Silane-based functionalization of synthetic antiferromagnetic nanoparticles for biomedical applications

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Synthetic antiferromagnetic nanoparticles (SAFNPs) have been successfully coated with two different kinds of silanes, 3-aminopropyltrimethoxysilane and 2-[methoxy (polyethyleneoxy)propyl]trimethoxysilane. The morphology of SAF particles is characterized by scanning electron microscopy and magnetic properties by alternating gradient magnetometry. The attachment of silane molecules is verified by Fourier-transform infrared spectroscopy and colloidal stability is studied using dynamic light scattering. These two silanes change the surface chemical properties of SAFNPs dramatically in different ways, which in turn affects the stability of these particles. © 2010 American Institute of Physics. [doi:10.1063/1.3355906]

I. INTRODUCTION

Magnetic nanoparticles are widely used in biology and medicine, such as cell separation,¹ magnetic resonance imaging,^{2,3} and biosensing.^{4–6} In most applications, large single particle magnetic moments are desired, but low remanence is required to prevent spontaneous aggregation. Commonly used superparamagnetic iron-oxide (SPIO) nanoparticles suffer from intrinsic size limitations since one cannot increase the magnetic moment by simply increasing the volume, because the magnetic anisotropy will transform superparamagnetism⁷ into ferromagnetism. Here, we use synthetic antiferromagnetic nanoparticles (SAFNPs),^{8,9} which do not face the same size limitation, to produce high moment magnetic particles. In addition, a recently developed stamp fabrication method,¹⁰ offer substantial improvements on the fabrication efficiency.

However, several problems still need to be solved before these particles can be fully utilized. These include further cost and size reductions, and development of methods for optimizing functionalized nanoparticle stability in aqueous solution and for conjugation of biomolecules. In this work we therefore adopt a trilayer resist method for defining the nanoimprinted templates,¹¹ thus eliminating the requirement for vacuum deposition of thick sacrificial Cu release layers previously used.⁸ Release is now readily accomplished with organic solvents, which is preferable to our prior use of a caustic Cu-ammine based etch. We have also explored surface functionalization and conjugation through silane based procedures, in place of previously used citrate stabilizers and carboxylate mediated binding of biological targeting molecules.⁹ The success of chemical bonding was verified by

FT-IR observations, but we have encountered difficulties with particle aggregation and particle interaction with tube walls. Therefore, we now more carefully explore surface charge (Zeta potential) in aqueous solution; which influences dispersity, colloidal stability, and nonspecific interactions.

Silanes have been used to modify many surfaces for decades, due to their excellent reactivity with silicon and other oxide surfaces, and for the flexibility they offer for attaching different functional groups to the surface. Here, we report results of coating SAFNPs with silanes bearing amine or polyethyleneoxy (PEG) functionalities. These molecules were chosen since amine groups are valuable in common reactions for attaching proteins and other biomolecules^{12,13} and PEGs are widely used as biocompatible molecules which reduce nonspecific binding, prolong *in vivo* circulation time, and increase aqueous solubility.^{14,15}

II. EXPERIMENTS

The fabrication process of SAFNPs was described elsewhere.^{8,11} This time, a stamp made according to a recently developed method¹⁰ is used to fabricate SAFNPs. Layers of elemental materials are thermally evaporated onto the wafer to constitute the nanoparticles in the sequence and thickness (nm): Ti 5/Fe 10/Ti 3/Fe 10/Ti 5 (bare SAFNPs) or Si 3/Ti 5/Fe 10/Ti 3/Fe 10/Ti 5/Si 3 (Si-capped SAFNPs). Finally, the particles are released by using 1165 stripper (primarily N-methyl pyrrolidone, shipley) and repeatedly washed and resuspended in either acetone or water. Bare SAFNPs are then dried on Si substrate for SEM and AGM measurements.

Acetone suspensions are used for silanization. Si-capped SAFNPs are used for all of the following experiments. Two kinds of silanes are used: 3-aminopropyl trimethoxysilane (APTMS, SIA0611.0, Gelest, Inc.) and 2-[methoxy (polyethyleneoxy) propyl] trimethoxysilane (PEG-silane, molecular weight: 460–590D, SIM6492.7, Gelest, Inc.). Either 10 μ L

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FIG. 1. (a) SEM image of released SAF nanoparticles. Samples are dried on a Si substrate; (b) hysteresis loop of released particles measured by AGM.

APTMS or 10 μL PEG-silane were added to vials containing $\sim 10^9$ SAFNPs in 1 ml acetone and incubated for one day with gentle agitation. After the reaction, these particles are washed with acetone and centrifuged at least two times to remove residual reagent.

For Fourier-transform infrared spectroscopy (FT-IR), aliquots of the acetone suspension, both before and after silane coating, were applied to and dried on stretched Teflon (PTFE) substrates. IR adsorption spectra were measured with a Nicolet 570 instrument. PTFE membranes were chosen to provide a clear background in the region from 2000 to 4000 cm^{-1} . Samples of the particles are also prepared for particle size distribution and Zeta potential measurements by dynamic light scattering (DLS, Brookhaven Zeta Pals). Zeta potential measurements used 10 μL of nanoparticle suspensions in 1.5 ml aqueous buffers with $\text{pH}=4, 7$, and 10. Hydrodynamic DLS radii were useful for detecting aggregation, but the results are not reported because aggregate size distributions were broad and numerical values depended strongly on weighting algorithms. We also examined particle aggregates using SEM (FEI XL30 Sirion). Aliquots of aqueous suspensions, both before and after silane coating (transferred from acetone by centrifuge), were dropped onto Si water coupons for 20 s and removed by aspiration.

III. RESULTS AND DISCUSSION

Figure 1(a) shows SAFNPs after they have been released and transferred to water. These particles are disk-shaped, with the diameter around 80 nm and thickness around 40 nm, with a noticeable diameter variation between the top and bottom sides. The particles appear to be randomly oriented.

FIG. 2. FT-IR spectrum for (a) uncoated SAFNPs, (b) PEG-silane coated SAFNPs, and (c) APTMS coated SAFNPs.

A hysteresis loop of solution deposited particles measured at room temperature shown in Fig. 1(b), resembles that of superparamagnetic nanoparticles, with a slight coercivity of tens of Oersted and low remanence. However, the effective magnetic volume of SAFNPs is much larger than that of SPIO nanoparticles, which usually have diameters less than 20 nm. Therefore, SAFNPs can attain much larger single particle magnetic moments, while maintaining hysteresis loops similar to small SPIO nanoparticles.

The FT-IR “adsorption” spectra of particles on PTFE membranes are shown in Fig. 2 for three samples: SAF before silane coating, after coating with PEG-silane, or APTMS. The particle concentrations on the membrane are similar to those used for Fig. 1(a). For all samples, there are two small peaks around 2350 cm^{-1} , due to residual CO_2 inside the FT-IR chamber, which diminish as the chamber vacuum improves. Except for these two peaks, the uncoated SAF spectrum shows a clear background throughout the region from 2000 to 4000 cm^{-1} [Fig. 2(a)]. However, for both PEG-silane and APTMS coated samples [Figs. 2(b) and 2(c)], broad peaks appear between 2700 and 3000 cm^{-1} , which can be attributed to the stretching mode of C–H bonds in alkyl chains,¹⁶ indicating hydrocarbon attachment to the particle surfaces which is not evident for the untreated sample. This peak is much stronger for PEG-silane than for APTMS, which can be explained by the fact that PEG-silane has a longer chain than APTMS. Moreover, the APTMS coated sample has a unique peak at 3350 cm^{-1} , which is typical for N–H stretching of the amine group. There is an additional peak at 3500 cm^{-1} for the PEG-silane coated sample, probably due to the hydroxyls.¹⁷ These results show that the desired functional groups have been coated onto the particle surface. Other characteristic peaks, such as the one corresponding to C–O–C bonding¹⁸ from PEG, are expected to appear in the region below 2000 cm^{-1} , but are masked by the PTFE membrane background.

Zeta potentials are key parameters for colloidal systems, as they influence colloidal stability¹⁹ and interactions with container walls and biological targets.⁴ Results shown in Figs. 3(a) and 3(b) illustrate the surface chemical state of

FIG. 3. (a) Zeta potential for uncoated SAFNPs (raw SAF), APTMS coated SAFNPs (amino SAF), and PEG-silane coated SAFNPs (PEG SAF) in aqua solution with different pH value; (b) schematic of the surface states of raw SAF, amino SAF, and PEG SAF; (c), (d), and (e) SEM images of different polydispersed SAFNPs on Si substrate: (c) raw SAF, (d) amino SAF, (e) PEG SAF.

differently coated SAFNPs. For all three samples, Zeta potentials increase with the decreasing pH since decreasing pH values facilitates adsorption of positive charge. For uncoated SAF, the Zeta potential stays negative for pH values ranging from 4 to 10. This is consistent with the fact that the surface of silicon exposed to air and water contains a remarkable number of hydroxyl groups. After coating with APTMS, there is a significant positive shift of the Zeta potential, indicating protonation of bound amines. Finally, for the PEG-silane coated SAF particle case, the surface charge is more nearly neutral, which might be expected when nearly neutral PEG chains replace surface hydroxyl groups. Also, the Zeta potential change with pH variation, though still apparent, is less than for untreated or aminated surfaces; which is probably caused by polymer chains attachment shifting the slipping plane outwards, where the absolute value of potential is reduced due to ionic screening.

These Zeta potential changes significantly affect the stability of nanoparticles in aqueous suspensions, which is not surprising since they alter the balance between attractive Van der Waal's forces and repulsive "steric" and electrostatic interactions. Some indication of the extent of aggregation is provided by the SEM images in Fig. 3(c)–3(e). Here SAFNPs are quickly deposited from solution in order to minimize aggregation induced by solvent evaporation. Uncoated particles, which have a relatively large negative potential, are often found isolated and sitting flat on the substrate, especially for dilute suspensions. However, after these particles are coated with silanes, which render Zeta potentials much less negative, remarkable particle aggregates are observed, as shown in Figs. 3(d) and 3(e). This indicates that our present functionalization method is not sufficient to prevent particle aggregation in water or saline solutions, even with PEG coatings.

IV. CONCLUSION

In summary, silanes with different functional groups have been successfully used to coat SAFNPs. Amine groups tend to produce positive charges, while PEG chains tend to neutralize the initially negative surface charge. Highly negatively charged particles are found to be more stable in aqueous solution.

Guided by this rule, we proposed to attach biomolecules to SAFNPs by maintaining highly negatively charged surfaces during functionalization. Excess charges will be neutralized after the polymer shell and desired biomolecules are in place.

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