Biomedical Engineered Ferrofluids

Birgit Fischer[1], Leidong Mao[1], Mustafa Gungormus[2], Candan Tamerler-Behar[2,3], Mehmet Sarikaya[2], and Hur Koser[1]
[1]Electrical Engineering, Yale University, New Haven, CT, 06520
[2]Materials Science and Engineering, University of Washington, Seattle, WA, 98195
[3]Molecular Biology and Genetics, Istanbul Technical University, Istanbul, Turkey

ABSTRACT

Ferrofluids have a wide application potential in the field of biomedicine, including cellular manipulation and sorting, hyperthermia and targeted drug delivery. For such applications to be practical, the colloidal suspension of magnetic nanoparticles within a ferrofluid needs to be stable under physiological conditions. Current approaches that utilize non-specific adsorption of surfactants onto nanoparticle surfaces do not provide stability against agglomeration under dilution or increased ionic strength. Here, we present a new approach to synthesizing water-based and bio-compatible ferrofluids using genetically engineered peptides for inorganics (GEPI's) that are selected for specific and strong binding to the surface of the nanoparticles. Initial results demonstrate the efficacy of GEPI's in significantly reducing agglomeration with increasing ionic strength when used in a ferrofluid comprised of cobalt-ferrite nanoparticles surrounded with a thin silica shell. The same approach could easily allow direct biological functionalization of the nanoparticles, rendering such ferrofluids useful in a wide range of applications.

INTRODUCTION

Ferrofluids are colloidal suspensions comprised of nanosized magnetic particles that are stabilized with a surfactant [1]. Their unique properties have led to a myriad of industrial and medical applications, including their use as liquid seals and bearings, in hard disk drives and speakers, as contrast agents in magnetic resonance imaging and as targeted drug delivery platforms [2]. In most of these applications, stability of the colloidal suspension is critical. Agglomeration of particles eventually leads to deterioration in product performance. In biomedical applications, particle agglomeration *in vivo* may even lead to dangerous scenarios, such as artery blockages and tissue damage. It is therefore not surprising that significant research effort in the field of ferrofluids goes to improving their stability.

Typically, surfactants that non-specifically adsorb to the surface of magnetic nanoparticles are used to stabilize water-based ferrofluids through steric and/or electrostatic mechanisms [1]. Either approach can create ferrofluids that can remain stable on their own for extended periods, but these colloidal suspensions end up being sensitive to dilution, as well as pH and ionic strength changes [3]. Often, biomedical applications require that ferrofluids are stable within a pH of 7.4 and a high ionic strength (up to 500 mM of salt concentration). Using large polymeric surfactants (such as Dextran [4]) for steric repulsion, it is possible to obtain a magnetic liquid that is stable at physiological conditions. In that case, however, the required surfactant layer is generally too thick and ends up substantially reducing the concentration of the magnetic phase, resulting in a rather weak ferrofluid.

In this work, we present a new approach to stabilizing water-based ferrofluids at neutral pH using genetically engineered peptides for inorganics (GEPI's). Such GEPI's selected for specific and strong binding to the surface of nanoparticles not only increase colloidal stability by acting as a thin surfactant, but they also enable an efficient route for rendering the ferrofluid bio-functional and bio-compatible. As part of this work, cobalt-ferrite magnetic nanoparticles were synthesized via a co-precipitation method [5] (as described in the next section) and covered with a thin silica shell [6]. The resulting ferrofluid was then mixed with silica-binding GEPI's and its stability was characterized both in an AC susceptibility measurement system [7], and using a ferro-microfluidic device [8]. Both approaches actuate a ferrofluid volume directly via magnetic fields alone, and their frequency spectra reveals information about the size of the magnetic nanoparticles [8]. An ideal ferrofluid with monodisperse particles displays a single and clear pumping peak; agglomeration can directly be observed as a broadening of the susceptibility and pumping spectra.

EXPERIMENTS AND RESULTS

Synthesis of cobalt ferrite with a thin silica shell

Cobalt ferrite particles were precipitated out of a boiling solution of sodium hydroxide by adding a solution of iron (III) and cobalt (II) [5]. The magnetic precipitate was separated with a strong magnet and washed twice with de-ionized (DI) water. Once the water was decanted, a 1 M nitric acid solution was added to the cobalt-ferrite precipitate and the mixture was continuously stirred at 80°C for half an hour [9]. Later, the particles were redispersed in DI water. To achieve a stable dispersion at neutral pH, the magnetic particles were dialyzed against a 0.005 M citrate solution. To cover the particles by an additional silica shell, a modified Stoeber Synthesis was used [6]. In this process, the citrate stabilized ferrofluid was diluted with a mixture of ethanol and ammonium hydroxide solution, and tetra-ethoxy-siloxane was added as a precursor for the silica shell. After stirring for 24 hours, the particles were covered with a thin silica shell. Ethanol and ammonium hydroxide were then removed and the ferrofluid was dialyzed against DI water for one week. In transmission electron microscopy (TEM) images, it was observed that the cobalt ferrite nanoparticles were covered with a 4 nm thick silica shell (Figure 1). The average diameter of the magnetic nanoparticles (without the silica shell) was determined to be around 11 nm.

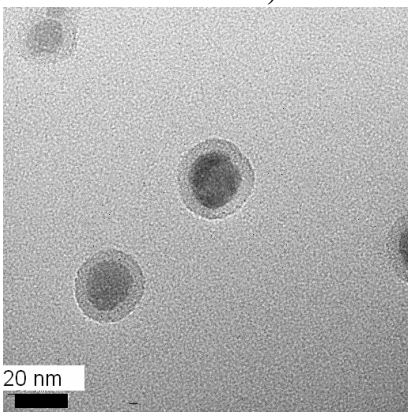

Figure 1. TEM image of cobalt ferrite nanoparticles with a thin silica shell.

Susceptibility measurements

The magnetic moment within the cobalt-ferrite nanoparticles responds to external magnetic fields with a characteristic time constant. For small nanoparticles, this magnetic moment may relax through an internal rotation within the particle (i.e., Neel relaxation). Above a critical size, the magnetic moment stays fixed within the nanoparticle and the entire particle rotates to align its magnetization with the applied field. For cobalt ferrite, the critical particle diameter is about 5 nm. A ferrofluid comprised of cobalt ferrite nanoparticles larger than this limit relaxes primarily via particle rotation (referred to as Brownian relaxation) [2], and this is indeed the case for the ferrofluid described above. A given size distribution of nanoparticles within the ferrofluid results in an average relaxation time constant under externally applied magnetic fields. This time constant, in turn, determines the hydrodynamics of ferrofluid actuation when sinusoidally varying fields are utilized. For instance, the time constant (and hence, the average particle size within a ferrofluid) may be inferred by measuring the susceptibility of the ferrofluid under different frequencies of alternating (AC) fields. The overall relaxation time constant results in a peak within the imaginary part of the AC susceptibility frequency spectrum, as depicted in Figure 2-a. The peak frequency for each curve is a measure of the overall time constant of that ferrofluid [10], whereas the specific curve shapes are indicative of the particle size distribution. Presence of agglomeration may be inferred through an increase in the low frequency component of the AC susceptibility, as observed in Figure 2-b for the 60% case. Notice that higher dilutions in that figure result in an actual increase in the peak frequency (corresponding to a reduced time constant and smaller particles overall), indicating the presence of some agglomeration that is already present in the original (100%) cobalt ferrite/citrate ferrofluid to begin with.

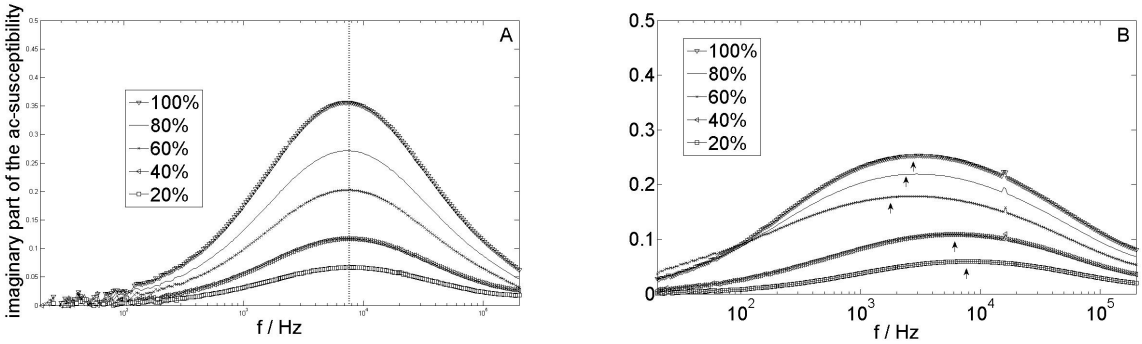

Figure 2. Normalized imaginary AC susceptibility curves for a cobalt ferrite ferrofluid stabilized with a thin silica shell (A) and.citrate (B) for different values of dilution with DI water. Normalization is performed such that the magnitude of the overall DC susceptibility is unity for the 100% (i.e., non-diluted) ferrofluid sample. The peak locations and curve shapes are maintained with the use of the silica coating, whereas the performance of the citrate-based surfactant appears to depend on the specific dilution.

It is clear from Figure 2-a that the cobalt ferrite/silica nanoparticles form a ferrofluid that is not compositionally sensitive to dilution. For this promising ferrofluid, a stability test against increasing ionic strength was performed using sodium chloride as the ionic solute (Figure 3-a). As a control, the same test was also conducted for the cobalt ferrite ferrofluid stabilized with citrate (Figure 3-b). Increasing ionic strength within the solution alters the effective zeta potential

at the surfaces of the nanoparticles, while providing a charge-screening effect that reduces the Coulomb repulsion between the magnetic nanoparticles. The typical result is that of increased colloidal agglomeration and particle precipitation, as is clearly evident in Figure 3 for both silica and citrate stabilized cobalt ferrite ferrofluids. Here, agglomeration results in an increase of AC susceptibility values at lower frequencies as the salt concentration is increased, whereas precipitation leads to an overall reduction in the AC susceptibility. Silica-stabilized ferrofluid samples show less agglomeration than their citrate-stabilized counterparts, though both ferrofluid types suffer from significant precipitation at elevated ionic strength levels.

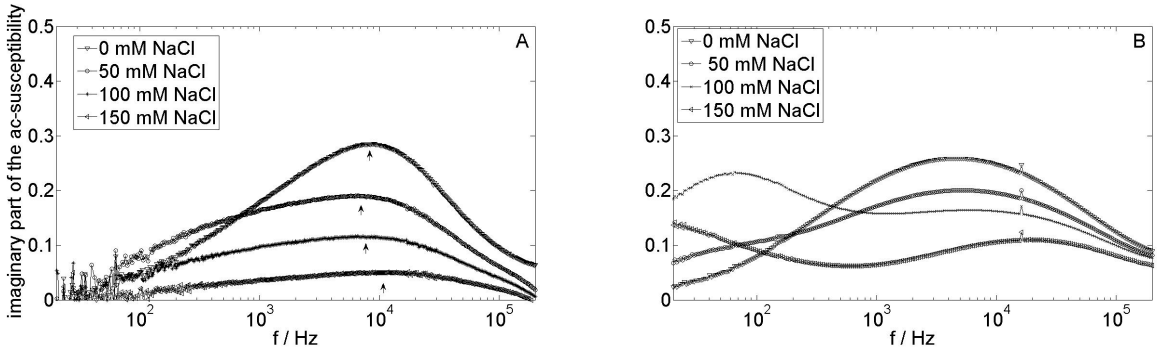

Figure 3. Normalized imaginary part of the AC susceptibility at different salt concentrations within a cobalt ferrite ferrofluid stabilized with a silica shell (A) and with citrate (B). In this case, normalization was performed in the same fashion as for Figure 2. The topmost curve in (A) is different than the one in Figure 2-a; the ferrofluid sample here in (A) contains about 1.25% (by volume) phosphate buffer solution (PBS), in order to make a comparison with results of Figure 4 below. The ionic content of PBS is enough to cause a small amount of agglomeration and precipitation, as is evident from the altered shape of the topmost curve in (A).

GEPI experiments

In order to improve the stability of the cobalt ferrite/silica ferrofluid at elevated ionic strength levels, we introduced silica-binding short peptides into the colloidal suspensions of Figure 3-a. The resulting AC susceptibility spectra of these samples are depicted in Figure 4. Notice that the main peak location in frequency remains virtually constant in Figure 4, and the spectra are somewhat narrower, indicating reduced agglomeration. The curve heights are also higher overall, suggesting a decreased level of precipitation.

We suggest that GEPI's selected for specific and strong binding to the surface of nanoparticles increase colloidal stability by acting as a thin surfactant. Since GEPI's are already genetically engineered to function optimally at physiological conditions, they would be ideal as surfactants that strongly and specifically bind to the nanoparticles in ferrofluids under elevated ionic strength conditions. What is more, such GEPI's also enable an efficient route for rendering a ferrofluid bio-functional through the insertion of various different proteins and linkers in their sequence. For instance, the ferrofluid-based pathogen detection scheme described in [REF-Nanotech] could be realized using functionalized GEPI's that carry specific binders for various different antigens present in solution. As such, GEPI's can be utilized as optimized linkers between the magnetic nanoparticles and various receptors for target antigens.

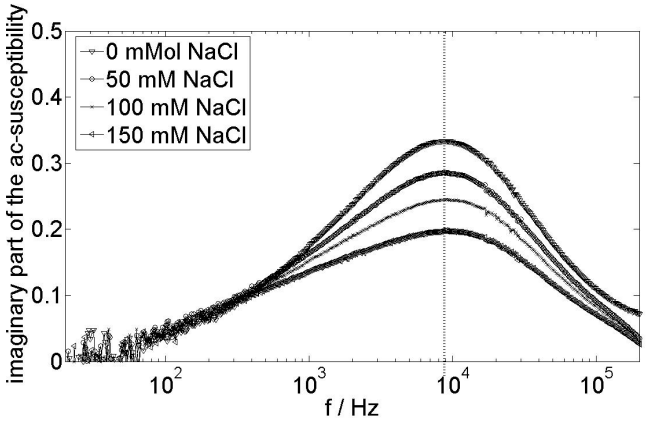

Figure 4. Normalized imaginary part of the AC susceptibility of cobalt ferrite/silica ferrofluid containing silica-specific GEPI's under different salt concentrations. GEPI's that are genetically engineered to specifically bind to silica result in significantly higher susceptibility curves with reduced agglomeration and precipitation (relative to Figure 3-a), especially at the maximum ionic strength level tested.

Results of light-scattering experiments confirm that the GEPI's are indeed binding to the magnetic nanoparticles and remaining bound even when the ferrofluids are significantly diluted; average nanoparticle hydrodynamic diameter increases slightly from 45.9 nm to 46.4 nm when the GEPI's are introduced into the ferrofluid.

Pumping experiment

For magnetic pumping experiments, a microfluidic device was prepared by conventional microfabrication and soft lithography as described in [8]. The ferrofluid sample is injected into a microfluidic channel that is 35 mm × 4 mm × 0.1 mm. Ferrofluidic pumping is characterized by a differential pressure sensor connected to either end of the microfluidic channel. A traveling wave is generated via integrated electrodes under the channel, creating a frequency-dependent pumping spectrum that resembles the imaginary AC susceptibility data described above. This device provides an alternative method to confirm our findings described above. Ultimately, this miniaturized ferro-microfluidic device could be used as a cheap and portable pathogen sensor [8, 11].

Figure 5 depicts a representative pumping spectrum that was obtained using a cobalt ferrite/silica ferrofluid, and the effect of using silica-binding GEPI's at a modest concentration (2.5% by volume). The increased pumping signal at the same magnetic particle concentration is indicative of improved ferrofluid stability.

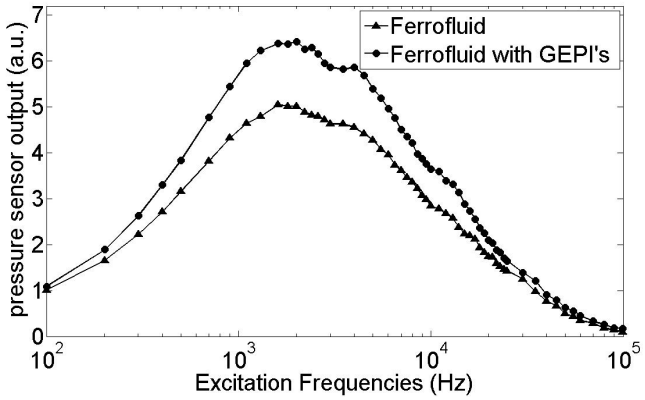

Figure 5. Pumping profile of the cobalt ferrite/silica ferrofluid with and without GEPI's.

CONCLUSIONS

This study is the first of its kind that demonstrates the utility of genetically engineered peptides in improving ferrofluid stability and bio-compatibility. GEPI's that strongly and specifically bind to the surfaces of nanoparticles enables ferrofluids to demonstrate improved immunity against agglomeration and precipitation under increased ionic strength conditions. Using a thin silica shell as a permanent surfactant around the magnetic nanoparticles helps to

achieve compositional stability against dilution of the ferrofluid. The silica shell around the nanoparticles also provides a large surface area for the silica-binding GEPI's to attach. If desired, the GEPI's could be engineered to be specific binders to other inorganic materials (such as magnetite), rendering different ferrofluid recipes stable and bio-compatible. Our future work will focus in detailed studies with the ferro-microfluidic device utilizing GEPI-stabilized ferrofluids as pathogen detectors.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (NSF – 0449364).

REFERENCES

1. R.E. Rosensweig, "Ferrohydrodynamics", Dover Publication: New York (1997).
2. B. M. Berkosvky, V.G. Bashtovoy, "Magnetic fluids and application handbook', Begell House: New York and Wellingford, UK, (1996).
3. A. K. Gupta, M. Gupta, *Biomaterials*, **26**, 3905-4021, (2005).
4. S. Mornet, J. Portier, E. Duguet, *J. Magn. Magn. Mat,* **293**, 127-134, (2005).
5. S. E. Khalafalla, G. W. Reimers, US Patent: 3.764.540 (1973).
6. J. Wagner, T. Autenrieth, R. Hempelmann, *J. Magn. Magn. Mat*, **252,** 4, (2002).
7. B. Fischer, B. Huke, M. Lücke, Hempelmann, *J. Magn. Magn. Mat*, **289,** 74, (2005)
8. L. Mao, H. Koser, *Nanotechnology*, **17**, 34-47, (2006).
9. R. Massart, *IEEE Trans Magn. MAG-*, **17**(2), 1247-48, (1981).
10. L. Mao, H. Koser, J. *Magn. Magn. Mat.,* **289**, 199-202, (2005).
11. L.Mao, H. Koser, *IEEE Proceedings Transducer and Eurosensors*, 1829, (2007).