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# Maghemite, silver, ceragenin conjugate particles for selective binding and contrast of bacteria

Mark A. Hoppens [a], Zaanan E.W. Wheeler [a], Ammar T. Qureshi [a], Katie Hogan [a], Ashleigh Wright [b], George G. Stanley [b], David Young [c], Paul Savage [d], Daniel Hayes [a,*]

[a] Department of Biological and Agricultural Engineering, Louisiana State University and Agricultural Center, 149 E. B. Doran, Baton Rouge, LA 70803, USA
[b] Department of Chemistry, Louisiana State University, 232 Choppin, Baton Rouge, LA 70803, USA
[c] Department of Physics & Astronomy, Louisiana State University, 202 Nicholson, Baton Rouge, LA 70803, USA
[d] Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84602, USA

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ABSTRACT

New synthesis techniques are providing increasing control over many inorganic nanoparticle characteristics, facilitating the creation of new multifunctional theranostics. This report proposes the synthesis and testing of a combination nanoparticle comprised of a maghemite core for enhanced T2 MRI contrast diagnostics, a colloidal silver shell acting as an antimicrobial and therapeutic vehicle, and a ceragenin (CSA-124) surfactant providing microbial adhesion. A polyacrylic acid functionalized maghemite nanoparticle is synthesized by a high temperature organic phase reduction followed by thiol functionalization and gold cluster seeding. A silver shell is formed through $AgNO_3$ reduction, and an oriented monolayer of the thiolated ceragenin, is bound through a self-assembly process. The process and products are characterized throughout synthesis through TEM, DLS, FT-IR, UV–Vis, ICP-OES, HPLC-ESI-TOF-MS, DC magnetization and susceptibility, X-ray diffraction, and *in vitro* MRI. Synthesized Diagnostic Antimicrobial Nanoparticles (DANs) were found to have a spherical morphology with a diameter of 32.47 ± 1.83 nm, hydrodynamic diameter of 53.05 ± 1.20 nm, maximum magnetic moment of 12 emu/g NP (54 emu/g Fe) with little variation due to temperature, and are predominantly paramagnetic. *In vitro* MRI studies show that DANs contrast well at concentrations as low as 9 ppm, and successfully adhere to *Staphylococcus aureus*. DAN MIC was determined to be approximately 12 ppm and 24 ppm against *S. aureus and Escherichia coli* respectively.



## 1. Introduction

As synthetic control, manipulation, and understanding of inorganic nanoparticles increase, so too does the interest, research, and development in the biomedical field. Inorganic nanoparticles are being synthesized and studied for use as antimicrobials, MRI contrast agents, CT contrast agents, thermal ablation targets, and delivery platforms for RNA, DNA, peptides, fluorescent markers, and other small molecules [1–7]. The highly specific tuneability of inorganic nanoparticles with respect to composition, morphology, shell thickness, resonance frequencies, and surface chemistries as well as their economical and facile bulk synthesis make them ideal for this wide range of applications [8–10]. Recently several hybrid nanoparticles have been developed to accomplish multiple functions, such as in theranostics wherein the particle both aids in the diagnosis and therapeutic treatment of disease [11,12].

Superparamagnetic iron oxide nanoparticles (SPIONs) are one such inorganic nanoparticle that has been used clinically with great success as a T2 contrast agent for magnetic resonance imaging (MRI). SPIONs are the primary active component of products such as Feridex, Resovist, and Combidex, which have been used in the diagnosis of spleen, liver, and bone marrow related ailments [13]. They can vary in size from 30 to 150 nm and may be coated in dextran, starch, albumin, silicones, polyethylene glycol, and many other hydrophilic surfactants [14]. One property common to all MRI contrast agents is that they must be paramagnetic, meaning

*Abbreviations:* NP, nanoparticle; CSA, cationic steroidal antimicrobial, aka ceragenins; MNP, magnetic nanoparticle; PAA, polyacrylic acid; HPC, hyroxypropyl cellulose; TEM, transmission electron microscopy; DLS, dynamic light scattering; MRI, magnetic resonance imaging; ICP-OES, inductively coupled plasma optical emission spectrometry; DTT, DL-Dithiothreitol; ACN, acetonitrile; HPLC, high precision liquid chromatography; ESI-TOF-MS, electrospray ionization -time of flight-mass spectrometer.

* Corresponding author. Fax: +1 (225) 578 2919.
*E-mail addresses:* mhoppe4@lsu.edu (M.A. Hoppens), zwheel1@lsu.edu (Z.E.W. Wheeler), aqures1@tigers.lsu.edu (A.T. Qureshi), Hogan.gecko@mail.com (K. Hogan), awrig14@tigers.lsu.edu (A. Wright), gstanley@lsu.edu (G.G. Stanley), dyoung@phys.lsu.edu (D. Young), paul_savage@byu.edu (P. Savage), danielhayes@lsu.edu (D. Hayes).

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they only possess a magnetic moment when in the presence of a magnetic field [13].

Among inorganic nanoparticles, silver has shown to be an affective antimicrobial as well as therapeutic carrier agent. Silver has been demonstrated to deactivate bacteria through several different mechanisms, thereby making it very difficult for an antimicrobial to develop resistance [15]. Silver Nanoparticles can act as a reservoir for silver ions which bind to proteins and cause structural modifications to bacterial cell wall and nuclear membranes resulting in cell death. As with all *in vivo* drug delivery applications, toxicity is a concern. A 28 day silver toxicity study was conducted by Il Je Yu et al. in which rats orally received between 30 and 1000 mg/kg/day silver nanoparticles. It was determined that only slight liver damage could be found after 300 mg/kg/day exposure and that there were no statistically significant differences in total erythrocyte numbers or ratios [16]. A similar study in 2011 exposed rats to between 4 and 40 mg/kg silver nanoparticles intravenously and found that exposure to under 10 mg/kg did not significantly affect WBC count, platelet counts, hemoglobin, or RBC counts and concluded that such doses would be safe for biomedical applications with no side-effects [17]. Because of its biocompatibility and antimicrobial nature at moderate concentrations, silver is currently being incorporated into many products such as dental resins, medical device coatings, wound dressings, and washing machines [2,10,18]. Furthermore, noble metal nanoparticles such as silver, also offer an attractive vehicle for small molecule delivery due to their robust functionalization chemistries, large surface area/volume ratios, readily tunable morphologies, and ease of bulk synthesis [19]. They also offer high-density surface ligand attachment and reduced degradation of the therapeutic agent [20–22].

Recent studies have examined the antimicrobial properties of silver applied in conjunction with a variety of other elements. Melaiye et al., developed a method of using silver's antimicrobial properties to prevent infections in burn wounds by encapsulating silver(i)-imidazole cyclophane gem-diol complexes in tecophilic nanofibers. The silver mats proved to be effective at killing bacteria and allowed for extended release of silver [23]. Similarly, silver-organoalkoxysilane nanomembranes containing silver nanoparticles were shown to inhibit *Escherichia coli*, *Bacillus anthracis*, *Staphylococcus aureus*, and *Brucella suis* within 30 min of application and prevent further colonization [24]. Silver nanoparticles have also been attached to magnetic hybrid colloids consisting of an iron oxide core and a silica shell, exhibiting substantial antimicrobial tendencies. As silver nanoparticles bind to the bacteria, released Ag+ ions rupture the bacterial membranes and thus effectively destroy the bacteria [25]. Magnetic-silica Janus nanorods decorated with silver nanoparticles were shown to be biocompatible, have magnetic sensitivity, and exhibit antimicrobial activity both in the short- and long-term [26].

Antimicrobial peptides, which provide bacterial membrane selectivity and novel modes of action, are a promising means of controlling bacterial growth because they are capable of circumventing traditional resistance mechanisms. However, several constraints to the general clinical use of these peptides are their high cost of bulk synthesis and poor structural stability in the presence of proteases. New mimics of antimicrobial peptides, ceragenins or CSAs, have been developed which mimic the selectivity and antimicrobial characteristics of antimicrobial peptides while offering increased stability and ease of synthesis. CSAs have been shown to be effective against tobramycin-resistant *Pseudomonas aeruginosa*, drug resistant strains of *Helicobacter pylori*, vancomycin-resistant *S. aureus (VRSA)*, and periodontopathic bacteria such as *Streptococcus mutans* and *Porphyromonas* species [27–29].

This report proposes a novel theranostic conjugate nanoparticle exhibiting selective binding of bacteria, T2 MRI negative contrast capability, and antimicrobial efficacy. These Diagnostic Antimicrobial Nanoparticles (DANs) harness the attributes of SPIONs, silver, and synthetic antimicrobial peptides, and conjugate them into a single spherical nanostructure. A synthesis procedure is provided for the creation of an iron oxide core, reduced silver shell, and CSA-124 surfactant. The synthetic process and resulting materials are characterized by several methods including transmission electron microscopy (TEM), dynamic light scattering (DLS), Fourier transform infrared spectroscopy (FT-IR), Inductively coupled plasma optical emission spectrometry (ICP-OES), ultraviolet–visible spectroscopy (UV–Vis), High Performance Liquid Chromatography – Electrospray Ionization tandem Time of Flight Mass Spectrometry (HPLC-ESI-TOF-MS), and DC magnetization and susceptibility. It is the objective of this study to present a viable synthesis procedure by which to consistently synthesize DANs as well as demonstrate their selective diagnostic potential.

## 2. Material and methods

### 2.1. Materials

The following materials were ordered from Sigma–Aldrich®: polyacrylic acid (average MW 1800), triethylene glycol (99%), iron acetylacetonate (99.9%), ethyl-dimethyl-aminopropylcarbodiimide, cysteamine hydrochloride (⩾98%), 2-(N-morpholino)ethanesulfonic acid, silver nitrate (>99%), ammonium hydroxide (28–30%), N-hydroxysulfosuccinimide (98.5%), sodium hydroxide (NaOH), tetrakis(hydroxymethyl)phosphonium chloride (THPC, 80% in water), tetrachloroaurate trihydrate ($HAuCl_4$), and dialysis membranes (10,000 Da). Acetic acid (99%) was obtained from Fisher Scientific®. Formaldehyde (36.5–38%) was obtained from Mallinckrodt Chemicals®. CSA-124 was prepared by functionalizing a ceragenin, CSA-13, with a thiol group on a short PEG tether. Synthetic details for CSA-124 will be reported elsewhere. All other chemicals were used as received unless otherwise noted. Mueller Hinton Broth, and Nutrient Agar, pH6.0 with 0.8%NaCl was purchased from Himedia®/VWR™.

### 2.2. Methods: DAN synthesis

#### 2.2.1. Polyacrylic acid-coated iron nanoparticle synthesis

Iron-polyacrylic acid nanoparticle (Fe–PAA NP) synthesis was based on the thermal decomposition methods employed by Ming Ling et al. [30]. All reactions were conducted in an argon atmosphere. First, 1.0 g of polyacrylic acid was added to 25 mL triethylene glycol, followed by the addition of 2.0 mM (706 mg) iron acetylacetonate ($Fe(acac)_3$) solution and stirred until dissolved. Using a Glas-Col 500 mL, 325 W heating mantle regulated by a Glas-Col power regulator the mixture was slowly heated to 190 °C over 30 min, then rapidly heated to reflux at 275 °C where the temperature was held for 20, 30, or 40 min.

To purify the resulting mixture, 30 mL ethyl acetate (EtOAc) was added. The mixture was shaken and then centrifuged at 5870*g* for 30 min. The supernatant (EtOAc and dissolved impurities) was removed and discarded. The black precipitate was further purified via liquid/liquid extraction by first re-suspending in 5–10 mL DI water and then adding 20–30 mL EtOAc, centrifuging once at 3000 g for 5 min, and removing the supernatant of EtOAc and dissolved impurities. The liquid/liquid extraction process was repeated for a total of two iterations. Removal of residual EtOAc was accomplished via dialysis (MW cutoff 10,000 Daltons) over a 24–36 h period. This procedure yielded a solution of 28.5 ppm iron content. The Fe–PAA NPs were characterized using TEM, DLS, ICP-OES, and FT-IR.