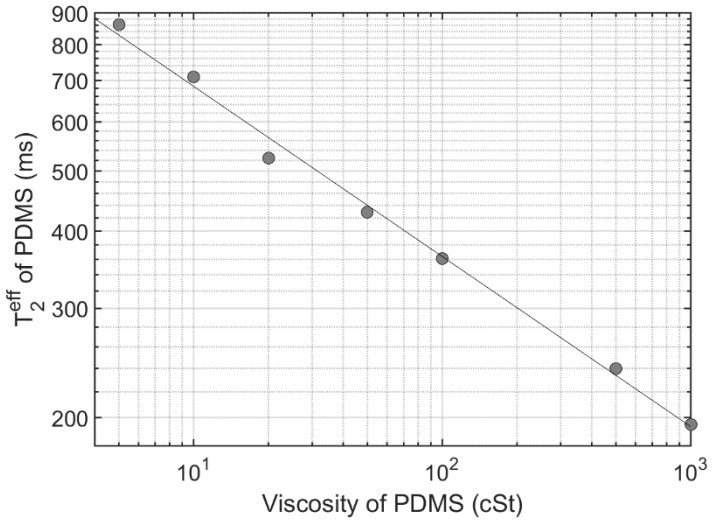

Figure 2. A logarithmic plot of the T_2^{eff} relaxation time of PDMS oil as a function of viscosity.

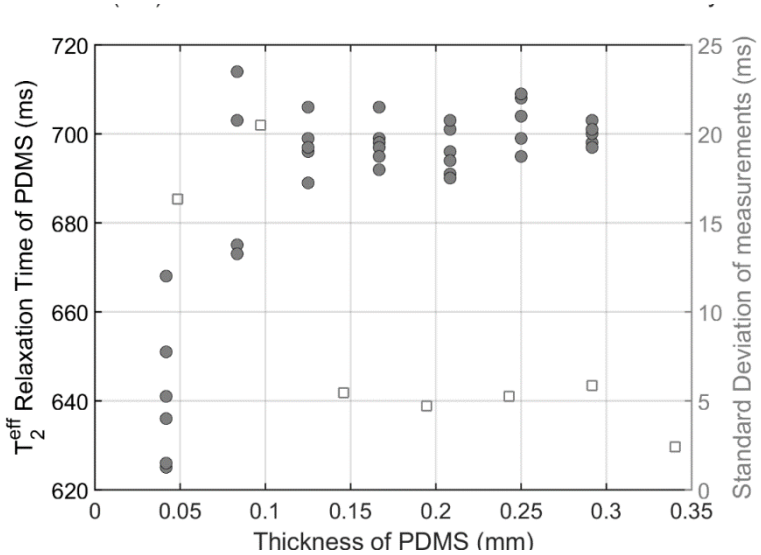

Figure 3. Repeated measurements of T_2^{eff} (filled circles) and the standard deviation of these measurement (open squares) for different thicknesses of 10cSt viscosity PDMS oil showing that is the measurements are unreliable below 125 µm thickness.

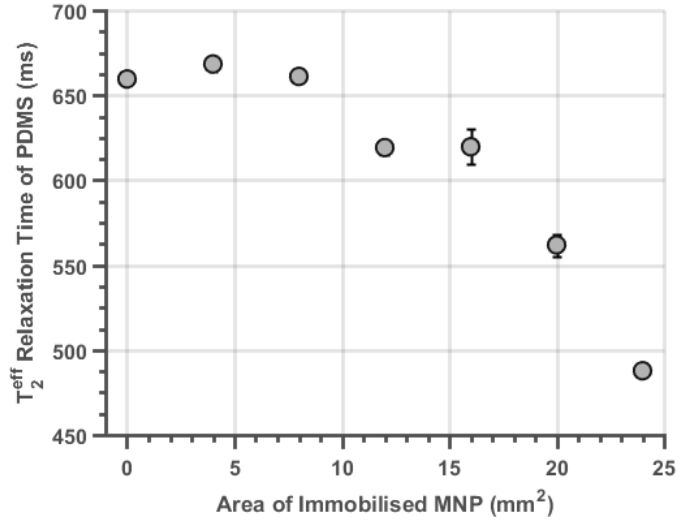

Figure 4. The T_2^{eff} relaxation time of the 10cSt viscosity PDMS oil as function of the area of immobilised MNP for a 250μm thick layer.

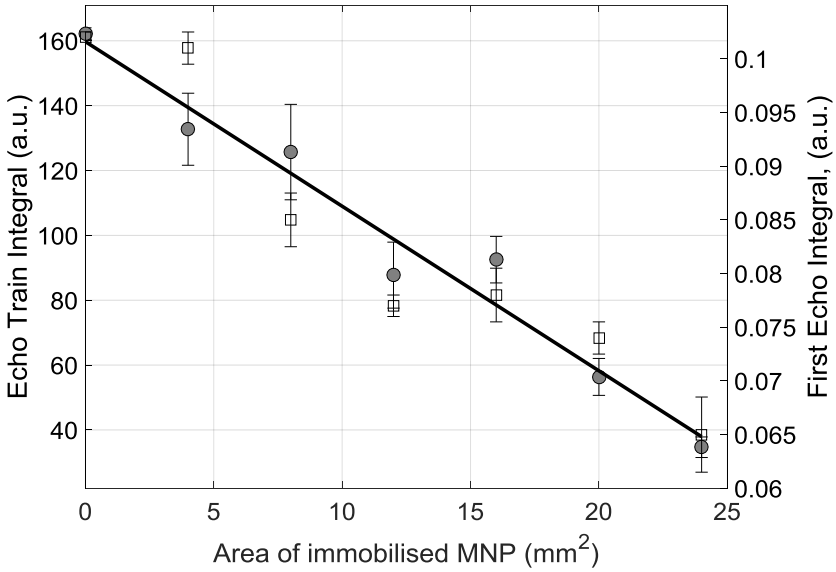

Figure 5. The integral of 4096 echoes (filled circles, left axis) and the integral of the first echo (open squares, right axis) as a function of the function of the area of immobilised MNP. The regression lines of both data sets lie on top of each other for these scales although their parameters are different.