GOLD-COATED MAGNETIC POLYMER MICROSPHERE SYNTHESIS AND

CHARACTERIZATION: APPLICATIONS IN BIOLOGICAL CONTEXTS

By

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FUNCTIONALIZATION AND APPLICTATION OF GOLD-COATED, MAGNETIC

POLYMER MICROSPHERES

Introduction

Magnetic polymer microspheres (MPMs) are a multicomponent inorganic and organic material that can be easily manipulated with a magnetic field. It was realized in the 1950's that MPMs could be coated with antigens and used to agglutinate in the presence of the corresponding antibody, which has lead to successful application in numerous biological assays.[1] The success of MPMs in biological assays has led to the synthesis of several forms of MPMs.

A MPM is composed of a magnetic and polymer component that can form one of the following morphs shown in **Fig. 1**: (a) a single magnetic particle surrounded by a
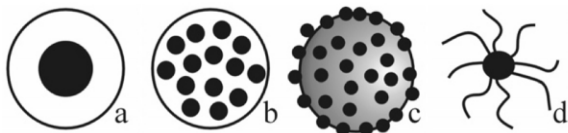
Figure 1.: Morphs of magnetic polymer microspheres.

polymer matrix, (b) numerous magnetic particles homogeneously distributed throughout a polymer matrix, (c) a polymer matrix coated with

nanoparticles, or (d) a magnetic particle with polymeric ligands extending from the surface. The most commercially available MPMs exhibit either a or b type morphs. Each type has its advantages and disadvantages and no one preparation method can satisfy all of the following criteria: functionalized, monodispersed, spherical particles completely encapsulating magnetic cores while controlling size and biocompatibility.[2]

The polymer matrix serves as a protective shell against the degradation of the magnetic particles and presents a robust surface that can be further functionalized with ligands that enhance the utility of MPMs.

With the advent of more powerful microscopic instruments, research into nanoscale materials has grown significantly in the last few decades. As materials are formed at the nanoscale, optical, electronic, and magnetic properties deviate from those observed in the corresponding bulk materials. Along with those interesting intrinsic properties, nanomaterials can encompass a range of forms and sizes, and they also have the composition of single or multicomponent structures. Multicomponent structures can be composed of a single element or multiple elements and compounds. This allows for greater customization of the nanoscale properties. The unique properties and structural diversity of the nanomaterials make them excellent candidates for a variety of applications in diagnostics, therapeutics, environmental testing.

This work will highlight properties of magnetism on the nano- and microscale and common issues associated with the use of magnetic particles. The issue of magnetic degradation can be overcome by careful consideration of magnetic nanoparticle synthetic strategy and method of surface passivation. The choice of polymer matrix can circumvent these issues related to magnetic degradation, but there is a trade off between the passivation and protection of the magnetic particles with ease of conjugation that must be considered. Common methods of microsphere synthesis will be reviewed culminating with a facile method of coalescence of gold to the MPM surface that alleviates ligand coupling issues that arise with organic coupling strategies.

Magnetism on the Nanoscale

These unique properties have intrigued scientist to ask not only how do the properties of the nanomaterials differ from their bulk counterparts but also, how can these unique properties be exploited for utilization in a variety of applications? Magnetic nanoparticles have been thoroughly researched. These are unique in that they provide easy manipulation with a magnetic field and can also be utilized for facile and efficient separations.

The spin of unpaired electrons causes atoms to have a net magnetic moment. If the spins orient in the direction of an externally applied magnetic field, the phenomenon, as illustrated in **Fig. 2**, is called paramagnetism. In ferromagnetism, all of the spins orient in the same direction. This violates the Pauli principle, which states that electron spins will orient in opposite directions to minimize repulsion and increase electrostatic energy; the difference being called exchange energy. Over long distances exchange energy is circumvented by the natural tendency for spin to align. The effect is that spins are similarly aligned in regions, but over thousands of atoms, the spins will actually begin to flip.

Figure 2: Two types of magnetic behavior in the absence and presence of an externally applied magnetic field.

Domain walls separate regions of similarly aligned spins. As magnetic material reduces in size to the nanometer scale (1 to 100 nm), the number of atomic spins reduces such that the material supports only a single domain. The energy required to form a domain wall is a balance between magnetostatic energy and the domain wall energy. A

collection of these single-domain particles in the presence of a magnetic field can align with the field. This phenomenon is called superparamagnetism. Superparamagnetic particles are ferromagnetic in nature, because all of the spins are aligned in each individual nanoparticle. They have the added benefit that when the field is removed; the nanoparticles exhibit no remanence, which allows for facile resuspension throughout a medium. There exist a critical volume below which it cost more energy to create domain walls than support the external magnetostatic energy of the single-domain state.

Magnetic Nanoparticle Synthesis

Magnetic nanoparticles can be composed of iron oxides (Fe_3O_4, α-Fe_2O_3, or γ-Fe_2O_3), pure metals (Fe or Co), ceramic spinel type oxides ($MgFe_2O_4$, $MnFe_2O_3$, or $CoFe_2O_3$), or alloys (CoPt, FePt). Magnetic nanoparticles can be synthesized by a top down or bottom up approach that offer differing degrees of morphology, stability, and dispersity control. Co-precipitation, thermal decomposition, and mechanochemical synthetic strategies are three techniques that are efficient and reproducible for scale up to larger systems. Although only a few techniques will be explored in depth, a host of other techniques are available to synthesize magnetic nanoparticles including: laser pyrolysis, hydrothermal synthesis, and microemulsions.

Co-precipitation is an in situ precipitation technique for iron oxides, Fe_3O_4, α-Fe_2O_3. The precipitation reactions are usually undertaken under inert atmospheres, basic conditions and ambient or elevated temperatures. In general, the dispersity can be controlled by the reaction temperature, pH, ionic strength of the solution, types of iron salts used, and the ratio of Fe^{2+} to Fe^{3+}. Without the use of stabilizing agents, the

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synthesized nanoparticles can be polydispersed. Using the co-precipitation method, particles on the order of 2 to 10 nm in diameter have been shown with relatively narrow size distributions. Although using surfactants increases monodispersity, the choice in surfactant can influence the shape of the particles.

Thermal decomposition is the degradation of organometallic compounds in the presence of high boiling organic solvents and stabilizing surfactants to yield magnetic nanoparticles. Using this method, alloy, pure metal, and iron oxide magnetic particles can be synthesized. In a general reaction, the organometallic compounds are synthesized followed by subsequent decomposition in the presence of surfactant. Size and shape are controlled by the ratio of starting reagents to solvent. Valency of the metal in the organometallic compound, influences whether the product will be a pure metal, alloy, or oxide. These reactions often have lengthy reactions times anywhere from 1 to 8 hours. Particles have been prepared at many sizes from 1.6 to 60 nm in diameter. This technique allows for good control over size and shape of the particles synthesized.

The ball-and-mill, mechanochemical, process is a top down approach of reducing size of magnetic powders to micro and nanometer scale using the angular momentum of a mill. In a typical reaction, hollow steel balls are charged with multiple hardened steel balls of much smaller diameter and a soft magnetic powder is added. The hollow steel ball is sealed, then milled at a constant angular velocity for a given amount of time in a planetary ball mill. Owing to the nature of the system, morphology control is not a key aspect of this mechanochemical process. Particle size is influenced by ball-to-powder mass ratio, milling time, milling environment, milling speed, and type of ball mill. The

ball and mill process is the most industrial technique that can be quickly and reproducibly extended for mass production of magnetic nanoparticles.

Owing of the high percentage of atoms at the surface of nanoparticles surface, electrostatic energy and interface reactions become increasingly appreciable. Many routes have been taken to coat magnetic nanoparticles for passivation or protection of the surface to decrease surface effects that may lead to loss of magnetization.[3] Coating strategies that have been utilized for magnetic particles include, but is not limited to: surfactant and polymer coating,[4] precious metal coating,[5] silica coating,[6] carbon coating,[7] and matrix dispersion.[7] Because the vast majority of research has been focused on polymer and silica coatings, this work will focus only on surfactant/polymer and silica coating techniques in broad detail.

Magnetic Particle Coatings

Applications of Magnetic Polymer Microspheres

As mentioned previously, one of the first major applications of MPMs was in the area of biological separations. MPMs can be utilized to capture a host of biological materials including: DNA, proteins, enzymes, cells, and biomolecules. This leads to applications in cell separating, counting, identification, imagining, and immunoassays.[1, 2] If those applications are viewed in a more general sense, the process involves positive or negative separation. Positive separations require the use of functionalized MPMs to capture and transport the target of interest away from a sample. Conversely, in negative separations, the unwanted material is removed to purify the sample. Examples of positive separations include: metal recovery quantification, nucleotide capturing,

detection, and gene separation. Examples of negative separations include: signal

amplification. The general view is that the particles capture and transport with ease of

manipulation of a magnetic field. This has inspired research in catalysis,[5] surface

enhanced Raman spectrometry (SERS),[8] ligand blotting,[9] molecular profiling,

spectrofluorometric assays, electrochemiluminescence, optical encoding, metal

chelation,[10] diagnostics, and therapeutics. MPMs can provide binding sites for chemical

or physical adsorption. The choice of polymer matrix has great implications on the

applications available to the MPM.

Silica Coating

Stability in aqueous conditions, well-understood silica chemistry, and easy to

control inter-particle interactions in solution and within structures make silica fitting for

magnetic nanoparticle coating. Homola and colleagues have shown that a silica coating

is used to tune the magnetic properties of nanoparticles, since the extent of dipolar

coupling is related to the distance between particles and this in turn depends on the

thickness of the inert silica shell.[3] Iron oxide, one of the most used magnetic materials,

has a strong affinity to silica. Most syntheses begin with the Stöber method to generate

silica nanoparticles.[6] The surface can then be modified to contain different

functionalities by reacting with silanated ligands. For example, 3-

aminopropyltrimethoxysilane (APTES) contains amine and silane terminal ends, which

allow silane to react with the hydroxyl group of the silanated nanoparticle. Once the

surface is amine-terminated, the particles can undergo organic coupling strategies for

further functionalization.

There are several difficulties associated with silica coatings. For example, nanometer scale thickness with uniform shell thickness is difficult to obtain. Deposition of pure metals is difficult due to lack to hydroxyl groups on the surface, and in some cases, surface grafting of the nanoparticle is required. Silica based coatings are instable under basic conditions and may contain pores that allow the diffusion of oxygen and other unwanted species, which can reduce the effectiveness of the magnetic core.

Surfactant and Polymer Coatings

Surfactant and polymer coatings use electrostatic and steric interactions to coat magnetic nanoparticles and passivate their surfaces and keep them in a stable colloidal state. These coatings can either be chemically or physically adsorbed to the magnetic nanoparticle to form a single layer or multiple layers of oppositely charged electrostatic interactions. These coatings can provide a range of functional groups at the surface depending upon the monomer reactants chosen. This allows for various functionalization strategies to be attempted based upon the exposed surface molecules. Although the there are many advantages, thin polymer coatings do not provided enough barrier to highly reactive metal particles and are not suitable for very reactive magnetic nanoparticles. Furthermore, at high temperature, polymer coatings exhibit lower stability. Their stability in a range of pH values and the ability to grow thick polymer shells with a lighter density than silica makes polymer coatings ideal for a variety of applications.

Preparation of Magnetic Polymer Microspheres

Two major routes exist for the preparation of MPMs. One is the mixing of separately prepared magnetic cores and polymers. Strategies under this heading include: phase separation, solvent evaporation, layer-by-layer processes, and sol-gel transitions. These strategies have been reviewed elsewhere.[2] The most frequently used techniques involve heterogeneous polymerization in the presence of magnetic nanoparticles. For the purpose of this work the more common techniques under this heading will be reviewed. They including: suspension polymerizations, microemulsion polymerizations, miniemulsion polymerizations, and dispersion polymerizations.

In all of these techniques, monomer subunits are polymerized in the presence of magnetic particles to produce magnetic polymer microspheres. Most routes are an extension of the bottom up approaches to magnetic nanoparticle synthesis outlined previously. The different techniques yield microspheres of diameters ranging from tens of nanometers to millimeters. The magnetic material should have the greatest attraction to the monomers and little affinity to the other components of the system. In all cases, two immiscible liquid phases are mixed and the MPMs form in the droplets of the emulsion. This can generate hydrophilic or hydrophobic surface charges depending upon the characteristics of the droplets. Microemulsions produce very tiny particles due to the nature of microemulsions; two immiscible solutions mix to yield stable isotropic and optically transparent solutions.

Magnetic Polymer Microsphere Functionalization

The previously mentioned applications would not be possible without the functionalization of the MPM surface. In this work, conjugation is defined as the process of adsorbing an arrangement of molecules or atoms onto a solid support. The definition highlights three aspects for consideration: (1) the type of solid support, (2) the properties of the molecules or atoms (ligand), and (3) the actual process of conjugation. Chapter II chronicles the formation of a gold-coated MPM solid support that was originally realized for application involving electromagnetic radiation then later used in SERS. Chapter III is dedicated to the properties of the ligand and the process of conjugation to MPMs.

Any ligand utilized for conjugation to the MPM, will be composed of (1) a head group, (2) the backbone (chain), and (3) a terminal group. The head group provides linkage to the MPM. For uniform and expedient processes, the head group conjugation should be facile. The chain controls the sterics and thereby density of the molecules on the solid support, allows for distance from the solid support, and stabilization of the molecule. The terminal group imparts the surface functionality of the particle. Each of these parts contributes not only the structural and physical properties of the conjugated particles, but also, the ease of synthesizing or the cost of the ligands.

Coupling reactions are essential reactions to organic chemistry that combine two hydrocarbons via reactions involving metal catalyst, organic catalyst, or activating compounds. The coupling techniques and reactions available for MPMs are dominated by two factors: (1) the surface functionalities available for purchase from the MPM manufacturer and (2) ligands with the available functionality amenable to the specific cross coupling. A host of surface functionalities are available from manufacturers.

Amine, carboxylic, epoxy, tosylactivated, and silanated are a few common types. Because of the carboxylic and amine terminated offered opportunities for well known coupling strategies, they will be explored in more detail.

Coupling of thiolated ligands via succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) is available. **Fig 3.** outlines the reaction of an amine-terminated particle with a thiolated ligand. The amine of the MPM attacks the ester of the SMCC to yield an activated SMCC intermediate and *N*-hydroxysulfosuccinimide (NHS) as a byproduct. A sulfhydryl containing compound can then react with the succinimide moiety followed by a proton transfer to yield a linked MPM and ligand of interest. Carboxyl and amine-terminated MPMs have access to *N,N'*-Dicyclohexylcarbodiimide (DCC)[11] and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling.[12] Both EDC and DCC are reagents used to couple amino acids during peptides synthesis reactions. EDC requires an acidic pH and is used as a carboxyl-activating agent for the coupling of primary amines to synthesize amide bonds. The synthetic strategy for EDC coupling with sulfo-NHS is shown in **Fig. 4**. Sulfo-NHS is used to stabilize the intermediate otherwise the EDC activated compound would be vulnerable to hydrolysis. The activated carboxylic intermediate is attacked by the terminal amines of the MPM. After a proton transfer and cyclization of sulfo-NHS, the amine is now coupled to the carboxy terminated ligand.

Bartczak and Kanaras recently showed the demanding task of optimization for a preparing peptide functionalized gold nanoparticles using EDC. In a typical reaction, 10 μL of peptide was added to the oligoethylene glycol nanoparticles (OEG-NPs), 15 μL of EDC and sulfo-NHS added simultaneously, the reaction was stirred for 24 hours then

purified by centrifugation. The concentration of EDC/Sulfo-NHS, peptide, and buffer was varied in the study, but in each case the volume added was kept constant. For the addition of EDC/Sulfo-NHS, the ratio was kept at 1:2 for EDC/Sulfo-NHS. Reaction time and morphology were two more parameters that were varied during optimization. After thirty reactions, the optimal conditions were determined for this microscale reaction. If the reactions were scaled up, the results may not be the same. Therefore, coupling of ligands to the surface of particles may not be the most efficient route.

Each organic coupling technique requires optimization of several different parameters. Although the SMCC and EDC coupling reaction have well known chemistries, the optimization and application of these reactions in a heterogeneous reaction systems is not as standard as expected. The success of these reactions requires optimization of pH, temperature, and reagent concentration and is not easily manipulated on a heterogeneous reaction system. Therefore, a more facile method of conjugation utilizing the efficient gold-thiol bond has been researched.
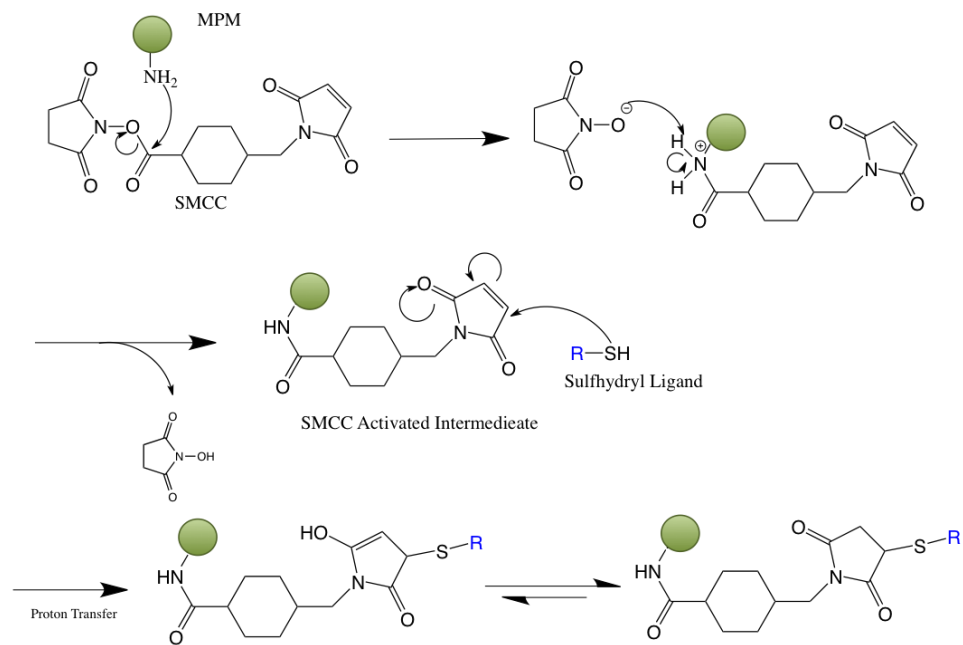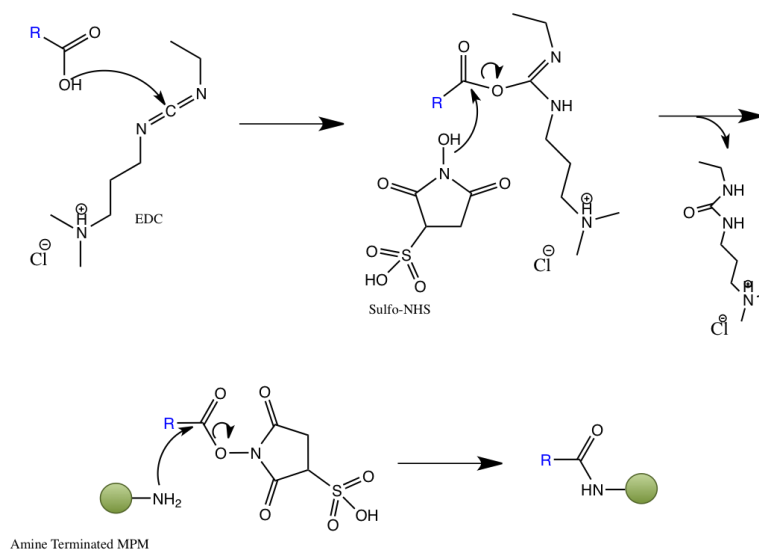
Figure 3: Mechanism of SMCC coupling

Figure 4: Mechanism of EDC coupling.

Electroless Plating of Magnetic Polymer Microspheres

Properties of Nanoscale Gold

A multitude of nanoscale research pertains to biological systems, because nanoscale objects have an increased ability to be taken up by cells and offer interaction with nano- and micro-size objects within the cell, such as DNA. Gold is a noble metal that is often employed in medical and biological applications not only for its intrinsically interesting nanoscale properties, but also the significant degree of biocompatibility of the material.[13] Gold nanoparticles are so small that electrons are not free to move about as in bulk gold which gives rise to the phenomena of surface plasmon resonance. This restricted movement causes the nanoparticles to react differently with light changing causing a red shift in the absorbance. This allows for applications in imaging, and detection that are based on electromagnetic properties.[14]

Electroless Plating

A nanoshell is an electronically conducting material encapsulating a dielectric particle core that is coated with a functionalizable layer.[15] Halas and colleagues set out to create a material capable of tailorable interactions with electromagnetic radiation to controllably tune optical characteristics over a wide wavelength range. The method was first realized with AuS particles with a conducting gold shell surrounding a dielectric sulfide core. They later moved to synthesizing particles with larger dielectric cores from silica that spanned the range of hundreds of nanometers. The silica particles were made via the Stöber method then functionalized as mentioned previously to present an amine-terminated surface capable of undergoing electroless plating.
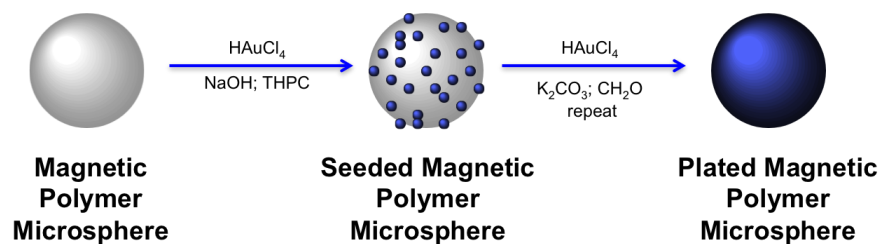
Figure 5. Seed and Growth (Plating) Schematic

Electroless plating (**Fig. 5**) utilizes molecular self-assembly and colloid reduction chemistry. In a typical electroless plating (seed and growth) scheme for MPMs, the reaction begins with an amine-terminated particle. Often the dielectric core is either silica or polystyrene and the coating layer is gold. The amine sites serve as electrostatic attachment points for negatively charged small colloidal gold particles. The subsequent growth of the thin layer comes from the coalescence of the colloidal gold at these nucleation sites. Using this method then is limited only by having an amine-terminated surface.

The colloidal particles for seeding can be synthesized in a variety of methods. One such strategy is the Turkevich method, which involves the reduction of chloroauric acid ($HAuCl_4$) with sodium citrate. Another method involves reduction of chloroauric acid with tetrakis(hydroxymethyl)phosphonium chloride (THPC). The distinction between the two methods is the size of the gold nanoparticles generated. The Turkevich and THPC methods yield on average 12 nm and 2 nm particles, respectively. It has also been shown that the reduction with THPC yields more uniformly spherical particles over the Turkevich method.[15] The THPC method yields a more uniform coating in a fewer plating steps. The gold coatings provide a functionalizable surface with interesting optical properties that have access to efficient gold-thiol chemistry

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Self-Assembled Monolayers

The discovery of self-assembled monolayers (SAMs), propelled the field of gold-thiol chemistry. Consequently, the general aspects of thiol SAMs on gold, the self assembly process, and the structure of the gold-thiol surface have been extensively researched and thoroughly reviewed.[16] The gold-thiol bond strength is 44 kcal/mol. Although the gold-thiol bond is on the order of covalent strength, the aspects of the gold-thiol bond degradation must be considered. The gold-thiol bond has been shown to degrade by oxidation and UV exposure to disulfides and sulfonates via the following reactions:

$$2 \text{ RS-Au} \rightarrow \text{RSSR} + 2 \text{ Au} \tag{1}$$

$$\text{RS-Au} + H_2O + O_3 \rightarrow RSO_3H + \text{HO-Au} \tag{2}$$
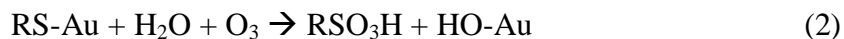
Degradation is heavily dependent upon size and structure of gold support. Two week stability in ambient conditions has been shown for gold-thiol substrates.[17,18] Thermal degradation is influenced by chain length and surface roughness among other things. It has been shown that the gold-thiol bond is stable up to 100 °C. Longer chain lengths tend to be stable at increased temperatures.

Carryover

The overarching goal is to build an easily reproducible system that has plug and play functionality and can be utilized in a variety of assays. A tubular extraction cassette has been introduced by Bordelon and colleagues that takes advantage of the capture and transport abilities of MPMs.[19] In the cassette, a polymer tube is filled such that liquid
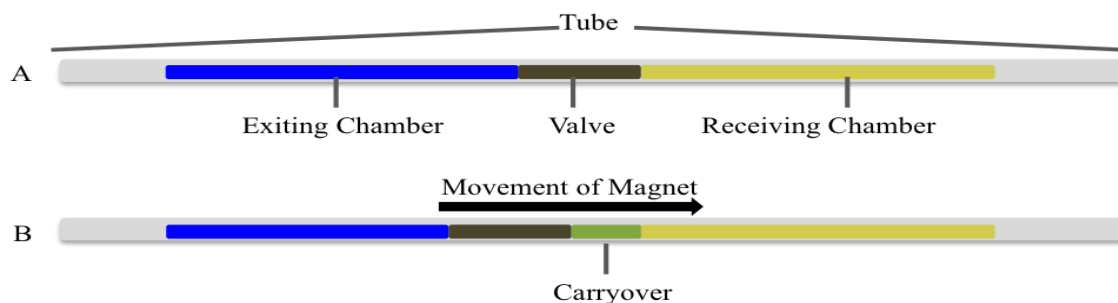
Figure 6: A) tubular extraction cassette. B) Schematic of fluid carryover after MPMs have traversed a valve.

solutions (chambers) are separated by either pockets of air or mineral oil. The separator either air or oil is referred to as a valve. The blue (exiting) chamber contains the target of interest and is the initial chamber in which the MPMs enter. After the MPMs and have been incubated to allow for maximal capture, the MPMs are magnetically pulled through the valve (**Fig 6**, B) into the next, receiving, chamber. Small amounts of liquid from the capture chamber can become trapped in the interstices of the magnet packed MPMs as the beads traverse the valve. The process of liquid being carried over from one chamber to the other across the mineral oil or air valve in the tubular extraction cassette is called Carryover.

Carryover can affect the purity of the receiving chamber. The volume of each chamber is usually on the order of hundreds μL. Therefore, if even microliters volumes of liquid are being carried over to the receiving chamber, the receiving volume is being altered by a significant percent. This can lead to inconsistencies in results and ambiguity about the actual concentration of the target being captured. The functionalized AuMPMs can be tailored to coordinate to a range of ligands. Provided the solutions are aqueous, the gold-coated magnetic particles can be tuned to have a more hydrophobic surface that in theory would have less carryover and circumvent issues of purity in the tubular extraction system. Therefore, AuMPMs were functionalized with the three ligands

representing a positive, neutral, and negative charges and their efficiency in the magnetic was tested. Changing the hydrophobicity of the surface structure could have an impact on the amount of force required for the beads to traverse the valves.

Aims

The aim of this research is investigate the fundamental aspects of the production of gold-coated MPMs. The ideal MPM for use in the extraction and purification cassette would be evenly coated with gold, spherical monodispersed, and easily synthesized. This work probes the uniformity and dispersity throughout the process of gold-coating and subsequent functionalization. The ease and conditions necessary for conjugation will be investigated. Finally, using gold-coated and functionalized MPMs, they will be applied to a tubular purification system to determine the effects of different charged surfaces on the ability to overcome electrostatic interactions at the surface of the liquid-air and liquid-mineral oil interfaces. Ultimately, a platform technology is being developed that can easily coordinate and bind different types of ligands and can be utilized in a variety of biological, environmental, and analytical contexts. The works provided in this document provide the foundation for more thorough investigations into tuning the gold surface for thickness and smoothing, the impact of the gold-thiol bond on magnetic properties of the MPMs, electronics associated with the gold-thiol bond, and chemical and thermal stability of the conjugated MMP.

CHAPTER II

GOLD-COATED MAGNETIC POLYMER MICROSPHERES

Introduction

As noted in Chapter I, MPMs can serve as an enabling technology in a number of

important applications. The key to successful application of the MPMs lies in their

ability to be functionalized with a variety of ligands specific to the application. MPMs

can be purchased with different surface functionalities. Some of which represent specific

interactions i.e., streptavidin coated MPMs utilized for streptavidin-biotin complexation.

Other functionalities present opportunities for conjugation and cross coupling techniques.

As mentioned in Chapter I, there are many difficulties associated with performing

organic coupling reactions with the particles. The use of well-known gold-thiol

chemistry might provide a useful approach to circumvent these problems.

The following experiments chronicle the synthetic strategy towards gold-coated

MPMs. The synthesized particles are characterized by TEM and SEM to probe dispersity

and homogeneity of gold coating. Experiments to examine the thermal stability of the

gold-coated MPMs is presented.

Experimental

Materials

Amine-terminated MPMS were purchased from Invitrogen Life Sciences at a stock

concentration of 2×10^9 MPMs per mL. Sodium hydroxide was obtained from Sigma-

Aldrich. Tetrakis(hydroxymethyl)phosphonium chloride (THPC) and chloroauric acid were obtained from Aldrich. All chemicals were used as received unless otherwise noted.

Preparation of Gold Nanoparticle (AuNP) Seeding Solution

To 45 ml of H_2O, 500 ml of 1 M NaOH and 1 ml of THPC solution (12 μL in 1 ml H_2O) were added. The solution was stirred for 5 min. Next, 2 ml of 1% $HAuCl_4$ added quickly resulting in a brownish color. The solution was stirred for 5 min then refrigerated at 4 °C when not used immediately.

Preparation of AuNP Plating Solution

A 1.8 mM solution of K_2CO_3 was made with one liter H_2O. After the solution had stirred for 10 min, 15.5 ml of a 1% v/v HAuCl4 solution was added. The solution stirred three min then was left quiescent for 30 min. The solution was stored at room temperature and shielded from light when not in use.

Seeding of Amine-Terminated Magnetic Polymer Microspheres

One hundred μL of stock MPMs were washed 3 times with H_2O. The MPMs were magnetically separated between washes. After washing, the MPMs were resuspended in 100 μL of H_2O. To the dispersed MPMs, 1 mL AuNP seeding solution was added. The solution was stirred for 5 min, then allowed to sit quiescently and unexposed to light. After 2 hr, the solution was magnetically separated and washed with H_2O three times. The particles were resuspended in 1 mL H_2O.

Plating of Seeded Magnetic Polymer Microspheres

To 100 μL of seeded particles, 1 ml of plating solution was added. The particles reacted in solution for 3 min. After reaction, the microspheres were was magnetically separated and washed with H_2O 2 times. The microspheres were resuspended in 100 μL H_2O. This procedure was repeated to achieve successive platings. After the particles were resuspended in 1 ml, 14 μL aliquots were removed for transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Thermostability Test

Two mg of gold-coated microspheres and amine-terminated MPMs at an equivalent concentration were heated separately to 800 °C in a thermogravimetic analyzer.

Results and discussion

Characterization

The seed and growth method was utilized with amine-terminated MPMs from Invitrogen Life Sciences. The growth (plating) step was repeated to provide a gold-coated MPM (AuMPM). Ten plating steps (Au(10)MPM) were sufficient to fully coat the surface of the particles. With more coatings, agglutination became apparent with the visible eye, and the particle density increased causing the particles to rapidly settle out of solution. Philips CM20 Transmission Electron Microscope and Hitachi S-4200 Scanning Electron Microscope were utilized to probe the physical aspects of the gold-coated and uncoated MPMs. In **Fig. 7**, the MPM (A) and seed (B) are marked by large pores. As the particles

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are successively plated from A to F, the surface becomes less porous and smoother. The particle diameter remains and monodispersity remain constant through successive platings. In **Fig. 8**, the TEM micrograph shows the decreased transmission of electron through the material equating to the attachment of gold. Branches appear at the surface of the particle, which also signifies the coalescence of gold at the MPM surface.
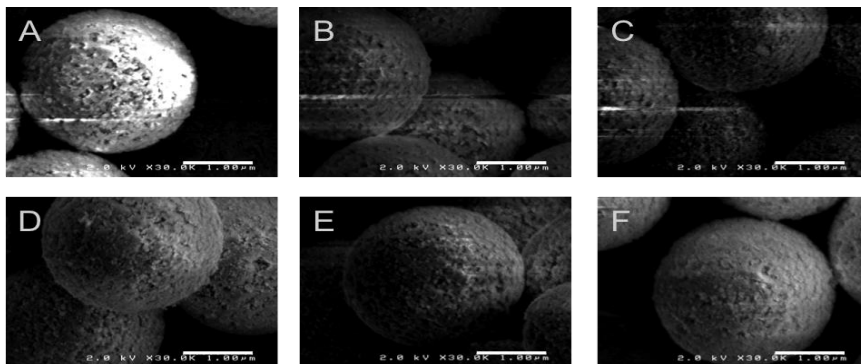

Figure 7: Plating monitoring via SEM. A) amine-terminated microsphere B) seeded amine-terminated polymer microsphere C) 3x plated microsphere D) 5x plated microsphere E) 7x plated microsphere and F) 10x plated microsphere. Scale bar 1 μm.
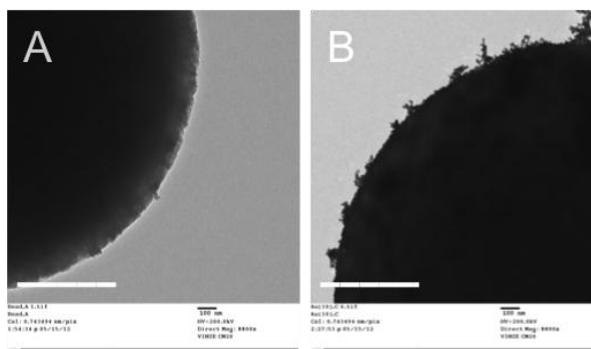

Figure 8: TEM images of A) uncoated MPM and B) Au(10)-MPM. Scale bar 500 nm.

Iron-Gold Monitoring

TEM and SEM provide evidence of gold coalescence, but the quantity of gold has yet to be measured. Using energy dispersive x-ray (EDX) capabilities of the SEM, the atomic

percentage of elements in the sample was monitored. In a typical EDX experiment, the sample was irradiated with X-rays at 20 µA. The instrument measures the incident x-rays from the sample and compares them against a standard to determine atomic composition. Assuming a fairly constant percentage of iron per microsphere, increased gold attachment to the particles would cause a decrease in the iron:gold ratio. In the EDX experiment, a 196 µm2 area of planar particles was scanned for 2, 4, 6, 8, and 10 platings (**Fig. 9**). As, expected, as the number of plating steps increased, the iron:gold ratio decreased.
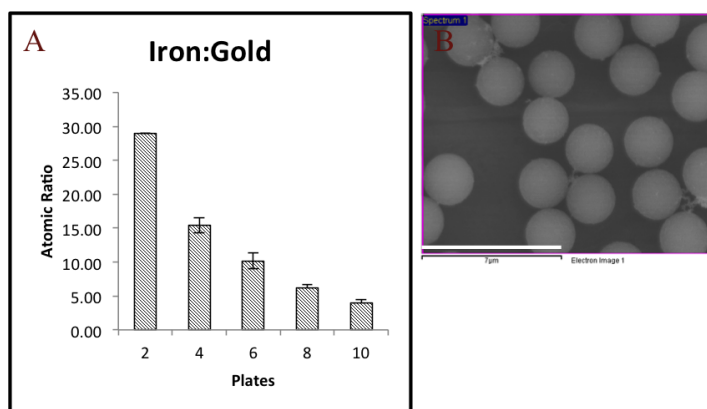

Figure 9: Iron and gold monitoring of plated MPMs using EDX. A) Iron to gold ratio and B) size of scan the yielded the results. Scale bar 7 µm.

Thermostability

In an effort to probe the robustness of the Au(10)MPMs, the beads were heated to 800 °C under a nitrogen atmosphere. SEM images for the manufacturers amine-terminated MPMs and Au(10)MPMs can be seen in **Fig. 10**. In both cases the MPMs retain monodispersity and spherical shape. The particles have undergone a reduction in size from the initial diameter of 2.8 microns to approximately 1.0 micron. This is attributable to the loss of the polymer matrix. Gold on the surface of the MPMs has the elemental state and remains attached to the particles.
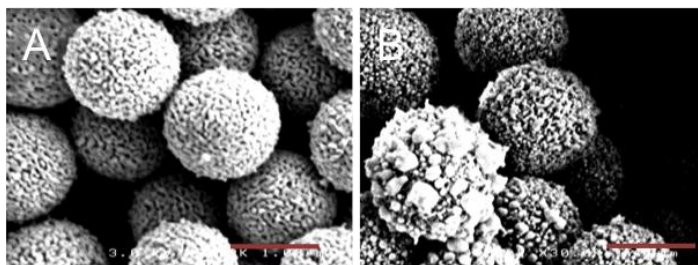
Figure 10: After heating to 800 °C, SEM images of A) Uncoated MPMs and B) Au(10)-MPMs. Scale bar 1 μm.

Conclusions

The MPMs have been successfully coated with gold using a facile seed and growth method. The MPMs remained monodispersed throughout both seeding and plating reactions. As the number of plating steps increase, the amount of gold is also increasing, leading to a thicker shell of gold. Ten platings were sufficient enough to have a fully coated gold surface. Furthermore, the microspheres are robust can be heated to extreme temperatures and retain dispersity and shape.

CHAPTER III

APPLICATION OF MAGNETIC POLYMER MICROSPHERES

Introduction

The force required to move the group of MPMs through the interfacial or surface tension valve in the tubing, where the movement is constrained to the x direction only ($F_{m,x}$), was approximated using the following equation (Gijs 2004)[20]:

$$F_{m,x} = \frac{V * \chi_v}{\mu_0} \left(B_x \frac{\partial}{\partial x} + B_y \frac{\partial}{\partial y} + B_z \frac{\partial}{\partial z} \right) B_x$$

(3)

where V is the bulk volume occupied by the MPMs, χ_v is the volume susceptibility, μ_0 is the permeability of free space ($4\pi \times 10^{-7}$), and B is the magnetic field along the axis of the tube through which we are pulling the MPMs (x axis). To measure the force required to pull the MPMs through the surface tension valve, an apparatus was developed to measure x, y, and z coordinates of the magnetic field (B) of a 2.54 cm cube permanent magnet along the axis of the tube using a F.W. Bell series 9900 gaussmeter. The values for the x, y, and z coordinates were plotted as a function of distance from the edge of the magnet.

The gradient of the magnetic field for the x, y, and z coordinates $B_{x,y,z} \frac{\partial}{\partial x,y,z}$ was roughly approximated for by using the slope of the lines between two measurements from the curves. Because the gradient of the magnetic field in the y and z coordinates was approximately zero, the $B_y \frac{\partial}{\partial y}$ and $B_z \frac{\partial}{\partial z}$ terms of the magnetic force equation were set to zero.

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To obtain the χ_v an Alfa Aesar Magnetic Susceptibility Balance Mark 1 was used. The balance provides reading of mass susceptibility, χ_g, which can be expressed as follows:

$$\chi_g = \frac{\ell * C_{Bal}}{m * 10^9} \ (R - R_0)$$

(4)

Where C_{Bal} is a constant proportionality, R is sample reading, R_0 is the blank reading, l is the sample length (cm), and m is the sample mass. Once χ_g has been resolved, the measurement can be can be converted to χ_v for use in equation 3 by the following equation:

$$\chi_g * d = \chi_v$$

(5)

Where d is the density under a magnetic field. The force will be used to determine how neutrally, positively, and negatively charged MPMs traverse the valves of the tubular extraction cassette.

The following experiments probe the dispersity, morphology, and surface charge of the gold-coated MPMs that have been functionalized with thiolated ligands. The effect of surface change on transit through a valve was examined by measuring a parameter called pull through.

Experimental

Materials

From Sigma-Aldrich, the following chemicals were purchased: 6-amino-1-hexanethiol hydrochloride, 6-mercaptohexanoic acid, and 6-mercapto-1-hexanol. Ethanol purchased from Pharco-aaper. Silica gel purchased from Dynamic Adsorbents. All chemicals used

without further purification. Cole-Parmer vinyl tubing (tygon) with and inner diameter 1/16 in and outer diameter 1/8 was utilized.

Ligand Conjugation

Each ligand was prepared at a concentration of 1 mM by dissolving in ethanol. One hundred microliters of gold-coated, MPMs at 3.32×10^{-13} M were added to a conical tube. To this tube, 900 µL of each stock ligand solution was added. The solution incubated while stirring for 8 hours. The solution was magnetically separated and washed with H_2O 3 times. After washing, the MPMs were resuspended at a concentration 3.32×10^{-13} M.

Zeta Potentials

To the 100 µL sample of AuMPMs, 650 µL of H_2O was added for a concentration of 6.65×10^{-14} M. This was found to be the optimal concentration to obtain the most reliable data. The zeta potentials were measured on a Malvern Zetasizer. All measurements were taken in water at a pH of 5.6.

Pull Through Measurement

To measure the force required to pull the MPMs through a surface tension valve, a preloaded tube containing 20 µL of a 3.32×10^{-13} M MPMs suspension. The tube slowly moved toward the 2.54 cm cube magnet along the *x* coordinate of the measured magnetic field until the point where the MPMs pulled through the valve. The distance at

which the MPMs pulled through was recorded and used to approximate the magnetic

field strength (B_x) and the magnetic field gradient ($B_x \frac{\partial}{\partial \mathrm{x}}$) at that distance.

Magnetic Susceptibility

Volume (*V*) was measured as the bulk volume that the particles occupied under the influence of a magnetic field. The values were calculated by measuring the cylindrical volume that a known mass of MPMs occupied in a short length of 1.6 mm inner diameter tubing. One mg of Invitrogen Life Sciences MPMs was diluted into 114 mg silica gel. To a small glass vial, 114 mg of silica gel was weighed. To this, 1 mg of dried gold-coated magnetic microspheres was mixed thoroughly. A blank sample of only 115 mg silica gel was also prepared. The magnetic susceptibility was measured using an Alfa Aesar magnetic susceptibility balance. The balance was zeroed and the calibration constant was calculated using the manganese chloride standard supplied by the manufacturer. The blank was made using 114 mg silica gel without MPMs added. The tube was rinsed with water between each sample, dried at 100 °C for 10 min, and measured empty to verify that residual magnetic MPMs had been removed after each wash. Each sample was measured three times, removing and repacking the MPMs between each measurement.

Results and discussion

Zeta Potentials

Confirmation of conjugation was obtained using zeta potentials. The uncoated magnetic polymer microsphere in **Fig. 11** shows a highly positive charge as is to be expected of

amine-terminated microspheres from the factory. Interestingly the Au(10) microspheres showed a highly negative charge that is consistent with the K_2CO_3 stabilizer in the plating solution that is apparently acting as a capping agent to the microspheres. The carboxyl microspheres fall within the range of the Au(10)MPMs considering that they are both capped with the same terminal functionality. More neutral measurements were expected for the hydroxyl terminated Au(10)MPMs. The hydroxyl ligand may not have sufficiently displaced the K_2CO_3 stabilizing agent. Also, there is no significant difference between the amine functionalized MPM and the MPM from the manufacturer.
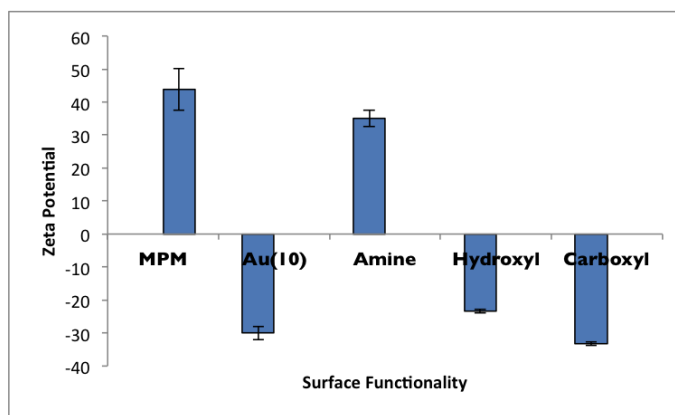

Figure 11: Zeta Potentials of functionalize Au(10)-MPMs.

Magnetic Pull Through Measurements

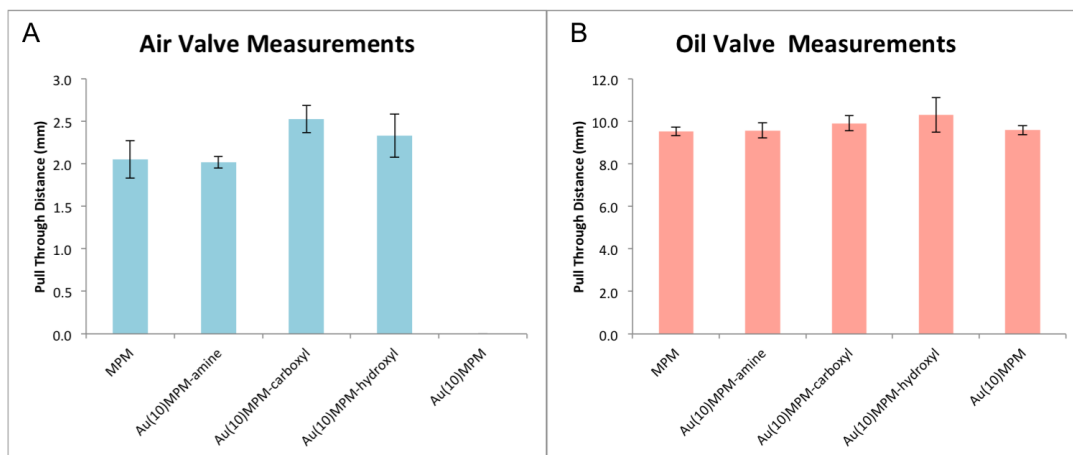The pull through for surface and interfacial tension valves were measured.

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Figure 12 Pull through measurements for A) surface tension (air valves) and B) interfacial tension (oil valves).

In the oil valve pull through experiments (**Fig. 12, B**) , all of the surface functionalities had statistically similar pull through measurements. For air valves (**Fig. 12, A**) the Au(10)MPMs, exhibited no pull through at the mass of MPMs used in the experiment. There is a significant difference between the amine surface functionality measurement and the carboxyl and hydroxyl measurements, but no significant measurement difference between the MPM and the amine surface functionality.

Conclusions

The gold-coated MPMs have shown successful and facile functionalization in an aggressive ethanol environment. The surface functionalities corresponded to the expected positive or negative charge. The surface functionality does not to play much of a role in increasing or decreasing the force required for transit of MPMs through either an oil or air valve.

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