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Accessible and Green Manufacturing of Magnetite (Fe₃O₄) Nanocrystals & Their Use In Magnetic Separations

By

Cafer Tayyar Yavuz

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APPROVED, THESIS COMMITTEE:

Lon J. Wilson, Professor of Chemistry

Pedro Alvarez, George R. Brown Professor and Chair of Civil and Environmental Engineering

Vicki L. Colvin, Professor of Chemistry and Chemical and Biomolecular Engineering, Director, Center for Biological and Environmental Nanotechnology (CBEN), Chair

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ABSTRACT

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This work describes the first size dependent magnetic separation in nanoscale. Magnetite (Fe₃O₄) nanocrystals of high quality and uniform size were synthesized with monodispersity below 10%. Magnetite nanocrystals of 4 nm to 33 nm (average diameter) were produced. Batch synthesis was shown to go up to 20 grams which is more than 10 times of a standard nanocrystal synthesis, without loosing the quality and monodispersity. Reactor design for mass (1 gram per hour) production of magnetite nanocrystals is reported for the first time. The cost of a kg of lab purity magnetite nanocrystals was shown to be $2600. A green synthesis that utilizes rust and edible oils was developed. The cost of a kg was brought down to $22. Size dependency of magnetism was shown in nanoscale for the first time. Reversible aggregation theory was developed to explain the low field magnetic separation and solution behavior of magnetite nanocrystals. Arsenic was removed from drinking water with magnetite nanocrystals 200 times better than commercial adsorbents. Silica coating was successfully applied to enable the known silica related biotechnologies. Magnetite – silica nanoshells were functionalized with amino groups. For the first time, silver was coated on the magnetite - silica nanoshells to produce triple multishells. Anti-microbial activity of multishells is anticipated.
ACKNOWLEDGEMENTS

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Chapter 1

Magnetic Separations: From Steel Plants to Biotechnology*

In this introductory chapter, the basic concepts behind magnetic separations are introduced and a few examples of its industrial and laboratory scale applications are summarized. High gradient magnetic separation (HGMS) and batch systems for magnetic separations have been developed largely in parallel by different communities and both approaches are compared and contrasted here so that readers can approach both key areas. New advances in magnetic materials, particularly on the nanoscale, as well as magnetic filter design for industries that have challenging separation problems, are also discussed.

1.1 Introduction and History

Magnetic separations have for decades been essential processes in diverse industries ranging from steel production to coal desulphurization. In such settings magnetic fields are used in continuous flow processes as filters to remove magnetic impurities. High gradient magnetic separation (HGMS) has found even broader use in wastewater treatment and food processing. Batch scale magnetic separations are also relevant in industry, particularly biotechnology where fixed magnetic separators are used

* This chapter is under review for publication at "Chemical Engineering Science"
to purify complex mixtures for protein isolation, cell separation, drug delivery, and biocatalysis.

The properties of magnetic materials were identified as early as the 6th century BC, but the means by which magnets could move material remained only a curious phenomenon until the late 18th century [1]. As Gauss, Helmhotz and others developed a framework for electricity and magnetism, the reasons that magnets could move materials such as lodestone became apparent. This once mysterious force was quickly put to use in the nascent chemical and mining industries. In 1792 a patent was filed by William Fullarton describing the separation of iron minerals with a magnet and the field of magnetic separations was born [2, 3]. The early applications relied on the intrinsic magnetic properties of sediments for separation. In 1852, magnetite was separated from apatite by a New York company on a conveyor belt separator [2]. Later, a new line of separators were introduced for separation of iron from brass fillings, turnings, metallic iron from furnace products and magnetite from plain gangue [2]. From these beginnings, magnetic separation technology has found its way into increasingly complex and diverse industrial processes (Figure 1.1).
The basic principle behind magnetic separations is remarkably simple and remains unchanged from these early examples. It relies on the simple fact that materials with differing magnetic moments experience different forces in the presence of magnetic field gradients; thus, an externally applied field can handpick out of physically similar mixtures those components with distinctive magnetic characteristics [4]. The use of this principle is straightforward in mixtures where a magnetic component is known to exist; an intrinsically magnetic material can be separated using electrically powered electromagnets or strong permanent magnets. The process is generally binary and results in a magnetically rich retentate (usually a solid) and the residual non-magnetic solvent.

The 1950s were a time of great expansion for the field of magnetic separations as the introduction of high gradient magnetic separation (HGMS) systems permitted faster and more general magnetic separation processes [5-7]. HGMS works through the application of large static fields (~1 Tesla) to columns containing ferrous matrices such as steel wool; these irregular surfaces give rise to magnetic gradients as high as $10^4$ T/m.
which generate forces large enough to capture even weakly magnetic particles in a flow stream [8, 9]. This new technology meant that magnetic separations could be applied more universally to separation problems where particulate matter was not strongly magnetic. The use of HGMS in water treatment, for example, provided a way to clarify water with high sediment loads, provided that the sediment has some weakly magnetic character. A larger market was found in the steel industry where HGMS separators became critical components for purifying high grade, low iron content steel.

More recently, separations using external magnetic fields have become commonplace in biotechnology where they are used for both protein purification as well as flow cytometry [10-12]. In these contexts, separators apply relatively small field gradients (500 T/M) to fixed volumes of solution with the aim of removing a valuable biological components. Such an approach to separations is more as a ‘batch process’, and it works well for biomedical research laboratories where solution volumes are small and separation speed is not a major issue. However, the small scale and time consuming nature of biomagnetic separators precludes their application in biological manufacturing processes. In addition, the separators themselves are standalone configurations of permanent magnets which require little upkeep or even power. This separator simplicity is offset by the need for magnetic beads that are coated so as to bind to particular biological components [13-15]. As a result, the technique is not that general and conventional biomedical researchers can only use magnetic separations if a commercial magnetic bead source is available for their targets. Commercial sources for magnetic beads have grown substantially in the past decade, however, and a wide variety of
proteins, cells and other bio-macromolecules may be selectively removed using these methods.

1.1.1 Magnetic separations: their unique position among separation technologies

The problem of separating materials, whether they are specialized chemicals, high purity steel or valuable pharmaceuticals, from product streams is a near universal one for any manufacturing process. When speed is not an issue and the materials of interest are solids or flocculated products, sedimentation or centrifugation is routinely employed. For faster processing, filtration is a well established method for removing waste or concentrating product. Given these standard methods it is not always obvious that magnetic separations could or should be applied to a given problem. Certainly, if part of a mixture is intrinsically magnetic then magnetic removal is often the best solution. In these cases, magnetic separations generally offer higher throughput with greater specificity than equivalent centrifugation or filtration methods [8, 16-18].

However, even in the absence of intrinsically magnetic components the use of designer magnetic beads - targeted to the product of interest - can make a magnetic separation feasible for virtually any system. Such processes offer very different kinds of trade-offs in speed and selectivity as opposed to the more conventional approaches. A consideration of the unique advantages of moving materials with external fields, as opposed to other conventional alternatives, is detailed in Table 1.1. For the purposes of this analysis we considered both the slower 'batch' model for magnetic separations as well as the faster flow separators based on the high gradient magnetic columns. We also
limit our comments to separation processes for materials in liquids, a case which captures
the majority of current application in this area.

Table 1.1 Industrial applications of magnetic separations (Adapted from [3])

<table>
<thead>
<tr>
<th>Application area</th>
<th>Objective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical and related industries</td>
<td>Tramp metal removal from machinery to avoid wear and malfunction</td>
</tr>
<tr>
<td>Food industries</td>
<td></td>
</tr>
<tr>
<td>Coal processing plants</td>
<td></td>
</tr>
<tr>
<td>Metals production and recycling industries</td>
<td></td>
</tr>
<tr>
<td>Raw materials processing plants, such as cement, glass, semiconductor.</td>
<td>Removal of iron and derivatives’ contamination</td>
</tr>
<tr>
<td>Mineral post processing industries</td>
<td>Separation and enrichment of magnetic ores (magnetite, hematite, ilminite, etc.)</td>
</tr>
</tbody>
</table>

An important feature of a magnetic separation is that the product stream meets
with virtually no flow resistance as it moves through a separator; this is in stark contrast
to filters which use a solid phase as the basis for the separation process. Filtration is
particularly challenging when sub-micron particles or biomolecules are the target
products; this requires filter pore sizes well under a micron (ultrafiltration) or even a sub-
micron (nanofiltration). The flow resistance that this solid phase introduces to the system
is significant, and industrial membrane filters often must operate with hundred of pounds
of solution pressure (many times the flow of a fire hydrant) to maintain flow rates of
several milliliters per minute. Such units require not just energy-intensive pumping
stations, but also expensive and high performance fittings and valves. Filters also can
become fouled with a wide variety of contaminants requiring backwashing procedures and ultimately replacement.

Conventional magnetic separators face few of these problems. In high-gradient flow systems, the columns are loosely packed with steel wools that offer virtually no resistance to flow. Reasonable operating pressures and conventional pumps are suitable for these systems, though the electromagnets that are needed for very high gradient separations do require significant energy. For separations in which speed is not an issue, then the batch magnetic separators are interesting options. Because they generate the fields with permanent magnets, they can operate without any external power; with the appropriate beads, such systems can remove a greater variety of substances than those accessed through sedimentation. This makes them particularly attractive for small-volume separations in low infrastructure settings.

While magnetic separations are in principle excellent choices for many problems, their widespread application has been limited by the complexity required in separator design and bead technology. In the case of flow separations, cumbersome and expensive electromagnets are used to create external fields in excess of a Tesla; the requirement for high gradients also limits column diameters and thus reduces throughput. In the batch separations favored in biotechnology, permanent magnets in a multipole configuration provide the relatively low gradients needed and their set-up and use is easy and immediate [19]. The trade-off here is that these low fields produce only very small magnetic forces on particles. Since the magnetic force is proportional to the particle
volume, the small field gradients are generally offset by the use of larger magnetic beads [16, 18, 20]. This lowers the available surface area for biomolecular recognition and adsorption, and has precluded application of this technology to biological separations on the larger scale [21]. Our group and others are working on ways to better design nanoscale magnetic beads so as to overcome these and other limitations. This materials design process must be guided by a more quantitative and formal description of the process of magnetic separations, the topic of the next section.

1.2 Modeling magnetic separations

The magnetic separation process is amenable to simple modeling and such treatments date back to the 1950s; most analyses assume that the materials being removed act independently and that macroscopic models for fluid dynamics are appropriate. These constraints make it straightforward to describe the many forces acting on particles in a flow, such as the magnetic force, Brownian motion, gravitation, and friction. What results is a universal equation that relates the net force on a particle to input parameters such as the particle size, magnitude of the field gradient, frictional coefficient and the magnetic moment of the particle. Obtaining precise values for these experimental data is difficult and limits the predictive power of these models. Still, these treatments provide an essential foundation for improving the materials and systems used for magnetic separations.

Perhaps the most important factor to include in magnetic separation models is the role of inter-particle forces such as Helmotz double layer interaction, dipole-dipole
interaction and Van Der Waals attraction. In this light, the magnetic, dipole-dipole interaction and Van Der Waals forces aid the process of separation, whereas, diffusion, double layer interaction and drag force act against the separation. Also, the particles are found to reversibly aggregate in presence of high field gradients, which would increase the net magnetic moment to a magnitude much stronger than the competing forces [22].

1.3 Magnetic separations use in column formats: examples

Magnetic separators used on the industrial scale are overwhelmingly high gradient systems which function as a column in continuous flow operations. The speed and efficiency of these systems have been exploited for decades and the early stages of the industry were marked by notable optimism: "Virtually every process in the chemical engineering industry is a potential application (for HGMS). Many previously unthinkable processes will now become practical, and many previously practical ones will become unthinkable. It has already happened in the kaolin industry, and is beginning to happen elsewhere." (Henry Kolm, September 1975) [8]. While these systems are not as ubiquitous now as their inventors anticipated thirty years ago, their applications have expanded notably.

1.3.1 Kaolin (clay) decolorization

Kaolin (a.k.a. china clay) is a clay mixture primarily consisting of kaolinite (Al₂O₃SiO₂·2H₂O or Al₂Si₂O₅(OH)₄) mineral [7]. It is named from the Kaoling Hills of the city of Ching-te chen where fine Chinese porcelains were produced; its resistance to acids and alkalis alike was greatly prized. Today, however, it’s mostly used in the paper
manufacturing industry where it plays dual role, as a filler between the pulp fibers and as a surface coating for a white glossy finish ([7, 23, 24], China Clay Producers Association)

Natural Kaolin has color as mined due to the iron containing micas, tourmaline, pyrite, anatase and rutile present in the material. To remove these impurities, kaolin can be magnetically cleaned with a continuous high gradient magnetic separator to produce highly white material suitable for paper or porcelain [7, 25-27] (Figure 1.2). Figure 1.3 shows kaolin mineral before and after the decolorization process. Because of the resistance of Kaolin impurities to other chemical cleaning methods, HGMS handles 75% of the world production of white porcelain and paper [26]. A typical plant would have an HGMS with a filter diameter of about 2 m and capacity up to 20 tons/h [28].
Figure 1.2 Metso® High Gradient Magnetic Separators (HGMS) are designed to recover weakly magnetic material from non-magnetic matter and can be used for many applications including the processing of clays, iron ores, rare earths and industrial minerals. In addition to the strongly magnetic minerals of Fe, Co, and Ni, a vast number of weakly magnetic minerals, which are not normally treatable by ordinary magnetic separators, may be processed by High Gradient Magnetic Separators. Metso HGMS separators are able to remove even weakly paramagnetic materials. (From Metso Minerals)
1.3.2 Steel factories and power plants

On average, generating one ton of steel requires 151 tons of water for cooling and cleaning purposes; the resulting wastewater is filled with many magnetic particulates and other iron-containing impurities (Table 1.2). Those particles, especially when present in gas and hot water streams, cause significant problems in processing and must be removed. Conventional methods for cleaning steel mill waste and process waters include sedimentation, flocculation followed by sedimentation, and fixed bed filtration. Such approaches require either large areas for settling tanks and clarifiers or expensive and short-lived filter systems [29]. Magnetic separation has emerged as an ideal solution for this industry, and it has offered great time, space and cost savings [7, 29, 30]. In a sample treatment at Kawasaki Steel Corporation of Japan, a 3 kOe field strength, 2.1 m diameter magnetic filter removes 80% of contaminants from the cooling wastewater of vacuum degassing process [31]. Similar use in treatment of wastewater can also be found in
power plants (both conventional and nuclear). For these cases, HGMS is used to remove ferromagnetic or paramagnetic particulates which extends the lifetime of cooling systems [7].

**Table 1.2** Sources of contaminants in a steel production process (Adapted from [29]).

<table>
<thead>
<tr>
<th>Source of contaminant</th>
<th>Contaminants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coke production</td>
<td>Non-magnetic particles, organics, oils</td>
</tr>
<tr>
<td>Iron manufacturing</td>
<td>Magnetic particles, organics</td>
</tr>
<tr>
<td>Steel production</td>
<td>Magnetic particles</td>
</tr>
<tr>
<td>Hot formation</td>
<td>Magnetic particles, oils, acids</td>
</tr>
<tr>
<td>Cold finishing</td>
<td>Magnetic particles, oils</td>
</tr>
</tbody>
</table>

Magnetic separations can also be used to treat pollution. Fly ash from coal power plants is 18% iron oxide. Magnetic filtration has been applied to capture 15% of waste fly ash, thus providing a means for recycling. Estimates show that this can replace some of the magnetite used in industry [28]. Figure 1.4 shows an example of a ball mill separator used in these operations.
1.3.3 \textbf{Enrichment of ores – Mineral Beneficiation}

The treatment of ores with magnetic separation is carried out primarily to enrich iron-containing ores. Conventional chemical and settling methods are not suited for this purpose given the similar density and reactivity of transition metal minerals. The magnetic nature of iron species, however, is unique and thus a natural target for magnetic separations. Among the iron ores taconite is most often subjected to magnetic treatments. From a taconite ore (33% iron) Kelland [32] was able to recover iron at 95% on a 5 cm/sec flow rate. Today, Metso Minerals, Inc. (formerly Sala International AB) offers
magnetic separators that can separate iron from ores with nearly 100% efficiency (depending on the particulate sizes, magnetic field and flow rate).

Figure 1.5 shows a successful continuous HGMS separator used for these purposes.

**Figure 1.5** Continuous High Gradient Magnetic Separation for many low susceptibility minerals that are associated with other minerals or have extra Fe in the crystals, and are hence often possible to separate. (From Metso Minerals)
Magnetic separation of pyrite (FeS₂) from coal for desulfurization is also a common process [33]. The weakly magnetic nature of pyrite, however, requires that the raw ore be pre-treated thermally to convert the pyrite (FeS₂, Mᵣ=0.3 emu/g) to more strongly magnetic pyrrhotite (Fe₇S₈, Mᵣ=22 emu/g). Up to 91% removal of sulfur from coal can be achieved by microwave heating followed by a magnetic separation [34]. Figure 1.6 shows an industrial scale drum separator used in large scale applications for powders such as coal.

![Diagram of industrial scale drum separator](image)

**Figure 1.6** An industrial scale drum separator used in large scale applications. From Eriez magnetics Inc.
1.3.4 Food industry

Strict food quality standards require the food products to be contaminant free, where mainly rare earth elements (REEs) constitute the majority. The food industry, therefore, has found magnetic separations to be an ideal method to remove REEs from food ingredients. Similar to the ore beneficiation or desulfurization, the target substances are weakly magnetic and require the high gradients of a magnetic field to be removed in a continuous food production line. Bunting Magnetics Co. offers magnetic metal separators and metal detectors for the quality of food and extended service life of the processing equipment, especially for cheese processing, chocolate plants, pet food processing, flour mills, spice plants, vegetable processing. Removal of both ferrous and nonferrous tramp metals is achieved by their line of food safety products for the food processing industry.

Figure 1.7 shows case studies from Greenwood Magnetics Ltd, another company that produces magnets and assembly systems for cleaning REEs off of the food production lines.
Figure 1.7 (Left) A single row easy-clean grid box which contains high-density rare earth easy-clean magnetic tubes filters loose tea with a flow rate of 5th. (Middle) A water-jacketed pipeline magnet was manufactured to suit a 4” pipeline pressure resistant up to 10 bar, nine high intensity rare earth magnets (11500 gauss) filter liquid chocolate flowing at 300 l/minute. The pressurized heated water-jacket maintains the temperature of the chocolate. (Right) The Bullet magnet that is used by a flour producer in which flour flows upwards through the 5” pipe and any ferrous contamination is removed by the high intensity rare earth bullet magnet (8500 gauss min).

1.3.5 Water treatment and metal removal

With the new, lowered maximum permissible concentration for arsenic in drinking water (10 μg/L), effective since January 2006[35], techniques for better arsenic remediation without much desorption have gained more importance. Currently, coprecipitation, adsorption in fixed-bed filters, membrane filtration, anion exchange, electrocoagulation, and reverse osmosis are of methods of interest[36], however, cost efficiency and waste quantity[37] requires further development that would aid in resolving the problem.
Arsenic adsorption and desorption are heavily influenced by adsorbent particle size [38, 39]. Nanoscale magnetite (Fe$_3$O$_4$, 12 nm) can remove 200 times better than its commercial counterparts (Table 1.3), which allows a significant cut in waste, instead of 1.4 kg of bulk iron oxide to remove arsenic (500 µg/L) from 50 liters of solution, 15 grams of nano magnetite can be used [22]. Apart from surface area increment, an obvious gain while going down to nanoscale, available open sites with the proper chemistry (free Fe on the surface) can be accounted for this unexpected result. Size dependent magnetic properties bring controllability and along with mobility, a critical nanoscale advantage, provides unique applicability, if put in a system, in especially household uses where electricity is not readily available.

Table 1.3 Arsenic removal by nano-sized magnetite. A comparison of As removal efficiency, assuming a treatment of 2 liters of As solution (500 µg/liter) with 1 g. Fe$_3$O$_4$ [22].

<table>
<thead>
<tr>
<th>Particle Size (nm)</th>
<th>As (V) or As (III)</th>
<th>Residual As concentration (µg/liter)</th>
<th>% removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>As (III)</td>
<td>3.9</td>
<td>99.2</td>
</tr>
<tr>
<td>20</td>
<td>As (III)</td>
<td>45.3</td>
<td>90.9</td>
</tr>
<tr>
<td>300</td>
<td>As (III)</td>
<td>375.7</td>
<td>24.9</td>
</tr>
<tr>
<td>12</td>
<td>As (V)</td>
<td>7.8</td>
<td>98.4</td>
</tr>
<tr>
<td>20</td>
<td>As (V)</td>
<td>17.3</td>
<td>96.5</td>
</tr>
<tr>
<td>300</td>
<td>As (V)</td>
<td>354.1</td>
<td>29.2</td>
</tr>
</tbody>
</table>
As early as 1970s Delatour and Kolm [5, 40] treated water samples from the Charles River (Fe$_3$O$_4$ seeding, 5ppm Al$^{3+}$) with a high flow velocity HGMS ($V_s = 136$ mms$^{-1}$, $H_c=1$T) and reduced coliform bacteria from $2.2\times10^5$/l to 350/l, turbidity by 75%, color by 95% and suspended solids by 78% [7]. Later, Bitton and Mitchell removed 95% of the viruses from water by magnetic filtration following a 10 minutes of contact period with magnetite (added to be 250 ppm) [7]. The following years, Boliden Kemi AB reduced phosphorus of water supplies at least 87% [7].

1.4 **Biotechnological (Batch) Applications**

The ability to control remotely inspired many biotechnologists and medical scientists to investigate magnetic solutions for several biochemical processes, such as protein and cell separations and purifications, magnetic drug targeting and delivery, and enzyme based bio-catalysis. Unlike industrial applications, in-lab or batch applications require tailor-made magnetic materials but remain fine with steady, not continuous, bench-top or batch, process solutions. First, we will give key components of a magnetic material to be used in vivo and then review some of the biological applications of magnetic separation.

1.4.1 **General Principle of Use**

In vivo applications of magnetic materials require biocompatibility. Thus, biochemists tend to use naturally existing minerals, such as magnetic iron oxides (magnetite, Fe$_3$O$_4$ and maghemite, $\gamma$-Fe$_2$O$_3$), due to their biologically safe nature i.e. in the ferrofluids [41]. Key requirements for a bio-magnetic separation material are
biocompatibility, suitable linkers, functional layers on magnetic core, protective layer, antigen detection, shape recognition, fluorescent signaling (Figure 1.8).

Figure 1.8 On a single particle, several necessary sections of a magnetic material that could be used in biological systems is summarized. (Reprinted with permission from [42])

1.4.2 Protein purification

Magnetic separation of biological entities had proven to be a rapid and effective process for over 30 years [17, 31, 43-45]. Proper coating and labeling of the magnetic particles and the target species provides simple and fast purifications with reduced costs.
[46, 47]. Although very effective, magnetic affinity separations need to be very specific. Immobilization of ligands on the magnetic adsorbents for the capture of the target species is crucial; this is perhaps one reason that conventional liquid column chromatography remains the gold standard for demanding purification processes.[47]. Recent studies on magnetic materials for protein separations relied on silica coated magnetite with amino functionality for salmon sperm DNA elution [48], phospholipid coated magnetite for myoglobin recovery [49], polyethylenimine coated magnetite for purification of plasmid DNA from bacterial cells [50], magnetic separation of erbia (III) attached biological particles [51], magnetic polyacrylamide-agarose beads for measuring rabbit antibody [45], magnetic polymer latexes for isolation of trypsin from pancreatic extract [11], ProtA-immobilized magnetic immunomicrospheres for immunoaffinity purification of antibodies IgG2a from mouse ascites [52], silica coated magnetite with iminodiacetic acid functionality for bovine hemoglobin (BHb) and bovine serum albumin (BSA) [53], streptavidin-functionalized magnetic nanoparticles for stimulant biotoxin [54], magnetite and nickel particles for the immobilization of a-chymotrypsin [55], Nickel-NiO-BSA-chymotrypsin for casein hydrolysis [56], magnetic affinity support for adsorption of lysozyme [57], streptavidin-biotin coated magnetic beads for DNA-RNA isolation [58], polyethyleneimine coated magnetite for virus capture [59], carboxyl-modified magnetic nanobeads for the isolation of genomic DNA from human whole blood [60], TeNT-linked iron oxide nanobeads with dextran coating for explaining the relative capacity of the specific compartments of a cell resulting from endocytosis through different receptors that promote antigen presentation and immune [61]. Figure 1.9 illustrates the standard
setup for a bench-top magnetic separation [41]. Figure 1.10 shows a summary of the available magnetic separators [47].

**Figure 1.9.** A simple, standard representation of a bio-magnetic batch separation. Red particles represent magnetic nanocrystals suitably functionalized for the desired species. Gray and spherical substances are the undesired species and the conical ones are the desired ones.
Figure 1.10 Examples of batch magnetic separators applicable for magnetic separation of proteins and peptides. (A) Dynal MPC-S for six microtubes (Dynal, Norway); (B) Dynal
MPC – 1 for one test tube (Dynal, Norway); (C) Dynal MPC – L for six test tubes (Dynal, Norway); (D) magnetic separator for six Eppendorf tubes (New England Biolabs, USA); (E) MagneSphere Technology Magnetic Separation Stand, two position (Promega, USA); (F) MagnaBot Large Volume Magnetic Separation Device (Promega, USA); (G) MagneSphere Technology Magnetic Separation Stand, twelve-position (Promega, USA); (H) Dynal MPC – 96 S for 96-well microtiter plates (Dynal, Norway); (I) MagnaBot 96 Magnetic Separation Device for 96-well microtiter plates (Promega, USA); (J) BioMag Solo-Sep Microcentrifuge Tube Separator (Polysciences, USA); (K) BioMag Flask Separator (Polysciences, USA); (L) MagneSil Magnetic Separation Unit (Promega, USA); (M) MCB 1200 processing system for 12 microtubes based on MixSep process (Sigris Research, USA); (N) PickPen magnetic tool (Bio-Nobile, Finland).

(Reprinted with permission from [47])

In an excellent review, Safarik and Safarikova discuss advantages and the equipment for a successful protein purification via magnetic means with a full scan of magnetic separation applications in isolation of enzymes, antibodies and proteins [47]. The efforts for the industrial scale applications are noteworthy and can be applied for a few biological molecules [62, 63]. Magnetic separation based protein analysis and detection systems on chips are of great interest for early diagnosis for fatal infections. Bio-barcoded magnetic beads [64], microfluidic biochemical detection system [65], micromachined magnetic particle separator [66] are prominent examples of this field. Recently, nanorods of Ni with Au edges were successfully used to remove His-tagged proteins with 90% recovery [67].

1.4.3 Cell separation

Similar to protein purifications, magnetic separations offer rapid quantification, high cell recovery when compared to the conventional methods, i.e. centrifugation [68]. As early as 1977, 99% recovery of neuroblasticoma cells was obtained in a matter of
minutes [69]. The same year, magnetic separation of red blood cells and lymphoid cells were also introduced [70]. Magnetic separation of cells is advantageous over the conventional methods mainly because it lets target cells to be isolated directly from the medium, i.e. blood, bone marrow, tissue homogenates, stool, cultivation, media, food, water, soil etc [71, 72].

Labeled cells, i.e. neural progenitor cells [14], red blood cells [13, 73-75], tumor cells [76], malarial parasites [77], baker's yeast [78], can be targeted to magnetic beads which can therefore be separated [79]. Magnetic moment or giant magnetoresistance of the magnetic particle-cell assembly can tell us about the location and even the count of the cells that are present [79].

With efforts to take magnetic cell separation to industrial level, Berger and coworkers were able to develop a micro cell separator [10], Haik introduced a magnetic device for continuous separation of red blood cells [80] and Zborowski applied a magnetic quadrupole flow sorter on a model cell system of human peripheral lymphocytes targeted with commercial monoclonal antibodies and iron-dextran colloid [81]. Figure 1.11 shows an example of magnetic devices for cell separations. Recently, magnetic nanowires were experimented with cell separation techniques and found to be four times better in purity (80 %) and recovery (85%) yields [82].
Figure 1.11 Magnetically labeled cells can be separated on a gravity feed through a high gradient magnetic separator (HGMS). (Reprinted with permission from [10])
1.4.4 Drug delivery

Bio-distribution of pharmaceuticals always faces a big challenge: Unspecific, evenly distribution of the drugs all over the body. This requires a large amount of the dose to get enough of it to the target which also brings a side effect of the non-specific toxicity in healthy sectors. Among other drug targeting methods, magnetic targeting offers one of the most viable solutions to the targeting problem [83]. To our knowledge, first applications in magnetic drug targeting date back to late 1970s [84-86].

For a successful delivery, a carrier must also be fully controllable. Aggregation, clogging or intrinsic, permanent magnetic behavior is completely unacceptable. Superparamagnetic iron oxides, therefore, offer both requirements for being an excellent shuttle for a successful drug delivery. Figure 1.12 explains how a magnetically targeted carrier would work. Researchers at FeRx Inc. were able to craft iron particles with activated carbon (1-2 µm) and attach Doxorubicin, an anti-cancer drug [87]. They used the magnetic particle – drug assembly to cure cancer tumor in a reversible drug release fashion [88, 89]. Sadly however, FeRx, Inc. is now out of business and laid off most of its employees because of their failure in phase II clinical trials [90, 91].
Figure 1.12 Magnetic Targeted Carriers (MTC) offer a target oriented drug delivery. (Reprinted with permission from [88])

As can be clearly seen in FeRx example, physical (magnetic properties to drug binding capacity) and physiological (target position to body weight) limitations for the in vivo studies resulted in unsuccessful medical therapies but also encouraged more in depth research [92]. For this reason, using epirubicin, an anticancer drug, Lubbe and coworkers identified the potential of ferrofluids [93]. More theoretical studies followed: a mathematical model for magnetic targeted drug delivery [94], a hypothetical magnetic drug targeting system using FEMLAB simulations with the high gradient magnetic separation (HGMS) principles [95], a two-step targeted drug delivery system [96], and a new method for locally targeted drug delivery with magnetic implants in the cardiovascular system [97] were developed. Regardless, anti-cancer drug delivery via magnetic carriers increases drug concentration at the tumor site and limits the systemic drug concentration, by which it enhances the drug activity to multiples of magnitude [98].
Recent treatments with magnetic drug targeting involved using of MTCs (Magnetic Targeted Carriers) in liver and lung [99], treatment of squamous cell carcinoma in rabbits with FFs (ferrofluids) bound to mitoxantrone (FF-MTX) that was concentrated with a magnetic field [100-102], preparation of magnetic liposomes containing submicron-sized ferromagnetic particles encapsulating the muscle relaxant drugs, diadony or diperony, for local anesthesia [103], an improved method for the physical delivery of rAAV vectors in vivo in which virion particles are conjugated to microsphere supports [104], thrombosis treatment using a composition of ferrofluid with fibrinolytic enzyme [105], and nucleic acid delivery with magnetically labeled non-viral vectors [106].

1.4.5 Biocatalysis and diagnostics

Biocatalysis is a newly developing field that has much to gain from the use of magnetic separations. For this area, magnetic beads are used to immobilize bio-catalysts, such as β-lactamase [107] and peroxidase [76], to permit the materials to be homogeneously dispersed and recovered after use. Figure 1.13 shows how an enzyme can be immobilized on a nano iron oxide.
Figure 1.13 Preparation of enzyme immobilized, silica coated nano iron oxide. (Adapted from [107])

Diagnostics is becoming increasingly vital for especially fatal and infectious diseases such as AIDS. Yager et al. developed microfluidic diagnostic technologies to replace highly sophisticated technologies which are specifically designed for climate controlled facilities with constant supply of calibrators and chemicals, stable electricity, adequate and rapid transportation and highly trained personnel [108]. bio-A bio-barcode assay within a microfluidic device was designed to carry out diagnostics of proteins at the attomolar sensitivity enabling early detection and improved treatment at the early stages of the epidemic [109] (Figure 1.14).
Figure 1.14 Implementation of the bio-barcode assay within a microfluidic device. First, magnetic particles functionalized with monoclonal PSA antibodies are introduced into the separation area of the chip. The particles are then immobilized by placing a permanent magnet under the chip, followed by introduction of the sample and gold nanoparticles that are decorated with both polyclonal antibodies and barcode DNA. The barcode DNA is then released from the gold nanoparticles and is transported to the detection area of the chip. The detection area of the chip is patterned with capture DNA. Salt and a second set of gold nanoparticles functionalized with complementary barcode DNA sequences are introduced into the detection area to allow hybridization. Finally, the signal from the gold nanoparticles is amplified using silver stain. (Reprinted with permission from [109])
1.5 Conclusions, further directions and challenges

Magnetic separations on the industrial scale are a well studied and well developed area; recent research applications based on the same principles have made these tools relevant for biotechnology. In these cases, bench top magnetic devices can be used to purify solutions in batch processing. With proper magnetic carriers that feature high quality nanocrystals, with greater surface areas and more responsive magnetic cores, we anticipate an expansion of the magnetic separations in biotechnology.

The field faces many opportunities for growth in the 21st century. For industrial scale applications, the magnetic strength of separator columns can permit more rapid and universal applications. Superconducting electromagnets are already in use but designing less expensive permanent magnets that could generate massive magnetic fields would be a key factor for further development. Batch applications, which by nature are highly specific, have the ongoing challenge of designing custom magnetic beads tailor made to recognize elements of interest. For every biological species that is desired to be separated, a different antibody or binding functionalization may be required. An improvement in more universal and magnetically responsive materials would be a breakthrough. For both industrial and batch scale systems, there is an ongoing need to lower the field strengths needed to move materials in liquids, as well as create simpler and more versatile systems.
References


Chapter 2

Experimental methods, tools and techniques

Aimed at the very basic knowledge of the experimental procedures, instruments, methods and techniques, this chapter will teach the average reader methods and tools used in this thesis for a successful reproduction. The following chapters will depend on the information described here. A well experienced reader is advised to skip this chapter and come back when needed by following the proper citation within the text.

2.1 Reaction and purification setup

Experimental setups often can be confusing, especially to those who do not have in-lab synthesis experience. Although trivial for many, the essential setups and methods will be introduced below.

2.1.1 Airless reaction setup

An airless setup is considered when the reactants (air sensitive) or reaction conditions (high temperature) would be affected by air (esp. O₂, and sometimes N₂) and moisture (H₂O). The goal is to keep the air out but still be able to carry out the experiments. There could be two types of airless setups: Vacuum or Inert gas atmosphere. Since vacuum cannot be used in a solvent - reactant system, inert gas atmosphere is very common in synthesis practice. An inert gas can be most noble gases (He, Ne, Ar, and
maybe Xe) and ultra high purity grade nitrogen (UHP-N₂). Since cost is often considered, UHP N₂ is very handy in routine reactions. Figure 2.1 and Figure 2.2 below shows the essential components of a temperature controlled airless setup.

**Figure 2.1** A typical high temperature airless setup. Drawn with Advanced Chemistry Development Inc.'s Chemschetch 8.0.
Figure 2.2 A picture of an airless setup with N\textsubscript{2} as an inert gas and a temperature controller for automatic heating.

2.1.2 Temperature Controller

A temperature controller is a programmable heating system with a mounted control of the temperature. Current models mostly have a digital display and a thermocouple (a temperature measuring rod wired to the device) for basic applications. More sophisticated ones contain a data port for a computer and/or timer with multiple settings. Generally, a temperature controller is expected to have reading temperature, setting temperature and
heating rate adjustment controls. Figure 2.3 shows Barnant Company ® Temperature Controller, used heavily throughout the studies reported in this thesis.

Figure 2.3 Barnant Company’s Temperature Controller. Shows current temperature (in °C) measured by the attached thermocouple. Lower line is the temperature that is set to stop heating once reached to. Below right shows the status of the device, in this case “stop”.

2.1.3 Reactor

The synthesis of magnetite (Fe₃O₄) nanocrystals, used in this thesis, was done primarily in one pot, airless setup described above (Section 2.1.1). However, mass production or fabrication was attempted and succeeded by a reactor coil made out of thick (10 mm diameter, 1 mm wall thickness, and 3 meters long) glass tubing. Figure 2.4 shows the reactor which is placed in a 4 L beaker.
Figure 2.4 Reactor for mass production (1g/hour) of magnetite nanocrystals. Coils are made of glass tubing (10 mm diameter, 1 mm wall thickness, and 3 meters long).

2.1.4 Centrifugation

In order to purify synthesized nanocrystals centrifugation offers great service. Very familiar to the biochemists, i.e. when purifying proteins, the centrifugation, as a method, comes with ease when as synthesized nanocrystals needed to be cleaned from unreacted precursor mixture. Due to the distinctive weight and size of those nanoparticles, it’s relatively easy to settle them out of a solution by two different centrifugation type: Low speed and high speed centrifugation. Both are used, depending on the circumstances and the stage of the chemical synthesis.
2.1.4.1 Low speed centrifugation

Centrifugation is compared by the g-factor of the devices. At lower speeds, you can still separate the species that can only be separated by higher speeds, only if run for days and weeks. For practical reasons, however, centrifugation instruments differed with speed and volume that they can spin and the accuracy of the balance they require.

When spoken of lower speeds, we used the ones with max speed of 3200 rpm (rotation per minute) and 4600 rpm (equals to 3600 g). Below, Figure 2.5, is a picture of the 4600 rpm instrument, Fisher Scientific’s Marathon 22K, that we used. For a typical run, Fisher Scientific’s FisherBrand ® 50 mL centrifuge tubes were used and cross-balanced within 0.1 g. for each pair placed.
Figure 2.5 Low speed centrifugation instrument, a Fisher Scientific Marathon 22K, was used in post-synthesis cleaning processes.

2.1.4.2 Ultra high speed centrifugation

Though identical in practice, ultra high speed centrifugators offer much more forceful and quicker settling than that of low speed ones because of the number of gs they produce. In our studies, this method was primarily used to force nanocrystals out of the solution while they are perfectly stable in a solution. Below,
Figure 2.6 is a Beckman-Coulter® Optima™ L-80 XP Ultracentrifuge, an ultra high-speed centrifugator that can settle any size of nanoparticles in couple hours. In this thesis, we used Optima L-80 with a rotor, Figure 2.7, type 70Ti which uses specialty vials, Beckman Polycarbonate Centrifuge Bottles with caps (for aqueous samples), Beckman Polyallomer Centrifuge Tubes with specialty metal caps (for non-aqueous samples). Pairs of vials were balanced within 0.1 g sensitivity with respect to one another.

Figure 2.6 Optima L-80 XP, an ultrahigh speed centrifugator from Beckman-Coulter.
2.1.5 Sonication

A vital step for water solubility (which will be discussed in Section 3.4) of nanocrystals is sonication. Although various sizes and types of sonicators with different features were used, we can classify them under batch or probe sonicators. Two parameters, power and the volume of the sample, are critical for a sonicator choice. In this thesis, 4 different sonicators, 2 batch, 2 probe sonicators, were used.

2.1.5.1 Batch Sonicators

With water as the shaking enhancer-transmitter, batch sonicators are designed to assist bulk samples but with low frequency and power. The following sonicators, Fisher Scientific’s FS6, Figure 2.8 (for smaller volumes), Branson’s Model 5510, Figure 2.9 (larger samples).
**Figure 2.8** Fisher Scientific’s FS6, a batch sonicator, for smaller volumes.

**Figure 2.9** Branson’s Model 5510, a batch sonicator, for larger volumes.
2.1.5.2 Probe Sonicators

Sonication with a probe provides more power to a localized, small volume of samples. In our water solubility studies (section 3.4), probe sonication proved usefulness by providing high power, high efficiency sonication. Below are the sonicators used for this purpose.

Figure 2.10 shows the older but more powerful one, an Ultrasonic Processor and Figure 2.11 shows the newer but lesser power probe sonicator, a Dr. Hielscher, GmBH’s Ultrasonicator. Both have advantages and disadvantages if compared, but proven useful in the respective water solubility experiments.

Figure 2.10 Ultrasonic processor
Figure 2.11 Dr. Hielscher, GmBH’s Ultrasonicator
2.2 Magnetic separation setup

Magnetic separation (MS), as discussed in Chapter 1, is a type of physical separation that utilizes magnetic field and field gradients. For an MS to work, a magnetic field generator and a magnetic substance is required. A matrix of ferromagnetic material would generate field gradients which would aid separation. One could list magnetic separation methods into two by means of the source of the magnetic field: separations done with permanent magnets and electromagnets.

2.2.1 Separations with permanent magnets

Magnetic separations (MS) with permanent magnets are also called “batch” MS in which a permanent magnet of any type or strength is stationed outside or bottom of the separation flask—usually placed adjacent to the walls-. Figure 2.12 has Dexter Magnetics LifeSep™ 50SX model which was measured to have 0.36 Tesla as maximum magnetic field. This type of MS is usually preferred when magnetic substances that are present in the steady batches are intended to be isolated rather than those that flow through a stream.
2.2.2 Separations with an electromagnet

Also classified as "column" magnetic separations, separations with an electromagnet rely on an electromagnet powered by electrical current, preferably tunable with Voltage and Ampere knobs. Figure 2.12 is an S.G. Frantz® Isodynamic Separator Model L-1CN. Figure 2.14 and Figure 2.15 also displays the same instrument. The dominant reason of using electromagnetic column for separation is to be able to collect magnetic content when there a continuous stream.
Figure 2.13 S.G. Frantz® Isodynamic Separator Model L-1CN (photo from sgfrantz.com).

Figure 2.14 Our own S.G. Frantz® Isodynamic Separator, Model L-1CN.
2.3 Characterization methods

Various methods for the analysis and characterization of samples obtained from the experiments were used throughout this thesis. For the simplicity and better use of the space, the ones that were used most are described below. The reader is encouraged to seek outside sources such as online libraries and tutorials from respective manufacturers.
2.3.1 Transmission Electron Microscopy (TEM)

TEM images are obtained from samples spotted on copper formvar grids using a JEOL 2010 electron microscope operating at 200 kV (Figure 2.16). Samples usually are prepared from several droplets (~0.02 mL) which are left to dry under air on a carbon only 400 mesh TEM grid (EMS CF400Cu-50) which also is placed on a filter paper. Visual cues such as color intensity of the solution and the dried colored rim formed around the grid signals a successful sampling.

Figure 2.16 A JEOL 2010 electron microscope was responsible for most of the TEM data in this thesis.
2.3.2 Super Quantum Interference Device (SQUID)

Magnetic measurements such as hysteresis loops and Zero Field Cooling/Field Cooling (ZFC/FC) obtained from a Quantum Design Multi Vu (Figure 2.17). This instrument measures the magnetization by reading the output of the SQUID (Superconducting Quantum Interference Device) detector while a sample moves up and down, from the initial position, through the superconducting coils. The magnet assembly is super cooled by liquid Helium and produces a strong magnetic field of up to 5 Tesla, at tunable temperatures from 400K down to 2K. Since the samples are required to be solid (in order to negate the immersion effects from the super cooled environment), substances dissolved in organic solvents are mixed with Polystyrene (with a molecular weight cut off 250k) and dried in a vacuum oven until a film is formed. Capsules are used to hold the wrapped film of samples and straws hosted the capsules at the tip of the rod that goes up and down the instrument’s sample chamber.
Figure 2.17 Superconducting Quantum Interference Device (photo from the Rice Shared Equipment Authority (SEA))

2.3.3 X-Ray Diffractometer (XRD)

Rigaku D/Max Ultima II Powder Diffractometer, a powder X-ray diffraction instrument, is used primarily to determine what crystal phases are present. The instrument is configured with a vertical Theta/Theta goniometer with a Cu source and has a graphite monochromator which allows only the Cu Kα radiation to be detected by the scintillation counter (Figure 2.18).
2.3.4 Cryogenic TEM (Cryo-TEM)

Solution behavior of nanomaterials may be different than their dry forms, for which a cryo-TEM micrograph is considered to be the ultimate solution along with the Dynamic Light Scattering (DLS) measurements. Samples are prepared thru freezing a liquid solution and cutting thin slices off of it to analyze under electron beams. JEM FasTEM 2010 is used to obtain such data (Figure 2.19).
2.3.5 Inductively Coupled Plasma (ICP)

The Perkin Elmer Inductively Coupled Plasma Optical Emission Spectrometer is a tool for conducting trace (ppb-ppm) elemental analysis (Figure 2.20). For more sensitivity, the Perkin Elmer ELAN9000 Inductively Coupled Plasma Mass Spectrometer was used, which is a wet system capable of analysis of ultra-trace (ppq-ppb) samples (Figure 2.21). Samples were prepared with dissolution in concentric HNO₃ and an
analytic dilution. By running against ICP standards which are purchased from companies, elemental content was obtained for different samples.

Figure 2.20 ICP Atomic Emission Spectroscopy (ICP-AES) (photo from the Rice Shared Equipment Authority (SEA))
2.4 Some key software

2.4.1 Image Pro ®

The features of the TEM images used in this thesis, which include average diameter and standard deviation, were measured using the sizing tools in Image Pro Plus 5.0 from Media Cybernetics. This software also had automated macros but they were used only when needed.
2.4.2 Jade 6.1 ®

JADE 6.1 (and later 8.0) software was used to analyze the XRD data and to determine the crystalline nature of the samples via comparison of respective crystal standards stored within the software. For advanced confirmation, American Mineralogist Crystal Structure Database (University of Arizona) was also used.

2.4.3 Origin 6.1 ® and Excel 2003

Sizing data exported from Image Pro Plus 5.0 was analyzed using two different software: Origin 6.1 (and later 7.0) and Microsoft Excel 2003. Origin was specifically used for visual purposes where histograms and plots were created. Excel was used primarily as a temporary storage of the data as well as standard deviation, mean size, count and size distribution calculations.
Chapter 3

Magnetite Nanocrystals

The most magnetic of all the natural minerals, magnetite (Fe₃O₄) in its nanoscale form - along with maghemite (γ-Fe₂O₃) - has been studied extensively because of its unique magnetic properties [1]. The distinctive size dependent magnetic behavior (see Chapter 4 for more information on this) has proven useful in applications such as environmental remediation [2-4], magnetic data recording [5], biomagnetic separation [6-8] and magnetic resonance imaging (MRI) [9]. Given its broad technological relevance, it is not surprising that the synthesis and mass production of magnetite nanocrystals has received great attention. Monodispersity in particle size and uniformity in shape have been major goals for this work, as these are essential parameters to control for industrial application [10]. Synthetic methods which enabled control of these features have helped to define the critical size (e.g. 13 to 17 nm) below which (Fe₃O₄) nanocrystals possess a magnetic dipole moment larger than that of the bulk mineral [2]. Equally important for the application of nanomagnetite is the development of manufacturing methods that are scalable as well as cost effective [11]. This is particularly true for the biological and environmental technologies discussed in this thesis which require both cheap and plentiful materials.
3.1 Historical view of nanoscale magnetite formation

The synthesis of nanoscale magnetite dates back to the early years of life on earth, when magnetotactic bacteria emerged as organisms [12]. More advanced life forms, such as fish, honey bees and pigeons, also produce magnetite nanocrystals of 15 to 50 nm average diameter where they are implicated in the sensing of the earth’s magnetic field and thus biological navigation [1]. Man-made nanoscale magnetite by comparison has appeared relatively recently [13]. The oldest synthetic methods use the reduction of iron salts in aqueous solutions to generate colloidal iron oxides; these techniques utilize the co-precipitation of aqueous solutions of Fe(III) and Fe(II) salts under basic conditions and yield often amorphous and typically agglomerated, these materials have been widely studied and in some cases applied for their unique magnetic properties [14-21]. One example of this is their use as ferrofluids, which has recently led to the application of nanoscale iron oxides as contrast agents for magnetic medical imaging [22, 23]. In 2002 Sun and coworkers demonstrated a new route to these materials which used the thermal decomposition of organometallic iron precursors in organic solvents [24].

3.1.1 Aqueous synthesis of Colloidal Iron Oxide

A method for the synthesis of magnetite nanocrystals is called “aqueous synthesis” if water (H₂O) is used as the primary solvent. In a typical aqueous synthesis, a mixture of known proportions (1:2) of Fe (II) and Fe (III) salts, is dissolved in a pH controlled (preferably by ammonium hydroxide) aqueous. This solution is then aged for various times yielding aggregated iron oxide nanoparticles which are aptly termed ‘colloids’ because their internal structure is not generally crystalline [14-21]. The
products of these simple reactions are commercial materials and have found applications as ferrofluids, e.g. Feridex [23].

This process has the advantages that it uses water as a solvent, and is simple to complete; however, the nanoscale materials that it produces are not of the highest quality. Most critically for scientific studies is the fact that the particles have a wide size (polydisperse) and shape (multiform) distributions [21, 22, 25]. Also, the nanocrystals will aggregate since they are only stabilized by weak electrostatic interactions. Because there is no proper control over their interfaces this soft aggregation can become permanent as the hydroxylated surfaces will eventually hydrogen-bond and eventually condense to form a permanent grain boundary [18]. Additionally, the low crystallinity of the product limits its use in various applications [26]. It is believed that the variations in the molar ratio of Fe$^{3+}$ and Fe$^{2+}$ reduce the quality of the nanocrystal and lead to mixed magnetic phases [27]. Modified sol-gel processes can afford slightly more control over the phase composition, as well as running at the increased temperatures possible in hydrothermal reactors. Still, the resulting materials have high levels of aggregation and consequently are not easily dispersed [28, 29]. Given the problems with these materials it is perhaps not surprising that their magnetic properties have never been well understood or characterized. For example, uncoated iron oxides have very different magnetic behavior at low temperatures that is their coercivity values increase as their grain sizes became smaller, indicating that interactions between grains dominate the magnetic properties and effectively preclude ‘single domain’ behavior [30].
3.1.2 High Temperature Synthesis of Nanocrystalline Magnetite

The methods for the synthesis of magnetite nanocrystals that do not employ water as the solvent are grouped here under the heading of a “high temperature” synthesis. This type of synthesis is relatively recent, and has its origins in the high temperature reactions used to make monodisperse quantum dots [31-33]. The formation of maghemite (γ-Fe₂O₃) nanocrystals was first reported by Alivisatos in 1999 [34] and then Hyeon in 2001 [35]. These methods were extended to magnetite (Fe₃O₄) by Sun and Zeng. They reported that Iron (III) acetylacetonate could be decomposed in mixtures of oleic acid, oleylamine, hexadecanediol and diphenyl ether under air-free conditions at high temperatures [24]. The resulting magnetite was of high quality and uniformity, and due to the presence of surfactants in the reaction mixture was not aggregated.

The high temperature synthesis of magnetite nanocrystals results in highly uniform crystals (size distribution under 10%) of excellent quality and uniformity (aspect ratio ~1 in most cases) [25, 26]. In January 2004, Sun and coworkers published a full paper on their magnetite synthesis with additions to make metal ferrites and water soluble nanocrystals [36]. Our group introduced an route which used iron oleate as a precursor (published online September 2, 2004) in which an acid-base reaction of FeOOH (Iron Oxo Hydrate) with oleic acid is completed in 1-octadecene (ODE) as a solvent under air-free conditions [10] (Table 3.1). Peng and coworkers followed (published online September 11, 2004) with their method of using Fe(oleate)₃ intermediate, which was made from FeCl₃ and oleic acid, in a solvothermal reaction that includes additional oleic acid and ODE [37]. Hyeon and his group came up with their method later in the year
(published online November 28, 2004) to present a method like Peng’s employing Fe(oleate)$_3$ complex which was made from FeCl$_3$ and Sodium Oleate, in a reflux with oleic acid and ODE [38].

Table 3.1 Comparison of the leading methods for non-aqueous synthesis of magnetite nanocrystals.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Date first</strong></td>
<td>September 2,2004</td>
<td><strong>June 20, 2002</strong></td>
<td>September 11, 2004</td>
<td>November 28, 2004</td>
</tr>
<tr>
<td><strong># of Steps</strong></td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong># of reactants</strong></td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>320°C</td>
<td><strong>265°C</strong></td>
<td>300°C</td>
<td>320°C</td>
</tr>
<tr>
<td><strong>Tunable size range</strong></td>
<td><strong>8 - 36 nm</strong></td>
<td>4 - 20 nm</td>
<td>8 - 30 nm</td>
<td>5 - 14 nm</td>
</tr>
<tr>
<td><strong>Initial Size</strong></td>
<td>Varies by time and stoichiometry</td>
<td>4 nm only</td>
<td>Varies by stoichiometry</td>
<td>Varies by stoichiometry</td>
</tr>
<tr>
<td><strong>Bigger sizes</strong></td>
<td>Varies by time and stoichiometry</td>
<td>Seed mediated growth</td>
<td>Varies by stoichiometry</td>
<td>Varies by stoichiometry</td>
</tr>
<tr>
<td><strong>Scalability</strong></td>
<td>Continuous Reactor: 1 gram/hour</td>
<td>Mentioned as scalable</td>
<td>Mentioned as scalable</td>
<td>Reported 40 g (scalable)</td>
</tr>
<tr>
<td><strong>Water solubility</strong></td>
<td>Bilayer and polymer</td>
<td><strong>Exchanging surfactant</strong></td>
<td>None reported</td>
<td>None reported</td>
</tr>
<tr>
<td><strong>Difficulty</strong></td>
<td>None</td>
<td>None</td>
<td>Purification of iron oleate</td>
<td>Purification of iron oleate</td>
</tr>
</tbody>
</table>
While the quality of the magnetite crystals made from the high temperature routes are unparalleled, the variations all share one critical problem: the as-synthesized magnetite nanocrystals are not soluble in water. The materials are coated strongly with oleic acid, which serves as both a surfactant to stabilize the iron oxide and a reactant in the process [25, 39, 40]. The nanocrystals are best described as hydrophobic balls since the hydrocarbon tails of the surfactants (oleic acid) stick out. This disadvantage poses limitations in biological applications where aqueous dispersions are required. To overcome this barrier, particles are solubilized in water by additional steps (see section 3.3). Some studies, however, came out recently claiming to eliminate the additional water dispersion steps, by changing the course of the synthesis. Caruntu et al., for example, introduced a method that involves dry co-precipitation of Fe(II) and Fe(III) salts with diethylene glycol and N-methyl diethanolamine producing nearly monodisperse, high quality magnetite nanocrystals [41]. Li et al suggested another method by producing water soluble magnetite nanocrystals from FeCl₃.6H₂O in 2-pyrrolidone without using any additional water [26].

The synthetic method we developed for magnetite nanocrystals, which utilizes the solvothermal decomposition of iron oxide hydrate (FeOOH) in the presence of oleic acid and 1-octadecene [10] (see section 3.1.2.1.1 for the experimental details) is one of the four reported methods of magnetite nanocrystal synthesis in non-aqueous media [25]. The strength of our method is that it is simple, and requires only 3 reagents which makes it a reasonable starting point for creating large-scale and cost effective manufacturing [36-38]. Of immediate concern for this goal is the cost and complexity of the production
process [2-4]. The goal of this work was to address these issues directly in a green process that utilizes rust and edible oils as well as other household items to produce high quality magnetite nanocrystals (see Chapters 4 and 6).

3.1.2.1 Proposed mechanism

As opposed to the aqueous methods, little is known about the mechanisms that control particle formation in the high temperature production of magnetite nanocrystals [25, 26, 42]. Thermal decomposition of iron precursors takes place at elevated temperatures in excess of the flash or smoke points of several ingredients; thus, the reagents themselves may be undergoing chemical transformation because of the high temperature (Figure 3.1). For example, oleic acid, a common surfactant for many nanocrystal production methods, changes its molecular structure from unsaturated to saturated species during the course of a reaction [39, 43]. To improve the understanding of our synthesis, controlled experiments on the components of the synthesis (Table 3.2) are, therefore, required and those will be discussed in Chapter 4. Before we describe these results, we present an optimized procedure of the synthesis.
Figure 3.1 Accepted mechanism of the solvothermal decomposition of iron oleate for magnetite nanocrystals. Inset is a digital picture from a batch setup.

Table 3.2 Components for the magnetite nanocrystal synthesis

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Surfactant</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeOOH (Iron Oxo Hydrate)</td>
<td>Oleic acid</td>
<td>ODE</td>
</tr>
<tr>
<td></td>
<td>((9Z)-octadec-9-enoic acid)</td>
<td>(1-octadecene)</td>
</tr>
</tbody>
</table>
3.1.2.1.1 High temperature synthesis of magnetite nanocrystals

The optimized procedure for the synthesis of magnetite nanocrystals is based on a method developed by Dr. W. Yu in our lab [10] and is described here. In a typical synthesis, 1 gram of FeOOH (Aldrich) is ground to powder in a mortar. In a 250 mL three neck flask, 12.71 grams of oleic acid (Aldrich, Tech Grade 90%, weight is taken instead of volume since it’s very viscous) is placed along with a magnetic stirbar. 50 mL of 1-octadecene (Aldrich, Tech Grade 90%) is mixed and solution is started stirring. Air-free conditions are generated with a standard Schlenk line and the reaction temperature is monitored with a digital temperature controller (see Chapter 2 for more information on experimental methods). The flask is fitted with a reflux condenser and a septum for precursor introduction and sampling. The thermocouple of the temperature controller is inserted through a septum with the help of a flame closed disposable Pasteur pipette; this serves as an adapter to protect the metal surface and the thermal probe. Nitrogen (UHP grade – Ultra High Purity) is used as the inert atmosphere. We note that we see little difference in our data when the reaction is performed open to air. A heating mantle is connected to the temperature controller and set to 320 °C; temperature controllers may need to be calibrated with mercury thermometers. The apparatus is insulated with glass wool to prevent heating loss and speed the process. We generally do not cool the reflux condenser as all of the substances have very high boiling points; in a few cases when we did do this there was no change in the results.

Samples are collected at various time points with a disposable syringe and a long steel needle (cleaned after every sampling) at sixty, seventy minutes and eighty minutes.
At ninety minutes heating is stopped and the solution is cooled immediately by the removing glass wool and the heating mantle. No additional cooling is necessary since air flow within the hood is adequate. After cooling to temperatures below 60 °C, the black mixture is transferred into 50 mL disposable centrifuge vials of approximately 10 mL each. To this, one to two mL hexanes (or chloroform) is added as well as 30 mL acetone. Balanced with each other to 0.1 gram, the vials are centrifuged for 10 minutes at 4500 rpm (see Chapter 2 for centrifugation instruments). Supernatant is discarded and the black precipitate is redispersed with minimal hexane (or chloroform) to 10 mL. 30 mL acetone is added to each of these vials and centrifuged again for 10 minutes at 4500 rpm. Final hexane dispersions are collected in a glass bottle and labeled for storage. A drop of the solution is used to sample for TEM (see Chapter 2 for more on TEM sampling) and Figure 3.2 shows a representative example of magnetite nanocrystals synthesized with this method (average size: 10.84 ± 0.55 nm, size distribution: 5.1 %).

Figure 3.2 A representative example of magnetite (Fe₃O₄) nanocrystals synthesized with high T method developed in our lab (average size: 10.84 ± 0.55 nm, size distribution: 5.1 %)
3.2 Large scale synthesis

A major drawback of the high temperature production of the magnetite nanocrystals is its presumed lack of scalability [44]. To be able to develop a new synthetic method for an industrial application, one has to offer mass production with affordable manufacturing and materials costs. Below, we'll discuss the large scale synthesis by dividing our strategies into two sections: a one-pot synthesis with larger iron concentrations and preliminary data on continuous production with a reactor.

3.2.1 Batch, one-pot synthesis

Any additional step or reactant or catalyst within a chemical process represents an expensive step that must be adapted by industry. For this reason, industrial chemical processes are often optimized to keep the steps involved to an absolute minimum. One can realize that magnetite nanocrystal synthesis by our method stands out as the best for an industrial adaptation by involving the least number of synthetic steps and reagents (Table 3.1). There are reports on multi gram scale magnetite nanocrystals synthesis [38] and one pot easy synthesis [45] with a clear goal of supporting the need for simplicity in all nanocrystal production techniques.

In our synthesis, we were able to scale up to 20 grams by increasing the relative amounts of reagents (Figure 3.3). It was concluded that there wasn’t any need to test larger scales since the monodispersity of the end product was independent of the scale, a behavior not observed in many nanocrystal synthesis techniques.
Figure 3.3  Scaling up the magnetite nanocrystal synthesis.

3.2.2  Continuous production with a reactor

As discussed earlier, a synthesis being one pot, one step with minimal number of reagents, is desired for the plant scale nanocrystal productions. The better system, however, would be the construction of a reactor that produces nanocrystals in a continuous manner. There aren’t many attempts in this regard, except those pilot size nanomanufacturing plants based on batch based “mix ‘n bake” systems. To our knowledge, the only reactors for nanocrystal synthesis were reported recently by Bawendi and Alivisatos [46, 47]. They demonstrated a silicon-based micro-reactor that incorporated both gas–liquid segmented flow and multiple temperature zones. Their reactor was claimed to be a general platform for high-temperature synthesis for very well defined reaction conditions.
For a continuous magnetite nanocrystal synthesis, a technique must not involve more than one step and should be fast enough to not require aging for longer time periods. Our synthesis provided both [10], enabling a reactor construction. From the designing viewpoint, we focused on having the reagent mixture spend 30 minutes in a tubular system that is kept at 320 °C at all times. Although metallic tubing was advised, we intended to construct a glass reactor to be able to monitor the liquid-gas interactions within the reaction chamber. The magnetite reactor was constructed by ChemGlass ® and used for several test runs (Figure 3.4). The output of the reactor was estimated to be 1 gram per hour.

Figure 3.4 Continuous reactor for the synthesis of magnetite nanocrystals with an output of 1 g/hr.
3.3 Water solubility

A disadvantage of magnetite nanocrystals formed in organic solvents is that they are not water soluble. Some methods were developed recently to overcome this problem [26, 41, 45]. These solutions are good first steps, but the quality of the materials is not generally as high as the organic synthetic routes. We have developed a suite of methods that are applicable to transferring magnetite nanocrystals into water: surfactant exchange, oxidative cleavage of surfactants, bilayer stabilization and polymer coating.

3.3.1 Surfactant exchange

In the transfer of a hydrophobic hydrocarbon ball (an oleic acid coated nanocrystal) into hydrophilic environments, the first method we tested was the replacement of the hydrophobic coating with a hydrophilic one. Sun et. al described a successful example of this that involved treatment with tetramethylammonium 11-aminoundecanoate, a bipolar surfactant [36]. Hatton and Lattuada developed this method further by screening a class of reactive surfactants, aiming to grow various water-soluble polymers and both cationic and anionic polyelectrolyte brushes on metal ferrite nanocrystal surfaces [48]. DePalma et al employed silane bearing surfactants that were used to replace the hydrophobic oleic acid on the surface, thus enabling further functionalization and water solubility [49]. For our magnetite nanocrystal systems, the commercially unavailable tetramethylammonium 11-aminoundecanoate was first synthesized and purified, then used to transfer the nanocrystals into water. We found the method unreliable, and abandoned this approach.
3.3.2 Oxidative cleavage of surfactants

Inspired by the popular olefinic oxidative cleavage in synthetic organic chemistry [50, 51], we pursued an approach which relied on the cleavage of the oleic acid coating at the double bond. Such a treatment yields a free carboxylic acid at that point, and a hydrophilic surface (Figure 3.5).

![Diagram showing the chemical reaction of Fe₃O₄ nanocrystal coated by Oleic acid reacting with TOAB and KMnO₄ to yield Fe₃O₄ nanocrystal coated by azelaic acid.]

**Figure 3.5** Chemical scheme for cleaving the oleic acid’s double bond with an oxidant, i.e. KMnO₄, while on the surface of a magnetite nanocrystal. TOAB represents tetra octyl ammonium bromide, a phase transfer agent.
In the following oxidative cleavage experiments, two common techniques for olefinic oxidation were employed: potassium permanganate (KMnO₄) and ozone (O₃).

KMnO₄ oxidation was carried out first by directly applying this oxidant to organic solutions of the magnetite nanocrystals: Poor organic miscibility of the reagents required that we incorporate a phase transfer agent, tetraoctylammonium bromide (TOAB). In parallel, potassium permanganate was also modified to form tetrabutylammonium permanganate, a bipolar molecule suitable for biphasic applications. We tried each reaction with varying success (Figure 3.6). Nanocrystals could be transferred into water, but the oxidants also reacted with the iron oxide materials as well. FT-IR data found distinctive carbonyl stretches consistent with anticipated structures (Figure 3.7). Further trials even generated interesting formations, such as nanoflakes (Figure 3.8) which are likely a form of MnO₂ colloids. The inconsistency and irreproducibility of the data led to a conclusion that permanganate was not suitable for oxidative cleavage of the surface oleic acid. This is because it also alters the form of the core nanocrystals.
**Figure 3.6** Phase transferred KMnO₄ oxidation of the surfactants of the magnetite nanocrystals in hexane dispersions.

**Figure 3.7** FT-IR spectrum of the permanganate treated magnetite nanocrystals. Two carbonyl stretches (1712.51 and 1731.69) fit to the carboxylic acid (1705-1720) and ester (1735-1750) functionalities proving the cleavage: a free acid and an ester on the nanocrystal surface [52].
Figure 3.8 Nanoflakes generated by uncontrolled oxidation of magnetite nanocrystals with KMnO$_4$ + TOAB.

**Experimental methods for permanganate oxidation:** For a KMnO$_4$ oxidation with a phase transfer agent, first a saturated solution of tetraoctylammonium bromide (TOAB) in ultrapure water is prepared. KMnO$_4$ is added to the TOAB solution to form a homogeneous solution and then mixed with the magnetite nanocrystals so that there is four times more oxidizing agent by weight than iron oxide. The reaction flask is shaken several times to ensure adequate mixing. With the help of magnetic stirbars, the solution is stirred overnight (8-10 hours) under rapid spinning and vortexing conditions. The solution is centrifuged down under the same conditions as used to pellet iron oxides in a synthesis. The supernatant is decanted and the pellet is treated with 5 ml of .1 M NaOH. If larger chunks of the material are observed in the solution after treatment, the solution is filtered with a syringe filter to remove them. If the solution is well dissolved, it is centrifuged and resuspended again. In the end, pellets are redispersed in pure water. For the oxidative cleavage with NBu$_4$MnO$_4$, first NBu$_4$Br (aq) and KMnO$_4$ (aq) is mixed to make NBu$_4$MnO$_4$ followed by drying under vacuum. The Fe$_3$O$_4$ particles are then mixed
with \( \text{NBu}_4\text{MnO}_4 \) in \( \text{CH}_2\text{Cl}_2 \). After \(~20\) h the solution is extracted from \( \text{NaOH} \), acidified and extracted finally with diethyl ether before being redispersed in water.

We reasoned that the phase transfer agent could facilitate the surface oxidation of the iron oxide, or stabilize a solid and reduced form of manganese oxide. As both of these outcomes were undesirable, we also tried Ozone as a reactant. Because it is a gas, it did not require the addition of a phase transfer reagent. In a typical ozonolysis reaction, magnetite nanocrystals in \( \text{CH}_2\text{Cl}_2 \) are exposed to \( \text{O}_3 \) for 45 minutes at temperatures approaching \(-78^\circ\text{C}\). The excess ozone (blue) is observed with the reaction of ozone (yellow). The blue color is found to be dominant at the end of 45 minutes. Then the solution is rapidly warmed to room temperature by removing the cooling bath. An electric ark ozone generator was used to generate Ozone from oxygen, and this reactant must be applied at \(-79^\circ\text{C}\) (because of its reactivity). This presented problem because at these low temperatures the magnetite solutions become frozen. This leads to a heterogeneous reaction with ozone reaching some parts of the reaction vessel and not others; interestingly, this oxidation was too strong and both the nanoparticles and surface coatings were changes in significant ways (Figure 3.9).
3.3.3 Bilayer stabilization

Our last approach relied on a bilayer stabilization method which encapsulates the as-formed particles with amphiphilic surface coatings. In this approach, there is no attempt to change the surface chemistry of the nanocrystals. Instead, a secondary surfactant is used to enclose the overall nanoparticle system. First introduced in 1980 [53, 54], the technique has been described over the years by numerous studies [18, 55-58]. The idea relies not on a transfer of the surface coatings from hydrophobic to hydrophilic, but rather the use of amphiphiles to encapsulate the materials and present an outer hydrophilic outer surface. In effect, surfactants form bilayers around magnetite nanocrystals through the intercalation of the hydrophobic ends of the surfactants of which the hydrophilic heads are bonded to the nanocrystals while their hydrophobic tails were sticking out. The hydrophilic ends of the secondary surfactants, therefore, form the outer
surface of the encapsulated nanocrystals and serve to render them water-soluble. The interaction between two layers is found to be predominantly Van der Waals. This approach is widely used to stabilize quantum dots prepared in a similar fashion.

Aside from other applications, this method was shown to be useful in a complex system [59]. Figure 3.10 shows the molecular models of the monolayer and bilayer systems. Figure 3.11 shows some of the secondary surfactants used in this study. In Table 3.3, the secondary surfactant tests and their results are shown.

**Figure 3.10** Molecular models of the monolayer and bilayer systems
Figure 3.11 Secondary surfactant molecular structures for bilayer studies.

Table 3.3 Secondary surfactant bilayer tests with magnetite nanocrystals

<table>
<thead>
<tr>
<th>Secondary Surfactant</th>
<th>Concentrations Used (% w/w)</th>
<th>Water Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 20</td>
<td>1, 5, 10</td>
<td>Successful</td>
</tr>
<tr>
<td>Tween 80</td>
<td>1, 5, 10</td>
<td>Successful</td>
</tr>
<tr>
<td>Igepal CO-520</td>
<td>10</td>
<td>Successful</td>
</tr>
<tr>
<td>Igepal CO-630</td>
<td>1, 5, 10</td>
<td>Successful</td>
</tr>
<tr>
<td>Brij 30</td>
<td>1, 5, 10</td>
<td>Successful</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>10</td>
<td>Successful</td>
</tr>
<tr>
<td>Triton N-101</td>
<td>1, 5, 10</td>
<td>Successful</td>
</tr>
<tr>
<td>Tween 85</td>
<td>10</td>
<td>Unsuccessful</td>
</tr>
<tr>
<td>Pluronic P123</td>
<td>10</td>
<td>Successful</td>
</tr>
<tr>
<td>Pluronic F-127</td>
<td>1 (small original quantity)</td>
<td>Unsuccessful</td>
</tr>
</tbody>
</table>
In a typical bilayer phase transfer process, the secondary surfactant is prepared first as water dispersions with concentrations of 1, 5, 10 % (w/w). Hexane (or chloroform) dispersions of magnetite nanocrystals are then mixed with 4 volume equivalent of surfactant solution and sonicated for 2 minutes at maximum power (See Chapter 2 for sonication instruments). The resulting solution (containing many bubbles) after twelve hours becomes clear with a water layer containing iron oxide materials. With some of the surfactants, additional sonication is necessary to achieve effective phase transfer. Sonication can heat up the magnetite solutions and to preclude any unwanted side reactions in some cases ice was applied to limit this effect. We found that that a curious crackling noise throughout the sonication went away right around the time the reaction is over. This is used as an indication of the completion of bilayer formation. Figure 3.12 shows stages of the bilayer method.

Figure 3.12 Photographic explanation of the steps of the bilayer approach

95
After we made transferred the material to water, we analyzed the materials under TEM to evaluate possible changes in the structure of the nanoparticles. Some geometric alignment of the nanoparticles could be observed, and whether this was due to drying effects or reflecting of the solution phase structure was not apparent. In Figure 3.13, an example of the drying patterns of the hexane dispersed magnetite nanocrystals and Tween-20® bilayered nanocrystals are shown.

**Figure 3.13** Tetragonal and hexagonal alignment by nanocrystal assemblies. **Insets** show the molecular model of the respective nanocrystal.

Stability in buffer solutions is very important for the potential biological applications of magnetite nanocrystals. Saline conditions (Figure 3.14) and other different common buffers (Figure 3.15) were tested with Sodium Dodecyl Sulfate (SDS) stabilized
4 nm magnetite nanocrystals. As seen in those figures, the water solubilized particles were stable up to 4 % NaCl solution and many other buffer solutions. This data shows the wide stability of these nanocrystals and their potential to be used in biological media.

**Figure 3.14** Stability of the Sodium Dodecyl Sulfate (SDS) coated magnetite in saline conditions

**Figure 3.15** More stability tests with various common buffers
3.3.4 Polymer coating

An alternative approach to make magnetite nanocrystals water soluble is to coat the particles with a polymer. Most techniques developed incorporate crosslinking in order to prevent the polymers from returning to the solution phase. Proven useful, there are a number of examples exist in the literature that includes our group’s recent work. Yu et al described this copolymer approach in two recent publications, being among the most prominent ones [60, 61]. Pellegrino et al is also noteworthy [62].

3.4 Future directions on synthesis

Synthesis of magnetite (Fe₃O₄) nanocrystals has come a long way since Haber et. al introduced the concept in the year 1900 [13]. Since 2002, the year when first organic synthesis was introduced [24], substantial development has been observed in this field, especially around 2004. There exist challenges however, and further research has to address them if these materials are to be manufactured widely.

Magnetite is particularly valuable in aqueous solution. We explored here several possible methods, and found that the phase transfer process using appropriate amphiphiles is the best option for our systems. It is simple and highly reproducible, and an essential procedure for making the highly uniform magnetite nanocrystals available in biological and environmental systems. Furthermore, nanocrystals with more reactive surfaces or a surface activation procedure need to be developed so that contaminant removal applications could be better implemented. Aggregation must be stopped by a proper and preferably universal surfactant, whose interactions with the surfaces are not so strong that sorbent interactions are precluded.
A major challenge for the non-aqueous, organic techniques shown earlier is the poor water solubility of the final products. The additional treatment is always required and this can be a significant disadvantage compared to magnetite produced in water. A universal synthetic route with minimal operation costs and simplicity is desired along with good water solubility. The other issues that need to be addressed are the environmental impact and scalability. Although magnetite is known to be non-toxic, some in depth toxicology studies should be done on nanocrystalline magnetite. Pilot scale production plans are underway, however, and for this systems a continuous reactor similar to that described in this chapter should be developed commercially.

There's no doubt that magnetite nanocrystals have superior properties, such as being natural, cheap, non-toxic and magnetic. These properties make them advantageous for many reasons. There's clearly a bright future for these materials and many more years of interesting and relevant chemical research.

References


Chapter 4

Accessible manufacturing of Magnetite Nanocrystals

In this chapter, we aim to transform the magnetite nanocrystal synthesis into a more accessible, more environmental friendly and cost effective method. In order to achieve this, it is first important to understand the importance of each of the synthetic components and reaction conditions. Table 4.1 summarizes the components of the magnetite nanocrystal synthesis we developed (see Chapter 3 for more information).

Table 4.1 Components for the magnetite nanocrystal synthesis

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Surfactant</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeOOH (Iron Oxo Hydrate)</td>
<td>Oleic acid</td>
<td>ODE (1-octadecene)</td>
</tr>
<tr>
<td></td>
<td>((9Z)-octadec-9-enoic acid)</td>
<td></td>
</tr>
</tbody>
</table>

4.1 Analysis of the components and parameters of the synthesis

In order to develop this process into one that is more accessible and cost-effective, it is first important to understand the importance of each of the synthetic components and reaction conditions.
**The precursor,** iron oxohydrate (FeOOH) is a hydrated iron (III) oxide generally referred to as rust [1]. For use in the synthesis it must be ground to a fine powder and dried. Our standard process uses a reagent grade source (Aldrich, Ferric hydroxide oxide, Fe(OH)O, catalyst grade, 30–50 mesh, cat. # 371254) which is ground in a mortar and pestle before use; the cost of this material is listed at $0.78/g. and in section 4.2 we show that natural rust is a useful alternative.

**The surfactant,** oleic acid (cis-9-octadecenoic acid) is a common fatty acid that can be derived from edible oils such as olive oil. Its use in nanocrystal synthesis is widespread as it results in monodisperse and uniform nanocrystals of materials as diverse as cadmium selenide and iron oxide [2-4]. To understand the role of oleic acid we tested stearic acid (a saturated version of oleic acid) and linoleic acid (double unsaturated version of oleic acid) in the magnetite nanocrystal synthesis (Figure 4.1). The reactions were remarkably similar in all cases, indicating that the presence or absence of a double bond in the fatty acid is not a critical feature; moreover, the longer chain double unsaturated fatty acid also gave comparable results. This data illustrates that the reaction works well for a range of fatty acids.
Figure 4.1 Effect of the surfactant in magnetite nanocrystal synthesis. (A) FeOOH + Oleic Acid + ODE (B) FeOOH + Stearic Acid + ODE (C) FeOOH + Linoleic Acid + ODE (D) – (F) Histograms for the above TEMs. The molecular structure of the surfactant used in each run is given below the data sets.

The solvent, 1-octadecene (ODE), a routine solvent for many synthetic techniques, when used as the only organic component (e.g. no surfactant) did result in the formation of nanocrystals. However, the grain sizes were quite large and the materials appeared to be aggregated. The materials were very polydisperse, however, as compared to nanocrystals formed with a surfactant (oleic acid) proving that the surfactant is essential to form highly uniform materials (Figure 4.2B). Conversely, when reactions were run absent any
solvent (e.g. in pure surfactant) nanocrystals also formed; however, they were very large (d > 40 nm) which is consistent with our prior observations that the size of the nanocrystals increases as the surfactant concentration increases (Figure 4.3). Figure 4.2C shows that when saturated version of ODE, octadecane, was used, the high uniformity of the nanocrystals is still preserved. Figure 4.2A summarizes these data.

**Figure 4.2** TEMs of the magnetite synthesis with pure solvents. (A) FeOOH + Oleic Acid + ODE, (B) FeOOH + ODE, (C) FeOOH + Oleic acid + Octadecane (D), (E), (F) Histograms of the above TEM picture
Figure 4.3 TEM micrographs showing the evolution of size with the increase in Fe/Oleic acid molar ratio. Scale bars are 50 nm. “Pure” represents the reaction without any diluent, and oleic acid being both solvent and stabilizer. Size distributions are as follows (left to right): $10.84 \pm 0.55$ nm, $9.41 \pm 0.92$ nm, $6.89 \pm 1.02$ nm, $66.80 \pm 13.56$ nm.

The molar ratio of surfactant to solvent, was also evaluated. This is an important parameter in controlling nanocrystal size. Figure 4.3 shows that when the oleic acid amount was increased, shape control is lost and size increases. Of particular note for our goals is that the reaction can work with pure surfactant. The sizes produced are large, and the shapes are not as uniform, but the size distributions are quite reasonable.

An important observation of all of these data is that the uniformity of the resulting iron oxide nanocrystals is relatively insensitive to solvent purity, precursor sources and most critically the time and temperature control of the process. These features will prove to be invaluable later in our attempt to make an accessible synthesis. However, these observations do contradict the conventional explanations for the origins of nanocrystal uniformity in these synthetic processes.

Burst nucleation is a concept that describes formation of many nuclei at a given instant and their further growth without additional nucleation [5]. It is widely believed that this process is responsible for the very narrow size distribution nanocrystals that form in many high temperature processes in organic solution [6]. According to the LaMer
model for crystal growth [5], if the concentration of a precursor exceeds the critical supersaturation for precipitation then nucleation starts almost instantly. This process uses up the precursor, and its concentration then drops below the solubility point, and this halts nucleation. If the conditions that initiate the first ‘burst’ are such that nucleation, and subsequent monomer depletion, are extremely fast – then nucleation occurs in only a small window of time. This creates a narrowly distributed set of seed nanocrystals which then grow at comparable rates to form equally narrowly distributed nanocrystals.

In this model, the most essential parameter to control in a reaction is the time at which nucleation is active. Many early processes that formed nanocrystals relied on rapid injection of precursors to achieve narrow size distributions; or temperature jumps timed to result in rapid decomposition processes. Kinetic studies of iron oxide nanodisks by Alivisatos and coworkers found that in the case of iron oxides, formation of nuclei is slower that the further growth which allows the reaction rate to be controlled by the nucleation rate [7]. “Focusing” of the size distributions, an effect of the time elapsed between the end of the (burst) nucleation and the start of growth, has also been introduced by Alivisatos group [8]. This model relies primarily on the burst nucleation concept to explain narrowly sized nanocrystals, but also introduces the important concept that nanocrystals can dissolve – partially or completely - and reform on timescales comparable to their growth. These ripening processes can be manipulated through addition of monomer, and can be used to make smaller nanocrystals unstable with respect to larger ones leading to even narrower ‘focused’ nanocrystal distributions [6]. Still, an essential feature of this work is that if the initial burst nucleation process is not well controlled then uniform materials are unlikely to result.
Whether these mechanisms work for iron oxide nanocrystal syntheses are not apparent. Most studies of the high temperatures nanocrystal growth processes rely on quantum dots as a model for nanocrystal growth; it is possible to correlate the mean particle size to the position of the exciton peak in the absorption spectrum and find the particle concentration from the optical density of the solution [9]. The crystallite diameter and the concentration of nanocrystals can be controlled in a variety of ways, and in general this work has highlighted the role of the stabilizing ligands on the nucleation and growth of nanocrystals [10].

Mulvaney and coworkers analyzed the kinetic effects of varying ligands in a CdSe nanocrystal synthesis [10, 11]. Their findings suggest that capping agents may act both as “nucleating agents” as well as “growth promoters” depending on concentrations, temperature and solvent. It is this last observation, that the surface stabilizing agents can assist in ripening processes, that suggests an important modification of the conventional burst nucleation model for nanocrystal uniformity. If the capping ligands are strongly bound to the starting material, then what results is a very stable precursor-ligand complex and thus slower nucleation rates at the beginning of the reaction. The other consequence of strong ligand interactions is that once formed, the availability of surface sites for nanocrystal growth would be few because the capping agent equilibrium would be shifted towards a bound state.

Yin et. al analyzed the heating rate effect along with the surfactant type on a magnetic nanoparticle synthesis [3]. 35 °C/min (max) was found to be the best when compared to 5, 15, 25 °C/min. They argue that a homogeneous nucleation is dominant in
this process, when the heating rate is relatively high, and therefore the nucleation is finished within a short time interval.

Figure 4.4 describes a sketch based on LaMer plot and adapted for the cadmium selenide case [5]. As the reaction mixture is heated to reflux temperatures, the dissolved precursor concentration also increases (red curve). Beyond the supersaturation limit, nucleation starts but is generally limited. The blue curve represents the number of nuclei as a percentage calculation and according to Mulvaney and coworkers, only 3-8% of the initial CdSe concentration forms nuclei. Very similar to magnetite nanocrystal growth, we can assume that after a certain time the nuclei number becomes steady. Also, we can plot the concentration of the net amount of precursor that is within the particles (growth - black curve). The shaded area “B” represents the amount of precursor that was added to growing nanocrystals and “A” represents the amount that formed nuclei. The ratio of B/A should be proportional to the grain size. In other words, if most of the precursor was consumed in growth then the nanocrystals would be larger; as opposed to a case where nucleation accounted for most precursor depletion.
Figure 4.4 Magnetite nanocrystal growth plot. Concentration of the species vs. time. A represents the percentage of precursor used for nucleation. B represents the percentage of precursor used in the growth of the particle. (Adapted from Mulvaney et al. [10])

In the case of a non-aqueous magnetite nanocrystal synthesis, formation of iron (III) oleate complex is considered to be the precursor for the iron oxide formation. [12-16]. The decomposition of the Fe(Oleate)$_3$ at elevated temperatures follows the complex formation shown in Figure 4.5. An alternative approach could be the formation of FeO(Oleate), especially in our method, since the precursor may be partially neutralized by one equivalent of oleic acid instead of three (Figure 4.6). Although not proven, the latter mechanism may very well be taking place (at least to a degree) since synthesis with
1:1 stoichiometry also produces nanocrystals of appreciable monodispersity. Further analysis is needed to verify these claims.

**Figure 4.5** Accepted mechanism of the solvothermal decomposition of iron oleate for magnetite nanocrystals. **Inset** is a digital picture from a batch setup.
Figure 4.6 Scheme describing an alternative mechanism of the magnetic nanocrystal growth. The organo-metallic complex, FeO(Oleate), builds inverse micelles by the increasing concentration of the oleate. Magnetite nanocrystal grows with the dropped [FeO]$^+$ units and further stabilized with the free oleates. (inset) FeOOH reacts with oleic acid and forms FeO-Oleate.
The higher the concentration of the precursor, the smaller and more number of particles are observed [7] since more nuclei can form at the burst nucleation step. As for the surfactant, the higher the concentration of the surfactant, then the slower the nucleation rate because the precursor-surfactant complex is more stable [17]. This leads to more precursor being directed into growth and thus larger particles and larger particles. When the ligand chain length was shortened, the synthesis proceeds faster because of the decreased reactivity of the surfactant in bonding to the precursor [12]. Solvent choice depends on the synthesis, however, it could be said that the higher the boiling point of the solvent, the larger the nanocrystal may form since elevated temperatures provide more energy and therefore facilitates in general more rapid reactions [17]. The result of this analysis is that the iron oxide size and size distribution is more a function of the strong interaction with the surfactants, and less a function of control over the time of the nucleation process. [18] As a result, the reaction is remarkably robust and able to produce high quality nanocrystals under a range of conditions [19].

4.2 Green synthesis: Rust and Olive oil

An alternative approach to reducing costs without sacrificing quality as much would be replacing the expensive reagents with affordable, accessible and relatively less pure ones. Our research with rust collected from refuse and a fatty acid mixture obtained from edible oils showed that costs of the starting materials can be reduced by two orders of magnitude. Table 4.2 compares the costs related to the magnetite nanocrystal synthesis with lab purity chemicals and the affordable, green and everyday chemicals.
Table 4.2 Cost comparison of the synthesis of magnetite nanocrystals with pure lab chemicals and the everyday chemicals. * The cost of a kg of rust is an estimate.

<table>
<thead>
<tr>
<th>Pure lab chemicals</th>
<th>Price per kg</th>
<th>Everyday chemicals</th>
<th>Price per kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeOOH</td>
<td>$ 778.00</td>
<td>Rust</td>
<td>$ 0.20*</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>$ 20.60</td>
<td>Edible oil (coconut oil)</td>
<td>$ 0.25</td>
</tr>
<tr>
<td>1-octadecene</td>
<td>$ 24.75</td>
<td>Crystal drain opener (NaOH)</td>
<td>$ 1.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vinegar</td>
<td>$ 0.65</td>
</tr>
</tbody>
</table>

Existing green and cost effective synthetic methods are unfortunately limited for nanotechnology. In the area of quantum dot production, both olive oil [20] and Therminol 66 [21] can be used directly as solvents. These substitutions resulted in little change in the nanocrystal quality, but substantial savings in cost. Here, we explore whether these same ideas can be adapted to magnetite nanocrystals produced by the solvothermal decomposition process. It is possible to replace reagents with alternates that are both more widely available as well as economical. Moreover, the insensitivity of the reaction to time and thermal control make it feasible for low infrastructure settings.

An overview of the mechanism of magnetite nanocrystal synthesis via non-aqueous methods was assessed earlier in the chapter (section 4.1) and in the literature [12-15]. In these syntheses, a major intermediate is shown to be Iron (III) oleate, a direct salt of iron (III) with oleate anions. Figure 4.5 described a schematic outline of a reaction
that's widely accepted to be happening in the process. Since all four major synthetic methods for non-aqueous magnetite nanocrystals are compatible with this explanation of the mechanism, we assumed the progress would follow that pattern regardless of the physical parameter changes.

In a greener, alternative synthetic route, key ingredients of the synthesis, iron precursor and the oleic acid, were substituted with everyday chemicals. Edible oils presented the opportunity via saponification in replacing oleic acid with a mixture of fatty acids. Rust from an iron or steel based tool was the affordable replacement of FeOOH, the iron precursor. The new chemical reaction of the substituted synthesis is shown in equation (1).

\[
\text{FeOOH (rust) + Fatty Acid Mixture (FAM) } \rightarrow \text{Fe}_3\text{O}_4 \text{ nanocrystals} \tag{1}
\]

A saponification reaction of edible oils produces Fatty Acid Mixture (FAM), which is the mixture of the long chain organic acids that are formed once the soap is acidified. There are a number of different fatty acids that constitute FAM but 4 of them are significant: Oleic acid ((9Z)-octadec-9-enoic acid), Linoleic acid ((9Z, 12Z)-octadeca-9, 12-dienoic acid), Stearic acid (octadecanoic acid), Palmitic acid (hexadecanoic acid) (Figure 4.7) are the most common ones [22].
We found a number of parameters affected the success of the reaction using everyday items and simple reaction conditions. The type of oil used to produce FAM yields different mixtures of fatty acids, for example. As described in Figure 4.7, there are 4 major fatty acids that constitute >90% of FAMs of 12 different oils [22]. Figure 4.8 shows the TEM micrographs of the magnetite nanocrystals that are produced with pure form of different fatty acids.
Figure 4.8 Magnetite nanocrystal synthesis from FeOOH and (a) oleic acid, 10.84 ± 0.55 nm, (b) conc. oleic acid, 9.41 ± 0.92 nm, (c) stearic acid, 8.90 ± 0.60 nm, (d) linoleic acid, 7.68 ± 1.47 nm, and 1-octadecene.

Second parameter is the nature of the solvent, typically 1-octadecene (ODE). In the magnetite synthesis procedure described before (section 3.1.2.1.1) [15] ODE is generally thought to be more than just an inconsequential solvent. Figure 4.9 shows that nanocrystals can result if in effect no solvent is used, and illustrates that it is not necessary. However, under these conditions – pure fatty acid – the particles that form are quite large.
Figure 4.9 Magnetite nanocrystal synthesis with FeOOH and oleic acid (a) in ODE, 12.04 ± 1.23 nm, (b) and without ODE, 66.80 ± 13.56 nm.

The third parameter that has an effect on the reaction is the composition of iron source. For this work we went as far as to collect rust from refuse, and use this ill-defined and impure source for our reactions. Although rust is predominantly FeOOH [1], it may contain unoxidized zero valent Iron. Figure 4.10 shows X-Ray Diffraction data on different green synthesis runs with two different rust sources. Note that both spectra fit to the standard magnetite/maghemite peaks perfectly and Figure 4.10C contains some Fe (0).
Figure 4.10 Rust composition effect on the synthesis.

The last key parameter is the control over the reaction conditions – particularly the time and temperature changes. In our everyday synthesis reactions, a stainless steel pan was used and the temperature was monitored with a mercury thermometer affixed to the side of the pan.

Figure 4.11 shows the time vs. temperature curve with some visual cues for a potential kitchen synthesis experiment. Further information on the details of this method will be available at opensourcenano.net.
A comparison of the results of the controlled process using reagent grade materials, with our kitchen synthesis is shown in Table 4.3. Remarkable reproducibility can be achieved with these conditions, as seen in the similar sizes formed in the two procedures.
Table 4.3 Comparison of lab vs. kitchen synthesis products.

<table>
<thead>
<tr>
<th></th>
<th>Average Diameter (nm)</th>
<th>Standard Deviation (nm)</th>
<th>Diffraction Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab 1</td>
<td>10.84</td>
<td>0.55</td>
<td>Magnetite</td>
</tr>
<tr>
<td>Lab 2</td>
<td>66.80</td>
<td>13.56</td>
<td>Magnetite</td>
</tr>
<tr>
<td>FAM 1</td>
<td>12.04</td>
<td>1.23</td>
<td>Magnetite</td>
</tr>
<tr>
<td>FAM 2</td>
<td>52.69</td>
<td>12.56</td>
<td>Magnetite</td>
</tr>
</tbody>
</table>

Open source nanotechnology, a new way to advance and foster technology transfer to the underdeveloped world, can be applied to improve the kitchen synthesis of magnetite nanocrystals and bring accessibility to the technique, especially in water treatment applications. Through an interactive website, opensourcenano.net, potential consumers are expected to leave feedback for the whole process after their own trials and help build a free-licensed technology which would address their immediate problems, i.e. arsenic poisoning.
4.2.1 Experimental details for green synthesis

Our accessible and green synthesis route begins with soaps that are produced from edible oils. Non-edible triglycerides can also be used since triglyceride is the essential ingredient. To make soap, 100 g. of the liquid or liquefied oil was weighed in a crystallization dish or a similar container. 15 g. of crystal drain opener (or caustic soda, sodium hydroxide, potash) was weighed in a 50 mL vial (or a cup) and 30 mL of tap water was added and shaken (or stirred) until all solid was dissolved. While still warm, the solution was poured into the liquid oil and stirred with a spoon (or a magnetic stirbar) for about 15 minutes. The thick slurry was left to sit open to air in a hood (or ventilated area) to dry and cure for couple days (preferably a week). This process (Figure 4.12) is nothing more than a simple saponification reaction, as illustrated below:
The soap produced above (or commercial bar soaps) was used for making Fatty Acid Mixture (FAM) for the magnetite nanocrystal synthesis. Household acids, such as vinegar and Coca-cola were found appropriate but in this study white, distilled vinegar was the acid of choice. 60 grams of the dried soap grated using a cheese grater; one mL of acid for every gram of soap (i.e. 60 mL of acid, 650 mL of commercial vinegar with 9% acidity) was added to the soap. The mixture was heated over low flame and stirred.
until all visible pieces of the soap were dissolved (15-30 minutes). After the solution was cooled, two layers formed. The top more yellowish layer was removed and collected into a clean glass or steel jar. Further heating and boiling resulted in a yellow, cloudy solution. This material is as discussed above a Fatty Acid Mixture (FAM). Figure 4.13 shows the entire chemical scheme for the production of FAM.
Figure 4.13 Production of Fatty Acid Mixture (FAM) from a triglyceride.
The other essential component of the green synthesis of magnetite, rust, was collected by shaving off of the rust from iron or steel tools found around the campus and further ground into fine powder before use. In a typical synthesis, 1 gram of rust powder was mixed with 20 grams of fatty acid mixture in a glass or steel container. The reaction mixture was covered and subjected to heat for 2 hours until a completely black solution was observed. A strong visual cue for this process is that smoking of the mixture starts and is completed during the course of the reaction. With this setup, larger iron oxide nanocrystals, fifty to ninety nanometers in diameter, could be produced. Fifteen to twenty nanometer diameter nanocrystals were synthesized with a closed system, i.e. steam or pressure cooker.

In many applications of interest in this thesis, water soluble magnetite nanocrystals are desired. In order to transfer the waxy black deposits into water, the mixtures were treated with soapy water (the chemistry of this reaction will be discussed in Chapter 6). 10 grams of graded soap was mixed with 100 mL of tap water. Upon boiling, the soap was dissolved and the remaining chunks were filtered off. 3-5 grams of black magnetite product was mixed with a 40 mL of the soap-water solution. Stirring and boiling for 30 minutes resulted a brown solution of magnetite nanocrystals in water. The solution was filtered to remove a any magnetite not effectively phase transferred. Nanocrystals were further purified by a magnetic separation on a permanent magnet. Once deposits were collected, the supernatant was removed and additional water was used to clean the deposits from excess soap. Water (or ethanol) was used to redisperse the deposits. A schematic of the entire process of forming magnetite nanocrystals synthesis can be found in Figure 4.14.
Figure 4.14 Scheme of the synthetic process: (a) Ingredients for a typical nanocrystal synthesis include: Oil, vinegar, pan, crystal drain opener™ and rust. (b-c) Synthesis begins with soapmaking. For this the oil is mixed with crystal drain opener™ and water. (d) After curing for a day the soap solidifies. (e) For efficient dissolution in subsequent steps, the soap is ground to a fine powder, (f) then mixed with vinegar (g-h) while heating on a stove. (i-j) Once all the soap is dissolved the solution forms two layers: a yellow top layer and a cloudy white/yellow bottom layer. (k) The top layer is the fatty acid mixture (FAM). This needs to be heated at 110 °C to remove excess water and vinegar by-products. (l) Clear yellow FAM is collected. (m-n) Rust was scraped off of rusted metals and was ground to a fine powder. (o) FAM and rust were mixed. (p-q) Mixture was heated for 2 hours at below and near boiling temperatures. The temperature was measured using a standard mercury thermometer. (r-s) Magnetite (black) began to form. (t) TEM micrograph was obtained after magnetic separation in chloroform. Scale bar is 50 nm.
References


Chapter 5

Size dependent magnetic separations and Arsenic removal*

Magnetic separations can be applied to new problems in biotechnology and water treatment by exploiting the unique size-dependent properties of iron oxide nanocrystals [1]. Of particular interest here are the problems of point-of-use water purification and the simultaneous separation of complex mixtures. High surface area and monodisperse Fe₃O₄ nanocrystals are shown to respond to low fields in a size-dependent fashion. The particles apparently do not act independently in the separation, but rather reversibly aggregate through the resulting high field gradients present at the surfaces. Using the high specific surface area of Fe₃O₄ nanocrystals 12 nm in diameter the mass of waste associated with arsenic removal from water can be reduced by orders of magnitude. Additionally, the size dependence of magnetic separation permit mixtures of 4 and 12 nanometer Fe₃O₄ nanocrystals to be separated by the application of different magnetic fields.

The removal of particles from solution with magnetic fields is more selective and efficient (and often much faster) than centrifugation or filtration [2-4] and as a result

* This chapter was published in Science, volume 314, page 964-7, (Nov. 10, 2006).
magnetic separations are used in areas as diverse as biotechnology and ore refinement [5-8]. Central to the process is the generation of magnetic forces on particles large enough to overcome opposing forces such as Brownian motion, viscous drag, and sedimentation [9]. In biotechnology, magnetic separators use relatively low field gradients in a batch mode to concentrate surface-engineered magnetic beads from a suspension [10, 11]. For manufacturing, magnetic materials are typically recovered from waste streams under flow conditions with high-gradient magnetic separators (HGMS) that use larger fields (up to 2 Tesla) and columns filled with ferromagnetic materials [12, 13].

Decreasing the particle sizes used in magnetic separations from microns to nanometers would increase the available sorptive areas by a hundred to a thousand times. Such material optimization, however, is not generally practical because the magnetic force acting on a particle in a field gradient is proportion to the particle volume. If particles are too small their magnetic tractive forces in a field gradient will not be large enough to overcome Brownian motion and no separation will occur [14-16]. For iron oxide, extrapolations from the behavior of the bulk material suggest that the critical size for separation is ~50 nm for the case of an isolated (non-aggregated) particle [14]. This treatment assumes very large applied fields and the latest designs for extremely high-gradient separators, both features that make magnetic separations prohibitively expensive in many settings [4, 17]. For simpler and less costly low gradient separators, the critical size for capture in magnetic gradients increases substantially.
Extrapolations from bulk properties to nanoscale materials are frequently problematic, and a more comprehensive analysis of nanoscale magnetic behavior suggests that nanocrystals could offer significant opportunity for low field magnetic separations. Below 50 nm diameter, nanoscale magnets exhibit a complex range of size dependent behaviors, including a transition below ~40 nm in size to single domain character [18, 19], magnetic susceptibilities greater than that of the bulk material [20, 21], and the emergence of superparamagnetic behavior [18]. Such systems could experience larger magnetic forces than expected from bulk behavior due to larger moments. Hutten and co-workers have suggested the advantages of higher susceptibility materials, such as FeCo, in which an increased magnetic moment could in principle enable high-gradient separations with isolated nanocrystals [8, 22]. Additionally, in external fields the large surface gradients present at the surfaces of single domain materials could induce transient aggregation, effectively forming larger and more magnetically responsive particles. Laibinis and co-workers suggested nanoparticle aggregation, even before field application, to explain their observation of the magnetic capture of polydisperse nanocrystals in a high-gradient separation (> 1000 T/m) using fields of 1 to 2 Tesla [17, 23].

5.1 Low field magnetic separation

We have discovered [1] that magnetite particles (Fe₃O₄) of a critical size (20 nm) can be removed from solution at very low field gradients (< 100 T/m). Our data suggest that particle aggregation caused by the high field gradients at the nanoparticle surfaces helps drive this process (Figure 5.1). To determine if there exists a critical size for this
low field separation, Fe$_3$O$_4$ nanocrystals were prepared that are highly monodisperse in size and non-aggregated in both aqueous and organic solutions [24, 25].

![Image](image_url)

**Figure 5.1** A magnetic batch separation of 16 nm water soluble Fe$_3$O$_4$ nanocrystals

### 5.1.1 Material synthesis and characterization for magnetic separations

Size dependent studies require high quality nanocrystals such that errors from aggregation, poor crystallinity or solution behavior can be minimized. Figure 5.2 shows the materials that were synthesized from the high temperature (320°C) decomposition of finely ground Fe(OH)$_2$ (0.178 g.) in oleic acid (2.26 g.) using 1-octadecene (5.00 g.) as a solvent [24]. Contrast differences in the images reflect the crystalline nature of the NCs and their random orientations with respect to the electron beam. Panel A shows particles of average diameter 12 ± 1.0 nm while panel B samples are 4.0 ± 0.3 nm. The smaller
sizes are synthesized by Sun’s method [25] which refluxes at 265°C a mixture of 2 mmoles of Fe(acac)_3, 10 mmoles of 1,2-hexadecanediol, 6 mmoles of oleic acid, 6 mmoles of oleylamine and diphenyl ether (solvent) under nitrogen. As seen in panel C, these samples have no magnetic moment unless an external field is applied; as expected, the larger size reaches its saturation magnetization at lower field than the smaller size. Illustration indicates that the surface coating adds about 3.6 nm to the core diameter in defining the hydrodynamic diameter. From the inset inscribed as panel E, both of these materials show no residual magnetization at zero applied field.

Figure 5.2 (A – B) TEM micrographs showing arrays of highly monodisperse Fe_3O_4 NCs. (C) Normalized magnetization (magnetization/maximum magnetization) vs. applied field (Oe) for two representative samples, 16 nm and 4.0 nm NCs. (D) A schematic of an oleic acid coated magnetite NC [circles are iron (black), oxygen (red) and carbon (blue) - hydrogens were omitted for clarity]. (E) Inset shows an expansion of the magnetization data near zero field (-100 Oe to 100 Oe).
In Figure 5.3 a sample library that shows various sizes of magnetite nanocrystals is given to secure that the necessary dispersions of magnetic nanocrystals exist for a successful size dependent study. Representative transmission electron micrographs of all materials used in this work are shown along with the histogram of their diameters. The average size as found from counting over 1000 nanoparticles is reported. For these values we used two significant figures which was the error imposed by the sampling error for a population of $N = 1000$. 
Figure 5.3 Sample Library.
Figure 5.4 shows the cryogenic transmission electron microscopy data of magnetite nanoparticle suspensions to prove their independent (non-aggregated) behavior within their solutions. Panels A and B show cryogenic TEM images of magnetic nanocrystal suspensions before magnetic separation. For these experiments water solutions of iron oxide nanocrystals were flash frozen to produce a thin film of amorphous ice, and this specimen was imaged using a JEOL-200 equipped with a cryogenic sample stage. This technique is widely used in structural biology and the freezing process has been shown to preserve the room temperature solution state structure of complex biomolecules [26, 27]. Panel A shows Igepal CO 630® coated nanoparticles similar to those used for arsenic experiments. This particular image is displayed because it contains many nanoparticles and the solution is necessarily much more concentrated suspension than that used in this work. These nanoparticles are not fused crystallites nor do they show any indication of large-scale (e.g. > 100 nm) clustering. Panel B shows a similar sample which has been stabilized with a thicker amphiphilic polymer coating that is also water soluble. Nanoparticles are well separated in this image and show no evidence of interparticle interactions. Both types of nanoparticles exhibit similar low gradient magnetic separations.
Figure 5.4 Cryogenic transmission electron microscopy of iron oxide nanoparticle suspensions.

Dynamic light scattering (DLS) of iron oxide nanocrystal suspensions is shown in Figure 5.5. The DLS data was collected on dilute suspensions of iron oxide nanocrystals using a Malvern Zetasizer Nano ZS machine; a column graph fit was used to calculate the nanoparticle size. Panels A, B and C show similar results for 4.0, 8.0 and 16 nm iron oxide cores; light scattering finds average particle sizes range from 10 to 20 nm. These results are quite good considering the semi-quantitative nature of DLS when applied to nanoscale systems. Most critically for this work is the complete absence of any aggregates in suspension (e.g. no DLS signals for larger sizes). This is consistent with cryogenic TEM images that show no hard aggregation of these materials.
Figure 5.5 Dynamic light scattering (DLS) of iron oxide nanocrystal suspensions.
Powder x-ray diffraction data for 4.0 and 6.0 nm Fe$_3$O$_4$ nanocrystals from a Rigaku D/Max Ultima II are shown in Figure 5.6. Black plot corresponds to 4.0 nm diameter iron oxide and red plot to 6.0 nm. The orange lines represent the theoretical diffraction pattern for a magnetite crystal from Jade 8 software’s library for crystals. XRD data for bigger sizes of nanocrystals are published in an earlier report [24].

**Figure 5.6** Powder x-ray diffraction data for 4.0 and 6.0 nm Fe3O4 Nanocrystals from a Rigaku D/Max Ultima II.

Having previously established that the surfaces of nanocrystalline magnetite are useful in the context of arsenic removal from solution, this problem will be used as an example for a high-throughput separation [28].
5.1.2 Batch magnetic separations at low fields

Nanocrystalline Fe$_3$O$_4$ could be removed from solution with a low gradient separator (23 T/m) similar to those applied to recovery of micrometer-sized beads in protein purification.

Figure 5.1 shows a magnetic batch separation of 16 nm water soluble Fe$_3$O$_4$ NCs with a conventional separator, Dexter Magnetic LifeSep 50SX. The field gradient at full field was 23.3 T/m. The initial rust colored solution contains Fe$_3$O$_4$ Nanocrystals of 16 nm diameter homogeneously dispersed in water. Panel A shows the appearance of the solution immediately after placement in the separator. After several minutes, the initially homogeneous solution becomes heterogeneous and a black deposit forms on the back wall where the gradient field is the highest (panel B). After removal from the separator, the solution can be restored to its initial state with a vigorous shake. Similar behavior is observed for all nanocrystals larger than ~10 nm, but the time for complete separation varies with solution concentration and nanocrystal size.

5.1.3 Reversible aggregation concept for the low field behavior

The removal of nanocrystals cannot be explained with a simple model of non-interacting particles. If we assume that the iron oxide particles act independently, we may calculate a size cut-off below which nanocrystals will not be removed. For a 23-T/m field gradient $\nabla B$, the largest magnetic force that can be applied to a single particle of diameter $d$ is

$$F_{mag} = \frac{4\pi}{3} \left(\frac{d}{2}\right)^3 M_{sat} \nabla B,$$

where $M_{sat}$ is the saturation magnetization of the
material. In order for these particles to separate effectively, this force must exceed the typical Brownian force, \( F_B \sim \frac{k_B T}{d} \). For magnetite, with \( M_{\text{sat}} = 4.69 \times 10^5 \text{ A/m} \), at 300 K, this implies that independent particles smaller than \( \sim 160 \text{ nm} \) in diameter will not separate in the low field gradients we used. The previous analysis ignores the fact that the application of an external field will generate extremely large field gradients (up to \( \mu_0 M_{\text{sat}} / d \)) at the surfaces of single-domain particles. Even in the absence of a field, magnetic nanocrystals may interact due to magnetic dipole-dipole interactions [27]. In the presence of a field, these surface gradients could generate chains or aggregates of nanocrystals. Once the field is removed surface gradients would disappear and particle interactions would diminish. This reversible aggregation provides the advantages of a high surface area nanocrystalline sorbent, without sacrificing the ability to separate the materials in a reasonable field gradient.

5.2 Size dependent magnetic separation

The size dependence of the nanocrystal separation process was characterized using a high-gradient magnetic separator. Unlike the simple batch separator used in Figure 5.1, this system uses an electromagnet to generate external fields of variable strength around a column packed with ferromagnetic wire. Exact measures of the field gradients in such a system are challenging, but most treatments predict that the gradient and applied external field are proportional and that at fields in excess of 1T, gradients of \( 10^4 \text{ T/m} \) are possible [29, 30]. \( \text{Fe}_3\text{O}_4 \) Nanocrystals of varying sizes were gravity fed into the 22.3-cm long column at various field strengths and the effluent collected and
analyzed for iron content using atomic emission spectroscopy. From such experiments, the fraction of material retained in the column was calculated at increasing magnetic field strengths, and compare these retention efficiencies between samples.

The results are shown in Figure 5.7 where panel A shows the size-dependent magnetic separation of 4.0, 6.0, 9.1, 12 and 20 nm Fe$_3$O$_4$ in a column separator. For this work, a hexane dispersion of nanocrystals was passed through a stainless steel column packed with 15 grams of stainless steel wool; solutions were introduced at 20 mL/min using gravity feed. The column was held in an S. G. Frantz ® canister separator (tunable field 0 to 1.6 Tesla). After each data point was taken, the packing was removed and the column was washed thoroughly, dried in an oven (60°C), and packed with clean, unused, stainless steel wool. Fractions collected at each data point were digested in conc. HNO$_3$ and diluted to 5.5 % HNO$_3$ before iron content analysis using ICP-AES. The % retention was calculated by dividing the atomic iron concentration in a solution by the concentration found for the starting (unseparated) suspension. Curves presented are complex polynomials meant to guide the display and are not reflective of any physical model. These data illustrate that the smaller the nanocrystal, the greater the magnetic field required retaining the nanocrystal in the column. The 20 nm diameter particles permanently affix to the column after removal of the field. Panel B displays the absolute field required to retain 100% of the nanocrystals loaded to the stainless steel column (black) versus the diameter of Fe$_3$O$_4$ nanocrystals is presented. Also shown (right axis) are the fractions of material that are unrecoverable after washing the column. The shaded area represents the optimal size for magnetic separations. For 4.0 nm and 6.0 nm,
materials which were not completely retained, the absolute field for complete retention was estimated from their low field behavior.

![Graph showing size-dependent magnetic separation](image)

**Figure 5.7** Size-dependent magnetic separation of 4.0, 6.0, 9.1, 12 and 20 nm Fe₃O₄ in a column separator. Panel B represents the absolute field required to retain 100% of the nanocrystals loaded to the stainless steel column (black) versus the diameter of Fe₃O₄ nanocrystals.

The amount of material retained in the column increases as the external field strength increases. For example, nearly 100% of the 12 nm diameter nanocrystals are retained in the column at applied fields of only 0.2 Tesla, well below the saturation
magnetization for stainless steel. This same field, however, cannot capture nanocrystals less than 8.0 nm in diameter. The inset in Figure 5.7 shows that for all particles, as the domain size becomes smaller more field is required to ensure their complete separation. This result parallels the observation (Figure 5.2C) that at low field strengths small nanocrystals are not fully magnetized. Without complete magnetization, the magnetic moments of nanocrystals would be quite small and would not generate enough tractive force with external field gradients [20, 31].

The size of nanocrystals can also influence their recovery after magnetic capture. At zero external field (after columns are magnetized) nanocrystals larger than 16 nm diameter cannot be removed from the column matrix even after repeated washes. This irreversible interaction is analogous to the fouling of a physical filter, and would limit the use of larger magnetic sorbents in a commercial setting. Smaller nanocrystals, however, do not show such behavior and can be concentrated and reused quite easily. This observation stems from the fact that below about 16 nm diameter, iron oxide nanocrystals behave as superparamagnets [18, 31-34]. In this limit, Nanocrystals have no remnant magnetism (Figure 5.2E) and thus experience no interactions with the very small stray fields present in the ferromagnetic column matrix [35]. This result is consistent with observations of commercial magnetic beads which found that even in micron-sized systems there was much value to using magnetic materials that were superparamagnetic [36, 37]. The data shown here reveals that superparamagnetism is only one of several properties that should be considered in material design. Indeed, if nanocrystals are too small then magnetic separations require very large critical field strengths to affect any
capture of particles. For optimal nanocrystal magnetic separations at low fields one should use the largest nanocrystals which still show superparamagnetic properties.

5.2.1 Bimodal separation

The size dependence of nanocrystal capture shown in Figure 5.7A now makes it possible to develop magnetic separation processes that can in one process remove several different types of materials. Such capability is particularly important in biotechnology where the simultaneous treatment of mixtures requires more sophisticated multiplexed separations [38].

Figure 5.8 demonstrates the principle for magnetic separations in which different field strengths recovered different populations of a bimodal distribution of iron oxide nanocrystals. 4.0 nm and 12 nm Fe₃O₄ nanocrystal solutions (both in hexanes) were mixed in a 1:3 ratio (v/v) to achieve a particle mixture that was roughly the same concentration of each size. Using an S. G. Frantz ® Canister Separator (Model L1-CN) the mixture separated into two size fractions depending on the field. Panel A shows the TEM micrograph of the initial bimodal mixture. In panel B, TEM micrograph of the high field (0.3 T) fraction is shown in which 94.4% of 4.0 nm recovered and less than 3% of the particles are larger. Size bar is the same as Figure 5.8A. Panel C displays a TEM micrograph of the low field (0.03 T) fraction where 98.3% of larger (12 nm) nanocrystals are collected. Panels D to F, size distribution histograms for panels A to C, respectively counted. In panel A two different populations are observed. In panel B the smaller size range is apparent as observed in the TEM micrograph. In panel C the larger sizes are
successfully recovered separation. For all size histograms > 1000 particles were counted in multiple images and measured automatically using the software package ImagePro®.

\[ \text{Figure 5.8 Multiplexed separation of nanocrystal mixtures.} \]

Initially, the sample consists of two monodisperse fractions of nanocrystals intentionally combined to create a test solution (Figure 5.8A); at low applied fields (0.3 Tesla), the effluent from the column contains >90% the smaller size, and the larger size is retained (Figure 5.8B). After the field is turned off, a column wash recovered the larger fraction (Figure 5.8C). Using monodisperse iron oxide nanocrystals it is thus possible to use magnetic separations in a multiplexed mode and recover different components of a mixture in one treatment.
5.3 Arsenic removal*

Since nanocrystals can be removed from batch solutions using permanent, handheld magnets, a question was raised whether these nanocrystals could act as effective magnetic sorbents for the removal of arsenic from water. Arsenic is a good model contaminant for these materials as its interaction with iron oxides is strong and irreversible even on the nanoscale particles [28, 39], and its practical and effective removal from groundwater remains an important and intractable problem in water treatment [40, 41]. Conventional high-gradient magnetic separators operating at 1 Tesla and higher already find use in water treatment processes, primarily to induce aggregation of intrinsically magnetic waste products not easily amenable to other methods of coagulation [13, 28, 42, 43].

Both As (III) and As (V) exhibit strong adsorption onto iron oxide nanocrystalline surfaces; as the concentration of arsenic increased in the standard solution, the amount of As bound to nanocrystals increased as well until the capacity of the material was reached. The interactions between iron oxide and arsenic are also irreversible, so Fe₃O₄ nanocrystals that have bound As should not release the material back into the environment during storage and further treatment. It was clear that as the size of sorbent nanocrystals decreases, sorption capacities increase substantially because of the increased surface areas in the samples.

* This section was made possible by the help of Drs. Sujin Yeon, Lili Cong, Amy Kan, Mason Tomson
Once arsenic was sorbed onto nanocrystal surfaces, magnetic separations provided a way to remove the materials from solution. The As concentrations in test solutions before and after removal of particles using a column magnetic separator (Table 5.1) show that As (III) and As (V) can be reduced to well below the current U.S. standards for drinking water [44]. Also, because the test nanocrystals are very small (12 nm) their very high specific surface areas provide extremely concentrated waste materials. For example, 300-nm iron oxide particles have a sorption capacity of only .002 % (w/w) and thus to treat 50 L of 500 μg/L arsenic generates 1.4 kg of waste; in contrast, for an equivalent treatment only 15 grams of 12 nm iron oxide sorbent is required.

### Table 5.1 A comparison of As removal efficiency assuming a treatment of 2 L of As solution (500 μg/L) with 1 g magnetite.

<table>
<thead>
<tr>
<th>Particle Size (nm)</th>
<th>As(V) or As(III)</th>
<th>Residual As Concentration (μg/L)</th>
<th>% Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>As (III)</td>
<td>3.9</td>
<td>99.2</td>
</tr>
<tr>
<td>20</td>
<td>As (III)</td>
<td>45.3</td>
<td>90.9</td>
</tr>
<tr>
<td>300</td>
<td>As (III)</td>
<td>375.7</td>
<td>24.9</td>
</tr>
<tr>
<td>12</td>
<td>As (V)</td>
<td>7.8</td>
<td>98.4</td>
</tr>
<tr>
<td>20</td>
<td>As (V)</td>
<td>17.3</td>
<td>96.5</td>
</tr>
<tr>
<td>300</td>
<td>As (V)</td>
<td>354.1</td>
<td>29.2</td>
</tr>
</tbody>
</table>
References:


Chapter 6

Green and open source nanotechnology*

Nanomaterials may enable technological solutions to critical problems now faced by the developing world in the areas of water treatment, energy production and healthcare [1, 2]. To see these technologies applied successfully, however, requires innovative manufacturing strategies that not only make these materials at low cost, but also in ways that are transferable to the minimal infrastructure found in the developing world. In this chapter, a new scheme for generating nanocrystals that relies on everyday items and simple tools of the type found in kitchens is presented. The method takes advantage of the particle growth mechanism found when metal salts are decomposed in fatty acid mixtures; surprisingly high quality magnetic nanocrystals can be generated even using starting materials such as edible oils (e.g., olive oil) and rust collected from refuse. Quantum dot materials formed in a similar fashion are not as uniform in size, but the products still display the striking optical features essential for their applications. The optical and magnetic properties of these systems can be verified using relatively simple tools, and this information can provide feedback on a given sample’s utility. Potential users of nanotechnology in the developing world could employ these methods as a

* This chapter is under review for publication at “Nature Nanotechnology”
starting point to develop nanomanufacturing for micro-businesses or possibly even household settings [3]. Ultimately, the methods described here are expected to be adapted and refined by the global community using open source techniques pioneered by hackers and software engineers.

6.1 Low cost and accessible nanotechnology

This work introduces a paradigm for creating nanoscale materials which not only lowers their cost, but also enables small-scale production and rapid innovation at sites with little to no conventional manufacturing infrastructure. Arsenic contamination in ground water is a severe global problem, most notably in southeast Asia where millions suffer from acute and chronic arsenic poisoning [4-6]. Removing arsenic from groundwater in impoverished rural or urban areas without electricity and with no manufacturing infrastructure remains a significant challenge [7]. Recent work suggests that low-field magnetic removal of nanoscale arsenic sorbents may be a viable solution [8]. A substantial barrier for transferring this knowledge, as well as other nanotechnologies designed for global use, is the difficulty with engaging local communities in the development and commercialization process. The inaccessibility and high cost of the essential material components — often extremely uniform nanocrystals — contributes significantly to the problem. Even if nanomaterials and systems are given away through charitable endeavors, end users still have little ability to adapt and ultimately commercialize the technologies through their local economies.
In order to develop users, who are active partners, and ultimately experts in the technology, requires that two different elements come together in a manufacturing strategy. The first is a production process which optimizes not only cost and scale, but also uses simple, available and transferable materials and techniques. Research on nanocrystal production has recently begun to emphasize the cost, efficiency and environmental impact of standard processes [9, 10]. For materials destined for the developing world additional consideration must be given to the simplicity and accessibility of a process. Similar to work in “appropriate technology”, this strategic element defines a successful manufacturing approach as one that makes use of everyday tools and materials, is appropriate for the local infrastructure, and is easily adapted by interested users [11, 12].

An equally important consideration is the means by which this manufacturing information is shared. Thirty years ago, the only way to imagine such information transfer would have been through a network of costly laboratories and facilities in different locales, each grappling with legal, social and communication issues. Now, access to the Internet, coupled with the principles of the Free and Open Source (FOS) movement, have shown that users all over the world can become innovators, manage systems complexity in new ways, and navigate the legal issues associated with peer production [13, 14]. The success of FOS has been demonstrated in software, and increasingly in other domains as well [15]. Its application to nanotechnology would be novel, and especially important for global technology as it is ideally suited to empower local users to define and ultimately adapt its content for their unique needs. Open-source
frameworks can only work, though, if scientists have developed appropriate nanomanufacturing processes, with reproducible and clear instructions, using widely available materials. Taken together, appropriate manufacturing processes and the FOS model result in what could be called a “vernacular nanotechnology” — or a nanotechnology developed by a shared and evolving set of manufacturing methods.

Applying this principle of vernacular nanotechnology presents a significant research challenge for materials chemists. Nanotechnology is based on the unique properties of highly complex materials whose performance often depends on accurate control over nanometer scale features. Researchers and industry alike rely on million dollar lithographic facilities, electron microscopes and state-of-the-art chemical laboratories to achieve their materials performance. Indeed, it may be that for some processes — particularly top-down fabrication — appropriate manufacturing methods could never be achieved. However, chemical methods for forming nanomaterials are reasonable targets for appropriate manufacturing. Research over the last few decades has provided a wealth of practical knowledge and understanding of the mechanisms governing nanocrystal formation in solution phase reactions [16, 17]. This base of knowledge suggests that under the right conditions, even with poor thermal control, impure solvents and ambient atmospheres, it may be possible to generate reasonably high quality and functional nanocrystals.
6.2 Exchanging reactants with cheaper substitutes

The focus was first on finding nanocrystal starting products that in the spirit of our vernacular approach would be simply generated from everyday and inexpensive items. Monounsaturated fatty acids, particularly oleic acid (cis-9-octadecenoic acid), are central components in the preparation of both semiconductor (CdSe) and iron oxide (Fe₃O₄) nanocrystals [16, 18]. These acids are referred to as ‘ripening agents’ because they stabilize the soluble forms of the metal which both limits crystal nucleation (leading to larger particles) and promotes particle growth at intermediate times [19]. They can serve as both the solvent and stabilizer, or they can be diluted with inert heat transfer fluids and combined with other additives to tune the nanocrystal growth process [16].

6.2.1 Fatty Acid Mixture (FAM) vs. Oleic acid

A common source of these organic acids are several edible oils found in kitchens worldwide. These oils are predominantly triglycerides (> 90 w/w%) which can be converted to their carboxylate salts (e.g. soap) through a simple saponification reaction. The resulting soap can then be acidified with vinegar forming an organic acid, and leaving the unwanted glycerin and other impurities behind in the aqueous phase. In the case of olive oil, the resulting fatty acid is approximately 70 % pure oleic acid, and its impurities include linoleic acid (12 %), palmitic acid (12 %) and stearic acid [20]. This mixture is referred as ‘FAM’ for fatty acid mixture throughout the chapter, noting that its composition is highly sensitive to the origins and quality of the starting oil [21]. The use of extra virgin olive oil, for example, increases the yield of oleic acid in the final mixture as compared to lower grade olive oil. While the focus is on oleic acid from olive oil,
edible oils from many other plants (including coconut oil, linseed oil and others) all contain significant amounts of fatty acids appropriate for these reactions [22].

6.2.2 Rust vs. FeOOH

The iron precursor for magnetite nanocrystal preparations can also be replaced with a common item: finely ground rust collected from refuse. In a conventional reaction, bulk iron oxides or salts are first dissolved at lower temperatures in fatty acids to form iron carboxylates; these are then decomposed at higher temperatures to yield nanocrystalline iron oxides [23]. Rust is a reasonable replacement since it is the corrosion product of iron-containing metals and itself is a mixture of iron hydroxides and iron oxide [24].

6.3 Variation in green synthesis

In Figure 6.1, synthesis of magnetite nanocrystals from everyday chemicals is summarized. Panel A shows the ingredients and tools for a typical nanocrystal synthesis: (left to right) Edible oil, vinegar, pan, crystal drain opener and rusted steel. Reaction equation is given on the top right where FAM stands for Fatty Acid Mixture. In panel B, TEM micrograph of 9.65 ± 1.85 nm magnetite nanocrystals after magnetic separation in chloroform is displayed. For the synthesis pure FAM was used with a diluent to generate smaller sizes, 0.1884 grams of iron from rusted stand from lab hood, and a temperature of 322 °C for 55 minutes. Scale bar is 50 nm. Panel C has an uncorrected X-Ray diffraction of magnetite nanocrystals synthesized from everyday chemicals. The inset also provides a TEM electron diffraction of as synthesized magnetite nanocrystals.
Figure 6.1 Synthesis of magnetite nanocrystals from everyday chemicals
6.3.1 Size control with diluent amount

When a rust/FAM solution is heated to above 270 °C, it produces a black suspension similar in appearance to that made with laboratory reagents. The electron micrographs of these samples (Figure 6.1B) indicate the presence of large and well-separated nanocrystals, and the electron diffraction and x-ray diffraction (Figure 6.1C) are consistent with the magnetite structure. The size of the iron oxide nanocrystals produced using impure FAM and rust can be controlled in much the same way as for laboratory reactions. If the FAM is used without dilution, the resulting nanocrystals are larger (67 nm) with a cubic habit [25]. The addition of a diluent (1-octadecene) lowers the effective ratio of fatty acid to iron resulting in more nucleation and ultimately smaller nanocrystals with a hexagonal shape [26, 27].

Figure 6.2 shows the size control with changing diluent amount in kitchen synthesis of magnetite nanocrystals. In panel a, ferric hydroxide oxide (FeOOH – Aldrich) was cooked with Fatty Acid Mixture (FAM) without any diluent to produce magnetite nanocrystals. Particle size is 67 ± 14 nm. Scale bar is 50 nm. As shown in panel b, monodisperse magnetite nanocrystals were achieved when FeOOH was cooked with FAM in the presence of 1-octadecene (ODE – Aldrich) as a solvent in a setup with condenser. Particle size is 12 ± 1.2 nm. Scale bar is 50 nm. For the data shown in panel c, rust (from rusted metal in Figure 6.1) was cooked with FAM with minimal amount of ODE in a setup without condenser (less or no diluent – dry product). Particle size is 47 ± 18 nm. Scale bar is 100 nm. In panel d, rust (from rusted metal in Figure 6.1) was cooked
with FAM with solvent amount of ODE in a setup with a condenser (solution integrity preserved). Particle size is 9.7 ± 1.8 nm. Scale bar is 100 nm.

Figure 6.2 Size control with changing diluent amount in kitchen synthesis of magnetite nanocrystals.
6.3.2 Temperature effect on the green synthesis

The nanocrystalline product of the synthesis is sensitive to the temperature of the FAM/rust mixture. Once temperatures are above ~280 °C the reaction will occur, and particle sizes increase as the temperature of the reaction approaches the boiling point of FAM (> 340 °C estimated for these mixtures). A conventional frying pan enclosed loosely by a cover or tin foil was suitable for relatively constant temperature control; the FAM may oxidize at these temperatures in air but it is found that oxidation has little impact on the reaction products [18, 26]. Using equipment commonly found in a kitchen, it was found that one can controllably vary the nanocrystal size over a wide range while retaining reproducibility batch to batch of 10% on the diameter. Restaurants and home kitchens often are equipped to heat frying oil to comparable temperatures, and it is noted that if lower temperatures are desired then edible oils that yield shorter chain fatty acids may be preferred [20, 22].

Figure 6.3 shows that with the increase in temperature of the kitchen synthesis average size increases proportionally. Note that there are no nanocrystals at 180 °C. Picture A shows 37 ± 7.9 nm magnetite nanocrystals which were obtained at 308 °C. Picture B has the 43 ± 16 nm magnetite nanocrystals that were achieved at 312 °C. In c, 86 ± 12 nm magnetite nanocrystals were collected when cooked at 350 °C (near boiling point). For all of these reactions rust was added to FAM, the mixture was heated to 312°C in 60 minutes and cooked for another 60 minutes. Additional experimental information can be found at opensourcenano.net (website).
6.3.3 **CdSe nanocrystal synthesis**

To explore the generality of the kitchen synthesis approach, quantum dots were also produced using laboratory precursors but with FAM from olive oil and in equipment with limited temperature control. These systems are a much more stringent test for appropriate manufacturing methods in that their size distributions are well known to be highly sensitive to solvent purity, reaction temperature and additives such as phosphonic acids [28, 29]. The process yielded crystalline cadmium selenide nanocrystals that are
functional in that they have size-dependent and strong emission characteristic of a quantum dot material (Figure 6.4). However, the nanocrystals are not uniform in size and as a result the optical absorption spectra lack a well-defined exciton peak (Figure 6.4). The incorporation of additives such as phosphonic acids are likely to improve the size distributions, but we note that some users may find the as-prepared optical materials useful as broadly absorbing films for sunlight [30].

Figure 6.4 Magnetite and CdSe nanocrystals from kitchen chemicals.
Figure 6.4A shows a visual magnetic separation of homemade magnetite nanocrystals with a horse shoe magnet (0.13 Tesla). Figure 6.4B has a TEM micrograph of 35 ± 16 nm home made magnetite nanocrystals dispersed in water. Shown in Figure 6.4C is the TEM micrograph of 12 ± 2.7 nm CdSe nanocrystals made by dissolution of Se in fatty acid mixture (FAM) with little trioctylphosphine (TOP) and heating, followed by an injection of CdO in FAM until the recovery at several minutes later and 4.7 ± 0.7 nm CdSe nanocrystals at 10 seconds after injection (Figure 6.4D). Figure 6.4E has the UV-Vis absorption plots of two different sizes (upper, red plot: c, lower, black plot: d). The inset displays the digital pictures of CdSe nanocrystals solutions (left:c, right:d) under UV light (366 nm).

6.4 Open source nanotechnology

An important element of the kitchen synthesis approach to nanocrystal production is the ability to obtain rapid feedback on the reaction products using simple tools; such information is central if these methods are to be adapted and improved by local parties. It was examined here whether the functional properties of these nanocrystals could serve as the basis for evaluating the material quality. Figure 6.4 shows that a handheld magnet applied to a solution of nanocrystalline magnetite results in particle collection, and can replace more expensive evaluations of magnetic properties that rely on magnetometers (Figure 6.4B). Its magnetic properties and solution color are an indication both of its size as well as crystalline quality [8]. Additionally, quantum dots can be evaluated using handheld ultraviolet lamps for their fluorescence yield, which is easily detected with the naked eye under normal light conditions [31].
6.4.1 Methods of involvement

The work in this chapter shows that it is possible to create functional and high quality nanocrystals using methods appropriate for manufacturing in diverse and minimal infrastructure. It is suggested that the transfer of this knowledge is best achieved using the open source movement for guidance. FOS has demonstrated in the case of software (e.g. Linux, Apache and GNU) that when a working solution is legally available and well-coordinated, volunteers can and will work in parallel to innovate and adapt the solution to a vast array of circumstances. FOS also works because of widely and cheaply available hardware; by analogy, nanotechnology’s “source code” cannot rely on expensive, scarce or extreme production methods, but must similarly make use of the most widely available platforms, as in our case of magnetite production. Taken together, these methods and the FOS system may provide an alternative technology transfer process that encourages local innovation and micro-business using knowledge that is secured in a legal commons for all to use. Based on this preliminary work in magnetite production, we have begun a small-scale version of open source nanotechnology, and we encourage interested readers, and members of the global scientific community to participate (opensourcenano.net).
References


[15] Since roughly 2001, the principles of open source have been successfully applied in the following domains: music and film (Creative Commons "creativecommons.org", Open Source cinema "opensourcecinema.org"), textbook production (Connexions "cnx.org", MIT's Open CourseWare "ocw.mit.edu"),
Robotics (Terk "terk.ri.cmu.edu"), Biotechnology (Cambia "cambia.org") and synthetic biology (BioBricks Foundation "biobricks.org").


[29] W. W. Yu and X. G. Peng, "Formation of high-quality CdS and other II-VI semiconductor nanocrystals in noncoordinating solvents: Tunable reactivity of


Chapter 7

Silica and Silver Coating of Magnetite Nanocrystals

As discussed in Chapters 3 and 4, a major limitation of the oil-based solvents for nanomagnetite synthesis is the resulting insolubility of the product in water. To exploit the many great features (e.g. narrow size, high crystallinity) of the materials in biological and environmental applications requires additional steps to render them functional in aqueous phases [1, 2]. Section 3.3 describes methods for a successful transfer of the magnetite nanocrystals into water, and in this chapter we expand upon that by applying more extensive functionalization chemistry.

Our general approach is to use conventional polymer [3-8] and silica chemistry [9-16] to modify the surface of nanomagnetite with a versatile chemical coating that separates and stabilizes the particles in water [13, 17-19]. This strategy has been applied to a wide range of nanoparticles already, and in this work we focus in particular on the use of silica as a platform for further functionalization [4, 17, 20-26]. We note that polymer coatings were briefly assessed in section 3.3.4. Here, we describe methods that permit magnetite-silica materials to be platforms for the attachment of anti-microbial silver nanoparticles [27, 28].
7.1 Silica coating: Nanoshells of Magnetite – SiO₂

Nanocrystals, when coated with contiguous layers of a second material, can offer new chemical [29], optical [30], magnetic [26], catalytic [31] and reactive properties [18]. For example, silica coating on FePt was reported to prevent sintering during annealing, a crucial step for obtaining the highly magnetic anisotropic phase, L₁₀ [21]. Gold nanoshells, with their unique plasmon resonances, have been a prime example of the optical properties a shell can bring to a nanocrystal system [32, 33]. The luminescence properties of CdSe particles can be enhanced when coated with CdTe or ZnTe [34]. Multishell metallodielectric particles with unique optical fingerprints were also constructed of a silica-silver-titania triple structure [35]. In a recent report, the synthesis of a novel water soluble hybrid material consisting of quantum dots (QDs) and magnetic nanoparticles (MPs) encapsulated in a silica shell was shown [22]. Multimodal imaging was achieved by the synthesis of water soluble, silica coated, ZnS passivated Cds:Mn (Cds:Mn/ZnS/SiO₂ core/shell/shell) fluorescent quantum dots which were further functionalized with Gd (III) ions to achieve the paramagnetic property required for an MR imaging contrast agent [36]. Selvan et. al described the synthesis of a bifunctional core-shell system consisting of γ-Fe₂O₃ nanoparticles and CdSe quantum dots for their use in the imaging of live cells and targeting cell membranes by biolabeling [37].

Silica is an ideal coating for nanoparticles, both because its surface functionalization chemistry is well established and because it is a good material to prevent nanoparticle aggregation. Attachment of a wide variety of biomolecules to silica
has been reported, and silica shells on nanoparticles offer the perfect platform for attachment of biological molecules such as avidin [22]. In another example, silica coatings are essential for forming isolated nanoparticles. For example, the anisotropic dipolar attraction of iron oxide nanocrystals can lead to particle aggregation and the loss of the size dependent magnetic properties. While capping molecules, such as surfactants, can block the interparticle contacts necessary for interactions, they must be applied at high concentrations and often are not irreversibly bound. Silica shells, on the other hand, are more stable and effective for this purpose [24]. In addition, one can preserve the shape of the original core in the initial stages of coating because of the slow deposition of silica in water [38]. Two major factors are found to be in play for the stability of silica sols: (i) the Hamaker constant ($A_H$) of silica is much lower than those of metals and other oxide particles, and, (ii) a polymeric silicate layer is present at the silica-water interface at pHs higher than 10.5 on which hydrated cations are very tightly bound [38, 39].

A particularly important reason to use silica as a coating is its well documented biocompatibility. A silica shell will also provide a chemically inert surface for any nanoparticle which will be applied in biological environments. Furthermore, it will improve the water solubility and prevent aggregation of nanocrystals in biological buffers [23]. For these reasons, silica coating of magnetite in particular has been important for its development in biomedical applications including guided drug delivery, tumor targeting and biomagnetic separation of cells and proteins [25, 40].
We adapted existing procedures for silica coating chemistry from this literature for our nanocrystalline magnetite [21, 23, 24, 41]. Because our materials are formed in organic solvents and except for FePt system [21] no silica coating procedure was reported to date on Fe$_3$O$_4$ that is synthesized via non-aqueous methods, some important modifications were necessary. Like other approaches, we relied on the hydrolysis of tetraethyl ortho silicate (TEOS) using microemulsion deposition system. By operating under the appropriate pH conditions we ensured that silica formation only occurred at magnetite surfaces, and not free in solution. Additionally, we used microemulsions to confine the silica coating chemistry to smaller solution environments.

Reverse micelle microemulsion technique was used to produce silica coated magnetite nanocrystals. Since magnetite nanocrystals were hydrophobic, reverse micelles were formed first by Igepal CO® 520, and then silica coating was carried out by microemulsion via pH controlled hydrolysis of TEOS. The final product, silica coated magnetite nanocrystals, were soluble in water and other highly polar solvents.

With this general chemistry, it was possible to coat silica onto monodisperse and highly crystalline magnetite nanoparticles (Figure 7.1).
Figure 7.1 Silica (SiO$_2$) coated magnetite nanocrystal system. Core (Fe$_3$O$_4$) is 16 nm and the shell (SiO$_2$) is 34 nm.

In a typical silica coating reaction, 10 mL Hexane dispersion of Fe$_3$O$_4$ nanocrystals (made via methods described in Chapters 3 and 4) was dried evaporating the organic solvent at room temperature. 10 mL of cyclohexane (Aldrich) was added to the dried residue and sonicated to produce a dispersion of the material. 0.5 mL Igepal CO®520 (Aldrich) was added to 10 mL cyclohexane, then 1 mL of the cyclohexane dispersion of Fe$_3$O$_4$ nanocrystals was added to this. After sonication, 100 µL NH$_4$OH (Fisher) and 80 µL TEOS (Aldrich) were introduced into the suspension. The mixture
was stirred for ~24 hours under ambient conditions. Then, a 3x volume of EtOH (Fisher Scientific) was added to precipitate the nanomaterials, and centrifugation (3200 rpm, 5 minutes) of the mixture yielded a well defined pellet. This precipitate was dispersed in ethanol or H₂O (DI water, Millipore) with some batch sonication (see Chapter 2 for the sonication techniques). By using a permanent magnet, the silica-coated magnetite could be easily purified and cleaned by successive separation and redispersion.

Important variables to control in this experiment are the base concentrations of TEOS and NH₄OH, as well as their molar ratio. Their manipulation led to the some tuning of silica shell thickness, and under some circumstances could increase the number of clustered magnetite materials. Figure 7.2 and Figure 7.3 show the Transmission Electron Micrographs (TEMs) from some of these reactions. In these cases, the starting magnetite nanocrystals are 9 nm and 16 nm in diameter showing that the basic chemistry works well for a variety of sizes. In all cases, an increase in the net amount of TEOS and NH₄OH leads to an increase in the thickness of the silica shells. The images also illustrate that as the materials for the coating are increased in the reaction, there are a greater number of ‘multiplet’ species. Here we use the term ‘multiplet’ to describe groupings of magnetite and silica which have more than one magnetic core.
Figure 7.2 Variations in silica coating of magnetite nanocrystals. Average core size is 9 nm. (A) 80 μL of TEOS and 100 μL of NH₄OH were used per 1 mL of magnetite nanocrystals solution. (B) 160 μL of TEOS and 200 μL of NH₄OH were used per 1 mL of magnetite nanocrystals solution.
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The microemulsion reactions that produce silica coating, developed initially by Stöber et. al [42], involve hydrolysis and condensation of tetra ethyl ortho silicate (TEOS, Si(OCH$_2$CH$_3$)$_4$) in alcoholic medium in the presence of ammonium hydroxide (NH$_4$OH) as the catalyst and pH buffer (Equations 6.1, 6.2) [18].

\[
\text{Si(OCH}_2\text{CH}_3\text{)}_4 + 4 \text{H}_2\text{O} \rightarrow \text{Si(OH)}_4 + 4 \text{CH}_3\text{CH}_2\text{OH} \quad (6.1)
\]

\[
\text{Si(OH)}_4 \rightarrow \text{SiO}_2 + 2 \text{H}_2\text{O} \quad (6.2)
\]

Different coating thickness could be achieved by the variation of nanocrystals, TEOS and NH$_4$OH amounts. Lower TEOS to nanocrystal molar ratio yields thinner coating, whereas, variation in NH$_4$OH concentration changes the porosity, uniformity as well as morphology [18, 43]. Shell thickness was seen to level off over the ratio of 200 μL TEOS/mg of nanocrystals [21]. Multiple core presence is also observed and the variations catalyst quantity is predicted to be responsible.

7.2 Silver coating on the magnetite-silica nanoshells

Silver shells on nanosystems are very well studied (see these reviews: Halas, Caruso). Besides its optical properties [44, 45], anti-microbial use is gaining grounds especially for water treatment systems [27, 28]. Through a collaboration with Pedro Alvarez group at the Civil and Engineering Department (Rice University), we constructed a multishell structure consisting of magnetite nanocrystals as core, silica coating as the intermediate and silver coating on the top (Figure 7.4). This multishell system is expected to bring magnetic controllability to anti-microbial applications of silver nanocrystals [18].
Magnetically navigated silver and gold nanoshells are also sought in this practice, which may generate a new class of materials for optical and biological applications. Silver nanoparticles, however, are found to have a stronger and sharper plasmon resonance band than that of gold particles of the same size [46]. More research is underway.

Figure 7.4 Silver coating on silica coated magnetite nanocrystals.
Experimental details Before silver nanoparticle attachment is attempted, magnetite nanocrystals are first coated with silica as described in section 7.1. To prepare the surface for this reaction, 150 μL of 3-aminopropyl trimethoxy silane (APTMS - Aldrich) is added to a 1 mL solution of the Magnetite-SiO$_2$ core-shell solution. A batch magnetic separation (see Chapter 2 for details on the method) is carried out to clean functionalized nanocrystals from excess APTMS. Once the deposits are collected, they are added to a dilute solution of 5.7 mg of NaBH$_4$ (Aldrich) in 98 mL DI water (Millipore) while vigorously stirring. Immediately after the addition of silver nitrate (AgNO$_3$; 1 mL, 0.002M), the solution changes its color to a grayish dark color. After 15 minutes, the materials are collected by a batch magnetic separation. These materials are easily dispersed in ethanol, and could be used without further purification.

Mechanism Silver deposits on silica surfaces with the help of surface functionalization, the amino functional groups [47-49]. Sodium borohydride that are on the surface of the silica coated magnetite reduces the silver ions (Ag$^+$) in the course of 24 hours. The chemical reaction that’s taking place is given in Equation 6.3.

$$8\text{AgNO}_3 + \text{NaBH}_4 + 3\ \text{H}_2\text{O} \rightarrow 8\text{Ag}^{(0)} + \text{NaNO}_3 + \text{H}_3\text{BO}_3 + 7\ \text{HNO}_3$$ (6.3)
References


