The need for stable, mono-dispersed, and biofunctional magnetic nanoparticles for one-step magnetic immunoassays

F Öisjöen¹, J F Schneiderman¹, A P Astalan², A Kalabukhov¹, C Johansson² and D Winkler¹

¹Department of Microtechnology and Nanoscience – MC2, Chalmers University of Technology, Göteborg, 412 96 Sweden.
²Imego AB, Arvid Hedvalls Backe 4, PO Box 53071, Göteborg, 400 14 Sweden

Abstract. We have developed a magnetic immunoassay system (MIA) using magnetic nanoparticle markers for biomolecule detection. We have magnetically characterized multi-core magnetic nanoparticles (MNPs) containing single-domain crystals of Fe₃O₄ and CoFe₂O₄ with our system using a high temperature superconducting quantum interference device as detector. We use a Helmholtz coil to excite the MNPs and study the AC-susceptibility. The data is fit to a model and information about the particle size distribution of the MNP system is extracted. We observe high stability of the unfunctionalized MNPs. However, our MIA measurements require stable functionalized MNPs. We have found a significant increase in hydrodynamic size of the functionalized MNP systems in the course of just a few days caused by agglomeration behaviour. Separate measurements performed at Imego AB with their AC-Susceptometer, DynoMAG®, confirm these findings. Without stable, functionalized MNPs MIAs of this kind are impossible.

1. Introduction

Magnetic nanoparticles (MNPs) have found their way into a variety of applications in biomedicine. In this article, we focus on the applicability of MNPs for immunoassays which has been demonstrated by several groups [1-3].

Gilchrist et al. [4] was already using MNPs in 1957 for targeted heating of tissue. The MNPs are localized to specific tissue via appropriate surface functionalization. The tissue is then heated by transfer of energy from the MNPs as they absorb energy from the applied AC magnetic field. This method has been used to induce hyperthermia for destruction of cancerous tumours and has been repeated several times throughout the past decades [5, 6].

It is also possible to physically manoeuvre MNPs with an external magnetic field. Such a technique is used for magnetic separation of cells and other biomolecules [7] as well as drug delivery [8]. Magnetic separation methods employ MNPs with capturing molecules on their surfaces that specifically trap the molecule of interest. A magnet can then be used to gather the MNPs and the attached molecules before removing the supernatant liquid containing the unwanted substances. In the context of drug delivery, the MNPs are coated with the drug and injected into the blood stream of the body. An external magnetic field is then applied in order to concentrate the MNPs at the target where the drug is to be released. A review of applications of MNPs in biomedicine can be found in [9, 10].
The above applications require MNPs that are surface-coated with capturing molecules that specifically target a molecule of interest. The size distribution of the MNPs, however, is not crucial except, perhaps, for applications where the MNPs are injected into the bloodstream [10]. Generally speaking, the smaller the particle, the larger the total surface area for a given concentration of magnetic material, and consequently, a higher concentration of target molecules is detectable.

In this article, we discuss the application of MNPs to magnetic immunoassays (MIA) and the need for colloidal stability of functionalized MNP systems of hydrodynamic diameters below 100 nm. We observe clustering of functionalized MNP systems in the course of a few days that introduces complications in our MIA measurements and reduces their sensitivity, speed, and reliability.

2. Magnetic nanoparticles for magnetic immunoassays

2.1. Requirements on magnetic nanoparticles
We use cobalt-ferrite (CoFe₂O₄) or magnetite (Fe₃O₄) MNPs suspended in distilled water for our experiments. The multi-core MNPs consist of several single-domain crystals embedded in a coating of starch. After exposure to an external magnetic field, MNPs relax via two processes: Brownian- and Néel-relaxation. In the Néel-relaxation process, the internal magnetic moments of the single-domain crystals in the MNPs rotate independent of particle rotation whereas in Brownian relaxation the full magnetic moment rotates with the particle itself.

In our measurements, we monitor Brownian relaxation of the MNPs which means that we need to suppress the Néel-relaxation such that it is significantly slower than the Brownian relaxation. The relative rates for these relaxation processes depend on the size of the MNPs and their single-domains [11]. These qualities are therefore critical for our application. For example, magnetite multi-core particles with hydrodynamic size ~100 nm with single-domain diameter larger than ~16 nm have relaxation dynamics dominated by Brownian relaxation [11]. For MNPs in our experiments, we require a median hydrodynamic size of roughly 100 nm or less.

In our MIA, we measure the relative shift in Brownian relaxation time induced by binding of biomolecules to the surfaces of the MNPs which increases the median hydrodynamic radius, \( r_H \) [12]. The Brownian relaxation time, \( \tau_B \), is proportional to \( r_H^3 \). With a broad distribution of sizes in a MNP system it becomes difficult to differentiate a small change in median hydrodynamic radius. Therefore, our MNPs would ideally be mono-dispersed, i.e. of a single hydrodynamic radius.

2.2. Magnetic immunoassays
The principle behind MIA is the detection of biomolecules with magnetic readout. In our system, we use a high temperature superconducting quantum interference device (HT SQUID) to measure the relaxation of the MNPs that are magnetically excited with a Helmholtz coil, details of which can be found in [13, 14]. A separate system we use is Imego AB’s commercially available DynoMAG® which is based on an induction coil principle [2, 12]. We employ MNPs functionalized with streptavidin and biotinylated capturing antibodies. The high binding affinity for biotin to streptavidin enables coating of the MNPs with antibodies, see figure 1. In practice, we apply an AC-magnetic excitation field and measure the AC-susceptibility. The hydrodynamic particle sizes are determined by fitting the data to a Debye model assuming a log-normal size distribution.

![Figure 1](image-url) Schematic of chemical procedure for binding of antibodies to the surfaces of MNPs.
3. Results

We have measured the AC-susceptibility for several samples of both magnetite and cobalt-ferrite MNPs coated with dextran or starch and functionalized with streptavidin. We show representative AC-susceptibility measurements of starch coated cobalt-ferrite MNPs in figure 2 (herein referred to as Batch 1). The decrease in the overall level of the real-part of the low-frequency susceptibility in figure 2 is a sign of agglomeration. The samples were stored at 4°C and measured accordingly.

In figure 3 we show the time-dependence of the median hydrodynamic radius of two different samples. We extracted the median particle radius of the MNPs in Batch 1 from the data in figure 2 and observed an increase by almost 14% (from 105 nm to 120 nm) in a week. Similar agglomeration behaviour was measured in another sample, Batch 2. The initial median hydrodynamic particle radius for Batch 2 was 154 nm and after two days it had increased by approximately 7% to almost 164 nm.

4. Discussion

The data presented in figures 2 and 3 show the median hydrodynamic of the size distribution of the functionalized MNP-systems increasing and the change in standard deviation. When these particles are unfunctionalized, they have a stable hydrodynamic radius of ~50 nm. Addition of streptavidin (size: 54x58x48 Å3 [15]) should result in a slight increase in the hydrodynamic size (assuming the manufacturer’s specification of 30 molecules per MNP, the increase would be less than 10 nm). However, the data in figure 3 illustrates an increase of the median radius by more than 100% at 0 days, suggesting the particles have already clustered immediately after functionalization (from ~50 nm to \( r_0 = 105 \) nm). The clustering behaviour then continues over the course of days.

In order to achieve a fast, sensitive and simple assay we need stable freely-floating MNPs functionalized with streptavidin in a colloidally stable solution. Clustering of the particles can introduce errors in the assay since it effectively increases the hydrodynamic radius of the MNPs which is the parameter that we measure in order to detect biomolecules.

An increase in hydrodynamic radius also makes the relaxation dynamics of the MNPs slower. In figure 2, the curves are shifted towards lower frequencies as they cluster in time. Consequently, we have to measure at lower frequencies in order to reliably extract the hydrodynamic volumes relevant to our measurements which substantially increases the overall measurement time.

The initial size of the functionalized MNPs also affects the apparent biomolecule sensitivity of our system. An increase in hydrodynamic radius of, e.g., 10 nm induced by binding of biomolecules to the surfaces of MNPs whose initial average hydrodynamic radius was 30 nm would yield a relative change of 33%. However, if the initial average hydrodynamic radius is 60 nm, the relative increase would only be 17%. Moreover, clustering of the MNPs decreases the surface area available for
biomolecule binding because many of the binding sites are buried inside a cluster. Such loss in available binding sites further decreases the specific sensitivity of our measurement system.

The applications of MNPs discussed in the introduction (and in [9, 10]) all require MNPs functionalized with a capturing molecule that enables tagging. Not all applications, however, require a nearly mono-dispersed size distribution. We would like to, with this article, emphasize that there is a need and a market for stable, functionalized, and mono-dispersed MNPs for MIAs of this kind.

5. Conclusions

We have quantified clustering behaviour of MNPs functionalized with streptavidin over the course of a week using AC-susceptometry, a powerful tool for characterization of, for instance, the stability of MNP systems. The bare MNPs show high low-frequency susceptibility which is desirable for our application but we conclude that the functional MNPs cluster too rapidly to employ them in a reliable point-of-care MIA. The clustering introduces problems in assays that require freely-floating functional MNPs with hydrodynamic diameters below 100 nm that is needed for a fast, simple and sensitive assay of our type. However, we do observe stability and reproducibility in the frequency-dependent susceptibility of unfunctionalized MNPs that are already available commercially.

Acknowledgements

The authors thank Staffan Pehrson for construction of experimental parts. This research was funded by the European Community’s 6th Framework Programme (FP6/2005-2008) under grant agreement nº NMP4-CT-2005-017002 (Biodiagnostics). The Swedish Foundation for Strategic Research, the Swedish Research Council, and the Knut and Alice Wallenberg fund are gratefully acknowledged.

References

[1] Grossman H L, Myers W R, Vreeland V J, Bruehl R, Alper M D, Bertozzi C R and Clarke J 2004 Proc. Natl. Acad. Sci. U. S. A. 101 129-34
[2] Astalan A P, Ahrentorp F, Johansson C, Larsson K and Krozer A 2004 Biosens. Bioelectron. 19 945-51
[3] Eberbeck D, Bergemann C, Wiekhorst F, Steinhoff U and Trahms L 2008 J Nanobiotechnology 6 4
[4] Gilchrist R K, Medal R, Shorey W D, Hanselman R C, Parrott J C and Taylor C B 1957 Ann. Surg. 146 596-606
[5] Borrelli N F, Luderer A A and Panzarino J N 1984 Phys. Med. Biol. 29 487-94
[6] Brusentsov N A, Nikitin L V, Brusentsova T N, Kuznetsov A A, Bayburtskiy F S, Shumakov L I and Jurchenko N Y 2002 J. Magn. Magn. Mater. 252 378-80
[7] Morisada S, Miyata N and Iwahori K 2002 J. Microbiol. Methods 51 141-8
[8] Alexiou C, Arnold W, Klein R J, Parak F G, Hulin P, Bergemann C, Erhardt W, Wagenpfeil S and Lubbe A S 2000 Cancer Res. 60 6641-8
[9] Pankhurst Q A, Connolly J, Jones S K and Dobson J 2003 J. Phys. D: Appl. Phys. 36 R167-R81
[10] Gupta A K and Gupta M 2005 Biomaterials 26 3995-4021
[11] Matz H, Drung D, Hartwig S, Gross H, Kotitz R, Muller W, Vass A, Weitschies W and Trahms L 1998 Appl. Supercond. 6 577-83
[12] Astalan A P, Jonasson C, Petersson K, Blomgren J, Ilver D, Krozer A and Johansson C 2007 J. Magn. Magn. Mater. 311 166-70
[13] Oisjoen F, Magnelind P, Kalabukhov A and Winkler D 2008 Supercond. Sci. Technol. 21 034004 (4pp)
[14] Oisjoen F, et al. 2009 IEEE Transactions on Applied Superconductivity. 19 848-52
[15] Hendrickson W A, Pahler A, Smith J L, Satow Y, Merritt E A and Phizackerley R P 1989 Proc. Natl. Acad. Sci. U. S. A. 86 2190-4