Metal Oxide Nanocrystals: Synthesis, Characterization and Surface Derivatization

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ABSTRACT

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The three major topics explored through the research reported in this thesis are:

1. Characterization of nanocrystal surface ligands

2. Expansion of surface ligand options to provide varied solubility – especially aqueous solubility for biological applications

3. Development of a straightforward, general, green and inexpensive method for the preparation of a variety of metal oxide nanocrystals

Chapter one provides an introduction to nanocrystals and methods of synthesis, with an emphasis on the synthesis of metal oxide nanocrystals. In chapter two we describe the characterization of iron oxide nanocrystal surface ligands. Oleic acid, when used as a capping ligand, produces highly uniform and monodisperse nanocrystals; however, the reason for this is not well understood. A combination of spectroscopy including \(^1\)H NMR, FTIR, XPS, TEM and GCMS were used to characterize the ligand itself and gain a better understanding of its interaction with the iron oxide surface. We observed a ligand structural change to occur during the preparation of iron oxide nanocrystals. We attribute this structural change to the high temperatures used to prepare iron oxide nanocrystals and note that the structural change may be the feature that allows oleic acid to form highly monodisperse nanocrystals.

Chapter three describes the use of magnetic iron oxide nanocrystals (MIONs) as biological labels and the methods developed to make them water-soluble. MIONs were
made water-soluble by encapsulation within phospholipids micelles. A variety of phospholipids were found to be capable of performing this transformation and the method is efficient and requires little purification. Functionalized phospholipids can be conjugated to antibodies via SMPT, a heterobifunctional crosslinker, and fluorescence microscopy was used to visualize this conjugation. Ultimately, we found this to be a general method that can be used to create water-soluble nanocrystals and conjugate them to biomolecules.

Chapter four describes a new and simple method for preparation of TiO$_2$ nanocrystals of highly uniform shape. Addition of aqueous HCl was found to be critical for obtaining crystalline products in situ. Several morphologies (spherical, rod-like and triangular) can be obtained by altering reaction conditions. In addition, various capping ligands can be used, which allows us to tune the solubility of the nanocrystals.

Chapter five discusses the development of a general method for the preparation of metal oxide nanocrystals and also the expansion of capping ligands, which can be used during their synthesis. Several new ligands were found that provide solubility in polar solvents, one in water. Metal acetylacetonate precursors were used in a very simple method to synthesize several different types of metal oxide nanocrystals, and it is thought that they can be generally used to prepare other metal oxide species.
# Table of Contents

1 INTRODUCTION ............................................................................................................. 2

1.1 Nanocrystals .................................................................................................................. 2

1.2 Nanocrystal Synthesis .................................................................................................... 5

1.3 Metal Oxide Nanocrystals .............................................................................................. 9

1.4 Characterization of Ligands on Nanocrystal Surfaces ................................................. 12

1.5 Panoramic Preparations of Nanocrystals ..................................................................... 14

1.6 References .................................................................................................................... 16

2 SPECTROSCOPIC CHARACTERIZATION OF THE IRON OXIDE NANOCRYSTAL SURFACE .................................................................................................................... 22

2.1 Introduction ................................................................................................................... 22

2.2 Experimental Methods ................................................................................................. 28

2.3 Results and Discussion ................................................................................................. 29

2.3.1 TEM Characterization ............................................................................................. 31

2.3.2 Characterization of γ-Fe₂O₃ Nanocrystals by NMR .................................................. 31

2.3.3 Infrared Spectroscopy .............................................................................................. 35

2.3.4 X-ray Photoelectron Spectroscopy (XPS) .............................................................. 37

2.3.5 Effect of Heating on Oleic Acid and Synthesis with Analogous Ligands .................. 38

2.3.6 Surfactant Removal and Characterization ............................................................. 46

2.3.7 ¹H NMR Relaxation Studies .................................................................................... 49

2.4 Conclusion .................................................................................................................... 51

2.5 References .................................................................................................................... 52

3 BIOCONJUGATION OF γ-Fe₂O₃ NANOCRYSTALS FOR CELL DETECTION BY MRI .......................................................................................................................... 56

3.1 Introduction ................................................................................................................... 56

3.1.1 Motivation ................................................................................................................ 56

3.1.2 Maghemite: Structure and Magnetic Properties .................................................... 58

3.1.3 Methods for Creating Water-Soluble MIONs ......................................................... 59

3.2 Experimental Methods ................................................................................................. 62

3.2.1 Preparation of Iron Oxide Nanocrystals ................................................................. 62

3.2.2 Creating Water-Soluble γ-Fe₂O₃ Nanocrystals ......................................................... 63

3.2.3 Conjugation of Nanocrystals to Antibodies ............................................................ 64
4 SYNTHESIS OF TiO₂ NANOCRYSTALS: CAPPING LIGANDS, MORPHOLOGIES, CRYSTALLINITY AND SURFACE CHARACTERIZATION .................................................................86

4.1 Introduction ...........................................................................................................87

4.2 Experimental Methods .........................................................................................94

4.3 Results and Discussion ......................................................................................96
  4.3.1 Synthesis of TiO₂ Nanoparticles ................................................................96
  4.3.2 Surfactants used in TiO₂ Synthesis .................................................................97
  4.3.3 Three Morphological Forms of TiO₂ ...............................................................98
  4.3.4 Temperature Optimization ............................................................................101
  4.3.5 UV–visible Spectroscopic Characterization of annealed TiO₂ ..................106
  4.3.6 Addition of HCl: Synthesis of TiO₂ Nanocrystals ........................................107
  4.3.7 Solvent effects ...............................................................................................117
  4.3.8 Addition of H₂O to Reaction .........................................................................120
  4.3.9 Varying Amount of Surfactant .....................................................................122
  4.3.10 GCMS Analysis of TiO₂ Surfactant ..............................................................124
  4.3.11 Study of TiO₂ Surface with DRIFT (Diffuse Reflection Infrared Fourier Transform) .................................................................125
  4.3.12 NMR Study of TiO₂ Surfactant ..................................................................131
  4.3.13 Thermogravimetric Analysis of TiO₂ ............................................................133
  4.3.14 Photoluminescence of TiO₂ .........................................................................136

4.4 Conclusions .........................................................................................................137

4.5 References: .........................................................................................................138

5 METAL ACETYLACETONATES AS PRECURSORS FOR THE SYNTHESIS OF EARLY TRANSITION METAL OXIDE NANOCRYSTALS ........................................142

5.1 Introduction .........................................................................................................142
  5.1.1 Synthesis of Metal Oxides ............................................................................142
  Motivation .............................................................................................................143
  5.1.2 Metal Acetylacetonates as Precursors to Metal Oxide Nanocrystals ........143
  5.1.3 Capping Ligands and Solubility .................................................................145
  5.1.4 Iron Oxides, Manganese Oxides and Chromium Oxides ........................146

5.2 Experimental Methods ......................................................................................147
  5.2.1 Synthesis of Iron Oxide Nanocrystals .......................................................147
5.2.2  Synthesis of Chromium Oxide Nanocrystals ................................................. 148
5.2.3  Synthesis of Manganese Oxide Nanocrystals ................................................ 148

5.3  Results and Discussion ..................................................................................... 149
  5.3.1  Synthesis of γ-Fe₂O₃ Nanocrystals ............................................................... 149
  5.3.2  Synthesis of Mn₂O₃ and Mn₃O₄ Nanocrystals .............................................. 157
  5.3.3  Synthesis of Cr₂O₃ nanocrystals ................................................................. 163

5.4  Conclusions ........................................................................................................ 167

5.5  References ......................................................................................................... 168
List of Figures

Figure 1.1 Fluorescence of CdSe nanocrystals of increasing size (left to right). The size dependent change in fluorescence is caused by quantum confinement effects.

Figure 2.1 Illustration of two possible binding modes for carboxylates on iron oxide nanocrystal surfaces. The left shows a symmetric binding mode where both oxygen atoms are attached to the surface, while the right shows an asymmetric binding mode, where only one oxygen atom is bound to the surface.

Figure 2.2 TEM image of 13 nm γ-Fe₂O₃ nanocrystals dispersed in hexanes and dropped onto a 400-mesh Cu grid coated with Formvar support resin. Solvent was removed under vacuum for 1 h.

Figure 2.3 ¹H NMR of oleic acid (A), oleic acid@γ-Fe₂O₃ nanocrystals (B), and the ligands (4) removed from γ-Fe₂O₃ surface (C). The experiments were run at RT and the locking solvent was CDCl₃. Concentration of nanocrystals is 1.5 mM.

Figure 2.4 ¹³C NMR of ligand (4) stripped from nanocrystal surface (A) and oleic acid (B). Samples were run at RT in CDCl₃ solvent.

Figure 2.5 DRIFT spectra of oleic acid (A), stripped ligand (4) (B), and oleic acid@γ-Fe₂O₃ (3) (C). Spectra are normalized. Samples (1-5% wt) were mixed with pre-ground KBr powder.

Figure 2.6 XPS characterization of ligand capped γ-Fe₂O₃ nanocrystals (3). A) Cls; B) O1s; C) Fe2p; D) Fe3p.

Figure 2.7 TEM of γ-Fe₂O₃ nanocrystals synthesized with stearic acid surfactant.

Figure 2.8 TEM of γ-Fe₂O₃ nanocrystals synthesized with 1-octadecanol as the surfactant.

Figure 2.9 TEM of γ-Fe₂O₃ nanocrystals synthesized with elaidic acid surfactant.

Figure 2.10 ¹H NMR of oleic acid (spectrum A) and oleic acid after heating at 350 °C for 4.5 hours (spectrum B). The spectra were obtained at room temperature in CDCl₃ solvent. The large peak at 2.05 ppm results from acetone.

Figure 2.11 ¹H NMR of stearic acid (spectrum A) and stearic acid heated(spectrum B) at 350°C for 4.5 h. Sample was examined at room temperature in CDCl₃ solvent. No major changes are apparent.

Figure 2.12 Comparison of integration values obtained from ¹H NMR spectra of aliquots taken from heating oleic acid slowly to 350 °C as they change with temperature and time. Clearly a structural change occurs rapidly after 325 °C.

Figure 2.13 FTIR of oleic acid and aliquots removed at 325 °C and 340 °C. Samples were diluted in CCl₄ and recorded between KBr salt plates at room temperature. Loss of alkene C-H stretch (3003 cm⁻¹), carbonyl stretch (1710 cm⁻¹) and several bands from 1350-1150 cm⁻¹ are observed after 325 °C.

Figure 2.14 Flowcharts illustrating details of nanocrystal isolation, surfactant removal, component separation and characterization.

Figure 2.15 EIMS of surfactant stripped from γ-Fe₂O₃ nanocrystals. Stripped surfactant (4) was diluted in ethyl acetate.

Figure 2.16 Bar graph displaying NMR T₁ measurements of two NMR resonances in various metal oxide nanocrystal samples and free oleic acid.

Figure 2.17 T₁ measurements of two NMR resonances in various metal oxide nanocrystal samples capped with oleic and elaidic acid.

Figure 3.1 The unit cell of γ-Fe₂O₃. Oxygen ions are in red, while octahedral Fe³⁺ ions are in yellow and tetrahedral Fe²⁺ ions are in green.

Figure 3.2 TEM (A) of γ-Fe₂O₃ nanocrystals coated in oleic acid surfactant, selected area electron diffraction pattern (B) highlights crystallinity, and cartoon (C) representing 3-dimensional structure of one oleic acid capped magnetite nanocrystal. The oleic acid prevents nanocrystal aggregation, which can be seen on the TEM image.

Figure 3.3 Phospholipids used to render MIONs water-soluble. Various combinations of these may be used successfully. Ultimately a ratio of 99:1 mPEG 750 PE to PTE was selected.

Figure 3.4 TEM of uncoated (A) and coated (B) MIONs. MIONs were coated with 60% DPPC and 40% mPEG 2000 PE. Image B was stained with 1% phosphotungstic acid to highlight the phospholipid coating. The upper left section of the image B is stained well and the lipids are visible as a white ring around each particle. Image A was dispersed in hexanes, he sample shown in image B was dispersed in distilled water. No aggregation is observed.
Figure 3.5  Cartoon illustration of a phospholipid coated MION. The orange ligands represent the oleic acid, and the green and blue show how the phospholipids interact with the oleic acid surface. The hydrophobic palmitoyl chains (in blue) are interdigitated with the oleic acid, while the more polar end (green) of the phospholipids interacts with the solvent. (Image made by Kristi Hultman, a collaborator on this project.)

Figure 3.6  TEM images of 5 nm MIONs coated with 80:20 (A) molar ratio of DPPC:PTE and 99:1 (B) ratio. Notice that in image (A) (more PTE) the nanocrystals are aggregated in a hexagonal supertetra, likely due to disulfide linkages between particles.

Figure 3.7  TEM of MIONs coated with 99:1 molar ratio of DPPC to PTE. The TEM grid is stained with 1% PTA and white rings are visible around the MIONs, indicating the phospholipids coating.

Figure 3.8  15 nm diameter MIONs were prepared and dispersed in hexanes (A) and attempted to be coated with phospholipids and dispersed in water (B). The sample was

Figure 3.9  TEM images of DPPC and PTE micelles prepared in different concentrations of NaCl salt solutions in THF/water. No salt (A), 0.01 M NaCl (B), and 0.1 M NaCl (C). A drop of 1% PTA stain was added to the grid to visualize the micelles.

Figure 3.10  TEM of γ-Fe2O3 nanocrystals encapsulated within DPPC/PTE phospholipid micelles. The micelle solution was 0.1 M NaCl in THF/water and the TEM grid was stained with 1% PTA. The micelles are spherical.

Figure 3.11  TEM image of uncoated (A) and phospholipid coated (B) 15 nm γ-Fe2O3 nanocrystals. The nanocrystals on the right are water soluble and coated with DPPC and PTE.

Figure 3.12  Molecular structure of IgG class antibody (left) and cartoon illustrating basic structure of an antibody (right). The heavy chains are in red, and the light chains in yellow.

Figure 3.13  Conjugation of MION to islet cell using two fluorophores to indicate binding. A fluorescein labeled phospholipid was added to the phospholipid mixture of mPEG 750 and PTE. The MION is conjugated to a Texas Red labeled secondary antibody, which will attach to any primary antibody on the islet cell. The fluorescent labels can be detected with fluorescence microscopy.

Figure 3.14  MIONs coated with mPEG 750, PTE and either 10, 20, 30 or 40% of fluorescein labeled phospholipids. The fluorescein labeled phospholipid is shown at right. The amount of fluorescein labeled phospholipids added to the reaction does not seem to make a difference in the fluorescence of the MIONs.

Figure 3.15  Fluorescence microscopy used to image fluorescent MION-Ab conjugates. MION has green fluorescent, and secondary antibody has a Texas Red fluorescent label. These MION-Ab conjugate tags have been used to label primary antibodies on islet cells as illustrated in Figure 3.13. Image A shows all color channels, Image B has the red channel off and Image C has the green channel off. Both green and red fluorescence are observed in the same location in B and C, indicating that the MIONs and Ab are conjugated.

Figure 4.1  Unit cell of anatase TiO2. Anatase is a tetragonal polymorph of TiO2 with an elongated unit cell along the c axis. Its unit cell dimensions are a = 3.785 Å and c = 9.514 Å. Titanium atoms are blue and oxygen atoms are red. Edges of the unit cell are shown by a blue dashed line.

Figure 4.2  For comparison to anatase, the unit cells of brookite (left) and rutile (right) TiO2 are shown. Brookite has an orthorhombic structure, while rutile has a tetragonal structure. The edges of the unit cell are shown with the blue dotted lines.

Figure 4.3  Surfactants selected to add to the reaction illustrated in Scheme 4.1.

Figure 4.4  TEM images of TiO2 nanoparticles prepared with different surfactants. 2-acetyl pyridine (A), p-anisaldehyde (B), propylene carbonate (C), γ-butyrolactone (D), 1-formyl piperidine (E). The particles were dispersed in CHCl3 and drop-cast onto a carbon coated 400-mesh Cu grid. The products are amorphous, as determined by XRD.

Figure 4.5  TEM image of amorphous TiO2 rods (A) and spheres (B) obtained from the reaction of TiOt(acac)2 with TMA solvent and propylene carbonate surfactant. The sample was dispersed in CHCl3 and drop-cast onto a carbon coated 400-mesh Cu grid.

Figure 4.6  XRD diffraction pattern of TiO2 before and after annealing at 500 °C for 4 h. The bottom line is the XRD of the sample before annealing and the top line is the XRD pattern observed after annealing. The sample was characterized as anatase.
Figure 4.7 TEM of TiO₂ nanoparticles prepared by heating TiO(acac)₂ and PC in TOA solvent. A bimodal size distribution is observed. Sample was dispersed in CHCl₃ and drop-cast onto a carbon coated 400-mesh Cu grid.

Figure 4.8 TEM and XRD (inset) images of triangular shaped nanoparticles resulting from synthesis of TiO₂ from TiO(acac)₂, TOA, BL and DMSO.

Figure 4.9 UV-visible spectra of TiO₂ nanocrystals synthesized with different surfactants. Both display an absorption peak near 242 nm, indicating the anatase form of TiO₂.

Figure 4.10 TEM images of anatase TiO₂ prepared with various surfactants with addition of HCl. γ-butyrolactone (A), propylene carbonate (B), 1-formyl piperidine (C), p-anisaldehyde (D), ethyl salicylate (E), and 2-acetyl pyridine (F). These nanocrystals were prepared by combining TiO(acac)₂, TOA, surfactant and 0.3 M HCl with heat. The most uniform nanocrystals were prepared with propylene carbonate surfactant.

Figure 4.11 Example XRD of anatase TiO₂ synthesized with addition of 0.5 M HCl to reaction mixture. Surfactant in this case is propylene carbonate.

Figure 4.12 HRTEM of TiO₂ prepared in propylene carbonate and TOA with 3 mL of 0.5 M HCl. Crystallinity is observed and d-spacing is 3.7 Å. Sample was dispersed in CHCl₃ and drop-cast onto a holey-carbon coated Cu grid.

Figure 4.13 X-ray diffraction pattern of TiO₂ synthesized with the addition of sulfuric acid (bottom) and nitric acid (top) instead of hydrochloric acid. Samples exhibit crystallinity and are identified as anatase.

Figure 4.14 TEM images of TiO₂ prepared with the addition of two different acids, 0.5 M sulfuric acid (A) and 0.5 M nitric acid (B).

Figure 4.15 TEM image of TiO₂ nanocrystals synthesized with different solvents. trietylamine (A), octyl ether (B), 1-decene (C), propylene carbonate (D). It was not possible to obtain a TEM image of TiO₂ synthesized in 1-hexadecanol.

Figure 4.16 XRD pattern for TiO₂ samples synthesized in different solvents. All are identified as anatase TiO₂. 1-decene (A), trietylamine (B), octyl ether (C), propylene carbonate (D) and 1-hexadecanol (E).

Figure 4.17 TEM images of TiO₂ synthesized with 1(A), 2 (B), 3 (C) and 4 mL (D) of H₂O instead of addition of HCl.

Figure 4.18 XRD of nanocrystalline TiO₂ synthesized with various quantities of water. All samples were characterized as anatase. Four mL water (A), three mL water (B), two mL water (C).

Figure 4.19 TEM images of TiO₂ synthesized with different ratios of PC:precursor. 1:1 (A), 2:1 (B), 3:1 (C), 4:1 (D) and 5:1 (E).

Figure 4.20 GC trace obtained from GCMS analysis of surfactant removed from TiO₂ surface.

Figure 4.21 GCMS data from surfactant species removed from TiO₂ surface. This MS trace corresponds with Peak B in Figure 4.20. The large peak at 355 is assigned to trietylamine.

Figure 4.22 DRIFT spectra of TiO₂ sample synthesized with different surfactants: trietylamine (A), 2-acetylpyridine (B), propylene carbonate (C), γ-butyrolactone (D), 1-formyl piperidine (E), p-anisaldehyde (F), ethyl salicylate (G).

Figure 4.23 DRIFT of TiO₂ nanocrystals synthesized with propylene carbonate surfactant and TOA solvent.

Figure 4.24 H NMR of surfactant removed from TiO₂ nanocrystal surface. This sample of TiO₂ was prepared with TOA solvent and PC surfactant. The surface species were removed by precipitating the nanocrystals with EtOH.

Figure 4.25 TGA and derivative obtained for TiO₂ capped with γ-butyrolactone and trietylamine.

Figure 4.26 Photoluminescence of anatase TiO₂. Signal is very weak, but does increase in the absence of air and at decreased temperatures.

Figure 5.1 Capping ligands/solvents chosen for nanocrystal synthesis. All solvents have carbonyl groups to bind to the metal oxide surface, and high boiling points that allow them to survive high reaction temperatures.

Figure 5.2 TEM images of γ-Fe₂O₃ nanocrystals prepared with various solvents; 2-acetyl pyridine (A), p-anisaldehyde (B), γ-butyrolactone (C), ethylene carbonate (D), and 1-formyl piperidine (E).

Figure 5.3 X-ray diffraction pattern for γ-Fe₂O₃ nanocrystals prepared with p-anisaldehyde.

Figure 5.4 TEM of FeO nanocrystals. Average size is 10 nm. Prepared by heating Fe(acac)₃ in oleic acid to 300 °C. Characterized as FeO by XRD.
Figure 5.5  Low-resolution TEM of Mn$_2$O$_3$ nanocrystals. Prepared by heating Mn(acac)$_3$ in acetone for 9 days at 200 °C.

Figure 5.6  HRTEM of Mn$_2$O$_3$ nanocrystals at 60,000 and 600,000 magnification. These images were obtained at Brookhaven National Laboratory.

Figure 5.7  XRD of Mn$_2$O$_3$ prepared from Mn(acac)$_3$ and acetone. Scherrer equation was used to calculate average particle size as 17.2 nm from (222) reflection.

Figure 5.8  TEM of Mn$_2$O$_3$ nanocrystals prepared at 250 °C.

Figure 5.9  Transmission electron micrograph of Mn$_3$O$_4$ nanocrystals prepared from Mn(acac)$_3$.

Figure 5.10  Powder x-ray diffraction pattern of Mn$_3$O$_4$ nanocrystals.

Figure 5.11  Unit cell of Cr$_2$O$_3$ has a rhombohedral structure and is isostructural to corundum (α-Al$_2$O$_3$). The Cr atoms are in blue and the O atoms are in red.

Figure 5.12  Photograph of Cr$_2$O$_3$ powder.

Figure 5.13  TEM of Cr$_2$O$_3$ nanocrystals.

Figure 5.14  X-ray diffraction pattern of annealed Cr$_2$O$_3$.

Figure 5.15  TEM images of particles synthesized with different solvents: tetrahydrofuran (A), ethanol (B), and cyclohexanone (C).
List of Tables

Table 1.1 Table showing change in surface area as length of cube edge decreases ........................................... 3
Table 2.1 Frequency of carbonyl stretches in carboxylate ions and carboxylic acids ............................................. 25
Table 2.2 Bands observed in DRIFT IR of technical grade oleic acid .................................................................. 35
Table 4.1 Tabular summary of a series of reactions run to determine optimal heating temperature in the synthesis of TiO₂ nanoparticles. Average particle size and size distribution were determined from TEM images. .................................................. 103
Table 4.2 Summary of a series of reactions investigating heating at different combinations of temperatures and times. Rods were found in product samples when reactions were heated for longer periods of time ........................................................................................................... 104
Table 4.3 Average TiO₂ nanocrystal size for each surfactant system as amount of HCl added to reaction is changed between 1 mL and 4mL .................................................................................................................. 114
Table 4.4 Dispersability of TiO₂ prepared in TOA and different surfactants in addition to 3mL of HCl. 'Yes' means that TiO₂ was fully dispersed in solvent; 'Some' means that some of the TiO₂ was dispersed, however a significant amount remained as precipitate; and 'No' means that TiO₂ remained a ppt and solvent remained clear and transparent. ........................................................................................................ 115
Table 4.5 Average nanocrystal diameter based on solvent choice ........................................................................... 118
Table 4.6 Molar ratio of Surfactant:TiO₂(acac)₂ compared to average nanocrystal size .................................... 124
Table 4.7 Assignment of IR bands shown in trioctylamine spectrum .................................................................. 127
Table 4.8 Infrared bands observed for TiO₂ nanocrystals prepared in TOA and PC .............................................. 128
Table 4.9 Results from calculations of number of surface ligands from TGA data. .................................................. 134
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To My Family
Chapter 1

Introduction
1 Introduction

1.1 Nanocrystals

Nanocrystals are discrete crystalline nanometer sized particles containing tens to thousands of atoms. They are broadly defined as particles that have one dimension of less than 100 nm. Nanocrystals are most often spherical, but can also form in the shape of rods, triangles, hexagons or cubes, among many others. Nanocrystals and nanoparticles (not fully crystalline) have attracted extensive attention during the past several decades from researchers and entrepreneurs alike for the mesoscopic phenomena found at this size regime that distinguish them from their molecular and bulk counterparts. Figure 1.1 illustrates the structural differences between molecular, nanocrystalline and bulk structures. Nanoscale structures often display new size-dependent electronic, magnetic, optical, or dielectric properties that make them of interest and of potential value for both purely academic research and potential technological applications.$^{1-4}$

![Diagram of molecular, nanocrystal, and bulk structures](image)

Figure 1.1 Illustration of molecular, nanoscale and bulk structures$^5$
The majority of the size-dependent properties observed in the nanoscale regime are related to the large surface area to volume ratio at this scale. Nanocrystals have a large percentage of atoms on the surface relative to the total number of atoms. As the size decreases, bulk-like properties of materials diminish, while surface properties are magnified and dominate the overall properties of the material. This allows chemists and material scientists the opportunity to change the electronic and chemical properties of a material simply by controlling its particle size. Table 1.1 highlights the change in surface area as a 1 cm$^3$ cube (bulk structure) is chopped into 0.1 Å$^3$ cubes. Almost unbelievably, the surface area changes from 6 cm$^2$ to 60,000 m$^2$ as the size of the cube edges is decreased.

<table>
<thead>
<tr>
<th>Length of Cube Edge</th>
<th>Number of Cubes</th>
<th>Total Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cm</td>
<td>1 (~ A sugar cube)</td>
<td>6 sq. cm. (Volume 1 cc)</td>
</tr>
<tr>
<td>1 mm = 1000 μ</td>
<td>$10^3$</td>
<td>60 sq. cm.</td>
</tr>
<tr>
<td>0.1 mm = 100 μ</td>
<td>$10^6$</td>
<td>600 sq. cm.</td>
</tr>
<tr>
<td>0.01 mm = 10 μ</td>
<td>$10^9$</td>
<td>6,000 sq. cm.</td>
</tr>
<tr>
<td>1 μ = 1000 nm</td>
<td>$10^{12}$</td>
<td>6 sq. m</td>
</tr>
<tr>
<td>0.1 μ = 100 nm</td>
<td>$10^{15}$</td>
<td>60 sq. m</td>
</tr>
<tr>
<td>0.01 μ = 10 nm</td>
<td>$10^{18}$</td>
<td>600 sq. m. (Football field)</td>
</tr>
<tr>
<td>0.1 nm = 1 Å (An atom)</td>
<td>$10^{24}$ (~ A mole of cubes)</td>
<td>60,000 sq. m.</td>
</tr>
</tbody>
</table>

**Table 1.1**  
*Table showing change in surface area as length of cube edge decreases*

The study of nanocrystals from synthetic methods, to applications, to characterization is currently a broad field of research that continues to expand and become more interdisciplinary as new applications arise. Cadmium selenide nanocrystals are the paradigmatic illustration of the size-dependent properties of nanocrystals. The fluorescence of these semiconductor CdSe nanocrystals can be tuned from red to blue by
altering the size of the nanocrystals (Figure 1.1). The absorbance of CdSe shifts to shorter wavelengths (blue) as the nanocrystal size decreases.

![Image](image.jpg)

**Figure 1.1** Fluorescence of CdSe nanocrystals of increasing size (left to right). The size dependent change in fluorescence is caused by quantum confinement effects.\(^7\)

The wide scope of applications for nanocrystalline materials spans many industries. Semiconductor nanocrystals such as CdSe with size-tunable fluorescence can be used for biological labeling and imaging techniques.\(^8\) Iron oxide nanocrystals (maghemite and magnetite) have magnetic properties which allow them to be used for magnetic resonance imaging, biological labeling and magnetic recording media.\(^11-13\) The high surface area of nanocrystals also makes them ideal for catalysis applications. Nanocrystalline titanium dioxide is often used as a photocatalyst,\(^14,15\) and manganese and copper oxides are used for oxidation of CO and NO\(_x\).\(^16-19\)

Control of the products of nanocrystal syntheses in terms of size, size distribution and morphology is well worked out; however, in terms of application-based nanocrystal research there is a need for a better understanding of the chemistry at the nanocrystal surface. Extensive research has been performed to examine the surface, yet much of the surface chemistry remains elusive.\(^20-27\) It is still difficult to tune the solubility of nanocrystals. Exploitation of nanocrystal properties for technological applications most
often requires further chemistry or modification of the nanoparticle surface, whereby knowledge of and insight into the structure and behavior at the nanocrystal surface is imperative. Spectroscopic characterization of these nanocrystalline surfaces remains challenging and for that reason one of the goals of this research was to expand upon what is known about nanocrystal surfaces. Many nanocrystals are coated with a monolayer of ligands to provide stability (inhibit nanoparticle aggregation) in solution, yet nanocrystal-ligand interactions are not well understood. The research efforts presented here attempted to leave a footprint in the area of nanocrystal surface research and therefore studied these nanocrystal-ligand interactions with several nanocrystal systems. Another avenue of research focused on general methods that could be applied to preparing a wide variety of nanocrystals. We have developed a straightforward route capable of producing several first-row transition metal oxides, which can likely be applied to other systems as well.

1.2 Nanocrystal Synthesis

Studying methods of nanocrystal synthesis is of major interest for understanding the properties of nanoscale materials. It is also interesting from a methodological and application-based standpoint since many of the properties exhibited by nanoscale materials make them of interest for technological applications. Synthetic control over size, size distribution and shape of nanocrystalline samples allows for control of the size-dependent properties of the nanocrystals. Research with this in mind has dominated recent efforts described in the literature. There is an abundance of methods available to synthesize metallic, binary and ternary metal oxide, semiconductor, and core-shell nanocrystals, among other types.
A wide variety of methods that are used to prepare nanocrystals including: arc discharge,\cite{28} preparation in polymer-blend membranes\cite{29} or block copolymers,\cite{30,31} aqueous precipitation,\cite{32-34} microemulsions,\cite{35-37} inverse micelles,\cite{38} hydrodynamic cavitation,\cite{39} liquid flame spraying,\cite{40} vapor condensation,\cite{41} sonochemical synthesis,\cite{42,43} pyrolysis of molecular organometallic precursors\cite{44-46} and sol-gel processes.\cite{47,48}

Methods pioneered by Murray and colleagues\cite{1,44} greatly advanced organometallic syntheses of semiconductor nanocrystals. A general method was developed for the controlled preparation of monodisperse CdE (E = S, Se, Te) semiconductor nanocrystals. In the synthetic preparation of CdSe, a solution of Me2Cd, TOPSe (trioctylphosphine selenide) and TOPO (trioctylphosphine oxide) are injected into a hot solution of TOPO coordinating solvent. CdS and CdTe were prepared by similar methods. Injection of precursor reagents into hot coordinating solvent was found to be critical for the formation of monodispersed and uniform shaped nanocrystallites. Rapid injection at high temperatures leads to an instantaneous nucleation event because the high concentration of reagents, which is followed by slow and controlled growth of nuclei as the heat is gradually elevated. The rapid nucleation event serves to attenuate the high concentration of precursor reagents. The products range in size from 12 Å to 115 Å and can be separated by size selective precipitation into narrower size-distribution samples. Larger crystallites flocculate first when a non-solvent (such as methanol), miscible with the solvent, is added to the colloidal solution. Addition of MeOH increases the average polarity of the solvent mixture, which decreases the energetic barrier to flocculation. The van der Waals attractive forces among these crystallites are stronger than small crystallites and thus they flocculate first. The as-made colloids capped in TOPO after size
selection are soluble in a number of solvents including, alkanes, aromatics, long chain aliphatic alcohols, chlorinated solvents and organic bases (amines, pyridines, furans and phosphines). At the time, this method was instrumental in furthering research on controlled synthesis of monodisperse nanocrystals. Similar methods using organometallic precursors have been widely developed since and have been used to prepare a wealth of different metal-based nanocrystals.

Leading up to this seminal work by Murray, was the preparation of colloidal II-VI semiconductors by aqueous precipitation by Rossetti,33,34 followed by more controlled methods using structured media such as inverse micelles38,49 to prepare II-VI semiconductor clusters. Much of this early work embarked upon and established by Steigerwald and co-workers brought about the use of organometallic precursor-based syntheses.38,46,49-52

Since Murray’s results, many syntheses have been developed which piggyback on this general method. Efforts since have focused on synthesis of other types of nanocrystalline materials: metals, metal oxides and complex oxides, among others. The number of organometallic precursor, solution-phase methods has grown and they are used extensively, if not nearly exclusively. Control of size, size-distribution and morphology have also been extensively studied. Peng in particular has used the CdSe model as a medium to further understand the growth mechanisms and kinetics of nanocrystals in-depth.45,53-57 Kinetic control over the reaction was achieved by using the characteristic band-edge photoluminescence exhibited by CdSe to probe and further understand the growth mechanisms of the CdSe nanocrystals. Average size and size-distribution were calculated from the PL spectra. Their observations led to the conclusions that two growth
regimes, focusing and defocusing, exist and depend upon the concentration of monomer. Focusing occurs when the monomer concentration is higher than the solubilities of the nanocrystals in solution; in this case all particles grow. At very high monomer concentrations, smaller particles will grow at a more rapid rate than larger ones, and this focuses the size distribution to a monodisperse one. However, when the monomer concentration is low, the high surface free energy of small nanocrystals lowers their stability and they shrink in size, while larger nanocrystals continue to grow. This “defocuses”, or broadens the size distribution.

Peng found that impurities (alkyl phosphonic and phosphinic acids) present in technical grade TOPO aid in slowing down the growth rate of CdSe nanocrystals, and under certain heating conditions lead to the production of nanorods, as opposed to spheres. Wurtzite CdSe has an inherently anisotropic structure, whereby it is possible to manipulate the growth mechanism by altering the ratios of coordinating solvent. If the ratio of phosphonic acid impurities present in TOPO are enhanced, growth of rods with aspect ratios as high as 10:1 is possible. Phosphonic acids bind strongly to Cd ions and can manipulate the growth rate and therefore the shape of the nanocrystals. Further control over size and shape was achieved by using CdO as a starting material in place of Me₂Cd. The method does not require size-selection and was expanded to the synthesis of CdTe and CdS as well. In addition to improved control of morphology and size, the new method offers a less toxic approach to the synthesis of CdSe. Further work replaced the toxic TOPO solvent and Me₂Cd species used in the traditional Murray method with fatty acids and Cd(Ac)₂ for a greener synthesis which produces high-quality CdSe nanocrystals in controllable sizes.
Efforts to control nanocrystal size and shape have dominated much of the recent literature on nanocrystal synthesis via organometallic precursors. Use of coordinating ligands is crucial in controlling particle growth. During nanocrystal synthesis at high temperatures the coordinating ligands dynamically adsorb to the surface of growing crystals and help control growth. After synthesis, at low temperatures, the ligands organically passivate the surface to prevent particle aggregation in solution. Ligand selection, which will be discussed more later, plays a large role in nanocrystal growth as does solvent choice. Other foci in this growing field are the development of green, non-toxic methods to prepare nanocrystals, as well as increasing the number of general synthetic routes that can be applied to the synthesis of many different types of nanocrystals.

1.3 Metal Oxide Nanocrystals

Metal oxide nanocrystals are of growing interest for their technological applications, which result from their interesting properties (magnetic, optical, electric) along with general thermal stability and chemical resistance. Silica (SiO$_2$) is widely used in glass, sand, silica gel desiccants and in the food industry.$^{58}$ Iron oxide ($\gamma$-Fe$_2$O$_3$) exhibits ferrimagnetism, which makes it useful in magnetic and data storage applications, and has also found uses as biological labels. Barium titanate (BaTiO$_3$) is a ferroelectric material; it is used in ceramic capacitors, transducers, actuators, and high-k dielectrics.$^{59}$ Many metal oxide nanocrystals have also been used in catalysis for their high surface area/volume ratio, including Cu$_2$O,$^{60,61}$ Mn$_2$O$_3$,$^{18}$ and TiO$_2$.$^{14,62,63}$

There are many methods available for the preparation of metal oxide nanocrystals. Some of the early methods used to prepare them in a controlled fashion include the
following: matrix mediated synthesis, \textsuperscript{64-66} hydrodynamic cavitation, \textsuperscript{39} hydrothermal, \textsuperscript{67} aqueous precipitation in salt solutions, \textsuperscript{68} sol-gel methods \textsuperscript{48,69} and mechanochemical processing. \textsuperscript{70}

Solution-phase methods using organometallic precursors have been used most widely, and provide controlled growth mechanisms that give rise to high-quality nanocrystals. Preparation of metal oxide nanocrystals in organic media, as opposed to aqueous media provide better control over size distribution, size, crystallinity and surface properties. \textsuperscript{71} Synthesis in organic solvents instead of aqueous solution reduces rates of reaction and leads to better control over synthesis and crystallization. Several types of organometallic precursors are commonly used in the preparation of metal oxide nanocrystals including the following: metal carbonyls, \textsuperscript{72} metal acetates, \textsuperscript{19,73,74} metal acetylacetonates, \textsuperscript{75,76} metal alkoxides, \textsuperscript{77,78} metal halides \textsuperscript{79-81} and metal cupferronates. \textsuperscript{82}

Hydrolytic methods for the synthesis of metal oxide nanocrystals are quite prevalent, and typically involve the hydrolysis of metal alkoxides or metal halides; however, they have several key disadvantages. The presence of water results in nanoparticles that have many hydroxyl groups dangling from the surface. Hydroxyl groups affect the reactivity, properties of the metal oxides, and potential surface derivitization. \textsuperscript{83} In addition, this method of preparation causes the monodispersity to be low and makes it more difficult to prepare small nanocrystals. \textsuperscript{58} For this reason, alternative, non-aqueous solution-phase synthetic methods are favored. The first method to prepare metal oxide nanocrystals via a non-aqueous solution-phase method was performed by Colvin and co-workers. \textsuperscript{81} They utilized a chemical reaction known to form amorphous Ti-O networks \textsuperscript{48} at lower temperatures and developed it for use with metal
oxides to form nanocrystals. Ti halides were reacted with Ti alkoxides to lose alkyl halides and form nanocrystalline TiO₂. Though the nanocrystals were not of uniform shape or monodisperse, this was a significant contribution to the field of metal oxide nanocrystal synthesis.

One recent method has made meaningful advances towards monodisperse metal oxide nanocrystals. Yin and co-workers¹⁹,⁷³ use metal acetate precursors in trioctylamine solvent, with oleic acid capping ligands to prepare monodisperse Cu₂O, MnO, and subsequently Mn₃O₄ nanocrystals in high yield and without the need for post-synthetic size selection.

One focus of our work was towards the advancement of methods for the synthesis of TiO₂ nanocrystals. Similar to metal oxide nanocrystals in general, TiO₂ was typically prepared by hydrolytic methods, which prevented crystallization in situ and also hindered the formation of monodisperse samples. Despite the previously mentioned advances, it is still challenging to produce a crystalline and monodisperse sample of TiO₂ in situ. We have developed a solution-based method for preparing crystalline TiO₂ without the need for annealing using TiO(acac)₂, a non-toxic precursor material. Reacting TiO(acac)₂ in highly-coordinating trioctylamine, a capping ligand, and HCl produces nanocrystalline anatase TiO₂. Several capping groups can be used, which alter the surface chemistry, and serve to tune the solubility of the TiO₂, however addition of one capping group in particular, propylene carbonate, gives rise to nanocrystals of highly uniform spherical shape. Uniform shape and hence monodispersity still remain challenging in the preparation of TiO₂. The TiO₂ nanocrystals contributed by this work are easily prepared and crystalline, and have a highly uniform, spherical shape.
Chapter 5 expands upon this work by realizing a general method for the preparation of metal oxide nanocrystals using metal acetylacetonate precursors. $\gamma$–Fe$_2$O$_3$ nanocrystals were prepared with various capping groups, giving us the ability to tune the solubility and surface chemistry of $\gamma$–Fe$_2$O$_3$. Iron oxide nanocrystals are used in various applications for their magnetic properties and the ability to tune solubility is important.

In addition, a simpler method was found to produce amorphous Cr$_2$O$_3$, Mn$_2$O$_3$ and Mn$_3$O$_4$ nanoparticles, which are crystalline after annealing. Analogous to the method used to prepare TiO$_2$ and $\gamma$–Fe$_2$O$_3$, $M$(acac)$_x$ ($M$ = Cr or Mn) is heated in acetone to form the aforementioned metal oxide nanocrystals. This reaction is uncomplicated and environmentally benign, since the only reagents are $M$(acac)$_x$ and acetone. Several methods are known for the preparation of manganese oxides, however the formation of Cr$_2$O$_3$ nanocrystals is reported$^{84}$ to be difficult, and for that reason there are few reports of its preparation. This contribution is significant in its simplicity and in its potential to be used for the synthesis of many other metal oxide nanocrystals. Metal acetylacetonates are widely available, and there are likely numerous other metal oxide nanocrystals that can be prepared by this general method.

1.4 Characterization of Ligands on Nanocrystal Surfaces

Nanocrystals are prepared with capping ligands that dynamically adsorb to the surface during growth to control growth mechanisms, and also serve to passify the surface after synthesis so that the nanocrystals are stable in solution. Ligand choice has a large effect on the post-synthetic solubility of the nanocrystals; however, an overwhelming majority of ligands used are hydrophobic and are thus soluble in non-polar, organic solvents. The preparation of metal oxide nanocrystals with capping ligands
mainly occurs in solution-based reactions at high temperatures, however there are some low-temperature routes.\textsuperscript{85} Pursuant to this near requirement of high temperatures in most solution-based routes, selected ligands must have a high boiling point. Some commonly used ligands are long alkyl chain carboxylic acids, phosphines, phosphates, amines or diols. Although most synthetic methods use these types of ligands, it is possible to perform ligand-exchange post-synthesis\textsuperscript{86} to substitute others.

Characterization of nanocrystals typically involves TEM and XRD to examine the core structure of the nanocrystal, however it often ignores any characterization of surface ligands. There are several examples of ligand and surface characterization, however.\textsuperscript{88-94} It is generally assumed that the ligand remains unchanged by the reaction conditions. Recent efforts to characterize nanocrystal surfaces have found that changes in ligand chemical structure can occur when oleic acid is used as a ligand.\textsuperscript{95,96} Chapter 2 discusses our surface characterization of $\gamma$-Fe$_2$O$_3$ nanocrystals by $^1$H and $^{13}$C NMR, FTIR, XPS, and elemental analysis, which found a significant change in the structure of oleic acid during nanocrystal preparation. This structural change was only observed for oleic and elaidic acid (the cis and trans isomers), however not for stearic acid (saturated oleic acid). It is our speculation that the structural change is what allows oleic acid to produce such monodisperse and uniform nanocrystals.\textsuperscript{19,72,73,97} Chapter 4 discusses the synthesis of TiO$_2$ with a range of different surface ligands and the characterization of those surfaces with FTIR, TGA, GCMS and $^1$H NMR. In most cases trioctylamine, the coordinating solvent, was found to be a stronger coordinating ligand than the surfactants added to the reaction.
Knowledge of the structure of ligands on the surface of nanocrystals and how they bind can provide an understanding of the dynamics and binding interactions at the nanocrystal-ligand interface. A recent study by Peng and co-workers\textsuperscript{98} examined the dissociation of thiolate ligands from CdSe nanocrystals as the solution pH was lowered by steady-state titration and UV-visible spectroscopy. They observed the dissociation pH to be size-dependent, and not related to the concentration of nanocrystals, as hypothesized.

1.5 Panoramic Preparations of Nanocrystals

Given the vast array of methods available for nanocrystal synthesis, there is a recent trend toward the development of general methods that can be used for the preparation of metal oxide nanocrystals. Development of general methods for controlling size and dimensionality of metal oxide nanocrystals is an important topic in the field of materials chemistry. For industrial applications, general methods that can be applied to the preparation of a variety of nanocrystals would be favored from a production standpoint. One of the first general methods towards the synthesis of nanocrystals is Murray's method for the preparation of CdSe, CdS and CdTe.\textsuperscript{44} Using identical reaction conditions and reagents (with the exception of the precursor) a series of semiconductor nanocrystals were prepared. Another early method used hydrodynamic cavitation (generated mechanically by a high-pressure fluid system) to prepare TiO\textsubscript{2}, ZrO\textsubscript{2}, NiO and CeO\textsubscript{2} from their metal nitrate precursors (ex. ZrO(NO\textsubscript{3})\textsubscript{2}•xH\textsubscript{2}O). This method produced nanoparticles of non-uniform shape that required calcination to achieve crystallinity.

More recently, several versatile generic solution-based methods have been reported for the synthesis of metal oxide nanocrystals, one of the most important classes
of nanocrystalline materials. Sun and co-workers\textsuperscript{99} heated Fe(acac)\textsubscript{3} in 1,2-hexadecanediol, oleic acid, oleylamine and phenyl ether to prepare Fe\textsubscript{3}O\textsubscript{4} nanocrystals. They extended this work to the preparation of MFe\textsubscript{2}O\textsubscript{4}, where M = Co or Mn by partial substitution of Fe(acac)\textsubscript{3} with Co(acac)\textsubscript{2} or Mn(acac)\textsubscript{2}. Another general method for the preparation of 1-D metal oxide nanorods was reported recently by Seo and co-workers\textsuperscript{79} and builds upon the method developed by Sun. Beginning with metal chloride precursors in oleic acid and oleylamine, heat is used to prepare metal oxide nanorods of W\textsubscript{18}O\textsubscript{49}, TiO\textsubscript{2}, Mn\textsubscript{3}O\textsubscript{4} and V\textsubscript{2}O\textsubscript{5}. Jana\textsuperscript{84} and co-workers have used self-prepared fatty acid metal salts (ex. Fe(II)-stearate) heated in octadecane to form nanocrystalline Fe\textsubscript{3}O\textsubscript{4}, Cr\textsubscript{2}O\textsubscript{3}, MnO, Co\textsubscript{3}O\textsubscript{4} and NiO of various morphologies, including rod-like, spherical, cubic and triangular. A significant contribution was made recently by Wang and colleagues,\textsuperscript{100} who reported a general “liquid-solid-solution” method for the preparation of twenty different types of nanocrystals, including some oxides (TiO\textsubscript{2}, CuO, ZrO\textsubscript{2}, SnO\textsubscript{2}, ZnO and some composite oxides). The experimental conditions are not described in detail; however, the method begins with metal ions in an aqueous solution, which move to a solid phase containing (RCOO)\textsubscript{n}M. When the metal ions move from the liquid to solid phase, the M\textsuperscript{+} dehydrates into oxides. The scope of this reaction is full and includes the synthesis of noble metal, semiconductor, magnetic, dielectric and even polymer nanocrystals.

Metal acetates and metal acetylacetonates are becoming more popular in the synthesis of metal oxide nanocrystals, and general routes to produce metal oxide nanocrystals popularized as well. Yin and O’Brien\textsuperscript{19,73,101} have found that metal acetates heated in the presence of trioctylamine and oleic acid form highly uniform and monodisperse MnO and Cu\textsubscript{2}O nanocrystals. More recently, metal acetylacetonates were
used as precursor compounds in a very simple method for the preparation of a variety of metal oxide nanocrystals.\textsuperscript{102} The preparation of $\gamma$-Ga$_2$O$_3$, ZnO and In$_2$O$_3$ nanocrystals was carried out by heating their respective M(acac)$_x$ starting materials with benzylamine at 200 °C for 2 days. A complex reaction mechanism was also proposed to account for the formation of nearly eight different side-products found in the final product solution. Very recently, Ba and co-workers\textsuperscript{71} reported the synthesis of indium tin oxide (typically 90% In$_2$O$_3$ and 10% SnO$_2$) nanocrystals by combining In(acac)$_3$ and Sn(OrBu)$_4$ in benzyl alcohol with heat. Based on these two publications, it appears that M(acac)$_x$ can be used to easily prepare a variety of metal oxide nanocrystals in simple Lewis basic solvent media.

Our methods have a similarly straightforward approach. In the first method, TiO(acac)$_2$ is combined with trioctylamine and a surfactant with HCl in the presence of heat to produce anatase TiO$_2$ nanocrystals. Another approach, only slightly altered, combines Fe(acac)$_3$ with one of a number of surfactants to give rise to $\gamma$-Fe$_2$O$_3$ nanocrystals. The preparation of Cr$_2$O$_3$, Mn$_2$O$_3$ and Mn$_3$O$_4$ nanocrystals was performed by combining Cr(acac)$_3$, Mn(acac)$_3$ or Mn(acac)$_2$, respectively, in acetone with heat for several days. These methods all provide simple, straightforward and cost-effective alternatives for the preparation of metal oxide nanocrystals.

1.6 References


Chapter 2

Spectroscopic Characterization of the Iron Oxide Nanocrystal Surface
2 Spectroscopic Characterization of the Iron Oxide Nanocrystal Surface

2.1 Introduction

Magnetic nanocrystals have attracted much interest recently because of their unique size-dependent properties, which are not observed in the molecular or bulk phases. The size dependent properties and biocompatibility of these magnetic nanocrystals give them potential for many biological applications, such as magnetic resonance imaging (MRI), DNA detection and drug delivery. In addition, maghemite ($\gamma$-Fe$_2$O$_3$) nanocrystals have been used to catalyze the formation of carbon nanotubes by CVD (chemical vapor deposition) growth methods and the diameter of the grown nanotubes is related to the diameter of the nanocrystal catalysts. Given the quantity of emerging applications for iron oxide nanocrystals, it is important to gain a full understanding of the nanocrystal structure, both in terms of the inorganic core and organic capping shell, in order to develop an accurate and universal description of the system.

Nanocrystals are isolated three dimensional nanometer scale units of materials, typically with symmetrical geometrical (spherical, cubic, triangular) morphologies and a well-formed crystalline core. The concepts of surface capping and solution stabilization have been developed to allow stable suspensions of nanoparticles to exist in a variety of aqueous and non-aqueous (organic solvent) media. The recent history of organometallic precursor-based synthesis has evolved significantly because it proved very successful in the preparation of nanoparticles organically passivated on the surface with capping groups. This type of procedure is best illustrated by the cadmium selenide system first conceived by Brus, Steigerwald and co-workers, and later developed by Murray,
Bawendi, Alivisatos and co-workers.\textsuperscript{5,14} The nanoparticles resulting from these procedures are stable in non-polar solvents (such as hexane or chloroform) and have non-polar capping groups. The stability in solution arises from the surface capping ligands, which prevent particle aggregation. These methods are distinct from sol-gel chemistry (which produce gels) and other more traditional methods of producing colloids as discerned by the nature of the product. The capping groups (also called ligands because they bind to the surface of the nanocrystal) are typically long chain alkyl surfactants with heteroatom or polar head groups that react with and bind to the nanocrystal surface via covalent, electrostatic or coordination bonds (or some combination of all three), generally to the metal atoms.

The nanoparticles remain highly stabilized in solution, because they have a surface that is mutually unreactive and repulsive towards other particles. Nanocrystals are stable with respect to aggregation only if the capping groups have an attractive interaction of sufficient strength to compete effectively with solvent for the nanocrystal surface, while still being freely soluble in the solvent. In this way, they can be dispersed in solvents and do not aggregate because there is too large an entropic penalty and little enthalpic gain in doing so.\textsuperscript{5} This can be considered as steric stabilization.\textsuperscript{15} During the solvent evaporation process, self-assembly of the nanocrystals can readily occur into ordered regions and ultimately self-assembled superlattices.

Long-chain fatty acids are widely used in metal oxide nanocrystal syntheses because they can form a dense protective monolayer by adsorbing to the nanocrystal surface through bonding of the carboxylic acid group to the metal oxide surface.\textsuperscript{16} Oleic acid \([\text{CH}_3(\text{CH}_2)_{17}\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}],\) which possesses a \textit{cis}-double bond at the 9,10
position, is a widely used ligand for passivation of $\gamma$-Fe$_2$O$_3$ nanocrystal surfaces because it produces nanocrystals that are highly uniform and monodisperse. Other ligands such as long chain fatty acids, alkyl amines, and diols are also commonly used ligands for iron oxides. Ligands are used to provide stability in solution, however, they are also instrumental in controlling growth and nanocrystal size. Yin$^{17}$ and co-workers compared the particle size as fatty acids of different chain length were used, and found that longer chain length surfactants give rise to larger particles. It is proposed that the lower dipole moment in longer chain fatty acids results in a weaker interaction with the nanocrystal surface leading to larger nanocrystal size.

There two different possible binding modes described in the literature for a carboxylate functional group interacting with or bound to a nanocrystal surface. FTIR is useful for studying the ligand/nanocrystal interface and is often the technique used to probe this interaction. Typical carboxylate ion C=O FTIR stretches are shown in Table 2.1. Shafi, et al.$^{18}$ report ionic bonding (formation of Fe$^{3+}$ salts with carboxylate) via the carboxylate head-group. The evidence for this is C=O asymmetric and symmetric stretching modes at 1538 cm$^{-1}$ and 1441 cm$^{-1}$ (which has been reported earlier for carboxylates bound to $\gamma$-Fe$_2$O$_3$)$^{19}$ and the absence of a C=O stretch (for a C=O stretch in a free carboxylic acid) at 1710 cm$^{-1}$, indicating binding of the carboxylate via one oxygen atom to the Fe$^{3+}$ ions at the nanoparticle surface. Ahn$^{20}$ describes the carboxylate binding interaction of stearic acid on silver nanoparticles as a symmetrical interaction with the carboxylate group bound to the silver surface through two oxygen atoms and therefore only a symmetrical C=O stretch at 1404 cm$^{-1}$ is observed. In addition, Kreller and co-workers$^{21}$ describes the interaction between gallic and tannic acid with iron oxide.
surfaces. They observe a band at 1365 cm\(^{-1}\) and attribute it to the chemisorption of a carboxylate head group to the oxide surface; suggesting a symmetric binding mode; however, they do not speculate whether the carboxylate is bound to the surface via 1 or 2 oxygen atoms in the carboxylate. An earlier report by Tao\(^{22}\) found differing results when observing the binding of various carboxylic acids to copper surfaces. They report the binding of carboxylic acids through only one oxygen atoms and observe both the symmetric C=O stretch (1441 cm\(^{-1}\)) and asymmetric C=O stretch (1537 cm\(^{-1}\)). Wu and co-workers\(^{23}\) recently described the symmetric bonding of the oleic acid carboxylate on Co nanoparticles. By FTIR, they did not observe a C=O stretch near 1700 cm\(^{-1}\), but did observe symmetric and asymmetric carboxylate (COO\(^{-}\)) bands at 1410 cm\(^{-1}\) and 1556 cm\(^{-1}\), respectively, suggesting symmetric binding of the carboxylate via two oxygen atoms to the Co surface. An illustration of symmetric and asymmetric binding modes of an oleate ion to the surface of \(\gamma\)-Fe\(_2\)O\(_3\) is shown in Figure 2.1.

<table>
<thead>
<tr>
<th>Carboxylic acid and carboxylate ion FTIR stretches</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxylate asymmetric stretch</td>
<td>1650-1550 cm(^{-1})</td>
</tr>
<tr>
<td>Carboxylate symmetric stretch</td>
<td>Near 1400 cm(^{-1})</td>
</tr>
<tr>
<td>C=O stretch in free oleic acid</td>
<td>1710 cm(^{-1})</td>
</tr>
</tbody>
</table>

Table 2.1  
*Frequency of carbonyl stretches in carboxylate ions and carboxylic acids*
Figure 2.1  Illustration of two possible binding modes for carboxylates on iron oxide nanocrystal surfaces. The left shows a symmetric binding mode where both oxygen atoms are attached to the surface, while the right shows an asymmetric binding mode, where only one oxygen atom is bound to the surface.

Binding interactions with γ-Fe₂O₃ surfaces have also been studied²⁴ with fluorescence spectroscopy by observing the interactions between functionalized (carboxylic acid, ester, alcohol) pyrenes and the surface of γ-Fe₂O₃ nanocrystals. In this report Turro and co-workers²⁴ find that the fluorescence of pyrene is quenched most efficiently by γ-Fe₂O₃ when a carboxylic acid linker is used. The quenching was not as effective for alcohol or ester functionalized pyrenes bound to the γ-Fe₂O₃ surface, indicating a weaker interaction with the surface. Quenching was partially reversed when a small amount of oleic acid was added, however it took a large excess to observe total reversal. This suggests two modes of interaction with γ-Fe₂O₃, physisorption (carboxylic acid adsorbed to the surface) and chemisorption (formation of covalent bond with surface).
NMR is a powerful and valuable tool that is used primarily for characterization of organic molecules, but rarely (because of line broadening) for organic molecules on surfaces like nanocrystals. In the case of $\gamma$-Fe$_2$O$_3$, $^1$H NMR of surface ligands has not been observed because paramagnetic metals, such as iron, cause peak broadening due to fast nuclear spin relaxation and incomplete averaging of dipole-dipole interactions.$^{26,27}$ Paramagnetic relaxation is caused by the large magnetic dipole moment of an unpaired electron, which is on the order of 700 times greater than the magnetic dipole moment of a proton.$^{26}$

Although NMR is not a common technique for characterization of nanocrystal ligands, there are a handful of examples.$^{28-33}$ When applicable, NMR can be used to further probe ligand/surface interactions in a way that other characterization methods do not allow. NMR provides information about the electronic environment of protons – from which we can understand more about the nanocrystal-ligand interactions. The binding of thiols to CdS, CdSe and In$_2$S$_3$ nanocrystal surfaces has been studied extensively by both solution and solid-state $^1$H, $^{113}$Cd and $^{77}$Se NMR.$^{28-33}$ Sachleben found thiophenol bound to CdS to have a significantly perturbed $^1$H NMR spectrum.$^{30}$ The $^1$H NMR spectrum of thiophenol bound to CdS contained peaks that were consistent with thiophenol, however shifted from where free thiophenol resonances are expected. Using $^{13}$C NMR, Zelakiewicz$^{34}$ found spectra of 1-octanethiol ligated to gold nanocrystals to be broadened and to have slightly shifted resonances that depended upon nanoparticle size. EPR methods have also been used to probe the surface interactions of nanocrystals. Recently, Zhang$^{35}$ used TEMPO nitroxyl radicals to observe a decrease in EPR signal due to
interactions of TEMPO with Au nanoparticle surfaces. Unpaired electrons are believed to interact with conduction band electrons in gold nanoparticles.

The chemistry of nanocrystal surfaces is still not well understood, in particular the binding interactions because the surfaces are difficult to characterize. Although oleic acid is frequently used to organically passivate the surface of iron oxide nanocrystals, a full characterization of the oleic acid/nanocrystal interface has not been performed, to the best of our knowledge. In this study we sought to characterize this interface in an attempt to shed light on why fatty acids give rise to such uniform, monodisperse $\gamma$-Fe$_2$O$_3$ nanocrystals.

### 2.2 Experimental Methods

Monodisperse iron oxide nanocrystals ($\gamma$-Fe$_2$O$_3$) were prepared following a standard literature procedure pioneered by Hyeon and co-workers.$^{36}$ Trioctylamine (14 mL, 32 mmol) and oleic acid (2 mL, 6.3 mmol) were combined under positive N$_2$ pressure, and heated at 180 °C for one hour. The temperature was lowered to 100 °C, and Fe(CO)$_5$ (0.4 mL, 3.0 mmol) was added to the solution and heated at 350 °C for one hour. During this process, the color of the solution changed from orange to black. After one hour the temperature was lowered to 70 °C and (CH$_3$)$_3$NO (0.68 g, 9.12 mmol) was added to the solution and heated at 130 °C for two hours, followed by refluxing at 350 °C for one hour. The solution was cooled to room temperature, dissolved in hexanes and centrifuged to precipitate the largest particles. Ethanol was added to the supernatant until the transparent brown solution appeared turbid and the solution was centrifuged 10 minutes at 3,800 rpm. The precipitate was collected and dispersed in hexanes. The diameter of the nanocrystals was 11 nm with 5% polydispersity (calculated by measuring
the diameter of 100 nanocrystals in a TEM image). The structure was confirmed by powder x-ray diffraction (Scintag X2), $^1$H and $^{13}$C NMR, FTIR and XPS. Images of the particles were taken on a JEOL cx100 transmission electron microscope (TEM) in bright field (BF) mode at 100kV. Samples were prepared by drying hexanes solvent dispersions of the nanoparticles onto Formvar© amorphous carbon backed 400-mesh Cu grids followed by drying under vacuum at RT. FT-IR experiments were performed on a Nicolet 8700 fitted with a DRIFT Smart collector. NMR experiments were performed on a Bruker 400 MHz spectrometer at RT. The locking solvent was CDCl₃. GC-MS was conducted with a VARIAN Saturn 2100. The XPS experiment was performed on PHI 5500 model spectrometer equipped with an Al K alpha monochromator X-ray source running at 15 kV, a hemispherical electron energy analyzer, and a multichannel detector. The test chamber pressure was maintained below $2 \times 10^{-9}$ Torr during spectral acquisition. A low-energy electron flood gun was used to neutralize the possible surface charge. The XPS binding energy (BE) was internally referenced to the aliphatic C(1s) peak (BE, 284.6 eV).

2.3 Results and Discussion

Highly crystalline and monodisperse nanocrystals of $\gamma$-Fe₂O₃ were synthesized according to the procedure outlined in Scheme 2.1, which also illustrates that the nanocrystals produced possess a ligand shell around the inorganic iron oxide core. This ligand shell is composed of one monolayer of organic molecules, calculated from TEM, spectroscopy and previous literature. The starting material for the preparation of the ligand shell is oleic acid. The ligand existing in the product shell is denoted (4). The ligand capped nanocrystals, oleic@$\gamma$-Fe₂O₃, will henceforth be denoted (3).
Scheme 2.1 Preparation of γ-Fe₂O₃ nanocrystals. Reagents were combined and heated at temperatures of up to 350 °C followed by size selection to remove small and large nanocrystals.

The synthesis of γ-Fe₂O₃ nanocrystals involves heating at temperatures as high as 350 °C. The heating of fatty acids is known to cause isomerization and loss of unsaturation. Indeed, it was recently claimed that oleic acid "polymerizes" when tethered to a calcite surface and heated. Wu and co-workers also recently reported on their surfactant characterization for Co nanoparticles synthesized with oleic acid capping groups. They used NMR and FTIR to study the interaction of the carboxylate head group of oleic acid with the Co nanocrystal surface. Based on this precedent, there is a concern that oleic acid might suffer a structural change during nanocrystal synthesis. In this study, we employed transmission electron microscopy (TEM), ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy as well as Fourier transform infrared spectroscopy (FTIR), high resolution mass spectrometry (HRMS), elemental analysis and x-ray photoelectron spectroscopy (XPS) to monitor the chemical structure of the surfactant changes of the ligand during nanocrystal synthesis. The observed spectroscopic results suggest a loss of unsaturation in oleic acid occurs during synthesis of γ-Fe₂O₃ nanocrystals.
2.3.1 TEM Characterization

Transmission electron micrographs of \( \gamma-\text{Fe}_2\text{O}_3 \) nanocrystals (Figure 2.2) synthesized with oleic acid as a surfactant, showed well-formed, monodisperse and spherical nanocrystals that self-assemble into ordered arrays when deposited onto a TEM grid. They are surrounded by an organic surfactant layer, which separates them from each other and prevents aggregation. The interparticle separation of 50 Å is approximately commensurate with two interdigitated layers of surfactant molecules of comparable length to oleic acid (23.0 Å).

![TEM image of \( \gamma-\text{Fe}_2\text{O}_3 \) nanocrystals dispersed in hexanes and dropped onto a 400-mesh Cu grid coated with Formvar support resin. Solvent was removed under vacuum for 1 h.](image)

2.3.2 Characterization of \( \gamma-\text{Fe}_2\text{O}_3 \) Nanocrystals by NMR

NMR of nanocrystals and nanocrystal surfactants has been used in the past to understand interactions at the nanocrystal-ligand interface and to characterize the topography of the surface of diamagnetic nanocrystals.\(^{29,31,43-45}\) However, examination of ligands on paramagnetic nanocrystals by \(^1\text{H} \) NMR has been difficult due to large broadening effects caused by the paramagnetic nanocrystals. Therefore it was interesting
to find relatively sharp NMR resonances when the $^1$H NMR spectrum of (3) was observed. To the best of our knowledge, this is the first example of a $^1$H NMR spectrum of a ligand bound to a paramagnetic nanocrystal and also a change in chemical structure of a ligand, oleic acid, during nanocrystal synthesis.

Figure 2.3 compares the $^1$H NMR spectra of free oleic acid (A), the oleicacid@γ-Fe$_2$O$_3$ nanocrystals (3) (B), and ligands (4) removed from γ-Fe$_2$O$_3$ surface (C). Iron, being paramagnetic is known to broaden the resonances in $^1$H NMR spectra. Thus, the sharp resonances were unexpected in the $^1$H NMR spectrum of surfactant bound to γ-Fe$_2$O$_3$ nanocrystals (Figure 2.3, spectrum B).

**Figure 2.3** $^1$H NMR of oleic acid (A), oleicacid@γ-Fe$_2$O$_3$ nanocrystals (3) (B), and the ligands (4) removed from γ-Fe$_2$O$_3$ surface (C). The experiments were run at RT and the locking solvent was CDCl$_3$. Concentration of nanocrystals is 1-5 mM.
Spectrum A shows the $^1$H NMR of technical grade oleic acid (90%) in CDCl$_3$. The $^1$H NMR of γ-Fe$_2$O$_3$ (Figure 2.3, spectrum B) is different than that of oleic acid (spectrum A). In particular, in spectrum B there is a lack of vinyl and allyl proton resonances, which appear at 5.5 ppm and 2.0 ppm, respectively, in the spectrum of oleic acid. We also observe a shift in the location of peak "e" in between spectra A and B. In addition, peak "g" in spectrum A does not appear in spectrum B. The movement of peak "e" and the disappearance of peak "g" in spectrum A suggest a change to the carboxylic acid. The large peak at approximately 2.05 ppm in spectrum B is a result of residual acetone remaining from the size selection process. The absence of the vinyl and allyl peaks suggests the reduction of the oleic acid double bond during nanocrystal synthesis.

The ligand (4) was removed from the nanocrystal surface by washing the nanocrystal hexane dispersion with an equal volume of either EtOH or acetone several times (see Figure 2.14). Spectra B and C are quite different in Figure 2.3. This is most likely a result of shifting and perturbation caused by binding of the ligand to the nanocrystal surface. The $^1$H NMR of the ligand (4) stripped from the nanoparticle surface (Figure 2.3, spectrum C) also shows an absence of the vinyl and allyl resonances that are present in the $^1$H NMR of free oleic acid. Furthermore, peak "g" in oleic acid is shifted slightly downfield in spectrum C, whereas peak "e" in spectrum A is shifted upfield in spectrum C.

When elaidic acid, the trans isomer of oleic acid, is used as a surfactant in the synthesis of γ-Fe$_2$O$_3$, the vinyl and allyl resonances are also absent in the $^1$H NMR of the surfactant capped nanocrystals, indicating that the configuration about the double bond is not important in this chemical transformation.
The stripped surfactant (4) was also characterized by $^{13}$C NMR (Figure 2.4) The $^{13}$C NMR of the removed surfactant (4) supports the conclusions made from the $^1$H NMR. In the carbon NMR spectrum of free oleic acid the double bond carbons have a peak at 130 ppm and the carboxylic acid carbon has a peak at 177 ppm. Both of these peaks are absent in the spectrum of the removed surfactant, indicating the loss of the double bond and a loss of the carboxylic acid. There is also a peak at 57.5 ppm, which appears in the spectrum of (4), however is absent in the spectrum of oleic acid. It could be due to the formation of an epoxide at the site of the double bond, and this will be addressed further in section 2.3.6.

![Figure 2.4](image_url) $^{13}$C NMR of ligand (4) stripped from nanocrystal surface (A) and oleic acid (B). Samples were run at RT in CDCl$_3$ solvent.
2.3.3 Infrared Spectroscopy

Infrared spectroscopy was used to characterize the nanocrystal-ligand interaction and also to further characterize the ligand itself. The very broad feature from 3500–2500 cm\(^{-1}\) in spectrum A (free oleic acid) is assigned to an O-H stretch; it is typical of carboxylic acids which dimerize due to strong hydrogen bonding. This feature is not as pronounced as in B or C, indicating that there is less free oleic acid to dimerize through hydrogen bonding because it is bound to the surface. Table 2.2 shows the IR bands in oleic acid for reference.

<table>
<thead>
<tr>
<th>IR bands in oleic acid</th>
<th>Wavenumber (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-H stretch</td>
<td>3500-2500</td>
</tr>
<tr>
<td>Alkene C-H stretch</td>
<td>3007</td>
</tr>
<tr>
<td>Alkane C-H stretches</td>
<td>2952, 2924, 2852</td>
</tr>
<tr>
<td>C=O stretch</td>
<td>1708</td>
</tr>
<tr>
<td>O-H bends</td>
<td>1460, 931</td>
</tr>
<tr>
<td>C-O stretch</td>
<td>1282</td>
</tr>
</tbody>
</table>

**Table 2.2 Bands observed in DRIFT IR of technical grade oleic acid**

The DRIFT IR spectrum of the synthesized \(\gamma\)-Fe\(_2\)O\(_3\) nanocrystals (3) is shown in Figure 2.5, Spectrum C. Two very weak carbonyl stretches are present at 1738 cm\(^{-1}\) and 1710 cm\(^{-1}\) confirm the existence of a mixture of ligand components. These resonances could result from physisorbed (1738 cm\(^{-1}\)) and chemisorbed (1710 cm\(^{-1}\)) ligand bound to the surface.\(^{24}\) Spectrum C in Figure 2.5 also has strong signature \(\gamma\)-Fe\(_2\)O\(_3\) bands at 460 and 600 cm\(^{-1}\). The broad peaks in spectrum C at 1527 cm\(^{-1}\) and 1430 cm\(^{-1}\) can be assigned to a carboxylate (COO\(^{-}\)) stretch. Overall, the broad appearance of the peaks in this spectrum further suggests the presence of a mixture of compounds on the nanocrystal surface.
Spectrum B in Figure 2.5 represents the DRIFT of the ligand stripped from the $\gamma$-Fe$_2$O$_3$ nanocrystal surface (4). Similar to spectrum C, the peaks are significantly broadened, which suggests a mixture of compounds. There is no evidence of a vinyl C-H stretch, similar to the IR of $\gamma$-Fe$_2$O$_3$ nanocrystals; however, there is a significant carbonyl stretch centered at 1710 cm$^{-1}$, which is slightly broadened and may suggest a mixture of ligands on the surface. The presence of this band conflicts with the $^{13}$C NMR results, which show a loss of the carbonyl carbon at 177 ppm. This is suggestive of a mixture of ligand species in the removed surfactant. In addition, the O–H stretch which extends from 3600–2500 cm$^{-1}$ has decreased in intensity, as well as the C–O–H bends at 1460 cm$^{-1}$ and 931 cm$^{-1}$, which suggests a loss of the OH functionality.

**Figure 2.5**  
DRIFT spectra of oleic acid (A), stripped ligand (4) (B), and oleicacid@$\gamma$-Fe$_2$O$_3$ (3) (C). Spectra are normalized. Samples (1-5% wt) were mixed with pre-ground KBr powder.
2.3.4 X-ray Photoelectron Spectroscopy (XPS)

XPS is a quantitative spectroscopic technique used to measure the elemental composition, and electronic state of the elements present within a material. The spectra are obtained by irradiating a material with a beam of X-rays while simultaneously measuring the kinetic energy (KE) and number of the electrons that escape from the top 1-10 nm of the material being analyzed.

Further characterization of the ligand coated $\gamma$-Fe$_2$O$_3$ nanocrystals (3) was performed with x-ray photoelectron spectroscopy (XPS), as shown in Figure 2.6. The C1s spectrum contains two peaks with binding energies of 284.8 eV and 287.9 eV. The peak at 284.8 eV is assigned to the carbons in the aliphatic chain and the shoulder peak at 287.9 eV is assigned to the carboxylate (-COO') carbon, which are both in agreement with previous literature reports of carboxylic acids attached to nanocrystal surfaces.\textsuperscript{25,47-49}

Neither of the peaks in the spectrum can be assigned to a carboxylic (-COOH) carbon, which demonstrates that there is little or no free oleic acid present on the $\gamma$-Fe$_2$O$_3$ nanocrystal surface. The O1s core level spectrum has peaks with binding energies of 529.6 eV and 531.1 eV. The peak at 529.6 eV corresponds to the $\gamma$-Fe$_2$O$_3$ nanocrystals, while the peak at 531.1 eV is assigned to the carboxylate on the nanocrystal surface. From this we can conclude that the two oxygen atoms must be symmetric and bound via two oxygen atoms. Had the carboxylate been bound to the surface through one oxygen, another oxygen peak would be present at 533 eV. This supports the IR data, which show the disappearance of the carbonyl peak when the ligand is bound to the $\gamma$-Fe$_2$O$_3$ nanocrystals.
The Fe(3p) spectrum has one peak with a maximum at 55.7 eV, and the Fe(2p) spectrum has peaks at 709.9 eV (Fe 2p\textsubscript{3/2}) and 723.5 eV (Fe 2p\textsubscript{1/2}) and this is in agreement with previous XPS analysis of γ-Fe\textsubscript{2}O\textsubscript{3} nanocrystals.\textsuperscript{50}

![Graphs A, B, C, D showing XPS characterization of ligand capped γ-Fe\textsubscript{2}O\textsubscript{3} nanocrystals (3). A) C1s; B) O1s; C) Fe2p; D) Fe3p.]

2.3.5 Effect of Heating on Oleic Acid and Synthesis with Analogous Ligands

It has been previously reported that long-chain fatty acids undergo loss of unsaturation when subjected to high temperatures.\textsuperscript{37-40} We have observed that when oleic or elaidic acid is heated slowly to 350 °C, a similar loss of unsaturation is observed by \textsuperscript{1}H NMR and FTIR. Aliquots of the acid were taken as heating progressed and analyzed by \textsuperscript{1}H NMR and FTIR. We observed a loss of unsaturation to begin at approximately 290 °C, as evidenced in both the \textsuperscript{1}H NMR and FTIR spectra. Previous literature reports have
found that the structure of oleic acid changes upon heating in excess of 300 °C; and long chain monoolefinic C₁₈ fatty acids are believed to dimerize and polymerize when heated above 300 °C.⁴¹,⁵¹-⁵³

The significance of the carboxylic acid head group in facilitating uniform nucleation and growth of γ-Fe₂O₃ nanocrystals, is clarified by substituting methyl oleate (the methyl ester of oleic acid) [CH₃(CH₂)₇CH=CH(CH₂)₇COO(CH₃)] for oleic acid in the synthesis of γ-Fe₂O₃ nanocrystals, and maintaining all other conditions. The products were obtained in low yield, and have a low monodispersity. Surface characterization of the nanocrystalline products was not performed, so it is unclear if or how methyl oleate was bound to the γ-Fe₂O₃ nanocrystalline surface.

Since the aforementioned results confirm a loss of unsaturation in oleic and elaidic acid during nanocrystal synthesis, an attempt was made to synthesize γ-Fe₂O₃ nanocrystals with stearic acid [CH₃(CH₂)₁₆COOH], the saturated analog of oleic acid, and also with 1-octadecanol [CH₃(CH₂)₁₆COH]. In both cases the nanocrystals produced were of irregular shape and low monodispersity, which suggests that unsaturated bond must exert some kind of influence over the quality of nanocrystals produced, either due to the change in chemistry, structure or solubility of the ligand. TEM images of γ-Fe₂O₃ nanocrystals synthesized with 1-octadecanol (Figure 2.8), stearic acid (Figure 2.7), and elaidic acid (Figure 2.9) are shown. The TEM image of particles coated in elaidic acid show them to be of high uniformity and monodispersity, signifying that the conformation about the double bond does not have an effect on the particle shape. Methyl oleate was also used as a surfactant and yielded products of similar appearance to those made with stearic acid and 1-octadecanol.
Figure 2.7  TEM of $\gamma$-Fe$_2$O$_3$ nanocrystals synthesized with stearic acid surfactant.

Figure 2.8  TEM of $\gamma$-Fe$_2$O$_3$ nanocrystals synthesized with 1-octadecanol as the surfactant.
Figure 2.9  TEM of γ-Fe₂O₃ nanocrystals synthesized with elaidic acid surfactant.

In a previous report,⁵⁴ varied chain-length carboxylic acids were employed in the synthesis of γ-Fe₂O₃ nanocrystals, and proved to be adequate for the synthesis of γ-Fe₂O₃ nanocrystals. Therefore, it is believed that the presence of the double bond is unlikely to have a strong effect on the chemistry preceding nucleation, although the carboxylic acid head group might. The presence of oleic acid was found to be critical in the synthesis of stable, monodisperse γ-Fe₂O₃ nanocrystals. The structure of the oleic acid is irreversibly changed during nanocrystal synthesis, and this is expected given previous studies of its thermal history. However, we note that the chemical transformation of this ligand is inextricably linked to the high quality of the nanocrystals produced.

Since much of the existing literature reporting on heating long-chain fatty acids dates back nearly 60 years, control experiments were performed to observe the effects of heating oleic acid at high temperatures with modern spectroscopic techniques. Figure 2.10 shows NMR spectra which illustrate the distinct changes that occur when oleic acid is heated at 350 °C for 4.5 h. Similar to the ¹H NMR spectrum of the surfactant (4) removed from the nanocrystal surface, a disappearance of the vinylic and allylic resonances at 5.5 ppm and 2.0 ppm is observed. This confirms the result obtained when
synthesizing $\gamma$-$\text{Fe}_2\text{O}_3$, and affirms that the loss of unsaturation results from extreme temperatures, and not the presence of other reactants. The peaks at 1.6 ppm and 2.4 ppm, which correspond the hydrogen atoms on the carbons $\alpha$ and $\beta$ to the carboxylic acid, have also diminished significantly. This suggests that decarboxylation occurs at high temperatures. Stearic acid (the saturated analog of oleic acid) was heated in the same manner and analyzed by $^1\text{H}$ NMR (Figure 2.11). There are not any significant changes in the $^1\text{H}$ NMR spectrum of stearic acid after heating.

![NMR Spectra](image)

**Figure 2.10** $^1\text{H}$ NMR of oleic acid (spectrum A) and oleic acid after heating at 350 °C for 4.5 hours (spectrum B). The spectra were obtained at room temperature in CDCl$_3$ solvent. The large peak at 2.05 ppm results from acetone.
Figure 2.11 $^1$H NMR of stearic acid (spectrum A) and stearic acid heated (spectrum B) at 350 °C for 4.5 h. Sample was examined at room temperature in CDCl$_3$ solvent. No major changes are apparent.

Tracking changes while oleic acid is heated. Aliquots were removed every 25 °C while oleic acid was heated to a final temperature of 350 °C and analyzed by $^1$H NMR and FTIR to track the changes in the structure of oleic acid. Most importantly, the disappearance of the vinyl and allyl resonances was observed and the changes in peak integration were plotted graphically (Figure 2.12).
Figure 2.12 Comparison of integration values obtained from $^1$H NMR spectra of aliquots taken from heating oleic acid slowly to 350 °C as they change with temperature and time. Clearly a structural change occurs rapidly after 325 °C.

The relative $^1$H NMR peak integrals of the vinyl and allyl resonances were plotted versus time and temperature. From these integrals it is clear that the loss of unsaturation begins as the temperature of oleic acid approaches 300 °C and increases more rapidly upon reaching 325 °C. The vinyl and allyl resonances disappear at the same rate, as expected.

The aliquots removed during heating were also analyzed by FTIR (Figure 2.13). The vinyl C-H stretch, which occurs slightly above 3000 cm$^{-1}$ disappears between 325 °C
and 340 °C, confirming the loss of unsaturation during heating. Furthermore, at 340 °C the carbonyl stretch at 1710 cm\(^{-1}\) begins to diminish, indicating the possibility of decarboxylation. There is also a noticeable decrease in the broadness of the feature between 3400 cm\(^{-1}\) and 2500 cm\(^{-1}\). The broadness, which results from hydrogen bonding and dimerization of oleic acid must decrease due to a loss of the carboxylic acid functionality. Moreover, peaks in the fingerprint region from 1350-1150 cm\(^{-1}\) disappear almost completely, suggesting a loss of the C–O stretch. This transformation, along with appearance of peak at ca. 1380 cm\(^{-1}\), which is a C–H bend from a methyl group, suggests loss of CO\(_2\), and formation of a structure resembling octadecane.

![FTIR spectrum](image)

**Figure 2.13** FTIR of oleic acid and aliquots removed at 325 °C and 340 °C. Samples were diluted in CCl\(_4\) and recorded between KBr salt plates at room temperature. Loss of alkene C-H stretch (3003 cm\(^{-1}\)), carbonyl stretch (1710 cm\(^{-1}\)) and several bands from 1350-1150 cm\(^{-1}\) are observed after 325 °C.
2.3.6 Surfactant Removal and Characterization

The surfactant was removed from the γ-Fe₂O₃ nanocrystals by precipitating the nanocrystals with EtOH several times to isolate the surfactant as illustrated in Figure 2.14.

![Flowchart](image)

**Figure 2.14** Flowcharts illustrating details of nanocrystal isolation, surfactant removal, component separation and characterization.

Separation of the observed mixture into its constituents was attempted by column chromatography, however the component R₁₆s were too similar to achieve separation. The removed surfactant was further analyzed by electron impact mass spectroscopy (EIMS). The EIMS, shown in Figure 2.15, suggests the presence of multiple products, similar to the conclusions from the FTIR. Although the molecular ion peak for oleic acid was not
detected, the spectrum shows evidence of small quantities of an oleic acid dimer. Peaks at 376.5, 362.5 and 348.5 may correspond to fragments of the oleic acid dimer. These peaks are very weak, however, suggesting that there is only a small amount of dimer in the surfactant mixture. This hypothesis is also supported by the $^1$H NMR and FTIR data, which show the presence of only trace quantities of unsaturated and carbonyl containing compounds. The spectrum also points towards the presence of stearic acid ($MI^+$ at 284.4, fragments at 270.4, 256.4). There is also evidence for the formation of a species possessing the composition of oleic acid plus an additional O atom. The formation of an epoxide at the 9,10 position on the oleic acid carbon chain would be consistent with this FW; there is evidence in the literature$^{30}$ that oleic acid can absorb oxygen to form an epoxide and destroy the double bond. In the EIMS spectrum the molecular ion of the epoxide appears at 298.4. Overall, the spectrum contains many peaks and few distinguishable patterns among them, which ultimately suggests a mixture of several compounds.

![EIMS spectrum of surfactant stripped from \(\gamma\)-Fe$_2$O$_3$ nanocrystals. Stripped surfactant (4) was diluted in ethyl acetate.](image-url)
The stripped surfactant (4) was further analyzed by C, H, and O elemental analyses. The result of the analysis was a surfactant composed of 78.3% carbon, 14.13% hydrogen, and 5.62% oxygen. Oleic acid is 76.54 % carbon, 12.13% hydrogen and 11.33% oxygen. The lower percentage of oxygen, in conjunction with the $^{13}$C NMR also favors the theory that an epoxidation is occurring. The experimental results do not confirm or identify the removed ligand as a single product, however some of the spectroscopic data favors the proposal of decarboxylation, in addition of loss of double bond. The $^{13}$C NMR of the stripped surfactant shows no evidence of carbonyl or olefinic carbons. The FTIR of oleic acid heated to 350 °C also gives strong evidence to favor the notion of possible decarboxylation of oleic acid at high temperatures. The results of the elemental analysis were compared to the theoretical ratios of potential products such as stearic acid, octadecanol, or a dimer of oleic acid and none matched closely. These results also favor the hypothesis that the composition of the surfactant is a mixture. The percentage of oxygen present in the stripped surfactant is much lower than what is expected for oleic acid, further suggesting a decarboxylation, and possible formation of an epoxide.

FeO, Fe$_2$O$_4$, MnO, and Mn$_3$O$_4$ nanocrystals were studied with $^1$H NMR, however not in as much depth as $\gamma$-Fe$_2$O$_3$. The preparation of all of these nanocrystals used oleic acid as a surfactant and all have $^1$H NMR spectra similar to that of the surfactant bound to $\gamma$-Fe$_2$O$_3$ as shown in Figure 2.3. Albeit preliminary, this result shows that this change in surfactant structure during nanocrystal synthesis is not unique to $\gamma$-Fe$_2$O$_3$, but is common to several metal oxide species.
2.3.7 $^1$H NMR Relaxation Studies

The paramagnetic nature of nanocrystalline $\gamma$-Fe$_2$O$_3$ is manifest in the $^1$H NMR spectra of its surface ligands by the broadening of the resonances. The paramagnetism of $\gamma$-Fe$_2$O$_3$ nanocrystals was also observed by EPR (not shown). Therefore, it was of interest to investigate the extent of these ligand/surface interactions further. $T_1$ relaxation measurements of pure oleic acid and also $\gamma$-Fe$_2$O$_3$ with its altered surface ligands (3) were measured. Figure 2.16 illustrates the effect that paramagnetic nanocrystals have on $T_1$ relaxation of surface bound ligands. Both resonances profiled show a significant decrease in $T_1$ relaxation time for ligands bound to paramagnetic nanocrystals. This suggests that the ligands are indeed bound to the surface. The different resonances were chosen to highlight how the effect on $T_1$ depends upon the distance of the atom in the ligand from the nanocrystal surface. The peak at 0.9 ppm, for example is due to the methyl hydrogen atoms at the end of the ligand, which are located farthest away from the $\gamma$-Fe$_2$O$_3$ nanocrystal surface. It is clear from the graph that the $T_1$ for these atoms is not affected as much as the 2.4 ppm peak, which is due to hydrogen atoms closer in distance to the nanocrystal surface, on the carbon alpha to the carboxylic acid.
Figure 2.16  *Bar graph displaying NMR T<sub>1</sub> measurements of two NMR resonances in various metal oxide nanocrystal samples and free oleic acid.*

Oleic acid has its alkyl chains in a *cis* configuration about its double bond. Elaidic acid, the *trans* isomer of oleic acid, was also investigated to help determine why these compounds are better ligands than stearic acid and other long chain carboxylic acids in the synthesis of iron oxide nanocrystals. Elaidic acid was found to be as good as oleic acid (as mentioned earlier), however being liquid, oleic acid is easier to work with in this preparation. Elaidic acid is a while crystalline solid and is recommended to be stored in an inert environment. The T<sub>1</sub> measurements of γ-Fe<sub>2</sub>O<sub>3</sub> nanocrystals synthesized with oleic and elaidic acid were compared.
Figure 2.17  $T_1$ measurements of two NMR resonances in various metal oxide nanocrystal samples capped with oleic and elaidic acid

Free elaidic and oleic acids both have comparable $T_1$ measurements. The $T_1$s of oleic and elaidic acid bound to $\gamma$-Fe$_2$O$_3$ and Mn$_3$O$_4$ were decreased significantly, as expected. There was no significant difference between the $T_1$s of bound oleic and elaidic acid.

2.4 Conclusion

We report the first example of a change in surfactant structure during $\gamma$-Fe$_2$O$_3$ nanocrystal synthesis. We have demonstrated that oleic acid is transformed during synthesis of $\gamma$-Fe$_2$O$_3$ nanocrystals. The NMR, FTIR and MS results suggest that the surfactant is transformed in the presence of a mixture of compounds. The results presented in this report suggest that the decomposition of oleic acid, which occurs at high
temperatures, may promote the formation of high quality $\gamma$-Fe$_2$O$_3$ nanocrystals. Although the spectroscopic results are somewhat conflicting, they suggest several things: a loss of unsaturation, decarboxylation, and a potential epoxidation. Based on this accumulation of spectroscopic data, and known behavior of heated oleic acid, we believe that a mixture of species exists on the $\gamma$-Fe$_2$O$_3$ surface. This mixture of ligands that forms has the unusual ability to give rise to $\gamma$-Fe$_2$O$_3$ nanocrystals of higher monodispersity and uniformity than most other ligands. So far this transformation has only been observed on $\gamma$-Fe$_2$O$_3$ and other metal oxide nanoparticles that use oleic acid as a surfactant, however it is possible that this phenomenon occurs when oleic acid is added as the surfactant in the synthesis of other types of nanoparticles.

2.5 References


(42) Elemental analysis was performed by Galbraith Laboratories, Knoxville, TN
(49) Korolev, V. V.; Ramazanova, A. G.; Blinov, A. V. Russian Chemical Bulletin 2002, 51, 2044.
Chapter 3

Bioconjugation of $\gamma$–Fe$_2$O$_3$ Nanocrystals for

Cell Detection by MRI
3 Bioconjugation of γ-Fe$_2$O$_3$ Nanocrystals for Cell Detection by MRI

3.1 Introduction

3.1.1 Motivation

Magnetic resonance imaging (MRI) has risen to be a highly important tool for both medical diagnosis and research. It is a non-invasive imaging technique, capable of 3-D imaging of opaque tissues with near cellular resolution for the determination of pathological alterations in tissues.¹ In the year 2000, 14.4 million MRI examinations were performed in the United States, which translates to roughly 51 examinations per 1,000 individuals. Contrast-enhancement is sometimes used to delineate areas of interest; traditionally the contrast agent injected into patients receiving an MRI examination is gadolinium. Gadolinium contrast agents accumulate in abnormal tissue areas and these areas appear “lit up” in the MRI image. Even though gadolinium has strong imaging capabilities, the element itself is toxic and must be surrounded by a thick ligand coating to prevent harmful effects in the human body. This project demonstrates the development of a non-toxic type of contrast agent, magnetic iron oxide nanocrystals (MIONs), for imaging of islet cells which are aggregates of insulin-secreting β cells located in the pancreas. Islets are small islands of several thousand β cells and in individuals that have diabetes, the insulin producing cells are destroyed by attack from the immune system, which leads to a deficiency of insulin. Recent research efforts have found that an islet
cell transplant can lead to normal insulin production. Currently, donated islet cells are injected into the patient; however, there is no diagnostic method that allows medical practitioners to follow the journey of the islets from injection point to their final destination.

Magnetic resonance imaging is a mature field that has methods for targeting and imaging of specific tumors and organs, research into targeting smaller organisms, like cells, is relatively new and there is still much research to be done. The overall goal of this project is to use $\gamma\Fe_{2}O_{3}$ nanocrystals conjugated to antibodies such that they can detect changes in concentration of the MIONs by MRI.

Our work on this project has led to the preparation of an improved MION that is water-soluble and easy to attach to biomolecules such as proteins, cell receptors, or antibodies. When nanoparticles are prepared, they cannot be dissolved in water; however, in order to be useful in any biological application they must be water-soluble. Traditionally, MIONs are made water-soluble and biocompatible by encapsulation within a thick layer of dextran (polysaccharide) sugar. This method produces water-soluble MIONs; however, extensive purification is required and further chemical modification must be performed for the MIONs to attach to cells. We are able to create water-soluble MIONs by using phospholipids, which are smaller than dextran and similar in structure to a cell membrane, making it appear less foreign to the body. Our method also requires little purification and the phospholipids utilized can be tailored such that they will easily attach to target molecules and will not require additional chemical modification.

The capability of the MION as a label for islet cells was demonstrated by creating a MION-antibody conjugate with an antibody that adheres to the surface of pancreatic
islet cells. This label attaches to the surface of islets and fluorescence microscopy is used to image this event. Both the MION and the antibody (Ab) are labeled with a different color fluorescent molecule and fluorescent microscopy is used to visualize the attachment of the MION-Ab label to islet cells in a cross-sectional slice of human pancreas.

3.1.2 Maghemite: Structure and Magnetic Properties

The material used to create the MRI contrast agents is $\gamma$-Fe$_2$O$_3$, called maghemite, a magnetic form of iron oxide that exists in nature as a reddish-brown material. It is typically formed from another iron oxide (like Fe$_3$O$_4$) compound and typically adopts the crystal structure of its precursor. Maghemite is isostructural with magnetite and has a cubic unit cell. The main difference between magnetite and maghemite is that maghemite has only trivalent Fe ions, whereas magnetite has divalent and trivalent Fe ions. Maghemite also contains cation vacancies to compensate for the oxidation of Fe(II). Each unit cell contains 32 O$^{2-}$ ions, 21 1/3 Fe$^{3+}$ ions and 2 1/3 vacancies. There are eight cations in tetrahedral holes with the others randomly distributed in octahedral holes. The unit cell of maghemite is illustrated in Figure 3.1.
Figure 3.1 The unit cell of $\gamma$-Fe$_2$O$_3$. Oxygen ions are in red, while octahedral Fe$^{3+}$ ions are in yellow and tetrahedral Fe$^{3+}$ ions are in green.

Maghemite is ferrimagnetic at room temperature. It has two sublattices, one octahedral and one tetrahedral, with opposed magnetic moments. The magnetic moments of ions in each individual sublattice are parallel, but antiparallel to the magnetic moments of neighboring lattices, giving rise to an overall net magnetic moment. While ferromagnetic in the bulk and larger nanocrystals, maghemite becomes superparamagenetic in nanocrystals of less than 10 nm diameter.

3.1.3 Methods for Creating Water-Soluble MIONs

A survey of recent literature shows that most methods towards the coupling of biomolecules to MIONs utilize a dextran or polysaccharide coating in order to achieve water-solubility and biocompatibility. Water-solubility is necessary to use MIONS in biological systems. In the majority of the literature on this topic, dextran coated MIONS are further functionalized by linker molecules that allow the MIONs to be conjugated to
biomolecules. Methods using dextran as a means of achieving water-solubility are often time intensive and require significant chemical modification to couple MIONs to biomolecules (proteins, DNA, immunoglobulins).

A recently published method\(^3\) for obtaining water-soluble quantum dots utilizes phospholipids to coat quantum dots instead of the well-established dextran method. Phospholipids have a long nonpolar hydrocarbon tail and a polar ionic head group. The phospholipids form micelle structures around quantum dots with the hydrophobic end on the inside of the micelle and the hydrophilic ionic head interacting with the water environment. This method for solubilizing quantum dots using phospholipids is very simple and can be performed within several hours. Little product purification is required, as is necessary in many of the dextran methods.

A variety of methods are used to create water-soluble nanocrystals for biological applications; however, the preponderance of MIONs described in the literature are modified with functionalized polysaccharide dextran coatings. Surface ligand modification and exchange, or encapsulation within a water-soluble shell (dextran,\(^2^\text{-}^5\) phospholipids,\(^6\) dendrimers\(^7\)) are used most commonly to provide solubility in water. Most methods for preparation of iron oxide nanocrystals use capping groups that are neither water-soluble, nor functionalized with biologically relevant functional groups. Since the methods that produce the most uniform and monodisperse nanocrystals do not provide water-solubility, the nanocrystal surfaces must be altered post-synthesis. The most biologically relevant functional groups are -COOH, -SH and -NH, and these are highly useful in linking nanocrystals to biomolecules.
Bäumle and co-workers\(^8\) prepared water-soluble CdSe nanocrystals at RT by addition of oligopeptide butathione (GSH) during CdSe nanocrystal synthesis. The GSH coated CdSe nanocrystals were coupled to streptavidin protein and coated in polyethylene glycol (PEG) to prevent aggregation. The high temperatures necessary for the synthesis of monodisperse $\gamma$-Fe\(_2\)O\(_3\), make addition of linkers such as this one difficult because of the possibility of decomposition. Högemann\(^9\) uses an amine functionalized dextran coating to attach via a crosslinker to transferrin protein. This method requires significant purification and surface modification in order to prepare an MR contrast agent. Shen and co-workers\(^{10}\) reports another method of modifying a dextran coated MION for bioconjugation. They take advantage of the strong streptavidin-biotin interaction to conjugate a streptavidin modified dextran coated MION to a biotinylated secretin protein. Kroll\(^{11}\) uses another polysaccharide, alginate, to provide stability in water to $\gamma$-Fe\(_2\)O\(_3\), for use as MRI contrast agents. The method is uncomplicated; however, crystals prepared are not monodisperse.

Our research focuses on the simplification of methods for creating water-soluble $\gamma$-Fe\(_2\)O\(_3\) nanocrystals, which can be applied to MRI contrast applications. Water-solubility and ease of functionalization for targeting of biological molecules were the major goals of this work. Phospholipids were chosen as a water-soluble coating, which can easily be attached to $\gamma$-Fe\(_2\)O\(_3\) nanocrystals. Biological targeting of pancreatic islet cells was performed to show the effectiveness of the method developed.
3.2 Experimental Methods

3.2.1 Preparation of Iron Oxide Nanocrystals

Monodisperse iron oxide nanocrystals (γ-Fe₂O₃) were prepared following a standard literature procedure pioneered by Hyeon and co-workers.¹² Triocetylamine (14 mL, 32 mmol) and oleic acid (2 mL, 6.3 mmol) were combined under a positive N₂ atmosphere, and heated at 180 °C for one hour. The temperature was lowered to 100 °C, and Fe(CO)₅ (0.4 mL, 3.0 mmol) was added to the solution and heated at 350 °C for one hour. During this process, the color of the solution changed from orange to colorless, indicating the decomposition of Fe(CO)₅ into Fe ions. As the temperature continues to rise the clear solution turns black, indicating the transformation of partially oxidized Fe/FeO particles. After one hour the temperature was lowered to 70 °C and (CH₃)₃NO (0.68 g, 9.12 mmol) was added to the solution and heated at 130 °C for two hours, followed by refluxing at 350 °C for one hour. During the final heating stage, the color changed from black to a dark reddish-brown and dark brown as the nanocrystals become oxidized. The (CH₃)₃NO was prepared by distillation of (CH₃)₃NO·2H₂O in toluene to remove water followed by drying under vacuum. The dehydrated material was maintained in a glove box. The nanocrystal solution was cooled to room temperature, dissolved in hexanes and centrifuged to precipitate the largest particles. Ethanol was added to the supernatant until turbid to precipitate medium diameter particles and centrifuged and the precipitated particles were re-dispersed in hexanes. The diameter of the nanocrystals was 11 nm with 5% polydispersity. They were characterized by TEM, with structure confirmed by X-ray diffraction (Scintag X2). Images of the particles were taken on a JEOL cx100 Transmission Electron Microscope (TEM) in bright field (BF).
mode at 100 kV. Samples were prepared by drying hexane dispersions of the nanocrystals onto Formvar amorphous carbon backed 400-mesh Cu grids and then evaporated under vacuum at RT.

3.2.2 Creating Water-Soluble γ-Fe₂O₃ Nanocrystals

To begin the coating procedure, a hexane dispersion of monodisperse MIONs of known concentration and volume were dried under vacuum. Typically the nanocrystals were 10 nm in diameter and a concentration of 10 mg/mL was prepared. For antibody conjugation, a combination of phospholipids were used, a 99:1 molar ratio of mPEG 750 to PTE. This amount was found to be optimal; however, other phospholipids and different ratios of phospholipids were used as well, which will be noted in section 3.3. Typically, 10 ml of a 10mg/mL hexane dispersion of nanocrystals was dried under vacuum and then combined with a 1 mL chloroform solution containing 3.9 x 10⁻⁵ mol of mPEG 750 PE (1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)- 750] and 4.1 x 10⁻⁶ mol of PTE [1,2-Dipalmitoyl-sn-Glycero-3-Phosphothioethanol (Sodium Salt)]. The phospholipids were purchased from Avanti Polar Lipids and stored at 4 °C. The phospholipid mixture was sonicated briefly (approximately 5 minutes) and the chloroform was allowed to evaporate. After chloroform evaporation, the remaining residue was heated to 80 °C and then 1 mL of distilled water at 80 °C was added. The aqueous solution was sonicated with heating (approximately 40 °C) for approximately 30 minutes, or until the nanocrystals were fully dispersed. The solution was purified of excess phospholipid micelles by ultracentrifugation at 28,000 rpm for 3 hours. A brown pellet formed in the bottom of the centrifuge tube during ultracentrifugation and after disposal of the supernatant, it was re-
dispersed in water by sonication with gentle heating. If the particles easily dispersed in water, it was assumed that they were fully coated with phospholipids; however, they were also characterized by TEM which showed them to be surrounded by phospholipids. Some reactions also added a fluorescein tagged phospholipid to the mixture for imaging purposes.

The phospholipid coating was imaged with TEM by negative staining of the TEM grid with 1% PTA (phosphotungstic acid, 12 $\text{WO}_3 \cdot \text{H}_3\text{PO}_4 \cdot x \text{H}_2\text{O}$). A drop of the aqueous nanoparticle dispersion was dropped onto the TEM grid and dried under vacuum for a minimum of 2 hours. Next, a drop of 1% PTA was added to the same TEM grid and dried again under vacuum for 2 hours. The PTA stains the area surrounding the phospholipid micelles a dark color, making the phospholipids visible, appearing white. The area surrounding the lipids appears dark under TEM because of the electron dense nature of the PTA, while the phospholipids appear white due to their low electron density. This contrast highlights the presence of the phospholipid coating.

### 3.2.3 Conjugation of Nanocrystals to Antibodies

The water-soluble $\gamma$-$\text{Fe}_3\text{O}_3$ nanocrystals were attached to antibodies via a heterobifunctional crosslinker molecule, SMPT (4-Succinimidylxycaronyl-$\alpha$-methyl-$\alpha$-(2-pyridyldithio) toluene). The first step of this process was to attach SMPT to the antibodies. The second step involved crosslinking the nanocrystals and antibodies via SMPT. SMPT (0.4 mg, 1 mmol) crosslinker was dissolved in 1 mL of acetonitrile and 30 $\mu$L of this stock solution was combined with 500 $\mu$L of a 2 mg/mL solution of antibodies. The antibodies obtained from Abcam were either unlabeled or Texas Red labeled Guinea pig IgG antibodies. The mixture was prepared in a refrigerated room at 4 °C and stirred
for approximately 4 hours and kept dark by wrapping the flask in aluminum foil. After 1 hour the solution was purified by filtration with Sephadex G-25 gel to remove any unreacted SMPT. The Sephadex slurry was prepared 3 days in advance in a pH 9 borate buffer, which was also used as the mobile phase in the column filtration. The Sephadex filtration was performed with the aid of a Peristaltic pump to control the flow of the mobile phase. The crosslinker bound antibodies were then conjugated to the γ-Fe₂O₃ nanocrystals encapsulated within mPEG 750 PE, PTE and fluorescein labeled phospholipids. Immediately after the filtration, 5 mg of phospholipids coated nanocrystals were combined with the Ab-SMPT conjugate and stirred for 2 hours. The leaving group in the conjugation, pyridine-2-thione can be detected by a UV absorption maximum at 343 nm. After reaction was complete, the conjugated solution was ultracentrifuged at 28,000 rpm for 5 hours at 10 °C. The supernatant was discarded and the precipitate was re-dispersed in a pH 9 borate buffer and kept refrigerated.

All conjugation procedures were carried out in a refrigerated room at 4 °C. Any conjugation performed with a Texas Red labeled antibody was performed in dark conditions.

3.2.4 Proof of Nanoparticle-Antibody Conjugation

Conjugation of NP-Ab and effectiveness of our MION tag was observed by attachment to the desired target, islet cells. A block of human pancreas tissue, treated with 10% neutral buffered formaldehyde for 16-24 hours, and impregnated with paraffin wax was obtained from Maxim Biotech, Inc. To test the effectiveness of the magnetic tag, a secondary antibody conjugated to the MION, was linked to a primary antibody on an islet cell. In the experiment developed, the secondary antibody targets the primary
antibody, while the primary antibody targets antigens on the islet cells (Figure 3.13). The pancreas tissue was sliced into 5 μ thin slices with a microtome and mounted onto glass slides by the CUMC Histology Lab. Deparaffination was performed with xylene (C₈H₁₆) followed by rehydration of the tissue with 100%, 90%, 75%, and 50% EtOH to PBS. The slides were washed in a 1:1000 dilution of proteinase K in PBS for 15 minutes. Proteinase K is a serine protease, which is used for unmasking antigens. During tissue fixing with formaldehyde protein crosslinking occurs, thereby blocking antigenic sites, which results in false negatives during staining. Slides were rinsed three times for three minutes in each PBS/0.1% Brij-35 (Polyoxyethylene glycol dodecyl ether) solution. Brij prevents non-specific binding. The slides were immersed in a 3% H₂O₂ solution in EtOH for 20 minutes to block endogenous peroxidase activity, which could lead to non-specific background staining if not quenched. The final step involves washing with PBS for 3 minutes before incubating with avidin followed by a 3-minute PBS rinse. Another incubation with biotin was performed afterwards. This treatment prevents random binding of avidin or other reagents used to biotin, which may cause a weaker signal. CAS (chromic acid Schiff) was used to bind and block non-specific binding sites. Next an insulin primary antibody solution (1:100 in CAS) was used to incubate the tissue and then it was washed twice with PBS, followed by incubation with the secondary antibody or MION-Ab conjugate, which was diluted 1:100 in PBS. Lastly, each slide was washed 2 times with PBS (3 minutes) and a coverslip containing DAPI dye was placed on top. DAPI (4’-6-Diamidino-2-phenylindole) is used to dye cell nuclei a fluorescent blue color. This helps to image the islets, which are a collection of many cells.
3.3 Results and Discussion

3.3.1 Synthesis and Characterization of γ-Fe₂O₃ Nanocrystals

Maghemite nanocrystals were synthesized by the same method (Hyeon) described in Chapter 2. The nanocrystals were dispersed in either hexanes or chloroform after size selection and characterized by TEM and SAED (selected area electron diffraction). The sample was also characterized by XRD (x-ray diffraction) to confirm the structure of the material obtained. Visually from TEM, the high monodispersity of the sample is evident by the self-organized hexagonal packing of the nanocrystals. The individual nanocrystals appear dark or pale depending upon the orientation of the planes of atoms in the crystal with respect to the electron beam. Certain angles diffract the beam strongly, while others transmit the beam through the material. The TEM, SAED and a cartoon of ligand-capped nanocrystals are shown in Figure 3.2.

Figure 3.2  TEM (A) of γ-Fe₂O₃ nanocrystals coated in oleic acid surfactant, selected area electron diffraction pattern (B) highlights crystallinity, and cartoon (C) representing 3-dimensional structure of one oleic acid capped maghemite nanocrystal. The oleic acid prevents nanocrystal aggregation, which can be seen on the TEM image.
3.3.2 Creating Water-Soluble γ-Fe₂O₃ Nanocrystals

One of the great remaining challenges in nanocrystal synthesis is achieving water solubility in an efficient manner. There are several methods; however, most require significant purification or post-synthetic surface functionalization.⁸,¹³⁻²⁰ In this case, phospholipids were chosen to render the γ-Fe₂O₃ nanocrystals water-soluble because the method is simple, requires little purification, and because functionalized phospholipids are available commercially (Avanti Polar Lipids), making biological targeting almost effortless. A method developed by Dubertret⁶ was modified to encapsulate iron oxide nanocrystals within phospholipid micelles. Phospholipids are relatively small, and available in varying lengths. Dextran creates a very thick coating around the nanocrystals, which is disadvantageous because it hinders the nanocrystal mobility in biological systems. Phospholipids on the other hand, are available in various lengths, and being very similar in structure to cell membranes, should not appear foreign to the human body. Phospholipids are amphiphilic, having a polar water-soluble head group at one end, and a non-polar hydrophobic chain at the other. Dextran is widely used to create water-soluble magnetic nanoparticles because of its biocompatibility and varied lengths available; however, significant purification and further surface chemistry is required to make it useful, making it a highly inefficient process. The only purification required is a short centrifugation at the end to remove excess phospholipids.

In this study, several phospholipids were used to produce water-soluble magnetic iron oxide nanocrystals (MIONs). Different combinations of mPEG 2000 PE (1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (Ammonium Salt)), mPEG 750 PE (1,2-Dipalmitoyl-sn-Glycero-3-
Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-750] (Ammonium Salt), mPEG 350 PE (1,2-Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-350] (Ammonium Salt), DPPC (1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine) and PTE (1,2-Dipalmitoyl-sn-Glycero-3-Phosphothioethanol (Sodium Salt) were utilized. The structures of these phospholipids are shown in Figure 3.3.

![Diagram of phospholipids](image)

**Figure 3.3** Phospholipids used to render MIONs water-soluble. Various combinations of these may be used successfully. Ultimately a ratio of 99:1 mPEG 750 PE to PTE was selected.

The first MION samples were coated with a 40:60 molar ratio of mPEG 2000 PE to DPPC. These are general non-functionalized phospholipids that were expected to work well based on Dubertret's method for coating CdSe. This ratio of mPEG and DPPC
worked very well for smaller MIONs (4-6 nm); however, larger MIONs were more difficult to encapsulate within these liposomes. We did find that using mPEG 2000 alone, with no DPPC or other phospholipids, allowed coating of a larger range (up to 12 nm in diameter) of nanocrystal sizes and easy dispersion in aqueous solutions. Figure 3.4 shows nanocrystals coated with 40:60 mPEG 2000 to DPPC.

![Figure 3.4 TEM of uncoated (A) and coated (B) MIONs. MIONs were coated with 60% DPPC and 40% mPEG 2000 PE. Image B was stained with 1% phosphotungstic acid to highlight the phospholipid coating. The upper left section of the image B is stained well and the lipids are visible as a white ring around each particle. Image A was dispersed in hexanes, the sample shown in image B was dispersed in distilled water. No aggregation is observed. The images in Figure 3.4 show both coated and uncoated nanocrystals. The uncoated nanocrystals are capped only in oleic acid and are dispersed in hexanes, whereas the MIONs encapsulated within phospholipids are dispersed in distilled water. The phospholipid coated MIONs were stained with 1% phosphotungstic acid on the TEM grid to highlight the lipid coating. The upper left section of the image is stained and the white circles surrounding each MION signifying the lipid coating are visible. The nanocrystals in the TEM above are an average of 4 nm in diameter. Larger nanocrystals]
were difficult to encapsulate by this method, because the micelles cannot accommodate them.\textsuperscript{6}

During the encapsulation process, the phospholipids are combined in chloroform and it is thought that micelles form as the chloroform evaporates, and once the solution has surpassed the CMC (critical micelle concentration). Chloroform is a moderately non-polar solvent with a relative polarity of 0.259 (water is 1.0).\textsuperscript{21} The phospholipids used, mPEG and DPPC form micelles in chloroform, which allow them later to be soluble in water. After the micelles form and the chloroform solution of MIONs is added, it is thought that the MIONs diffuse into the micelle core as the chloroform evaporates; once this occurs, the MIONs are water-soluble. The cartoon illustrated in Figure 3.5 illustrates the phospholipid coating surrounding the $\gamma$-Fe$_2$O$_3$ core.

![Cartoon illustration of a phospholipid coated MION. The orange ligands represent the oleic acid, and the green and blue show how the phospholipids interact with the oleic acid surface. The hydrophobic palmitoyl chains (in blue) are interdigitated with the oleic acid, while the more polar end (green) of the phospholipids interacts with the solvent. (Image made by Kristi Hultman, a collaborator on this project)](image-url)
With the MIONs successfully encapsulated within water-soluble phospholipids, bioconjugation was the next step. A commonly used heterobifunctional amine and sulphydryl reactive cross-linker, SMPT, was chosen for conjugation of MION to antibody. The sulphydryl reactive end of SMPT can react with thiol-functionalized phospholipids and the amine reactive end can bind to the antibody. A thiol labeled phospholipid, PTE, is available commercially and was selected for this application. Knowing that thiols can react with each other to form disulfide bonds, an 80:20 molar ratio of DPPC and PTE were used to coat the MIONs. DPPC was chosen instead of mPEG 2000 because its length is similar to that of PTE.

\[ \text{Figure 3.6} \quad \text{TEM images of 5 nm MIONs coated with 80:20 (A) molar ratio of DPPC:PTE and 99:1 (B) ratio. Notice that in image A (more PTE) the nanocrystals are aggregated in a hexagonal superlattice, likely due to disulfide linkages between particles.} \]

A molar ratio of 80:20 DPPC to PTE did create water-soluble nanocrystals; however, the TEM image showed the nanocrystals tend to aggregate (Figure 3.6). This is thought to be due to the formation of disulfide linkages during the coating process. It is also interesting that the aggregated particles are organized into an almost hexagonal superlattice. Given the ‘stickiness’ of the PTE coated MIONs the ratio of PTE was
lowered to 99:1 DPPC to PTE, and in this case less aggregation was observed, as shown in Figure 3.7.

![Image](image-url)

**Figure 3.7** TEM of MIONs coated with 99:1 molar ratio of DPPC to PTE. The TEM grid is stained with 1% PTA and white rings are visible around the MIONs, indicating the phospholipids coating.

A sample of MIONs of 15 nm average diameter was prepared and coated with a mixture of 50:40:10 mPEG 350 PE:DPPC:PTE, as shown in Figure 3.8. This sample could not be re-dispersed in water after the coating procedure and did not appear surrounded by lipids when the TEM grid was stained with 1% PTA.

![Image](image-url)

**Figure 3.8** 15nm diameter MIONs were prepared and dispersed in hexanes (A) and attempted to be coated with phospholipids and dispersed in water (B). The sample was
difficult to disperse in water, and by TEM with staining, shows no evidence of a lipid coating.

Using these methods, smaller particles (4-6 nm) can be coated with several combinations of phospholipids and made water-soluble. They stay in solution for weeks at a time; however, doing the same with 15 nm MIONs is more difficult. The micelle core is not large enough to accommodate a 15 nm nanocrystal and therefore this method, as is, is limited to small nanocrystals.

3.3.3 Enlarging Phospholipid Micelles with Salts

It is known that addition of salts to micelle solutions can decrease the strength of electrostatic interactions in the micelle result in large micelles.\textsuperscript{22} Larger spherical, elongated and other shapes can occur under salty conditions.

To test this theory NaCl was added to micellar solutions in various concentrations to observe the size effects by TEM. Solvent conditions were changed since NaCl is not soluble in chloroform. After trying various solvents and combinations of solvents THF with a small amount of water (enough to dissolve the NaCl) was selected. NaCl stock solutions of 0.1 M and 0.01 M in THF/water were prepared and 99:1 molar ratio of DPPC:PTE (12.0 mg DPPC, 1.5 mg PTE) was dispersed in 1 mL of these solutions and solvent was allowed to evaporate. The solutions were re-dispersed in 1 mL THF and imaged by TEM. One drop of micelle solution was put on a TEM grid and dried under vacuum 1 hr, followed by 1 drop of 1% PTA stain and drying under vacuum 1 hr.
Figure 3.9  TEM images of DPPC and PTE micelles prepared in different concentrations of NaCl salt solutions in THF/water. No salt (A), 0.01 M NaCl (B), and 0.1 M NaCl (C). A drop of 1% PTA stain was added to the grid to visualize the micelles.

The TEM images in Figure 3.9 show round, spherical micelles in white, and stained areas on the grid in the background. The diameter of the micelles was measured from the TEM and the average micelle size was found to increase with concentration. In the control specimen where no salt was added to the micelle solution, the average micelle diameter was 5 nm. When the concentration of NaCl was 0.01 and 0.1 M, the micellar diameters are 9 nm and 13 nm. Larger NaCl concentrations were not tested because NaCl was difficult to dissolve in non-polar solvents.

The micelles prepared in 0.1 M NaCl solution were used to encapsulate γ-Fe₂O₃ nanocrystals because those micelles were the largest. Lipid solutions were evaporated to form micelles and then re-dissolved in 1 mL THF/water followed by addition to dried γ-Fe₂O₃. The mixture was vortexed to disperse and allowed to evaporate. The remainder of the procedure is unchanged. The product was soluble in distilled water after vortexing and stayed in solution for weeks at a time.
Figure 3.10  TEM of $\gamma$-Fe$_2$O$_3$ nanocrystals encapsulated within DPPC/PTE phospholipid micelles. The micelle solution was 0.1 M NaCl in THF/water and the TEM grid was stained with 1% PTA. The micelles are spherical.

The next step was to solubilize larger, 15nm $\gamma$-Fe$_2$O$_3$ nanocrystals. The larger nanocrystals were coated in the exact same manner as the 10 nm nanocrystals and were dispersed in distilled water with ultrasonication for approximately 10 minutes. The TEM of these nanocrystals coated in phospholipids is shown in Figure 3.10 and also Figure 3.11.

Figure 3.11  TEM image of uncoated (A) and phospholipid coated (B) 15 nm $\gamma$-Fe$_2$O$_3$ nanocrystals. The nanocrystals on the right are water soluble and coated with DPPC and PTE.
Image A in Figure 3.11 shows the uncoated 15 nm $\gamma$-Fe$_2$O$_3$ nanocrystals that were dispersed in hexanes and drop-cast onto a Formvar backed Cu TEM grid. Image B shows the same sample of nanocrystals after coating with DPPC and PTE. The TEM image on the right shows the white phospholipid ring surrounding each nanocrystal, highlighted by the 1% PTA stain.

These experiments show that salts can effectively be used to enlarge micelles and that larger DPPC/PTE phospholipids micelles can be used to encapsulate 10-15 nm oleic acid capped nanocrystals and render them water-soluble.

3.3.4 Conjugation of MION to Cells

3.3.4.1 Antibodies

Antibodies are proteins made up of two identical heavy chains and two identical light chains. They are produced by the immune system to identify and neutralize the presence of foreign objects such as viruses and toxins, to mark them for elimination. Detection of antibodies is highly specific and is useful in diagnostic medicine because of the binding specificity. For this reason, antibody targeting was used to detect the binding of MIONs to $\beta$ cells in the islets of Langerhans.

All classes of antibodies have a common structure of four peptide chains: two heavy chains, and two light chains (Figure 3.12). The total molecular weight of an antibody is approximately 150,000 Da. The light chains are bound to heavy chains by disulfide bonds and other non-covalent interactions such as salt linkages, hydrogen bonds and hydrophobic bonds. Both heavy and light chains contain constant and variable regions. Constant regions are basically unchanged for antibodies within a specific
subclass (IgG, for example); however, the variable regions, which consist of 100-110 amino acids on the end of the heavy and light chains, vary from one antibody to the next, and determine which targets the antibody can bind to. Since the constant region of antibodies does not change drastically between antibody classes, and do not participate in binding to targets, we chose to attach MION labels to the constant regions. In addition, the chemistry of the phospholipid coatings is also highly malleable, given the array of functionalized phospholipids commercially available. The variety of functional groups available on phospholipids makes it easy to conjugate water-soluble MIONs to most biomolecules. Therefore, the ability to tune the surface chemistry of the coated MION makes this a general method that could be applied to numerous bioconjugation applications.

![Diagram of antibody structure](image)

**Figure 3.12** Molecular structure of IgG class antibody (left) and cartoon illustrating basic structure of an antibody (right). The heavy chains are in red, and the light chains in yellow.
3.3.4.2 Bioconjugation Method

The MIoN-Ab conjugation was facilitated through a crosslinker molecule called SMPT. SMPT is a heterobifunctional amino and sulfhydryl reactive crosslinker typically used to conjugate immunotoxins and antibodies. Benzene and methyl group hinder the disulfide bond providing it with in-vivo stability. This crosslinker is also stable to hydrolysis, making it quite robust in aqueous environments. Scheme 3.1 illustrates the method of conjugation using SMPT crosslinker.

Scheme 3.1 Conjugation of a secondary antibody to SMPT crosslinker and then to MIoN. Conjugation takes place at 4 °C in acetonitrile. Excess crosslinker is removed by Sephadex gel filtration in pH 9 borate buffer.

This is the general method used for conjugation of MIoN to antibody. The success of this reaction can be observed by the release of UV active pyridine-2-thione during the second step of the reaction. This compound has an absorption max at 343 nm in the UV -visible range.

Subsequent experiments performed to show the binding of the MIoN-Ab conjugate to a primary Ab on islet cells used a Texas-Red labeled secondary Ab and a small amount of fluorescein labeled phospholipids in the lipid coating. This conjugation sequence of this experiment is shown in Figure 3.13.
**Figure 3.13** Conjugation of MION to islet cell using two fluorophores to indicate binding. A fluorescein labeled phospholipid was added to the phospholipid mixture of mPEG 750 and PTE. The MION is conjugated to a Texas Red labeled secondary antibody, which will attach a primary antibody on the islet cell. The fluorescent labels can be detected with fluorescence microscopy.

First, the fluorescein labeled MIONs were prepared, and their labeling was confirmed by fluorescence microscopy. Four samples were prepared to determine if the concentration of fluorescein labeled phospholipids affects the outcome. The experimental coating procedure was unchanged, except, either 10, 20, 30 or 40 μL of 1 mg/mL fluorescein labeled lipids in CHCl₃ was added to the lipid mixture. The MIONs were soluble in water and one drop of each MION solution was imaged on a glass microscope slide. The fluorescence microscopy images obtained are shown in Figure 3.14 along with the structure of the fluorescein labeled phospholipid.
Figure 3.14  MIONs coated with mPEG 750, PTE and either 10, 20, 30 or 40μL of fluorescein labeled phospholipids. The fluorescein labeled phospholipid is shown at right. The amount of fluorescein labeled phospholipids added to the reaction does not seem to make a difference in the fluorescence of the MIONs.

The addition of fluorescent labeled phospholipids to the mPEG 750 and PTE mixture was successful and green fluorescence is visible. The next step was to conjugate the fluorescent labeled MIONs to a secondary antibody labeled with Texas Red and then to an islet cell, as illustrated in Figure 3.13. This experiment was designed to show that the MIONs and the antibodies are binding together in the same location, and that the antibodies remain functional. The hypothesis was that the green and red fluorescence from the fluorescein and Texas Red should be in the same location, producing a yellow/orange combined fluorescence. If the nanoparticle was not bound to the secondary antibody, only Texas Red fluorescence would be seen. Figure 3.15 shows the results of this experiment.
**Figure 3.15**  Fluorescence microscopy used to image fluorescent MION-Ab conjugates. MION has green fluorophore, and secondary antibody has a Texas Red fluorescent label. These MION-Ab conjugate tags have been used to label primary antibodies on islet cells as illustrated in Figure 3.13. Image A shows all color channels, Image B has the red channel off and Image C has the green channel off. Both green and red fluorescence are observed in the same location in B and C, indicating that the MIONs and Ab are conjugated.

Figure 3.15 shows the successful conjugation of MION and Ab. An orange/yellow fluorescence is observed in image A, indicating a combination of green and red fluorescence from fluorescein and Texas Red. Images B and C highlight the individual green and red fluorescence by turning off the red (B) and green (C) fluorescence channels. The success of this experiment shows that the planned conjugation occurs, and that it does not interfere in any way with the function of the primary or secondary antibodies.

### 3.3.5 Magnetic Resonance Imaging (MRI) with MIONs

The motivation behind developing the phospholipid coated MIONs was to use them as non-toxic MRI contrast agents to diagnostically detect islet cells during transplants and follow their paths. The phospholipid coated MIONs act as contrast agents by binding to target biological molecules and influencing the relaxation rate of water molecules (protons, H\(^+\)) when a magnetic field is applied. Magnetic iron oxide
nanocrystals enhance the relaxation of water molecules nearby, making the image appear dark where they are present. Efforts in this project are now moving towards using these MION MRI contrast agents to target cells in rats.

3.4 Conclusions

A simple and safe method was developed for creating water-soluble $\gamma$-Fe$_2$O$_3$ nanocrystals (called MIONs) post-synthesis. A variety of phospholipids, of different lengths and functionalities, were used to provide water-solubility. Small nanocrystals were easier to encapsulate within phospholipids; however, salt solutions were used to enlarge phospholipid micelles to allow encapsulation of larger (15 nm) nanocrystals. TEM imaging with 1% phosphotungstic acid staining of the TEM grid was used to provide visual evidence of the phospholipid coating. The potential to use water-soluble $\gamma$-Fe$_2$O$_3$ nanocrystals as biological tags was explored through the targeting of pancreatic islet cells. SMPT, a heterobifunctional crosslinker was used to facilitate conjugation of MION to antibody to islet cell. Fluorescent markers which tagged the MION and antibody gave proof of the conjugation.

This is a general method that can be used to simplify the creation of water-soluble nanocrystals and the subsequent conjugation to biomolecules. We have demonstrated the ease with which this method can be utilized and applied. The potential to use these MION MR contrast agents for MR imaging has been pursued by collaborators on this project and the MIONs have proved to be good contrast agents for MR imaging applications.
3.5 References

Chapter 4

Synthesis of TiO$_2$ Nanocrystals: Capping Ligands, Morphologies, Crystallinity and Surface Characterization
4 Synthesis of TiO₂ Nanocrystals: Capping Ligands, Morphologies, Crystallinity and Surface Characterization

4.1 Introduction

Research interest in nanocrystalline titanium dioxide has flourished during recent years due to its diverse and far-reaching applications in photocatalysis,¹ photodegradation of organic molecules for water and air purification,² catalyst supports,³ anti-fogging⁴ and pigments,⁵ and others. Recently there has been a higher demand for monodispersed nanocrystalline TiO₂ in particular for its use in solar cells and photocatalysis due to its high energy conversion efficiency, low cost, and catalytic stability.¹ More recent research efforts have therefore focused on providing inexpensive and straightforward routes to TiO₂ nanocrystals.

The high surface area that nanocrystals possess increases the usefulness of TiO₂ for photocatalyst applications where a high surface area is critical to gain improved efficiency. Non-aqueous routes to TiO₂ are highly desirable because of the tendency of TiO₂ precursors to undergo hydrolysis, by which control over reaction products is diminished. Aqueous syntheses of TiO₂ result in hydroxylated TiO₂ surfaces, which is expected to have an effect on the surface chemistry and application potential of TiO₂ nanocrystals.

There are three distinct crystalline polymorphs of TiO₂ (shown in Figure 4.1 and Figure 4.2): anatase (tetragonal), brookite (orthorhombic) and rutile (tetragonal). Anatase is kinetically favored and exhibits more photoactivity than rutile or brookite; however, the photoactivity of anatase is size-dependent.⁶ Rutile is the thermodynamically favored
product and is more stable at high temperatures. It is commonly used in the pigment industry for its white color and high refractive index. Small TiO$_2$ nanocrystals tend to take on the anatase structure, most likely due to their ability to achieve lower surface energy. Rajh$^7$ and coworkers found that when anatase TiO$_2$ nanocrystals are smaller than 20 nm they adjust the coordination of their surface Ti atoms from hexacoordinated (octahedral) to pentacoordinated (square pyramidal) and shorten the length of the Ti-O bond to compensate for the curvature of the nanocrystal surface. Curtiss$^8$ has also reported that anatase converts to rutile upon reaching a certain nanocrystal diameter, in the range of 12-18 nm.

![Image of anatase TiO$_2$ crystal structure](image.png)

**Figure 4.1** Unit cell of anatase TiO$_2$. Anatase is a tetragonal polymorph of TiO$_2$ with an elongated unit cell along the c axis. Its unit cell dimensions are $a=3.785$ Å and $c=9.514$ Å. Titanium atoms are blue and oxygen atoms are red. Edges of the unit cell are shown by a blue dashed line.
Figure 4.2 For comparison to anatase, the unit cells of brookite (left) and rutile (right) TiO₂ are shown. Brookite has an orthorhombic structure, while rutile has a tetragonal structure. The edges of the unit cell are shown with blue dotted lines.

Hydrolytic methods, in both aqueous and organic media are commonly used to synthesize TiO₂ nanoparticles. These methods are less than ideal because the resulting nanoparticles are often of irregular shape and uniformity, and need to be calcined to obtain crystalline products. Nanoparticles obtained by aqueous methods are also covered with surface hydroxyl groups, which change the properties of the material and diminish its usefulness in certain applications. During hydrolytic methods, water acts as an oxygen donor to metal alkoxides or halides. The methods used to synthesize nanocrystalline TiO₂ are typically hydrothermal or sol-gel techniques. In addition, sol-gel methods are common because they allow for improved control of purity, uniformity and morphology of the final product. Sol-gel reactions can occur at low temperatures and typically use metal alkoxide or metal halide precursors. They nearly always require post-synthetic calcination. Several non-hydrolytic methods have recently been developed and applied to the synthesis of other metal oxides. Tang and co-workers have synthesized nanocrystalline brookite by decomposing bis(cycooctatetraene)titanium with DMSO in organic solvents and TOPO to produce TiO₂. Guo and co-workers recently described a non-aqueous preparation of TiO₂ nanopowders by acylation/deacylation of Ti alkoxides.
in supercritical CO₂. While it does generate nanoscale TiO₂ by a non-hydrolytic method, the product is not crystalline. Another quite powerful method was described by Garnweitner and co-workers¹⁴ whereby nanocrystalline anatase is synthesized by reacting Ti(OiPr)₄ with simple ketones or aldehydes. This reaction is ideal in that it is unrivaled in its simplicity, circumvents the need for calcination and produces TiO₂ in high yields (48-100%). The products are phase-pure anatase, and larger nanocrystals are observed when aliphatic ketones or aldehydes are used as solvents instead of aromatic ones. They propose that aromatic species ligate more strongly to the surface, thereby restricting growth. They speculate that the reaction begins with coordination of acetone to a Ti center followed by the release of isopropanol. Unfortunately, however, the nanocrystals are not monodisperse. Pinna and co-workers¹⁵ have reported a fascinating and simple non-aqueous route to metal oxide nanocrystals (γ-Ga₂O₃, ZnO, In₂O₃, γ-Fe₂O₃/Fe₃O₄) via solvothermal reduction of metal acetylacetonates in benzylamine solvent. It is a notable method that can likely be applied to the synthesis of many other types of transition metal oxide nanocrystals, and perhaps TiO₂. The side-products were analyzed by NMR to determine the reaction mechanism. The final reaction solution, which was found to include eight organic side-products, was analyzed to propose a complex reaction mechanism. This route most closely resembles our method which will be described in this chapter for the synthesis of TiO₂; however, the reaction mechanisms are likely quite different since one solvent is a primary amine and the other tertiary. During recent years many clever and simple methods have been reported which use non-aqueous routes to synthesize nanocrystalline TiO₂; however, control of growth mechanisms and achieving monodispersity is still a challenge. Our work was approached with the idea in mind that
better control over nanocrystal shape and size may be achieved by exerting more control over reaction rate. To aid this challenge, we chose precursors containing bidentate ligands and used a strongly coordinating solvent in addition to strongly coordinating surfactant species during the reaction to achieve slower crystal growth.

While the method of preparation (solvothermal, hydrolytic, etc.) was found to be an important factor in determining the characteristics and properties of nanocrystals, so too is the reaction pH. Several studies$^{8,16-18}$ have found the pH and more specifically, acidic conditions to be critical to the formation of nanocrystalline TiO$_2$. Scolan$^{18}$ reported that experimental conditions, including reaction acidity is important in the hydrolytic reaction of titanium $n$-butoxide in the presence of acetylacetone and $p$-toluenesulfonic acid if crystalline TiO$_2$ is to be obtained. They also reported that acidic conditions provide for efficient protonation of titanium-oxo bridges and elevated temperatures help to promote the structural reorganizations, which give rise to crystallization to form of TiO$_2$. Sugimoto$^{19}$ reported that the rate of formation of anatase is highly dependent on pH. Barnard et al.$^8$ studied the surface dynamics of TiO$_2$ nanocrystals synthesized in acidic and basic solutions and found anatase to have surface molecules containing a large amount of hydrogen, while rutile surface molecules contain a large amount of oxygen. Pinna$^{15}$ and co-workers reported that HCl is known to promote alcoholysis of acetylacetone in alcohol media to form acetone and acetic esters. They found that an analogous reaction occurs with acetylacetone in amine solvents to give rise to simple amide side products.

Nanocrystalline TiO$_2$ is known to exist in several interesting morphologies including spheres,$^{6,9,12}$ nanorods,$^{9,20}$ truncated rods,$^{21}$ nanotubes,$^{2,22}$ nanofibers$^{23}$ and
triangles.\textsuperscript{21} Eder and co-workers\textsuperscript{2} report the synthesis of rutile nanotubes by a lengthy CVD method involving synthesis of carbon nanotubes followed by coating CNTs with titania, heating to convert to anatase, then heating in N\textsubscript{2} atmosphere to convert to rutile, followed by heating at a higher temperature to remove CNTs. Wang and co-workers\textsuperscript{9} found that morphology could be altered between fibers, rods and spheres when TiCl\textsubscript{4} was heated in various combinations of alcohols and acetic acid. Rod-like or elongated nanomorphologies are formed when two or more solvents are present which can result in selective binding of solvent to certain lattice planes, hindering growth along some lattice planes resulting in an elongated morphology.

Surfactants or capping ligands are often added to nanocrystal preparations to stabilize nanocrystal surfaces and control growth mechanisms, and also to help solubilize the nanoparticles in solution. Surfactants are often added to reactions where solution applications are important for the nanocrystals being synthesized. Unfortunately, there are few surfactants that can both bind to the surface of metal oxide nanocrystals and survive the harsh temperatures required for thermal decomposition reactions, which are the most common. Surfactants such as oleic acid, TOPO ( trioctylphosphine oxide) and other long chain organics are most common in the synthesis of metal oxides and semiconductor nanocrystals; however, surfactants such as these limit solubility to non-polar organic solvents. These long-chain ligands have made it possible to synthesize a range of monodisperse and crystalline samples; however, make the study of ligand/surface chemistry difficult in that they are difficult to remove or exchange. Ligands that are more easily removed could be used in these preparations, which would allow for easier study of ligand/surface interactions; however, this would likely lead to
the production of samples of lower monodispersity and possibly poorer crystallization. There is a need for a greater variety of surfactants that offer varied solubility of the synthesized nanocrystals because all too often the ligand available is inappropriate for the desired application of the nanocrystalline material. Kominami has reported recently that surfactants may directly affect the nucleation of anatase. Surfactants are often used in the synthesis of nanocrystals such as γ-Fe₂O₃ or CdSe, which have extensive applications and can be synthesized with a high degree of monodispersity. It is interesting to note that TiO₂ is rarely synthesized with a surfactant meant to stabilize growth and provide for specific solubilities. The work described here explores the hypothesis that if ligands are used, improved control over reaction products may be possible.

Historically speaking, titanium alkoxides and titanium halides are the most commonly used precursors in the synthesis of TiO₂. These reactions are often hydrolytic and one of the major drawbacks to these methods is the lack of control over the hydrolysis-condensation rates of reaction. The reactions typically run to completion quickly, leading to low monodispersity and an absence of crystallinity, unless annealed at high temperatures. A more judicious choice of organometallic titanium precursors that have ligands that are more difficult to hydrolyze and more strongly bonded to titanium may aid in the controlled synthesis of crystalline TiO₂. Scolan has utilized titanium n-butoxide modified with acetylacetonate ligands to alter the ligand sphere and make it less susceptible to fast hydrolysis during preparation of monodisperse nanocrystals. Multidentate ligands such as acetylacetone are bound more strongly to the metal center and therefore are much less labile than alkoxide ligands. Pinna and co-workers found that using metal acetylacetonate precursors allowed formation of several late transition-
metal oxides that were nanocrystalline. A combination of GCMS and \(^1\)H NMR analysis of the product reaction solution also allowed them to propose a complex reaction mechanism based on their observation of seven organic species in the final reaction mixture. It’s apparent from these two reports that metal acetylacetonate precursors are an attractive group of potential precursors to metal oxides because they can provide for a more controlled reaction rate. Moreover, they are widely available commercially and can be easily prepared.\(^{35}\) For these reasons, TiO(acac)\(_2\) was explored in this study as a potential precursor to TiO\(_2\) nanocrystals, which was ultimately found to have significant promise.

In summary, the field devoted to synthesis of titanium dioxide nanoparticles and nanocrystals is mature and many applications for the different polymorphs of TiO\(_2\) have come to fruition. There are, however, unexplored topics within this ripe area of research. Surfactants are not commonly used in the synthesis of TiO\(_2\) nanocrystals, and it was thought that their use could provide control over reaction products and perhaps a more varied solubility, in addition to better monodispersity and crystallinity. Another goal of this research was to utilize a precursor with less labile ligands to help control the speed of reaction kinetics.

**4.2 Experimental Methods**

*General Methods.* Unless otherwise described the synthesis of TiO\(_2\) was performed in a 4-neck or 3-neck European style flask capped with rubber septa and vented to the air via a needle piercing one septum.
Chemicals. Trioctylamine (TOA), 0.5 N HCl, γ-butyrolactone (BL), propylene carbonate (PC), 1-formyl piperidine (FP), ethyl salicylate (ES), 2-acetyl pyridine (AP), p-anisaldehyde (PA), octyl ether, 1-decene, and 1-hexadecanol were used as received from Sigma-Aldrich. Oxobis(2,4-pentanedionato-O,O')titanium (TiO(acac)₂) was purchased from Strem Chemicals, Inc. and used as-is.

Characterization. Products were characterized by powder X-ray diffraction on an Inel X-ray diffractometer. Low-resolution transmission electron microscopy images were taken on a JEOL 100cx microscope (accelerating voltage 100 kV) equipped with a CCD camera. TEM samples were prepared by drying solvent dispersions of nanoparticles onto carbon coated 400-mesh Cu grids and then dried under vacuum at RT. Thermogravimetric analysis of TiO₂ samples was recorded on a TA Q50 Thermogravimetric Analyzer. FTIR experiments were performed on a Nicolet 8700 fitted with a DRIFT Smart collector. ¹H NMR experiments were performed on a Bruker 400 MHz spectrometer at RT. The locking solvent was CDCl₃. GC-MS was conducted with a Varian Saturn 2100. UV-Visible spectra were obtained on an Agilent 845x UV-Vis Chem Station. Photoluminescence (PL) spectroscopy was performed at room temperature (RT), using the 325 nm emission from a He-Cd laser (the maximum excitation intensity, Iₘₐₓ = 28 W/cm²). Galbraith Laboratories in Knoxville, TN performed elemental analysis. HRTEM was performed at Brookhaven National Laboratories.

Synthesis of TiO₂. TiO₂ powders were typically prepared by combining TiO(acac)₂ (2 mmol, 0.52 g), TOA (15 mL), 0.5 N HCl (3 mL unless otherwise noted), and 4 mmol of a polar surfactant (BL, PC, FP, ES, AP, or PA) in a centrifuge tube and sonicated briefly (5-15 minutes) before heating at 250 °C for 3 h followed by 150 °C for 2 h. These are the
conditions used most often. Any variations in solvent, surfactant, or amounts added will be noted. The nanocrystalline product was isolated by dissolving the product solution with 30 mL CHCl₃ followed by centrifugation at 3,800 rpm for 10 minutes. Supernatant containing TOA and other soluble species were discarded and the precipitate was washed and centrifuged repeatedly until the supernatant was colorless.

4.3 Results and Discussion

4.3.1 Synthesis of TiO₂ Nanoparticles

Titanium dioxide (TiO₂) nanoparticles were prepared via a simple route utilizing a TiO(acac)₂ precursor species in trioctylamine solvent and a polar surfactant. Under typical conditions TiO(acac)₂ was combined with one of the aforementioned polar surfactants in addition to TOA and stirred at 250 °C for 3 h and 150 °C for 2 h (See Scheme 4.1 and Figure 4.3). These conditions resulted in amorphous, mostly spherical nanoparticles (Figure 4.4). The nanoparticles were calcined at 500 °C for 4 h to produce nanocrystalline anatase TiO₂, identified as such by x-ray diffraction.

Scheme 4.1  Synthesis of TiO₂ nanocrystals. TiO(acac)₂ was reacted with a surfactant and trioctylamine solvent at 250 °C for 3 h and 150 °C for 2 h.
**Figure 4.3**  
*Surfactants selected to add to the reaction illustrated in Scheme 4.1.*

### 4.3.2 Surfactants used in TiO$_2$ Synthesis

TiO$_2$ nanoparticles were prepared using a variety of surfactant molecules to coordinate to the nanoparticle surface and provide solubility. Polar surfactants with carbonyl functionalities were chosen because it is known that the C=O oxygen coordinates strongly to the surface of TiO$_2$.$^{26,27}$ The surfactants chosen also all exhibit high boiling points, which is necessary due to the high temperatures required for nanoparticle synthesis by thermal-decomposition methods. A number of high boiling-point surfactants were examined to determine their effectiveness in TiO$_2$ synthesis and five were found to be successful. These surfactants are also less expensive and less toxic than more commonly used surfactants such as TOPO. The ability to use a wide variety of surfactants also provides for a greater range of solubility. Figure 4.4 shows TEM images of TiO$_2$ synthesized with several different surfactants.
Figure 4.4 TEM images of TiO$_2$ nanoparticles prepared with different surfactants. 2-acetyl pyridine (A), p-anisaldehyde (B), propylene carbonate (C), γ-butyrolactone (D), 1-formyl piperidine (E). The particles were dispersed in CHCl$_3$ and drop-cast onto a carbon coated 400-mesh Cu grid. The products are amorphous, as determined by XRD.

4.3.3 Three Morphological Forms of TiO$_2$

Heating conditions were varied to select the optimal heating environment and also to determine changes in nanoparticle size, size distribution and morphology. For example, when using propylene carbonate as the surfactant species, a mixture of spherical and rod-like morphologies was observed (Figure 4.5). The products obtained from different heating conditions suggest that heating at 250 °C for longer than 3 h results in a combination of spherical and large rod-like morphologies.
Figure 4.5   TEM image of amorphous TiO$_2$ rods (A) and spheres (B) obtained from the reaction of TiO(acac)$_2$ with TOA solvent and propylene carbonate surfactant. The sample was dispersed in CHCl$_3$ and drop-cast onto a carbon coated 400-mesh Cu grid.

It was previously reported$^{28}$ that crystal morphology depends upon relative growth rates along different crystal lattice planes and that the coordination ability of the solvent can have a significant effect on crystal morphology. In this study trioctylamine was chosen as the solvent, because it is known to be a strongly-coordinating, particularly in the synthesis of metal oxide nanocrystals.$^{28,30}$ The formation of rods suggests that there are two solvent species coordinating to the surface of TiO$_2$ and promoting its preferred growth along one plane, in this case propylene carbonate and trioctylamine. The use of two coordinating solvents$^{31}$ is a common technique in the growth of nanorods; in this way one can obtain selective preferential growth along one lattice plane and slower growth along another. Here we find that it is longer durations of heating that gives rise to a population of nanorods as opposed to solely nanoparticles. Rods were present when the reaction mixture was heated at 250 °C for longer than 3 h, which suggests that after a certain period of time growth along two planes is inhibited, while growth along a third lattice plane continues. The presence of rods, however does not presuppose crystallinity, as evidenced by the XRD, shown in Figure 4.6. The presence of rods could result from a difference in relative growth rates that develops later in the nanoparticle synthesis, or
could also result from a change in surfactant structure resulting from the high temperature methods used in this reaction. Recent investigations\textsuperscript{32-34} have shown that surfactant structure can be altered during nanocrystal synthesis and result in distinct nanocrystalline structures that are otherwise difficult to produce.

The x-ray diffraction patterns of the as-prepared TiO\textsubscript{2} nanoparticles showed the sample to be amorphous (Figure 4.6); however, after annealing the prepared powders for 4 h at 500 °C, the XRD pattern corresponds with the anatase form of TiO\textsubscript{2}. The diffraction pattern shown in Figure 4.6 illustrates the broadness of the peaks, which agrees with the small size of the nanoparticles exhibited by the TEM image. The average size of the annealed nanocrystals is 10.4 nm as calculated by the Debeye-Scherrer equation,\textsuperscript{35} which agrees with the average size observed by TEM. In addition, all of the peaks in the diffraction pattern can be assigned to anatase.

Sherrer derived an equation to account for the broadening of x-ray diffraction peaks caused by small crystallite sizes. The Sherrer equation is:

\[ B_{\text{crystallite}} = \frac{(K\lambda)}{(L \cos \theta)} \]

**Equation 4.1**

Wavelength of x-rays used is \( \lambda \), \( \theta \) is the Bragg angle, \( L \) is the average crystallite size and \( K \) is a constant. The constant \( K \) is 0.94 assuming uniform small cubic crystals. However, in most cases \( K \) is set at unity (1.0) since the equation is mostly used to calculate an average crystallite size for cubic and non-cubic crystals, however it has been calculated to vary between 0.89 and 1.39.
Figure 4.6  XRD diffraction pattern of TiO$_2$ before and after annealing at 500 °C for 4 h. The bottom line is the XRD of the sample before annealing and the top line is the XRD pattern observed after annealing. The sample was characterized as anatase.

4.3.4 Temperature Optimization

Various heating temperatures were examined to investigate whether reaction temperature affected the size, size distribution, and shape of the product. Reaction temperatures from 165 °C to 305 °C were studied by maintaining at a fixed temperature for 2 h. The results of this are highlighted in Table 4.1. The products were characterized by TEM and there appears to be a bimodal size distribution. The average particle diameter was measured, but does not change significantly with heating. Higher temperatures, however, yielded products of lower size distribution, so a temperature of 250 °C was chosen for future experiments. The average nanoparticle size was determined by measuring the size of a population of at least 100 particles in a TEM image. The large size distribution suggests that the time over which nucleation occurs is broad and therefore leads to a broad distribution of sizes.
Figure 4.7  TEM of TiO$_2$ nanoparticles prepared by heating TiO(acac)$_2$ and PC in TOA solvent. A bimodal size distribution is observed. Sample was dispersed in CHCl$_3$ and drop-cast onto a carbon coated 400-mesh Cu grid.
<table>
<thead>
<tr>
<th>Exp #</th>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>Avg. particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>165</td>
<td>2</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>2</td>
<td>185</td>
<td>2</td>
<td>17 ± 10</td>
</tr>
<tr>
<td>3</td>
<td>205</td>
<td>2</td>
<td>15 ± 8</td>
</tr>
<tr>
<td>4</td>
<td>225</td>
<td>2</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>5</td>
<td>245</td>
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<td>17 ± 7</td>
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<td>265</td>
<td>2</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>7</td>
<td>285</td>
<td>2</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>8</td>
<td>305</td>
<td>2</td>
<td>12 ± 4</td>
</tr>
</tbody>
</table>

**Table 4.1** Tabular summary of a series of reactions run to determine optimal heating temperature in the synthesis of TiO₂ nanoparticles. Average particle size and size distribution were determined from TEM images.

The synthesis of metal oxide nanocrystals often involves heating at several different temperatures to achieve desired results. It is common to heat at a relatively high temperature in the beginning of a thermal-decomposition reaction and lower the temperature towards the end of the reaction. High temperatures are critical for achieving efficient nucleation and particle growth with low size distribution. A detailed study was performed to investigate the effects of heating at different combinations of temperatures and times. The results of this study are shown in Table 4.2.
<table>
<thead>
<tr>
<th></th>
<th>Temp(C), Time</th>
<th>Particle size (nm)</th>
<th>Rod size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>180, 30 min</td>
<td>250, 2 h</td>
<td>150, 2 h</td>
</tr>
<tr>
<td>2</td>
<td>250, 2 h</td>
<td></td>
<td>150, 2 h</td>
</tr>
<tr>
<td>3</td>
<td>250, 3 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>250, 4 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>250, 5 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>250, 2 h</td>
<td>150, 2 h</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>250, 3 h</td>
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<td>8</td>
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<td>250, 1 h</td>
</tr>
<tr>
<td>9</td>
<td>250, 2 h</td>
<td>150, 2 h</td>
<td>250, 2 h</td>
</tr>
<tr>
<td>10</td>
<td>250, 3 h</td>
<td>150, 2 h</td>
<td>250, 2 h</td>
</tr>
</tbody>
</table>

Table 4.2  Summary of a series of reactions investigating heating at different combinations of temperatures and times. Rods were found in product samples when reactions were heated for longer periods of time.

The results from this series of reactions (Table 4.2) illustrate that shorter periods of heating results in primarily spherical nanoparticles, while extended heating times often results in the formation of nanorods. Each reaction was performed once, so it is possible that the observation of rods in reaction 3 is aberrant, as they were not observed when the reagents were heated for 4 h and 5 h at 250 °C. The ratio of spheres to rods was not quantified; however, visual examination by TEM showed a higher ratio of rods in reactions 7-10 when the heating time was longer. Although reaction 7 in Table 4.2 did not produce spherical nanocrystals, it did result in the highest yield. The heating conditions were selected for future use because they produced the highest yield. Future reactions using these conditions were found to yield spherical particles exclusively.

A third, faceted and triangular morphology was synthesized under a different set of conditions. TiO(acac)$_2$, γ-butyrolactone (BL) and TOA were stirred at 120 °C under
positive argon pressure for one hour followed by addition of DMSO and stirring at 250 °C for 5 hours. This reaction was performed in an effort to obtain crystalline TiO₂ in situ, without the need for annealing.

**Scheme 4.2**  *Reaction conditions used to produce triangular shaped TiO₂. DMSO was injected into reaction flask at 120 °C.*

The reaction product was a brownish orange powder that dispersed well in CHCl₃ after sonication. TEM (Figure 4.8) revealed a product that contained mainly spherical particles, however a small number of equilateral nanotriangles were present as well. The TEM image shows that the triangles are not flawless in shape, and many have distinct edges. Also present in the image are smaller, hexagonal shaped nanoparticles.

**Figure 4.8**  *TEM and XRD (inset) images of triangular shaped nanoparticles resulting from synthesis of TiO₂ from TiO(acac)₂, TOA, BL and DMSO.*
The majority of the product contained spherical shaped nanoparticles, which had a low level of crystallinity when the diffraction pattern was recorded, as shown in the inset of Figure 4.8.

It is unclear which polymorph of TiO$_2$ the XRD pattern (Figure 4.8) represents. The peaks are broad, which suggest either a sample that is amorphous or made of very small nanocrystals. The TEM images collected show that the average size of the spherical nanoparticles is 17 nm and one nanotriangle had an edge length of 60 nm, suggesting that the sample is amorphous and not composed of very small crystallites. A similar reaction using DMSO as a reactant which led to the production of brookite was reported recently by Tang.$^{12}$ One known method for synthesizing nanotriangles was reported by Chemsedine and co-workers$^{21}$ however the triangles in that report are acute in shape and not equilateral as we observe.

4.3.5 UV–visible Spectroscopic Characterization of annealed TiO$_2$

The annealed TiO$_2$ nanoparticles were also characterized by UV-visible spectroscopy (Figure 4.9), which can be used to distinguish between anatase and rutile phases of TiO$_2$. A recent study by Li$^{6}$ reported that anatase and rutile phases have absorption maxima at 242 and 272 nm, respectively. Samples of TiO$_2$ synthesized using AP and PC as the surfactant were examined by UV-vis and characterized as anatase. As can be seen in the spectra the TiO$_2$ synthesized with AP surfactant has an absorption maximum at 244 nm and the sample prepared with PC surfactant has an absorption maximum at 246 nm. Both samples also display an absorption band edge around 350 nm, which is consistent with the literature.$^{26}$
**Figure 4.9** *UV-visible spectra of TiO$_2$ nanocrystals synthesized with different surfactants. Both display absorption peaks near 242 nm, indicating the anatase form of TiO$_2$.***

### 4.3.6 Addition of HCl: Synthesis of TiO$_2$ Nanocrystals

A different product is obtained when reaction conditions are altered by adding 0.5 M HCl to the reaction mixture. When HCl is present at the start of the reaction, the product is highly crystalline anatase TiO$_2$ nanocrystals. This is an important discovery that obviates the need for post-synthetic annealing, which is commonplace in most TiO$_2$ nanocrystal synthetic methods.$^{2,3,6,11}$ The products were characterized with TEM (Figure 4.10). The crystallite size is different when HCl is present, producing nanocrystals of approximately 3 nm, instead of approximately 15 nm. Shape is variable as well; most nanocrystals are spherical when this method is utilized; however, distorted spherical shapes were often produced as well. It has been previously reported that reaction pH is an important factor in the synthesis of TiO$_2$ and therefore no surprise that it was found to be an important factor in this reaction.$^{8,17,19,38}$

In general, it was found that addition of HCl gave rise to spherical nanocrystals that did not require annealing; however, addition of NaOH did not give rise to crystalline
TiO$_2$. All samples prepared with addition of NaOH were amorphous. In line with the previous results (reactions carried out without HCl and with post-synthetic annealing), it was found that crystalline TiO$_2$ could be synthesized with a variety of polar capping groups with the addition of HCl.
Figure 4.10  TEM images of anatase TiO$_2$ prepared with various surfactants with addition of HCl. γ-butyrolactone (A), propylene carbonate (B), 1-formyl piperidine (C), p-anisaldehyde (D), ethyl salicylate (E), and 2-acetyl pyridine (F). These nanocrystals were prepared by combining TiO(acac)$_2$, TOA, surfactant and 0.5 M HCl with heat. The most uniform nanocrystals were prepared with propylene carbonate surfactant.
The images shown in Figure 4.10 illustrate the effectiveness and variability of this method. The nanocrystalline products have a higher degree of monodispersity than when HCl is not added to the reaction, and the crystals are smaller. Also of note is the high uniformity of the nanocrystals synthesized with propylene carbonate as the surfactant. One distinct property that propylene carbonate has is a dielectric constant that is higher than any of the other surfactants included in this study. Propylene carbonate has a permittivity of $\varepsilon = 66.14$\textsuperscript{39} whereas a surfactant like ethyl salicylate has a permittivity of $\varepsilon = 8.48$. The stronger polarity and Lewis basicity of propylene carbonate may allow it to interact more strongly to the nanocrystal surface, therefore providing for more controlled crystal growth. While the surfactant does only constitute a minor amount reaction mixture, and therefore is not expected to have a large effect on nanocrystal size or shape, it has been reported that small changes in surfactant are known to make differences in nanocrystal shape.\textsuperscript{32} Propylene carbonate is also known to undergo ring-opening when subject to heat and acidic conditions, and this may play a role in the effectiveness of its ability to form highly spherical nanocrystals.\textsuperscript{40} The samples were also characterized by XRD) and found to be anatase TiO$_2$. The diffraction patterns were similar, regardless of which surfactant was added so only one is shown in Figure 4.11.
Figure 4.11  Example XRD of anatase TiO$_2$ synthesized with addition of 0.5 M HCl to reaction mixture. Surfactant in this case is propylene carbonate.
Further examination of the crystalline TiO$_2$ was performed with HRTEM (Figure 4.12). A sample prepared in propylene carbonate, TOA and 3 mL of 0.5 M HCl was dispersed in CHCl$_3$ and drop-cast onto a holey-carbon film copper TEM grid. A d-spacing of 3.7 Å was calculated from the TEM and is in agreement with an anatase TiO$_2$ structure. Most crystals appear spherical, although the one at the center of the image has a slightly elongated structure. Measurements were performed in collaboration with Brookhaven National Laboratory.
The quantity of 0.5 M aqueous HCl added to each reaction system was varied (1-4mL) to determine the optimal amount of HCl. For each surfactant system, amounts between 1 mL and 4 mL of HCl were added and the products were examined by TEM and XRD. For reference, the amount of TOA solvent is 15 mL. Overall, the amount of 0.5 M HCl added to the reaction was found to have very little effect on the outcome of the nanocrystal size or shape.

<table>
<thead>
<tr>
<th>mL HCl</th>
<th>2-Acetyl Pyridine</th>
<th>Butyrolactone</th>
<th>Propylene carbonate</th>
<th>p-anisaldehyde</th>
<th>1-formyl piperidine</th>
<th>Ethyl salicylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mL</td>
<td>3.9 nm</td>
<td>3.9 nm</td>
<td>3.2 nm</td>
<td>2.8 nm</td>
<td>2.6 nm</td>
<td>2.0 nm</td>
</tr>
<tr>
<td>2 mL</td>
<td>5.0 nm</td>
<td>3.9 nm</td>
<td>2.1 nm</td>
<td>2.8 nm</td>
<td>2.9 nm</td>
<td>2.9 nm</td>
</tr>
<tr>
<td>3 mL</td>
<td>4.9 nm</td>
<td>3.5 nm</td>
<td>2.9 nm</td>
<td>2.7 nm</td>
<td>2.9 nm</td>
<td>2.3 nm</td>
</tr>
<tr>
<td>4 mL</td>
<td>2.9 nm</td>
<td>3.7 nm</td>
<td>n/a</td>
<td>3.1 nm</td>
<td>3.3 nm</td>
<td>2.4 nm</td>
</tr>
</tbody>
</table>

Table 4.3: Average TiO$_2$ nanocrystal size for each surfactant system as amount of HCl added to reaction is changed between 1 mL and 4 mL.

The data in Table 4.3 show that as the amount of HCl added to the reaction is changed between 1 mL and 4 mL, there is no significant trend in size change, indicating that its role is catalytic in nature. The average nanocrystal size was determined by measuring the fwhm (full width at half maximum) peak broadening of the (101) reflection and calculating the crystallite size using the Scherrer equation. These values were also in agreement with what was observed by TEM. This result was of interest, since it appears that the mere presence of HCl, regardless of the amount gives rise to crystalline TiO$_2$. Although there was little change in size as the amounts of HCl was changed, addition of 3 mL of HCl with propylene carbonate surfactant resulted in nanocrystals that were most spherical and uniform, so these conditions were chosen as optimal. A similar result was observed by Kominami and co-workers$^{24}$ when preparing brookite TiO$_2$. They synthesize brookite by combining TiO(acac)$_2$, ethylene glycol,
sodium laurate and a small amount of water, and find that the presence of sodium and water are necessary – although they are unsure why – in order to prepare phase-pure brookite. LaMer and Dinegar\textsuperscript{41} describe a theory of nucleation which postulates that nucleation occurs when a critical concentration is reached, upon which self-nucleation occurs to relieve supersaturation. They also describe supersaturation occurring when solubility is decreased – this can happen by lowering temperature or adding a non-solvent. This theory may explain why we observe more monodisperse and crystalline TiO\textsubscript{2} when aqueous HCl is added to the reaction mixture, but not otherwise. The presence of aqueous HCl could lead to a rapid initial hydrolysis and a supersaturation of hydroxyalted titanium, followed by fast nucleation and formation the metastable anatase nanocrystallites.\textsuperscript{42}

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Dispersability</th>
<th>EtOH</th>
<th>Acetone</th>
<th>EtOAc</th>
<th>CHCl\textsubscript{3}</th>
<th>Hexanes</th>
<th>THF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>No</td>
<td>Some</td>
<td>Some</td>
<td>Yes</td>
<td>Some</td>
<td>Yes</td>
<td></td>
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<tr>
<td>PC</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td></td>
</tr>
<tr>
<td>FP</td>
<td>Some</td>
<td>Some</td>
<td>Some</td>
<td>Some</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>PA</td>
<td>Some</td>
<td>Yes</td>
<td>Some</td>
<td>Some</td>
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<tr>
<td>ES</td>
<td>Some</td>
<td>Some</td>
<td>No</td>
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<td>Some</td>
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</tr>
<tr>
<td>AP</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Some</td>
<td>Some</td>
<td>Some</td>
<td></td>
</tr>
</tbody>
</table>

*Table 4.4 Dispersability of TiO\textsubscript{2} prepared in TOA and different surfactants in addition to 3mL of HCl. ‘Yes’ means that TiO\textsubscript{2} was fully dispersed in solvent; ‘Some’ means that some of the TiO\textsubscript{2} was dispersed, however a significant amount remained as precipitate; and ‘No’ means that TiO\textsubscript{2} remained a ppt and solvent remained clear and transparent.*

Varying surfactant species added to reaction mixture was expected to have an effect on the dispersability of the product in solution. TiO\textsubscript{2} samples prepared with different surfactants were tested for dispersability in solvents of varied polarity. The
results of this test are shown in Table 4.4, and it can be seen that the dispersabilities are quite different. Some samples, prepared in PC or AP, are barely dispersable in any of the solvents tested. TiO$_2$ prepared in BL and ES can be dispersed in chloroform, which is relatively non-polar, while TiO$_2$ prepared in PA can be dispersed in acetone. TiO$_2$ prepared in FP, on the other hand, can be dispersed in both hexanes and THF.

The specificity of the reaction conditions were further probed by the addition of acids other than HCl, namely HNO$_3$ and H$_2$SO$_4$ to examine the results of a change in anionic species. Propylene carbonate was chosen as the model surfactant with which to test the altered conditions because the nanocrystals produced with PC are of higher uniformity than with other surfactants. Addition of 3 mL of 0.25 M H$_2$SO$_4$ resulted in irregularly shaped nanocrystals measured to be an average size of 2.7 nm. When nitric acid (HNO$_3$) replaced HCl in this reaction the particles were spherical in shape and had a size of approximately 3.2 nm. It is possible that monoprotic acids favor the formation of spherical nanocrystals, whereas diprotic acids do not. The XRD pattern for both samples showed evidence of crystallinity and all major peaks in the XRD diffraction patterns can be assigned to anatase. The major peaks shown in the XRD patterns in Figure 4.13 are (101), (112) and (200). TEM images of these samples are shown in Figure 4.14.
Figure 4.13  *X-ray diffraction pattern of TiO₂ synthesized with the addition of sulfuric acid (bottom) and nitric acid (top) instead of hydrochloric acid. Samples exhibit crystallinity and are identified as anatase.*

Figure 4.14  *TEM images of TiO₂ prepared with the addition of two different acids, 0.5 M sulfuric acid (A) and 0.5 M nitric acid (B).*

4.3.7 Solvent effects

Solvent choice plays an important role in the synthesis of nanocrystals. There tend to be fewer choices because many nanocrystal synthetic methods require sufficiently high temperatures that most commonly used solvents are immediately excluded. Solvents with a low volatility such as TOA (triocytamine) and TOPO (triocetylphosphine oxide) are known to work well; however, it is worthwhile to explore others in search of
alternatives that may avoid the toxicity and price of TOA and TOPO. The coordination ability of a solvent can have significant effects on the size and/or shape of nanocrystals. In the case of ZnO, the coordination ability of the solvent was found to affect whether the nanocrystals were spheres, rods or prisms. Several solvents were investigated in this study: TOA, octyl ether, 1-decene, propylene carbonate and 1-hexadecanol. The solvents chosen represent a wide variety of polarities and each has a distinct functional group. For each of the reactions in this series, 0.5 M HCl was added to the reaction. The average nanocrystal size for samples prepared in various solvents is shown in Figure 4.15.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Avg. nanocrystal diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trioctylamine</td>
<td>2.9 nm</td>
</tr>
<tr>
<td>octyl ether</td>
<td>2.9 nm</td>
</tr>
<tr>
<td>1-decene</td>
<td>2.7 nm</td>
</tr>
<tr>
<td>propylene carbonate</td>
<td>2.4 nm</td>
</tr>
<tr>
<td>1-hexadecanol</td>
<td>2.9 nm</td>
</tr>
</tbody>
</table>

Table 4.5  Average nanocrystal diameter based on solvent choice.

Interestingly, the solvent choice made little difference in the size of the nanocrystal. For each solvent profiled, the final product was composed of spherical nanocrystals in the range of 2.4–2.9 nm. Unlike the ZnO system, these nanocrystals all form spherical or slightly distorted spherical nanocrystal regardless of solvent choice.
**Figure 4.15**  TEM image of TiO₂ nanocrystals synthesized with different solvents. triocytlyamine (A), octyl ether (B), 1-decene (C), propylene carbonate (D). It was not possible to obtain a TEM image of TiO₂ synthesized in 1-hexadecanol.

**Figure 4.16**  XRD pattern for TiO₂ samples synthesized in different solvents. All are identified as anatase TiO₂. 1-decene (A), triocytlyamine (B), octyl ether (C), propylene carbonate (D) and 1-hexadecanol (E).

The XRD pattern Figure 4.16 illustrates the ability to produce anatase TiO₂ in a variety of solvents. All of the solvents tested gave rise to crystalline anatase in situ with the exception of 1-hexadecanol. The TiO₂ sample prepared in 1-hexadecanol was
annealed for 4 h at 500 °C and the diffraction pattern reveals it to be a mixture of anatase and rutile. A reflection at approximately 27 degrees is assigned to the (110) peak in rutile (PDF 77-0446). It remains unclear why 1-hexadecanol is an unsuitable solvent (no TEM image could be obtained) for this application, as solvents with alcohol functionalities, such as benzyl alcohol, are known to be good solvents in the synthesis of metal oxide nanocrystals.¹¹

4.3.8 Addition of H₂O to Reaction

It is known that addition of an aqueous acidic solution is required in the synthesis of TiO₂ to obtain crystallinity in situ; however, it is not known whether it is the water, the acid or some combination that is responsible for the crystallinity. For that reason, a control reaction was performed to investigate the effects of adding only water, and no acid to the reaction. Hydrolytic methods are common in the synthesis of TiO₂; however, the nanoparticles obtained are often not crystalline nor do they have well-defined spherical shapes. In these reactions TiO(acac)₂ was combined with TOA, AP and 1-4 mL of H₂O and heated in the previously described manner. TEM images are shown in Figure 4.17.
Figure 4.17  TEM images of TiO$_2$ synthesized with 1 (A), 2 (B), 3 (C) and 4 mL (D) of H$_2$O instead of addition of HCl.

The average size of the nanocrystals is 4.5 nm when 2 mL was added, 4.1 nm when 3 mL was added and 3.8 nm when 4 mL of H$_2$O was added to the reaction mixture. Given that measurement of crystallite size by XRD is subject to a 10% margin of error,$^{35}$ we can say that the size does not vary with amount of water added. All of the samples were crystalline anatase as determined by XRD; however, the samples prepared when 3 mL and 4 mL of water were added have sharper peaks and less noise in the diffraction patterns. The diffraction patterns for some samples are shown in Figure 4.18. It can be seen that the crystallinity increases in the presence of larger quantities of water. An x-ray diffraction pattern was not observed for the sample prepared with 1 mL of H$_2$O.
Figure 4.18  

XRD of nanocrystalline TiO$_2$ synthesized with various quantities of water. All samples were characterized as anatase. Four mL water (A), three mL water (B), two mL water (C).

4.3.9 Varying Amount of Surfactant

It is well known that metal oxide nanocrystal size can be controlled by altering the molar ratio of surfactant to precursor.$^{29,43-45}$ Typically, the more surfactant present, the smaller the nanocrystals will be. We investigated this by varying the molar ratio of surfactant to precursor from 1:1 to 5:1 in this study. In these reactions, TiO(acac)$_2$ was combined with TOA, PC and 3 mL 0.5 M HCl and heated as described previously. The products were analyzed by TEM (Figure 4.19) and XRD (not shown). It was interesting to find that in contrast to previous studies,$^{29,43-45}$ the size of the resulting nanocrystals varied very little as the molar ratio of surfactant:precursor was varied. Table 4.6 shows the average nanocrystal size as the molar ratio of surfactant is increased. At higher surfactant concentrations (3:1, 4:1 and 5:1) the average nanocrystal size was slightly smaller. This suggests that in this reaction the surfactant plays a different role than expected, or perhaps none at all. During nanocrystal synthesis the role of the surfactant is to bind to the surface during nucleation and provide for controlled growth of the
nanocrystal. If a higher ratio of surfactant is present, slower growth occurs because more surfactant molecules are bound to the surface of the growing crystal and this leads to smaller nanocrystals than when less surfactant is present. A larger presence of surfactant leads to a denser monolayer on the surface, thereby hindering nanocrystal growth and producing smaller nanocrystals. It is unclear how the surfactant identity affects the products in this reaction; however, it is clear that the identity of the surfactant added to each reaction is important, as the nanocrystals produced with propylene carbonate surfactant are of more uniform shape than nanocrystals synthesized with other surfactants.

**Figure 4.19**  TEM images of TiO$_2$ synthesized with different ratios of PC:precursor. 1:1 (A), 2:1 (B), 3:1 (C), 4:1 (D) and 5:1 (E).
Moles of Surfactant:TiO(acac)$_2$  Average Nanocrystal Size

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>2.7 nm</td>
</tr>
<tr>
<td>2:1</td>
<td>2.9 nm</td>
</tr>
<tr>
<td>3:1</td>
<td>2.3 nm</td>
</tr>
<tr>
<td>4:1</td>
<td>1.9 nm</td>
</tr>
<tr>
<td>5:1</td>
<td>2.3 nm</td>
</tr>
</tbody>
</table>

**Table 4.6**  Molar ratio of Surfactant:TiO(acac)$_2$ compared to average nanocrystal size.

### 4.3.10 GCMS Analysis of TiO$_2$ Surfactant

To examine the role of surfactant further, a closer look at the ligands was performed. The surfactant was removed from TiO$_2$ prepared with PC surfactant and analyzed by GCMS. The GCMS shows that at least seven major peaks in the GC trace, which likely correspond to seven different species on the TiO$_2$ surface.

**Figure 4.20**  GC trace obtained from GCMS analysis of surfactant removed from TiO$_2$ surface.
The MS trace from peak A shown in Figure 4.20 has an M-H\(^+\) peak at 355, which corresponds to TOA. This result, strongly confirms the presence of TOA on the surface of the TiO\(_2\) nanocrystals. Several of the other large peaks in the GC trace have MS peaks at 355, further suggesting a strong presence of TOA on the surface. In addition, propylene carbonate is known to ring open at high temperatures, which could lead to further chemistry with other reactants resulting in numerous surface species, as observed by GCMS. Pinna and co-workers\(^{15}\) report many side-products while preparing metal oxide nanocrystals from M(acac)\(_x\) and benzylamine, similar to what is observed here.

![GCMS data from surfactant species removed from TiO\(_2\) surface. This MS trace corresponds with Peak B in Figure 4.20. The large peak at 355 is assigned to trioctylamine.](image)

**Figure 4.21**

**4.3.11 Study of TiO\(_2\) Surface with DRIFT (Diffuse Reflection Infrared Fourier Transform)**

To further probe the surface ligands, infrared spectroscopy was used. Infrared spectroscopy is a valuable technique that is sometimes used to study the interactions of ligands on a nanoparticle surface. Transmission infrared techniques often make it difficult to study nanoparticles or colloids because sample preparation can be challenging. Nanoparticles are often difficult to disperse in solution, which thus makes it hard to
prepare a thin film on a salt plate. In addition, nanoparticle absorbance is often too high to obtain an adequate transmittance spectrum. DRIFT is a technique that allows examination of small amounts of solid samples by grinding them up with KBr powder. It is useful for looking at rough, powder samples because the infrared beam must enter the sample and bounce around within it so that absorption can occur before it leaves the surface of the sample for collection and further analysis. The Kubelka-Munk unit is commonly used in DRIFT spectra in place of absorbance or transmittance units. It is not possible to directly compare reflectance spectra such as diffuse reflectance to absorption spectra due to the fact that weak signals in absorption spectra are enhanced in reflectance spectra when absorbance units are used. The Kubelka-Munk unit is a conversion unit that make reflectance spectra appear similar to absorption spectra for easier comparison.

DRIFT samples were prepared by making a 1% mixture of TiO₂ powder in pre-ground KBr powder. The DRIFT spectra of TiO₂ synthesized with various surfactants are shown in Figure 4.22 and Figure 4.23.
Figure 4.22  DRIFT spectra of TiO₂ sample synthesized with different surfactants: trioctylamine (A), 2-acetylpuridine (B), propylene carbonate (C), γ-butyrolactone (D), 1-formylpiperidine (E), p-anisaldehyde (F), ethyl salicylate (G).

<table>
<thead>
<tr>
<th>Assignment for TOA peaks</th>
<th>Wavenumber (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-H stretch ν₉₉ CH₃</td>
<td>2954</td>
</tr>
<tr>
<td>C-H stretch ν₉₉ CH₂</td>
<td>2924</td>
</tr>
<tr>
<td>C-H stretch ν₈ CH₃</td>
<td>2867</td>
</tr>
<tr>
<td>C-H stretch ν₈ CH₂</td>
<td>2854</td>
</tr>
<tr>
<td>C-H bend ν₉₉ CH₃</td>
<td>1465</td>
</tr>
<tr>
<td>C-H bend ν₈ CH₃</td>
<td>1377</td>
</tr>
<tr>
<td>Methylene twisting and bending</td>
<td>1350-1150</td>
</tr>
<tr>
<td>C-N stretch</td>
<td>1094</td>
</tr>
</tbody>
</table>

Table 4.7  Assignment of IR bands shown in trioctylamine spectrum.
Figure 4.23  DRIFT of TiO_2 nanocrystals synthesized with propylene carbonate surfactant and TOA solvent.

<table>
<thead>
<tr>
<th>Assignment for TiO_2 prepared in TOA and PC</th>
<th>Wavenumber (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physisorbed H_2O</td>
<td>3225</td>
</tr>
<tr>
<td>Chemisorbed H_2O</td>
<td>3077</td>
</tr>
<tr>
<td>C-H stretch (\nu_{as} ) CH_3</td>
<td>2957</td>
</tr>
<tr>
<td>C-H stretch (\nu_{s} ) CH_3</td>
<td>2857</td>
</tr>
<tr>
<td>C-H stretch (\nu_{s} ) CH_2</td>
<td>2854</td>
</tr>
<tr>
<td>Adsorbed H_2O</td>
<td>1634</td>
</tr>
<tr>
<td>C-H bend (\nu_{as} ) CH_3</td>
<td>1459</td>
</tr>
<tr>
<td>C-H bend (\nu_{s} ) CH_3</td>
<td>1377</td>
</tr>
<tr>
<td>Methylene twisting and bending</td>
<td>1350-1150</td>
</tr>
<tr>
<td>C-N stretch</td>
<td>1088</td>
</tr>
</tbody>
</table>

Table 4.8  Infrared bands observed for TiO_2 nanocrystals prepared in TOA and PC.

The most obvious feature of the spectra in Figure 4.22 is that many look strikingly similar, which is intriguing, since each was synthesized with a different surfactant species. Spectrum A represents a sample of TiO_2 prepared in TOA solvent without additional surfactant. Table 4.7 shows the assigned IR bands in triocytalamine. This
experiment was performed as a control and to provide a reference to define which peaks in the other DRIFT spectra can be assigned to TOA. The close similarity of spectra A through E suggest that the major species existing on the surface of these TiO₂ nanocrystals is trioctylamine, and not the surfactant added to the reaction. Given the small amount of surfactant added to the reaction compared to TOA solvent, this seems plausible. It is known from the literature⁴⁸ that TOA is a strongly coordinating ligand for metal oxide nanocrystals, so it is not surprising to find that there is a significant amount on the nanocrystal surface. The broadness of the spectral features has been observed before on the surface of nanocrystals³² and strongly suggests a mixture of species bound to the surface. It is interesting however that spectra F and G are quite different and do not resemble the spectrum of TOA coated TiO₂. This suggests that p-anisaldehyde and ethyl salicylate are more strongly coordinating ligands than the other surfactants used in this study.

Let us now consider the spectra in greater detail. The peaks observed in spectrum A of Figure 4.22 all correspond well with what is expected of TiO₂ nanocrystals coated in TOA. The broad band extending from 3500-2500 cm⁻¹ represents physisorbed and chemisorbed water molecules hydrogen bonding to surface hydroxyls on the TiO₂. The bands at 2956, 2926 and 2856 cm⁻¹ are all typical C-H stretches due to the presence of TOA. Symmetrical and asymmetrical CH₃ bending and a methylene scissoring band are observed at 1375 and 1465 cm⁻¹, in addition to a C-N stretch observed at 1091 cm⁻¹. The strong, broad peak centered at 1633 cm⁻¹ could result from acetylacetonate groups still bound to Ti atoms, in addition to water molecules hydrogen bonded to surface hydroxyls. β-diketonates typically exhibit broad peaks in this region. In addition, a series of three
vibrations at 1122, 1088 and 1054 cm\(^{-1}\) can be assigned to Ti-O-C vibrations from acetylacetonate ligands and is consistent with the literature.\(^{49}\)

Spectrum C represents the DRIFT of TiO\(_2\) synthesized with PC surfactant in TOA solvent and is also shown enlarged in Figure 4.23. The assigned IR bands are shown in Table 4.8. The DRIFT is very similar to that of spectrum A, and therefore it can be concluded that there is mostly TOA on the surface. This is an interesting result since the nanocrystals of most uniform shape were synthesized with propylene carbonate; however, there does not seem to be any PC present on the surface. Had propylene carbonate been present on the surface a carbonyl stretch would be anticipated somewhere slightly lower than 1789 cm\(^{-1}\) due to binding to the surface and a likely reduced C=O bond length. This spectrum is nearly identical to spectrum A and therefore it is concluded that this sample has mainly TOA on the surface.

Spectra C, D and E are all quite similar to A and B and therefore it can be concluded that mainly TOA exists on the surface of these TiO\(_2\) nanocrystals.

The DRIFT spectrum of TiO\(_2\) prepared with PA surfactant (spectrum F) absorbed to the surface is representative of that and has strong bands due to \(p\)-anisaldehyde, suggesting that there is a significant amount on the surface. Evidence of water bound to surface hydroxyls on the TiO\(_2\) is present given the broad peak ranging from 3600-2500 cm\(^{-1}\). Aromatic C-H bands from \(p\)-anisaldehyde are visible at 3071 and 3005 cm\(^{-1}\). Other evidence for the presence of \(p\)-anisaldehyde are the asymmetric and symmetric C-O-C stretches at 1255 and 1036 cm\(^{-1}\). Bands resulting from C=C ring stretches are located at 1589, 1511 and 1418 cm\(^{-1}\).
Spectrum G represents TiO$_2$ nanocrystals that have surface bound ethyl salicylate. Again, a broad band from 3600–2500 cm$^{-1}$ is the signature of water bound to TiO$_2$ surface hydroxyls and there is also a band at 3061 cm$^{-1}$ due to aromatic C-H stretches from ES. Bands at 1568 and 1526 cm$^{-1}$ are probably assignable to C-C ring stretches on the ES aromatic ring. Symmetric and asymmetric methyl stretches from TOA are located at 1468 and 1378 cm$^{-1}$ respectively. An acetate stretch from ES is present at 1256 cm$^{-1}$. In addition to the acetate stretch, an O-C-C asymmetrical stretch is found at 1142 cm$^{-1}$ and assigned to ES.

A recent study$^{50}$ reported on the strong interactions of pyridine with the surface of anatase TiO$_2$. Pyridine is known to form hydrogen and coordination bonds with the surface of TiO$_2$ via surface hydroxyls and electron acceptor centers, respectively. The lone pair on nitrogen can form hydrogen bonding interactions with the dangling hydroxyls and also form donor-acceptor pairs when nitrogen donates its electrons to vacant Ti orbitals. An analogous interaction is likely to occur with trioctylamine, which possesses a more basic (sp$^3$ vs. sp$^2$) electron pair on its nitrogen atom. It is reasonable to conclude that this may be why there is such a strong presence of TOA on the surface of TiO$_2$ in this study.

4.3.12 NMR Study of TiO$_2$ Surfactant

The surfactant bound to TiO$_2$ nanocrystals prepared with propylene carbonate as surfactant was removed from the surface by washing the nanocrystals in EtOH. The nanocrystal/EtOH solution was sonicated for approximately 30 minutes to remove the surfactant, followed by centrifugation to separate the nanocrystals. The supernatant was reduced under vacuum to isolate the surfactant and remove EtOH. $^1$H NMR revealed
evidence of a surfactant that contained mainly trioctylamine. Given the nature of the GC trace and MS data, it is confirmed that the surfactant removed from the surface of the TiO₂ nanocrystals is composed of a mixture of many species; however, most of the peaks in the NMR spectrum correspond to TOA. The resonances at 0.9 ppm, 1.26 ppm and 2.36 ppm are all assigned to TOA. Another peak at 1.65 ppm is also present in the NMR of 98% trioctylamine obtained from Aldrich and is assigned to whatever impurity that may be. The peaks at 1.26 ppm and 3.7 ppm are due to EtOH, which was used to remove the surfactant; however, not completely removed by rotary evaporation. Propylene carbonate does not appear to be present in this sample. If it had been present, its resonances would show up at 1.5 ppm, 4.0 ppm, 4.6 ppm and 4.9 ppm. The ratio of PC to TOA present in the reaction mixture is quite low at 2:17, and likely would not have appeared in a ¹H NMR spectrum if present in this amount on the surface. A mixture of PC and TOA was prepared in this ratio and examined by ¹H NMR and indeed, there was no evidence of PC in the ¹H NMR spectrum.
Figure 4.24 $^1H$ NMR of surfactant removed from TiO$_2$ nanocrystal surface. This sample of TiO$_2$ was prepared with TOA solvent and PC surfactant. The surface species were removed by precipitating the nanocrystals with EtOH.

4.3.13 Thermogravimetric Analysis of TiO$_2$

Thermogravimetric analysis (TGA) was used to further characterize the surfaces of the TiO$_2$ nanocrystals prepared with different surfactants (Figure 4.25). Each sample was heated at 5 °C/min up to 600 °C. In general, the thermograms show two very broad overlapping mass losses illustrated by the derivative of the mass loss. This suggests the presence of two or more ligands on the surface of the TiO$_2$ nanocrystals which are released from the TiO$_2$ surface at similar temperatures. One mass loss is observed at approximately 298 °C, and the second at 389 °C. The broad nature of the derivative is suggestive of a mixture of species on the surface. TOA, which has a boiling point of
365–367 °C is expected to burn off somewhere in this temperature range. Based on the DRIFT analysis, it can be assumed that the majority of the ligands on the nanocrystalline surface are TOA. With this in mind, an approximate calculation was performed to obtain a general idea of the number of ligands on each nanocrystal and the number of ligands per nm\(^2\). The calculation method described by Visintin\(^{51}\) was used as a model to perform these computations, and assumed a spherical particle shape. Since the DRIFT results showed that most samples contained mostly TOA on the surface, and for the sake of simplicity in the calculations it was assumed that only TOA was on the surface. For the TiO\(_2\) prepared with PA and ES, it was assumed that only PA and ES were on the surfaces, respectively. The calculations performed are crude in nature, since it is known that a mixture of ligands exist on the surface; however, they provide a general idea of the number of ligands per particle and per nm\(^2\). The results of these calculations are shown in Table 4.9.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Avg NP size</th>
<th>No. surf groups (per nm(^2))</th>
<th>No. surf groups (per particle)</th>
<th>No. TOA groups (per nm(^2))</th>
<th>No. TOA groups (per particle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-butyrolactone</td>
<td>3.9 nm</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>95</td>
</tr>
<tr>
<td>2-acetyl pyridine</td>
<td>4.9 nm</td>
<td>-</td>
<td>-</td>
<td>0.9</td>
<td>68</td>
</tr>
<tr>
<td>Propylene carbonate</td>
<td>2.9 nm</td>
<td>-</td>
<td>-</td>
<td>1.2</td>
<td>34</td>
</tr>
<tr>
<td>p-anisaldehyde</td>
<td>2.7 nm</td>
<td>1.4</td>
<td>32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1-formylpiperidine</td>
<td>2.9 nm</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>13</td>
</tr>
<tr>
<td>Ethyl salicylate</td>
<td>2.4 nm</td>
<td>0.6</td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trioctylamine</td>
<td>3.4 nm</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>47</td>
</tr>
</tbody>
</table>

Table 4.9 Results from calculations of number of surface ligands from TGA data.

Figure 4.25 shows a representative TGA thermogram obtained for a sample of TiO\(_2\) prepared with BL in TOA solvent. Two labeled mass losses are observed for this
sample, and the results were similar for all other samples examined by TGA. The
derivative (dmass/dT) was found and is shown below to highlight the temperatures at
which the mass loss is decreasing most. The dmass/dT line is quite broad, indicating that
there is probably a mixture of species being combusted between 200 and 500 °C. The
second derivative was also obtained, however did not delineate the mass loss peaks any
better than the first derivative.

Thermogravimetric analysis provides an understanding of what percentage of the
nanocrystal mass is due to surfactant. Knowing this allows for a more accurate
calculation of the percent yield of a reaction. For example, in the TGA shown in Figure
4.25, approximately 19% of the mass of the prepared TiO₂ is due to the organic surfactant
molecules on the surface. Given this value, we can calculate a percent yield for the
reaction, which for this sample was found to be 77%. Typical yields for this reaction
(TiO(acac)₂ in TOA, surfactant and HCl) were between 50 and 80 percent.
4.3.14 Photoluminescence of TiO$_2$

The anatase TiO$_2$ prepared by decomposition of TiO(acac)$_2$ was examined by photoluminescence spectroscopy$^{52}$ to determine its photoactivity. The sample was prepared with propylene carbonate as the surfacant; however, it is thought to be capped with mainly TOA. Room temperature, as well as 77 K and 10 K photoluminescence spectra were recorded at the maximum excitation of a He-Cd laser. At RT there is nearly no PL, however at 77 K and 10 K the PL increases slightly and maxima are observed at 2.2 and 2.4 eV at 77 K and what appears to be two overlapping peaks centered at approximately 2.4 eV (or 517 nm) when measured at 10 K. This corresponds well with an example in the literature$^{53}$ which reports PL of TiO$_2$ from 450-550 nm. The photoluminescence observed here is very weak. The spectra at 10 K and 77 K were recorded under vacuum since adsorption of oxygen can decrease the intensity of photoluminescence.
Figure 4.26  Photoluminescence of anatase TiO$_2$. Signal is very weak, but does increase in the absence of air and at decreased temperatures.

4.4 Conclusions

A straightforward new method for the synthesis of crystalline anatase TiO$_2$, without the need for post-synthetic annealing was developed. The synthesis of TiO$_2$ with PC as the surfactant produced highly uniform spherical anatase nanocrystals, which is rarely observed in other methodologies. TiO(acac)$_2$ also provides a less costly starting material which has the ability to produce TiO$_2$ of controllable shapes and sizes. When HCl is present in the reaction mixture, the reaction is thought to proceed by fast nucleation resulting from hydrolysis of TiO(acac)$_2$ to form metastable, crystalline anatase. The fast heating rate (35 °C/min) should cause the water to boil off after less than 3 minutes, which suggests that the nucleation is a fast event. Efficient nucleation –
and from that, crystallinity – does not result without the presence of aqueous HCl. The presence of HCl in the reaction mixture gives rise to smaller nanocrystals than if not present; this suggests that the nanocrystal growth is more hindered, perhaps due to surface hydroxyls that form.

Spherical, rod-like and triangular shaped nanocrystals can be synthesized by varying the reaction conditions. The equilateral nanotriangle shape, is novel for anatase TiO$_2$; however, this shape has been observed in other metal oxides such as ZnO. The TiO$_2$ surface was studied by standard techniques and the major component of the ligand mixture on the surface was found to be TOA, which is supported by NMR, IR, GCMS and TGA data. The broadness of the NMR and IR data, along with the many peaks in the GC trace make it known that there are many species in existence on the surface of these TiO$_2$ nanocrystals.

4.5 References:


(52) Performed by Yinyan Gong.

Chapter 5

Metal Acetylacetonate Precursors for the Synthesis of Early Transition Metal Oxide Nanocrystals
5 Metal Acetylacetonates as Precursors for the Synthesis of Early Transition Metal Oxide Nanocrystals

5.1 Introduction

5.1.1 Synthesis of Metal Oxides

Transition metal oxide nanocrystals represent a broad class of materials that have been researched extensively due to their interesting properties (magnetic, electronic and catalytic), nanoscale behavior, and wide scope of potential applications. There are numerous applications for these materials; however, several notable applications are MRI imaging,\(^1\) solar cells\(^2\) and heterogenous catalysis.\(^3,4\) The literature is flush with approaches to synthesizing metal oxide nanocrystals from various inorganic or organometallic precursors, resulting from the rapid development of this field during the past two decades. Thermal decomposition procedures are widely popular; and typical starting materials can include, metal acetylacetonates,\(^5\) metal cupferronates,\(^6\) metal alkoxides,\(^7\) metal carbonyls,\(^8\) and metal halides.\(^9\) Numerous other methods abound; however, controlled thermal decomposition routes have produced the most uniform products.

Methods for preparation of metal oxides are well-developed; however, recently there has been a focus on developing more general methods\(^5,10-14\) that can be applied to the preparation of a variety of metal oxides. This is important from an industrial scale-up perspective because many oxides could be prepared using one individual reactor as opposed to separate set-ups. We have focused our efforts on developing a method that can be used to prepare a variety of metal oxide nanocrystals from similar precursors –
metal acetylacetonates. A simple reaction for the preparation of \( \gamma \)-Fe\(_2\)O\(_3\) was found and expanded to other oxides. Through this work we have prepared several types of metal oxide nanocrystals and report on a general method for the preparation of \( \gamma \)-Fe\(_2\)O\(_3\), Mn\(_2\)O\(_3\), Mn\(_3\)O\(_4\), and Cr\(_2\)O\(_3\), which can likely be applied for synthesis of other metal oxide nanocrystals as well.

Chromium and manganese oxide nanocrystals can be prepared from Cr(acac)\(_3\), Mn(acac)\(_2\) and Mn(acac)\(_3\) precursor compounds, respectively. We found that acetone, cyclohexanone and ethanol are good solvents and sources of oxygen in this reaction. In addition, the starting materials (with the exception of cyclohexanone) are environmentally benign and relatively inexpensive. The method of preparation is simple, and products require little work-up. This is an easy, simple and safe route to chromium and manganese oxide nanocrystals. In addition, \( \gamma \)-Fe\(_2\)O\(_3\) nanocrystals were prepared in a similar way by refluxing Fe(acac)\(_3\) in the presence of a coordinating capping ligand/solvent.

**Motivation**

The motivation of the research presented in this chapter was two-fold: to develop a general method for the synthesis of metal oxide nanocrystals utilizing metal acetylacetonate precursors and to nanoengineer the surfaces of nanocrystals with new ligands and influence solubility.

**5.1.2 Metal Acetylacetonates as Precursors to Metal Oxide Nanocrystals**

Recently, several methods have been developed for the synthesis of metal oxide nanocrystals using metal acetylacetonate precursor compounds. Metal acetylacetocetates
represent an interesting class of compounds that bring many benefits to metal oxide nanocrystal synthesis. Metal alkoxides are used more commonly as precursors to metal oxide nanocrystals; however, they react rapidly to give rise to amorphous, non-uniform shaped particles. Rapid reaction rates also lead to low crystallinity, requiring that products be calcined post-reaction. In addition, metal alkoxides are not as widely available and when available are more costly than metal acetylacetonates. Metal halides, also used quite often as precursors to metal oxide nanocrystals, are bothersome because the halide impurities found in the final products and are difficult to remove. M(acac)_x precursors also have the benefit of being relatively non-toxic as well as having the benefit of not being air-sensitive, unlike many organometallic compounds such as metal carbonyls, which are often used to prepared nanocrystals.

During the past decade this area of research has developed rapidly and a number of metal oxides have been prepared from metal acetylacetonates. Several of the oxide nanocrystals prepared with M(acac)_x precursors are TiO_2, γ-Fe_2O_3/Fe_3O_4, γ-Ga_2O_3, ZnO, In_2O_3, V_2O_5, Nb_2O_5, Ta_2O_5, HfO_2 and SnO_2. One excellent method, pioneered by Niederberger and co-workers using only M(acac)_x precursors and benzyamine solvent has produced many of the aforementioned metal oxides. Sun and co-workers have synthesized Fe_3O_4 by heating Fe(acac)_3 with oleylamine, oleic acid and 1,2-hexadecanediol. In comparison to other acac or acetate precursors, Fe(acac)_3 is less costly and the nanocrystalline products are of comparable uniformity and monodispersity. Metal acetylacetonates also bring to the table low moisture sensitivity, rendering them less susceptible to hydrolysis, which is a common disadvantage of metal alkoxides and halides.
5.1.3 Capping Ligands and Solubility

The capping ligands on nanocrystals have several purposes. They provide a dense monolayer, which coats the nanocrystal surface to prevent aggregation and control nanocrystal growth mechanisms. Capping ligands also provide nanocrystals with stability in solution, and have the potential to modify the surface for further use in application-based chemistry. Highly elevated temperatures are commonplace in metal oxide nanocrystal syntheses, which limits ligand selection to only those which can withstand temperatures over 200 °C. For this reason, there is a focus on developing low-temperature nanocrystal synthetic procedures.23 Many of the more commonly used ligands such as oleic acid and TOPO (triocylphosphine oxide) are hydrophobic and limit solubility to non-polar solvents. Given these limitations, it is challenging to choose a surface ligand that can provide a desired surface chemistry and survive reaction conditions. The ability to tune solubility by altering surface chemistry would enhance the usefulness of metal oxide nanocrystals for technological applications. Currently ligand-exchange is often used to alter the solubility of nanocrystals post-synthesis.24 This post-synthetic modification involves removal of the ligand placed on the surface during nanocrystal preparation, followed by grafting the desired ligand to the surface. There are many ligands that have low volatility and can form covalent or ionic binding interactions with oxide surfaces; however, only a few, like oleic acid, deliver highly monodisperse and uniform nanocrystals. Part of the research described in this chapter sought to overcome these obstacles by screening for new ligands that could tune the solubility of nanocrystals beyond the realm of organic solvents.
5.1.4 Iron Oxides, Manganese Oxides and Chromium Oxides

In this chapter, a general method will be described which was developed for the synthesis of metal oxides nanocrystals and aptly utilized to prepare $\gamma$-Fe$_2$O$_3$, Mn$_2$O$_3$, Mn$_3$O$_4$ and Cr$_2$O$_3$ nanocrystals from acetylacetonate precursors. Preparation of iron oxide nanocrystals has been studied extensively and many applications for them have been developed, most resulting from unique size-dependent magnetic properties. Methods exist to prepare maghemite and magnetite from Fe(acac)$_3$; however, our studies found a different route to $\gamma$-Fe$_2$O$_3$, while also using various organic capping ligands to tune the surface chemistry and solubility of the nanocrystals.

Manganese oxides are known catalysts that can remove carbon monoxide and nitrogen oxide from waste gases expelled from sources such as automobiles and cigarettes.$^{25-28}$ Monodisperse manganese oxide nanocrystals have been prepared from manganese acetate in TOA and oleic acid with applied heat.$^{29}$ Our method uses metal acetylacetonate precursors and only acetone, cyclohexanone or ethanol as solvent.

Chromium oxide (Cr$_2$O$_3$) is a green powder used in pigment dyes,$^{30}$ magnetic materials$^{31}$ and catalysis.$^{32}$ There are few reports describing preparation of Cr$_2$O$_3$ nanoparticles, which is perceived to be difficult.$^{31}$ Typical methods reported for Cr$_2$O$_3$ nanoparticle synthesis are mechanochemical processing,$^{33}$ precipitation-gelation,$^{34}$ precipitation of Cr(OH)$_3$ followed by calcination,$^{35}$ arc-discharge of Cr followed by annealing,$^{36}$ and gas-condensation.$^{37}$ Not until Peng’s method recently,$^{31}$ which involves the thermal decomposition of metal fatty acid salts, did a solvothermal preparation exist. They described the reproducibility of this method to be difficult while producing triangular shaped nanoparticles.
5.2 Experimental Methods

5.2.1 Synthesis of Iron Oxide Nanocrystals

Unless otherwise noted, iron(III) acetylacetonate (2 mmol) from Strem Chemicals, Inc. was combined with 20 mL of surfactant/solvent (2-acetyl pyridine (AP), p-anisaldehyde (PA), γ-butyrolactone (BL), ethylene carbonate (EC) or 1-formyl piperidine (FP), all obtained from Aldrich) and refluxed for 30 minutes under positive N₂ pressure. The solvent also acts as the surfactant. The solution was cooled to room temperature and dissolved in hexanes to disperse followed by centrifugation at 3,800 rpm for 10 minutes. The precipitate was discarded and supernatant was collected. The workup for each reaction product is dependent upon the solvent used. When 2-acetyl pyridine or ethyl salicylate was the solvent the nanocrystals were precipitated from solution with water, centrifuged 10 minutes to collect the precipitate, which was then re-dispersed in chloroform. Nanocrystals prepared with p-anisaldehyde or γ-butyrolactone were collected by precipitating with hexanes and redispersed in ethanol or acetone. Ethylene carbonate solvent requires precipitating nanocrystals with ethanol, centrifuging to collect the precipitate, which is then dispersed in chloroform. With 1-formyl piperidine, the nanocrystals were isolated by precipitation with hexanes and dispersable in water. TEM imaging and structure determination by XRD (Inel X-ray diffractometer) were performed to characterize the samples. Images of the nanocrystals were taken on a JEOL cx100 Transmission Electron Microscope (TEM) at 100 kV with CCD camera attachment. Samples were prepared by drying solvent dispersions of the nanocrystals onto carbon backed 400-mesh Cu grids followed by drying under vacuum at room temperature.
5.2.2 Synthesis of Chromium Oxide Nanocrystals

Chromium oxide (Cr₂O₃) nanocrystals were prepared by heating Cr(III) acetylacetonate (1 mmol, 0.35 g) (Strem Chemicals, Inc.) with 20 mL acetone (or cyclohexanone or ethanol) in a Teflon-lined Parr acid digestion bomb at 200 °C for 72 h to one week. The bomb was cooled to RT before opening. The product was a dark green precipitate in a green solution that was dissolved in chloroform and centrifuged for 10 minutes. A green solid precipitated and was dried for 12 h under vacuum, followed by annealing 4 h at 500 °C in air. The product is soluble in chloroform. Percent yield increased with longer durations of heating. The dark green material was characterized by TEM, XRD and elemental analysis.

5.2.3 Synthesis of Manganese Oxide Nanocrystals

Manganese oxide (Mn₂O₃) nanocrystals were synthesized by heating Mn(III) acetylacetonate (1 mmol, 0.35 g) in 20 mL acetone or ethanol in a Teflon lined Parr acid digestion bomb at 200 °C for a minimum of 72 h. The dark product solution was dispersed in chloroform and centrifuged for 10 minutes. The black precipitate was isolated and dried at RT under vacuum for 12 hours, followed by calcination at 500 °C for 4 hours. The product was characterized by XRD, TEM and elemental analysis.

Mn₃O₄ nanocrystals were prepared by heating Mn(II) acetylacetonate (1 mmol, 0.25 g) in cyclohexanone in a Parr acid-digestion bomb, lined with Teflon for 4 days at 200 °C. The product was collected by adding chloroform to the product solution and centrifuged 10 minutes. The black precipitate was collected and dried under vacuum for 12 h at RT.
5.3 Results and Discussion

5.3.1 Synthesis of $\gamma$-Fe$_2$O$_3$ Nanocrystals

Metal acetylacetonate precursors have not become as common as metal alkoxides or metal halides for the synthesis of metal oxide nanocrystals, despite the fact that they are widely available commercially and inexpensive. Their $\beta$-diketonate bidentate ligands coordinate to the metal center strongly, and are believed to provide for more controlled reaction rates than monodentate ligands in the synthesis of metal oxide nanocrystals.$^{22}$

Through this research, metal acetylacetonate precursors were used to synthesize four different types of metal oxide nanocrystals using very straightforward and simple methods. It is thought that the methods developed can be generally applied to the synthesis of other metal oxide nanocrystals. Iron(III) acetylacetonate, Mn(III) acetylacetonate, Mn(II) acetylacetonate and Cr(III) acetylacetonate were all used to prepare the following metal oxide nanocrystals: $\gamma$-Fe$_2$O$_3$, Mn$_2$O$_3$, Mn$_3$O$_4$ and Cr$_2$O$_3$, respectively.

Iron oxide nanocrystals ($\gamma$-Fe$_2$O$_3$, maghemite) have magnetic properties which make them useful in a wide variety of biological, data recording and data storage applications. The methods available for synthesizing iron oxide are extensive; however, most methods use capping agents that are hydrophobic. The majority of capping ligands are long chain organic molecules containing functional groups that can bind to iron oxide nanocrystal surfaces, such as amines, diols, phosphates, carboxylic acids or carbonyl groups. Many methods used to synthesize monodisperse iron oxide nanocrystals require high temperatures and therefore the choice of ligands is limited to non-volatiles that can
withstand temperatures of up to 350 °C. Biological applications dominate the uses for γ-
Fe₂O₃ nanocrystals, therefore producing water-soluble γ-Fe₂O₃ is necessary. Ligand-
exchange is often used to alter particle solubility after synthesis; however, there is a need
for more efficient in situ methods. One goal of this research was to provide a method to
for tuning the solubility of iron oxide nanocrystals. This problem was approached with
the idea that surfactants of different polarities and solubilities could extend the solubility
of iron oxide nanocrystals to a wider array of solvents.

Maghemite nanocrystals were prepared simply and quickly by heating Fe(acac)₃
in one of a number of polar, high boiling point solvents. The solvents also act as capping
ligands, providing further simplicity, were selected based on several criteria. High
temperatures are required for this reaction and therefore a high boiling point is imperative
in the solvent. Since there are a large number of capping ligands that are capable of
providing solubility in non-polar organic solvents, solvents with a stronger polarity were
chosen, in an effort to provide solubility in more polar and aqueous solvents. Carbonyl
groups are known to interact strongly with the metal oxide surfaces, and for that reason,
solvents containing carbonyls were selected. The capping ligands/solvents selected are
shown in Figure 5.1.
Figure 5.1  Capping ligands/solvents chosen for nanocrystal synthesis. All solvents have carbonyl groups to bind to the metal oxide surface, and high boiling points that will allow them to survive high reaction temperatures.

Recently Li\textsuperscript{18} and coworkers reported synthesizing water-soluble iron oxide nanocrystals by refluxing Fe(acac)\textsubscript{3} in 2-pyrrolidone for approximately 15 minutes. This method also uses Fe(acac)\textsubscript{3} and contributes another capping group that produces water-soluble iron oxide via an uncomplicated route.

Our method is similar to this one; however, it provides more ligands that can be used, leading to a greater variety in solubility options. The route developed is also very short and rapid. Under favorable conditions the reaction can be completed within one hour. A reaction scheme illustrating highlighting the proposed binding of FP to the surface of γ-Fe\textsubscript{2}O\textsubscript{3} nanocrystals is shown in Scheme 5.1.
Scheme 5.1  Synthesis of water-soluble iron oxide nanocrystals by heating Fe(acac)$_3$ in 1-formylpiperidine solvent. The 1-formylpiperidine is proposed to bind to the metal oxide surface through the carbonyl group.
Figure 5.2  TEM images of γ-Fe₂O₃ nanocrystals prepared with various solvents: 2-acetyl pyridine (A), p-anisaldehyde (B), γ-butyrolactone (C), ethylene carbonate (D), and 1-formyl piperidine (E)

TEM images of γ-Fe₂O₃ nanocrystals prepared in different solvents are shown in Figure 5.2. The average diameter of the nanocrystals was determined from the TEM image and ranged from 3-8 nm, depending upon which solvent was added. The average nanocrystal diameters are as follows: 2-acetylpyridine (3nm), p-anisaldehyde (4nm), γ-butyrolactone (4nm), ethylene carbonate (8nm) and 1-formyl piperidine (3nm).
The nanocrystals prepared in 2-acetylpyridine (Figure 5.2A) are mostly spherical and cubic in shape; however, p-anisaldehyde (Figure 5.2B) gives rise to a bi-modal size distribution of spherical nanocrystals. Iron oxide nanocrystals prepared in \( \gamma \)-butyrolactone (Figure 5.2C) were difficult to image with TEM, but appear to be quite spherical in shape. When ethylene carbonate (Figure 5.2D) is the solvent in this reaction, the nanocrystals are hexagonal. Interestingly, nanocrystals prepared in 1-formylpiperidine (Figure 5.2E) are nonuniform in shape; however, are soluble in water. It is unclear why 1-formylpiperidine capped nanocrystals are soluble in water. Ethyl salicylate and propylene carbonate solvents were also tested; however, no nanocrystals were observed by TEM.

The products were characterized by x-ray diffraction and found to be highly crystalline. The reflections in the diffraction pattern correspond to \( \gamma \)-Fe\(_2\)O\(_3\); however, Fe\(_3\)O\(_4\) has a nearly identical diffraction pattern and may be present. The low angle reflections, (210) and (211), are a sign that \( \gamma \)-Fe\(_2\)O\(_3\) is present as well. An example of the x-ray diffraction pattern obtained during this method of preparation is shown in Figure 5.3. All were superimposable, and therefore only one is shown.
Figure 5.3  X-ray diffraction pattern for $\gamma$-Fe$_2$O$_3$ nanocrystals prepared with p-anisaldehyde.

One important goal of this research was to tune the solubility of iron oxide nanocrystals in order to make them more readily used in applications that require polar or aqueous solvents. It was found that nanocrystals capped with AP and EC are soluble in non-polar solvents such as chloroform. However, iron oxide nanocrystals capped with PA or BL are soluble in highly polar solvents like ethanol or acetone. Even more exciting is the fact that nanocrystals capped with FP are soluble in water.

Oleic Acid Solvent

Oleic acid is commonly used as a capping ligand in the synthesis of iron oxide nanocrystals. The most widely used method was developed by Hyeon$^8$ and uses a Fe(CO)$_5$ precursor. Iron pentacarbonyl is highly toxic, air sensitive, and also much more expensive than Fe(acac)$_3$. We prepared iron oxide nanocrystals by simply refluxing Fe(III) acetylacetonate in oleic acid. Fe(III) acetylacetonate (2 mmol) was heated to 210
°C in 20 mL oleic acid for approximately 30 minutes. The temperature was increased to 300 °C and stirred at that temperature for 1 h. Maintaining one temperature, for example 200 °C or 300 °C, did not produce nanocrystals of comparable quality to those in Figure 5.4. Characterization by TEM found the particles to be highly monodisperse and uniform in shape. The average size is 10 nm, which was found from the TEM image shown in Figure 5.4. The nanocrystals are spherical in shape and self-organize in two dimensions on a TEM grid.

![TEM Image of FeO Nanocrystals](image)

**Figure 5.4** TEM of FeO nanocrystals. Average size is 10 nm. Prepared by heating Fe(acac)₃ in oleic acid to 300 °C. Characterized as FeO by XRD.

The nanocrystals were characterized as FeO by XRD, which presumably can be further oxidized to Fe₃O₄ and γ-Fe₂O₃ with heat. This reaction works very well and produces highly monodisperse nanocrystals; however, is successful approximately 20% of the time.
5.3.2 Synthesis of Mn$_2$O$_3$ and Mn$_3$O$_4$ Nanocrystals

Metal acetylacetonate precursors were explored further to show that other transition metal oxide nanocrystals could easily be prepared by this method. Manganese exists in various oxidation states, and for that reason Mn(acac)$_2$ and Mn(acac)$_3$ are available commercially. Several manganese oxides are common, including MnO, Mn$_2$O$_3$ and Mn$_3$O$_4$.

Manganese oxides are of major interest for both battery and catalysis applications.$^{39,40}$ MnO is a model system for studying the magnetic and electronic properties of rock-salt oxides.$^{28,41}$ Mn$_2$O$_3$ is a catalyst that can remove carbon monoxide and NO$_x$ from waste gases.$^{4,26,27,42}$ The increased surface area of nanocrystals over bulk manganic oxide gives them a higher capacity for oxygen absorption and therefore the oxidation of more carbon monoxide to CO$_2$. Mn$_3$O$_4$ also acts as an oxidation catalyst and has been used to catalyze the oxidation of methane and carbon monoxide.$^3$

Mn$_2$O$_3$ and Mn$_3$O$_4$ nanocrystals were synthesized in a extraordinarily simple manner by heating either Mn(acac)$_3$ or Mn(acac)$_2$ in acetone in a steel autoclave for more than 72 hours. When Mn(acac)$_3$ and acetone were combined in a steel autoclave and heated in a 200 °C oven for 9 days and the dark brown powder annealed at 500 °C for 5 h, the XRD (Figure 5.7) showed the product to be nanocrystalline Mn$_2$O$_3$. The average nanocrystal size was determined from the Scherrer equation to be 17.2 nm by measurement of the (222) peak, which is in agreement with what was observed by TEM. The yield for this reaction was 62%, which is rather high for a metal oxide nanocrystal synthesis. LRTEM and HRTEM of annealed Mn$_2$O$_3$ nanocrystals is shown in Figure 5.5
and Figure 5.6, respectively. The nanocrystals are an average of 30 nm in diameter, as determined by the (222) peak in the x-ray powder diffraction pattern.

100nm

**Figure 5.5**  Low-resolution TEM of Mn$_2$O$_3$ nanocrystals. Prepared by heating Mn(acac)$_3$ in acetone for 9 days at 200 °C.
Figure 5.6  HRTEM of $\text{Mn}_3\text{O}_4$ nanocrystals at 60,000 and 600,000 magnification. These images were obtained at Brookhaven National Laboratory.
Figure 5.7  XRD of Mn$_2$O$_3$ prepared from Mn(acac)$_3$ and acetone. Scherrer equation was used to calculate average particle size as 17.2 nm from (222) reflection.

The products were amorphous post-synthesis (determined by XRD) and were annealed for 5 h at 500 °C. After annealing, the x-ray diffraction pattern (Figure 5.7) proved that the black powder was indeed Mn$_2$O$_3$, with all major reflections assigned to that structure. The nanocrystals are spherical and cubic in shape. Elemental analysis was performed by ICP (inductively coupled plasma) at Galbraith Laboratories to determine the percent manganese in this sample and further confirm it as Mn$_2$O$_3$. Experimentally, the sample contained 69.6 % Mn, which is also the theoretical percent of Mn in Mn$_2$O$_3$. Based on the XRD and ICP results, it can be said that the method developed produces phase-pure Mn$_2$O$_3$.

This reaction was repeated at a slightly higher temperature, 250 °C, for 4 days, which gave rise to Mn$_2$O$_3$ after annealing at 500 °C for 5 h. The yield for this reaction
was 10%, which is much lower than when the reaction was run at 200 °C. The particle shape is not as uniform, which suggests that heating at lower temperature for a longer period of time is better for obtaining more uniform crystal growth. The TEM is shown in Figure 5.8 and the average particle size was calculated to be 23.6 nm from the XRD pattern, by using the Scherrer equation, by measurement of the (222) peak.

![TEM of Mn$_2$O$_3$ nanocrystals prepared at 250 °C.](image)

**Figure 5.8**  *TEM of Mn$_2$O$_3$ nanocrystals prepared at 250 °C.*

In attempt an to replicate the reaction performed with Fe(acac)$_3$, Mn(acac)$_3$ was heated at 300 °C for 1 h in TOA with oleic acid surfactant; however, no nanocrystals were observed by TEM. From this experiment, we can ascertain that the reactivity of Mn(acac)$_3$ is quite different than Fe(acac)$_3$. Performing this reaction in a Parr acid-digestion bomb seems to produce better results than a shorter reaction time and at higher temperatures.

Mn$_3$O$_4$ nanocrystals were formed when Mn(acac)$_2$ was heated with cyclohexanone in a Parr acid-digestion bomb at 200 °C for 4 days. The product was isolated by centrifugation and found to be amorphous by XRD. The dark powder was annealed at 500°C for 5 h and re-examined by XRD, which showed it to be crystalline.
Further characterization by TEM revealed that the nanocrystals are spherical; however, there are several cubic shaped crystallites as well, as shown in Figure 5.9. The material was identified as Mn$_3$O$_4$ by XRD (Figure 5.10). Calculation of average nanocrystal diameter using the width of the (211) peak in the Scherrer equation gives an average diameter of 11.4 nm.

Figure 5.9  Transmission electron micrograph of Mn$_3$O$_4$ nanocrystals prepared from Mn(acac)$_2$.

Figure 5.10  Powder x-ray diffraction pattern of Mn$_3$O$_4$ nanocrystals.
In summary, manganese oxide nanocrystals can be prepared from both Mn(acac)$_2$ and Mn(acac)$_3$. Acetone, cyclohexanone and also ethanol (not reported) were used as solvents and, in addition to the acac ligands, provide potential sources of oxygen. The starting materials (with the exception of cyclohexanone) are environmentally benign and inexpensive. The method of preparation is simple, and products require negligible work-up post-reaction. This is an easy and safe route to manganese oxide nanocrystals.

5.3.3 Synthesis of Cr$_2$O$_3$ nanocrystals

Synthesis of Cr$_2$O$_3$ nanoparticles or nanocrystals has been rarely reported in the literature and is thought to be a difficult procedure.$^{31}$ There are few methods mentioned in the literature on preparation of Cr$_2$O$_3$ nanoparticles.$^{33,37,43,44}$ Chromic oxide has a wide variety of applications as green pigments,$^{30}$ catalysts,$^{32}$ and coating materials for thermal protection.$^{45}$ The lack of simple methods to prepare this useful material led us to pursue its preparation by way of Cr(acac)$_3$. 
Chromium oxide nanocrystals were prepared much in the same way as the manganese oxide nanocrystals. Cr(acac)$_3$ and acetone were combined in a Teflon-lined Parr acid-digestion bomb and heated for 3 days at 200 °C. Cr(acac)$_3$ sublimes at low temperatures (100 °C) and therefore the reactions were performed in bombs to prevent reagent escape. The product solution was green and contained a dark green precipitate; it was isolated by centrifugation followed by drying under vacuum. The product was a deep green powder (Figure 5.12).
Figure 5.13  TEM of Cr$_2$O$_3$ nanocrystals

The Cr$_2$O$_3$ nanoparticles prepared by this method are spherical, polydisperse, and amorphous as determined XRD and TEM (Figure 5.13). The green powder was annealed in a 500 °C furnace overnight and found to be crystalline Cr$_2$O$_3$ from the X-ray diffraction pattern, which is shown in Figure 5.14. After annealing the powder was a very dark green, almost black, and soluble in chloroform. Average crystal size is 18.5 nm, and was determined using the Scherrer equation.

Figure 5.14  X-ray diffraction pattern of annealed Cr$_2$O$_3$
The annealed powder was further analyzed by elemental analysis at Galbraith Laboratories to determine the percent Cr content. The method used for detection is ICP. The sample prepared in acetone solvent was found to have 64.1% chromium. Theoretically, pure Cr₂O₃ should be 68.4% chromium. Our experimental result is slightly lower than this, indicating a marginally lower ratio of chromium to oxygen in the sample. It is possible that some organic material from the precursor still remains; however, we expect it would have burned off during the annealing. While the elemental analysis is in slight disagreement with what is expected theoretically, the XRD analysis confirms that only Cr₂O₃ is present, and not the other oxides such as CrO₂, Cr₃O₄ or CrO₃.

A variety of solvents (all containing oxygen) were explored to see if product yields, shape, morphology or size distribution changed as the solvent species changed. Other solvents including benzaldehyde, cyclohexanone, ethanol and tetrahydrofuran were used in place of acetone; however, the nanocrystal shape was not as uniform. Benzaldehyde and THF did not produce Cr₂O₃, as determined by XRD, even after annealing, but cyclohexanone and ethanol did. The particles formed in THF are aggregated in the TEM and the sample was obtained in low yield. Ethanol produced micron size spherical particles, and cyclohexanone gave rise to non-uniform shaped chromic oxide. XRD analysis of the product obtained from the of the reaction of Cr(acac)₃ in benzaldehyde could not be assigned to any of the common CrₓOᵧ structures. TEM images of Cr₂O₃ prepared in other solvents are shown in Figure 5.15.
Figure 5.15  TEM images of particles synthesized with different solvents: tetrahydrofuran (A), ethanol (B), and cyclohexanone (C)

Reaction duration was varied from 2 days and 1 week and found to have little effect on the percent yield or quality of nanoparticles. In fact, heating for two days produced a higher yield of Cr$_2$O$_3$ than heating for one week. A 16% yield was obtained by heating for two days, and a 6% yield for heating seven days. No difference was noticed in the uniformity or shape of particles.

Since Cr(acac)$_3$ sublimes at low temperatures, reactions were also performed in Parr reaction bombs under air pressure at 200 psi and 160 °C for 3 days in attempt to increase reaction yield. The bomb was submersed in an oil bath to provide heat. Unfortunately the reaction yield was 1% under these conditions. The temperature may have been a factor; however, it was the maximum temperature sustainable in an oil bath.

5.4 Conclusions

A general method for the synthesis of metal oxide nanocrystals has been developed. Metal acetylacetonate precursors are environmentally friendly and can be used to prepare metal oxide nanocrystals by simple, straightforward methods using only a polar, oxygen-containing solvent and applying heat. γ-Fe$_2$O$_3$, Cr$_2$O$_3$, Mn$_2$O$_3$ and Mn$_3$O$_4$ can be prepared from their M(acac)$_x$ precursors. Maghemite, γ-Fe$_2$O$_3$, of varied solubility
can be obtained by preparing in different solvents/surfactants. These materials were characterized by TEM, XRD and elemental analysis.

5.5 References

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