Synthesis and Characterization of Superparamagnetic Silica--Homopolypeptide Composite Particles

Sibel Turksen
Louisiana State University and Agricultural and Mechanical College, sturks1@lsu.edu

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_dissertations

Part of the Chemistry Commons

Recommended Citation
https://digitalcommons.lsu.edu/gradschool_dissertations/4055

This Dissertation is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Doctoral Dissertations by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.
SYNTHESIS AND CHARACTERIZATION OF SUPERPARAMAGNETIC SILICA-HOMOPOLYPEPTIDE COMPOSITE PARTICLES

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemistry

by

Sibel Turksen
B.S., Middle East Technical University, 1998
May, 2005
Dedicated to the loving memory of my mother,

(1946-2002)
ACKNOWLEDGEMENTS

First, I would like to express my deepest gratitude to my mother for her heartfelt support and encouraging me to come back and pursue my degree even when she needed me the most. I would also like to thank my family for supporting my decision to come to the United States and trying to do everything to keep me away from the problems they have been going through.

I would like to express my sincere appreciation to a great teacher and a very supportive advisor for putting up with me throughout these troubles, long sickness leaves and for the family support he provided in time of need. His presence in the lab, day and night, whenever I needed help with the science, was the greatest input for the outcome of this dissertation. I gratefully acknowledge National Science Foundation and Coates Research Award for funding this work. I should also thank Cindy Henk from LSU Socolofsky Microscopy Center, Guangyu Li from NMR Facility, Song Guo from LSU Physics Department, and Jiechao Jiang from LSU Materials Characterization Center for their expert help with different techniques used for characterizations.

My warmest appreciation is extended to my colleagues in Dr. Russo’s research group. I would especially thank to Nadia, Randy, Garrett, Jirun, Jason, Derek, Erick, Jianhong, Grigor and Dr. Rafael, without all your help and support I wouldn’t have done it. Also, I’d like to thank all my friends, especially Selen, Yilmaz, Ozan, and Firat, for their endless support all these years. Finally, my special thanks go to Ozan Selcuk, who supported me throughout the latest and final efforts of graduation. I could not have finished without you.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS.................................................................................................................. iii

LIST OF TABLES .................................................................................................................................. vi

LIST OF FIGURES .................................................................................................................................. vii

LIST OF SYMBOLS ........................................................................................................................ xii

LIST OF ABBREVIATIONS................................................................................................................ xiv

ABSTRACT ........................................................................................................................................... xvi

CHAPTER 1 GENERAL INTRODUCTION ......................................................................................... 1
  1.1. Introduction............................................................................................................................. 1
  1.2. Dissertation Overview ......................................................................................................... 5

CHAPTER 2 BACKGROUND AND LITERATURE REVIEW ....................................................... 7
  2.1. Colloidal Silica ..................................................................................................................... 7
  2.2. Magnetism and Magnetic Particles.................................................................................... 12
    2.2.1. Superparamagnetic Iron Oxide (Magnetite) Nanoparticles ....................................... 20
    2.2.2. Superparamagnetic Cobalt Nanoparticles .................................................................. 22
  2.3. Synthetic Polypeptides......................................................................................................... 25
    2.3.1. Poly(α-Amino Acids) ..................................................................................................... 29
  2.4. Surface Modification ........................................................................................................... 39
    2.4.1. Silanization .................................................................................................................... 40
    2.4.2. Grafting with Synthetic Polypeptides ........................................................................... 45
  2.5. Cross-Linked Particles......................................................................................................... 50

CHAPTER 3 EXPERIMENTAL ........................................................................................................... 52
  3.1. Materials ............................................................................................................................... 52
  3.2. Preparation of the Superparamagnetic Silica-Homopolypeptide Particles......................... 52
    3.2.1. Synthesis of Colloidal Silica......................................................................................... 52
    3.2.2. Synthesis of Magnetic Iron Oxide Particles Coated with Silica .................................. 54
    3.2.3. Superparamagnetic Cobalt Particles Coated with Silica ............................................ 55
    3.2.4. Silanization of the Particle Core .................................................................................. 55
    3.2.5. Synthesis of Amino Acids and their Corresponding NCA Monomers ....................... 56
    3.2.6. Grafting of the Polypeptide Shell ................................................................................. 60
    3.2.7. Crosslinking of Particles ............................................................................................. 60
  3.3. Characterization Techniques............................................................................................... 61
    3.3.1. Light Scattering ............................................................................................................. 61
    3.3.2. Electron Microscopy ..................................................................................................... 62
    3.3.3. X-Ray Photoelectron Spectroscopy (XPS) ................................................................. 63
    3.3.4. Optical Rotatory Dispersion Spectroscopy (ORD) ..................................................... 63
    3.3.5. Optical Microscopy (OM) ........................................................................................... 64
3.3.6. Infrared Spectroscopy (IR) ................................................................. 64
3.3.7. Nuclear Magnetic Resonance Spectroscopy (NMR) ......................... 64
3.3.8. Thermogravimetric Analysis (TGA) ................................................... 65
3.3.9. Visible Spectroscopy ........................................................................... 65
3.3.10. Superconducting Quantum Interference Device (SQUID) .......... 65

CHAPTER 4 RESULTS AND DISCUSSION ................................................. 66
  4.1. Synthesis and Characterization of Particle Cores .............................. 66
  4.2. Characterization and Effect of Water on N-Carboxy Anhydrides ...... 79
  4.3. Synthesis and Characterization of Silica-Homopolypeptide Particles 90
  4.4. Characterization of Superparamagnetic Silica-Polypeptide Particles 97

CHAPTER 5 FINAL CONCLUSIONS AND FUTURE WORK ..................... 119

REFERENCES .............................................................................................. 124

APPENDIX A: COPYRIGHT PERMISSION ................................................. 146

APPENDIX B: DESCRIPTION OF SAMPLES ......................................... 147

VITA ............................................................................................................... 150
LIST OF TABLES

Table 2.1. Magnetic behavior and properties of different magnetic groups. ............... 16
Table 2.2 Magnetic properties of materials used for ferrofluids. ............................... 20
Table 3.1. List of materials used for the syntheses. .................................................... 53
Table 3.2. Functional groups used for the silanization reaction. ................................. 56
Table 3.3. 1H NMR data for NCAs. .............................................................................. 59
Table 4.1. Effect of EtOH, NH₂OH, and TEOS concentrations on Stöber silica particle radius. ............................................................................................................. 66
Table 4.2. Effect of citric acid concentrations on particle size of silica-coated cobalt particles from reference 221. ................................................................. 72
Table 4.3. Composition of samples from XPS. a collected via magnet, b collected by precipitation. .......................................................................................... 113
LIST OF FIGURES

Figure 1.1. Hierarchical construction of complex organized functional materials.9 ....... 2
Figure 2.1. Synthesis of Stöber silica particles (not drawn to scale; the particles are large compared to the surface layer thickness). .................................................................. 9
Figure 2.2. Schematic demonstration of silane coupling agent deposition onto colloidal silica particle. ...................................................................................................................... 11
Figure 2.3. Hysteresis plot (M vs. H) of a typical magnetic material with hysteresis... 14
Figure 2.4. Synthesis of silica-coated cobalt particles. .................................................. 24
Figure 2.5. General structure of amino acids. ............................................................... 26
Figure 2.6. Formation of a peptide bond through condensation reaction............... 27
Figure 2.7. Configuration of the peptide bond. (a) trans-peptide bond, (b) cis-peptide bond......................................................................................................................... 27
Figure 2.8. Basic structures of amino acids: (a) α-helix confirmation, (b) β-sheet confirmation, (c) random coil confirmation......................................................... 28
Figure 2.9. General structure of poly(α-amino acids). R group is (CH₂)₂CO₂CH₂C₆H₅ for PBLG, (CH₂)₂CO₂(CH₂)₁₈ for PSLG, and (CH₂)₄NHCO₂CH₂C₆H₅ for PCBL............................................................................................................ 30
Figure 2.10. General structure of N-carboxyanhydride derivatives of α-amino acids with ring numbering system. ......................................................................................... 30
Figure 2.11. The synthesis of α-amino acid NCAs with triphosgene. ....................... 33
Figure 2.12. Amine mechanism for ring opening polymerization of NCAs. ......... 34
Figure 2.13. Carbamate mechanism for ring opening polymerization of NCAs. ...... 35
Figure 2.14. Activated monomer mechanism of the polymerization of NCAs. 68;235 ....... 36
Figure 2.15. Polymerization of NCAs by zerovalent nickel complex, bpyNi(COD)...... 37
Figure 2.16. (γ-amino-propyl)triethoxysilane molecule...................................... 41
Figure 2.17. Reaction of silanes with a substrate. Methoxy group is used as a representative of the X group and a flat surface as a substrate. ......................... 42
Figure 2.18. Schematic of grafting methods: (a) Grafting onto (b) Grafting from .... 48
Figure 2.19. Crosslinking of poly(γ-dec-1-enyl-L-glutamate) with Grubbs catalyst. (a) structure of PDLG, (b) structure of Grubbs catalyst

Figure 4.1. Calculated radius (from DLS) of synthesized Stöber silica particles (ST.4.19A-E) from Table 4.1 plotted against scattering vector magnitude. The average $\mu_2/\Gamma^2$ was calculated to be 0.025 for all samples.

Figure 4.2. Negative scans of the electron micrographs of silica spheres (ST.4.19C) prepared via Stöber method. SEM images (a) 1 μm scale bar, (b) 200 nm scale bar; TEM images (c) 1 μm scale bar, (d) 0.5 μm scale bar.

Figure 4.3. Negative scans of TEM micrographs of Fe$_3$O$_4$ particles coated with silica (ST.2.40A). (a) 1 μm scale bar, (b) 0.3 μm scale bar, (c) 0.1 μm scale bar, (d) 0.1 μm scale bar.

Figure 4.4. Dynamic light scattering results of Fe$_3$O$_4$ (ST.1.103A), silica-coated Fe$_3$O$_4$ (ST.1.106A) and latex (ST.1.104A) particles. Calculated particle radius plotted against scattering vector magnitude.

Figure 4.5. Negative scans of TEM images of silica-coated cobalt particles (ST.3.46A). (a) scale bar 50 nm, (b) scale bar 100 nm, (c) scale bar 50 nm, (d) X-ray diffraction pattern of a single particle.

Figure 4.6. Comparison of magnetic properties of silica-coated cobalt particles (ST.3.93Aa) with commercial superparamagnetic latex (ST.4.17A). Both samples were dried in a vacuum oven for the measurements.

Figure 4.7. High field (300 MHz) TOCSY NMR of (a) 3-aminopropyltriethoxy silane (ST.2.41A), (b) functionalized silica (ST.2.36A) particles in DMSO-$d_6$.

Figure 4.8. FTIR spectra of (——) AEAPTM functional group (ST.4.29A), (——) bare silica particles (ST.3.59A), (——) functionalized silica (ST.3.59B) particles.

Figure 4.9. Schematic presentation of the reaction of water with NCAs.

Figure 4.10. MR labeling for (a) CBL-NCA, (b) BLG-NCA. Each letter corresponds to the carbon and proton atom at that location.

Figure 4.11. High field (500 MHz) (a) $^1$H NMR spectra, (b) $^{13}$C NMR spectra for CBL-NCA in DMF-$d_6$ (ST.3.92A). Temperature was gradually increased after the addition of water.

Figure 4.12. High field (500 MHz) (a) $^1$H NMR spectra, (b) $^{13}$C NMR spectra for BLG-NCA in DMF-$d_6$ (ST.3.131A). Temperature was gradually increased after the addition of water.

Figure 4.13. High field (500 MHz) HSQC spectra for CBL-NCA in DMF-$d_6$ (ST.3.92A) at 298K, before the addition of water.
Figure 4.14. High field (500 MHz) HMBC spectra of CBL-NCA in DMF-d\textsubscript{6} (ST.3.92A) at 298K, before the addition of water. ......................................................... 83

Figure 4.15. High field (500 MHz) HSQC spectra for CBL-NCA in DMF-d\textsubscript{6} (ST.3.92A) at 323K, after the addition of water. ............................................................ 84

Figure 4.16. High field (500 MHz) HMBC spectra of CBL-NCA in DMF-d\textsubscript{6} (ST.3.92A) at 323K, after the addition of water. ............................................................ 84

Figure 4.17. High field (500 MHz) COSY spectra of CBL-NCA in DMF-d\textsubscript{6} (ST.3.92A) at 323K, after the addition of water. ............................................................ 85

Figure 4.18. High field (500 MHz) TOCSY spectra of CBL-NCA in DMF-d\textsubscript{6} (ST.3.92A) at 323K, after the addition of water. ............................................................ 85

Figure 4.19. High field (500 MHz) HSQC spectra of BLG-NCA in DMF-d\textsubscript{6} (ST.3.131A) at 298K, before the addition of water .......................................................... 86

Figure 4.20. High field (500 MHz) HSQC spectra of BLG-NCA in DMF-d\textsubscript{6} (ST.3.131A) at 298K, before the addition of water .......................................................... 86

Figure 4.21. High field (500 MHz) HSQC spectra of BLG-NCA in DMF-d\textsubscript{6} (ST.3.131A) at 323K, after the addition of water. ............................................................ 87

Figure 4.22. High field (500 MHz) HMBC spectra of BLG-NCA in DMF-d\textsubscript{6} (ST.3.131A) at 323K, after the addition of water. ............................................................ 87

Figure 4.23. High field (500 MHz) COSY spectra of BLG-NCA in DMF-d\textsubscript{6} (ST.3.131A) at 323K, after the addition of water. ............................................................ 88

Figure 4.24. High field (500 MHz) TOCSY spectra of BLG-NCA in DMF-d\textsubscript{6} (ST.3.131A) at 323K, after the addition of water. ............................................................ 88

Figure 4.25. FTIR spectra of CBL-NCA in anhydrous DMF (ST.3.92A). (—) at 0°C no water added and (—) at 15°C, (—) at 25°C, (—) at 35°C, (—) at 50°C with water............................................................................................................. 89

Figure 4.26. SEM picture of PCBL-coated silica particles (ST.2.7A). ........................................... 91

Figure 4.27. Calculated radius of PCBL-coated silica particles (ST.2.7A) plotted at different scattering vectors. Inset is $\Gamma$ vs. scattering vector plot......................... 92

Figure 4.28. Photomicrographs of colloidal crystals of PCBL-coated silica particles (ST.1.128), between crossed polarizers. (a) and (c) variety of colors due to different domain orientations; (b) and(d) detail of bands due to crystal twinning. ...................................................................................................... 93
Figure 4.29. Transmitted light intensity vs. wavelength for PCBL-grafted silica composite particles (ST.3.128A). Imaged region includes 3 domains. ...... 94

Figure 4.30. Silica core (ST.4.19A) and PCBL-coated silica particles dispersed in anhydrous pyridine with increasing monomer concentrations (ST.4.25A-E). (a) Monitoring of particle shell thickness via controlled addition of the monomer. Radius was calculated from DLS plotted at different monomer concentrations. Inset is $\mu_2/I^2$ vs. [M] plot, each measurement were carried at different angles. (b) Radius vs. scattering vector of particles measured at different monomer concentrations. .............................................................. 95

Figure 4.31. PCBL coated silica particles dispersed in anhydrous pyridine with increasing monomer concentrations (ST.25A-E). The particles radius of gyration were measured at different scattering angles (30°-80° and 90°) after 2 months. ....................................................................................................... 96

Figure 4.32. Thermogravimetric analysis of the Fe$_3$O$_4$ magnetic silica particles (ST.1.109A) with PCBL shell. (—) weight % vs. temperature, (—) derivative weight vs. temperature. ................................................................................................................. 98

Figure 4.33. Thermogravimetric analysis of magnetic cobalt-silica particles coated with PCBL (ST.3.94B). (—) weight % vs. temperature, (—) derivative weight vs. temperature ................................................................................................................. 100

Figure 4.34. DLS results of PCBL-coated cobalt particles (ST.3.47A) in m-cresol. (a) Temperature dependence of hydrodynamic radius of PCBL particles. (b) $m^2/G^2$ vs. temperature plot, each point is average of all heating and cooling cycles form a, (c) Radius of the particles measured at three 15°C, 25°C, and 50°C plotted at different scattering angles ................................................................................................................. 102

Figure 4.35. ORD calibration curves for (a) aqueous sucrose (ST.4.27A-D), (b) PBLG-110K MW (ST.4.28A-C) in DMF, measured at 546 nm. ................. 105

Figure 4.36. Temperature dependence of the optical rotation of Co-PCBL (ST.3.47A) in m-cresol measured at different wavelengths. ......................... 106

Figure 4.37. The infrared spectra of Co-PCBL particles in m-cresol (ST.3.47A) at different temperatures. (a) Amide I-II region (b) Amide A region. Dashed line is drawn as a reference to eye. ......................................................... 108

Figure 4.38. X-ray photoelectron survey spectra of (a) silica particles (ST.3.59A) (b) amino functionalized silica particles (ST.3.59B) (c) Co-PCBL particles collected via magnet (ST.94Bb) (d) Co-PCBL particles collected via precipitation (ST.3.94Ba) ................................................................................................................. 110

Figure 4.39. The XPS detail scans corresponding to (a) Si 2p (b) C 1s (c) N 1s (d) O 1s; (—)Silica particles (ST.3.59A), (—) Functionalized silica particles
(ST.3.59B), (→) Co-PCBL collected via magnet (ST.3.94Bb), (→) Co-PCBL collected via precipitation (ST.3.94Ba).......................................................... 112

Figure 4.40. Magnetic hysteresis curve for PCBL-coated cobalt particles (ST.3.94B) at 300K. Sample was dried in vacuum oven................................................. 114

Figure 4.41. M/H vs. H plots for (a) Silica-coated cobalt core particles (ST.3.93Aa) and (b) PCBL-coated cobalt core-shell particles (ST.3.94B) dispersed in m-cresol.......................................................................................................... 115

Figure 4.42. Magnetization vs. temperature plots of PCBL-grafted cobalt particles (ST.3.94B) dispersed in m-cresol (a) all of the applied fields are plotted, (b) only selected fields are shown .......................................................... 117

Figure 4.43. Magnetization vs. temperature plots of silica-coated cobalt particles (ST.3.93Aa) dispersed in m-cresol (a) all of the applied fields are plotted, (b) only selected fields are shown.......................................................... 118

Figure 5.1. Proposed vapor deposition polymerization to produce flagella-like core-shell composite particles..................................................................................... 122
LIST OF SYMBOLS

\[ B \] Magnetic induction, flux
\[ C \] Curie temperature
\[ c \] Concentration
\[ D_{\text{app}} \] Apparent diffusion coefficient
\[ D_0 \] Diffusion coefficient
\[ g^{(1)}(t) \] Normalized electric field correlation function
\[ H \] Applied magnetic field
\[ H_c \] Coercive field
\[ k \] Boltzmann constant
\[ n \] Refractive index
\[ M \] Magnetization
\[ M_s \] Saturation magnetization
\[ M_{\text{rem}} \] Remanent magnetization
\[ m \] Magnetic moment
\[ q \] Scattering vector magnitude
\[ R_h \] Hydrodynamic radius
\[ T \] Absolute temperature
\[ V \] Volume
\[ \Gamma \] Decay rate of \( g^{(1)}(t) \)
\[ \mu \] Magnetic permeability
\[ \mu_0 \] Permeability of free space
\( \eta_0 \)  Solvent viscosity

\( \lambda_0 \)  In vacuo wavelength

\( \theta \)  Scattering angle
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEAPTMS</td>
<td>3-aminopropyl-aminoethyl triethoxysilane</td>
</tr>
<tr>
<td>APDMS</td>
<td>(3-aminopropyl)dimethylethoxysilane</td>
</tr>
<tr>
<td>APTES</td>
<td>(γ-amino-propyl)triethoxysilane</td>
</tr>
<tr>
<td>APTMS</td>
<td>(γ-amino-propyl)trimethoxysilane</td>
</tr>
<tr>
<td>BLG</td>
<td>Benzyl-L-glutamate</td>
</tr>
<tr>
<td>CBL</td>
<td>Carbobenzyloxy-L-lysine</td>
</tr>
<tr>
<td>CRA</td>
<td>Chemical reaction alignment</td>
</tr>
<tr>
<td>DCA</td>
<td>Dichloroacetic acid</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DCE</td>
<td>Dichloroethylene</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethyl alcohol</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transformation infra-red spectroscopy</td>
</tr>
<tr>
<td>MTMS</td>
<td>Methyltrimethoxysilane</td>
</tr>
<tr>
<td>NCA</td>
<td>N-carboxy anhydrides</td>
</tr>
<tr>
<td>NEXAFS</td>
<td>Near-edge X-ray absorption fine structure spectroscopy</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>ORD</td>
<td>Optical rotatory dispersion spectroscopy</td>
</tr>
<tr>
<td>PBLA</td>
<td>Poly(β-benzyl-L-aspartate)</td>
</tr>
<tr>
<td>PBLG</td>
<td>Poly(γ-benzyl-L-glutamate)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PCBL</td>
<td>Poly(ε-carbobenzyloxy-L-lysine)</td>
</tr>
<tr>
<td>PGA</td>
<td>Poly(glutamic acid)</td>
</tr>
<tr>
<td>PMLG</td>
<td>Poly(γ-methyl-L-glutamate)</td>
</tr>
<tr>
<td>PSLG</td>
<td>Poly(γ-stearyl-L-glutamate)</td>
</tr>
<tr>
<td>RAIR</td>
<td>Reflection absorption infrared spectroscopy</td>
</tr>
<tr>
<td>SAM</td>
<td>Self-assembled monolayers</td>
</tr>
<tr>
<td>SAXS</td>
<td>Small angle X-ray scattering</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SPDP</td>
<td>N-hydroxy succinimide ester of 3-(2-pyridylthio)propionic acid</td>
</tr>
<tr>
<td>SQUID</td>
<td>Superconducting quantum interference device</td>
</tr>
<tr>
<td>TCF</td>
<td>Trichloromethyl chloroformate</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TEOS</td>
<td>Tetraethylorthosilicate</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMA</td>
<td>Tetramethylammonium hydroxide</td>
</tr>
<tr>
<td>TNBSA</td>
<td>Trinitrobenzene sulfonic acid</td>
</tr>
<tr>
<td>VDP</td>
<td>Vapor deposition polymerization</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
</tbody>
</table>
ABSTRACT

Hierarchical construction and characterization of core-shell composite particles of interest are presented. The research described shows interest in new types of polymers that provide chirality and responsiveness which have been ignored for so long. The general synthetic design described in this document can be readily used as a model for other systems.

Core-shell composite systems of interest consist of silica or silica-coated cobalt cores grafted with a homopolypeptide shell, mostly poly(ε-carboxybenzyloxy-L-lysine) or poly(γ-benzyl-L-glutamate). Core particles were decorated with amino groups through a silylation reaction. The amino groups initiated the polymerization (with attachment) of N-carboxyanhydride (NCA) monomers, resulting in a homopolypeptide shell.

Homopolypeptide-grafted particles were considered as hierarchical systems due to the two levels of responsiveness. The first level of responsiveness is due to the superparamagnetic nature of the core and the second level of responsiveness is through the thermally responsive polypeptide shell. Both levels of response were confirmed with several characterization methods.

Characterization by electron microscopy and dynamic light scattering (DLS) confirmed the uniformity of the particles. Magnetic properties of the silica-coated cobalt core and polypeptide-grafted particles were investigated with a superconducting quantum interference device (SQUID). DLS and digital polarimeter demonstrated helix-coil transitions of the particles through continuous heating and cooling cycles in m-cresol.
A general approach to chemistry is the connection of atoms and molecules into functional components. With time, the rules of connection have been redefined and reconstructed to extend the generation and connection of larger objects, with dimensions of nanometers. Throughout this process, self-assembly, molecular structures, and interactions within the newly formed structures played a significant role. In the late 90s researchers from different fields used larger units to construct structures instead of using smaller atoms and molecules. This brought the concept of integrated chemical systems of colloidal structures. Core-shell particles are significant examples of such structures. They consist of a defined core mostly coated with a polymeric shell. Silica\(^1,2\) is among the most popular colloids used as a core in such particles. The core can be a metal such as gold\(^3-5\) or silver\(^6-8\) as well as another polymeric gel or aggregate.

Figure 1.1 summarizes the concept of hierarchical construction of complex, organized functional materials as they would form in nature.\(^9\) The integration of nucleons to atoms then to molecules is followed by the functionalization of molecules to form more complex structures or systems. The first level of hierarchy, system 1, can be followed by series of further steps which results in higher complexity. Functionality can be chemical, based on the number and arrangement of functional groups or physical, based on the anisotropy of shape or electronic and magnetic properties. Particles with
structural hierarchy can be generated through the appropriate arrangement of functional groups.

Scientists organize and connect functionalized building blocks, often polymers with colloids. Uniform colloidal particles play a crucial role in various fundamental
research areas. Nanoparticles,\(^9\) colloid-polymer interactions,\(^{10-17}\) probe diffusion in polymer solutions and gels,\(^{18-27}\) particle stability,\(^{28-30}\) interparticle interactions,\(^{11;31-33}\) surface properties,\(^{34-39}\) and characterization\(^{31;37;40-44}\) of such particles are some of the challenging areas under active investigation. There is an increasing demand for functional colloidal particles, especially in materials science and biomedical applications.\(^{45}\) The need for controlled drug release, artificial muscles and organs, biosensors, electromagnetic devices, and photonic gap materials, not to mention applications such as tunable filters or optical limiting devices and magnetic separations adds impetus to the design and production of functional particles. Such particles can greatly improve the understanding of fundamental issues. Better model particles will be needed to answer important questions, such as the transition from amorphous-to-crystalline state or diffusion in complex fluids.

It is clear that there is a huge enterprise with core-shell particles. The research described within this dissertation shows interest in new type of polymers that provide chirality and responsiveness which has been ignored for so long. While many conventional polymers\(^{17;46-48}\) have been grafted to colloidal particles, it is surprising that so little is known about polypeptide-coated particles, especially since they offer several desirable features. In relatively recent years, polypeptides have been attached to flat surfaces\(^{49-63}\) but, not often attached to colloidal particles. Dietz \textit{et al.} used fumed silica and Tsubokawa \textit{et al.}\(^{64}\) used carbon black to attach the homopolypeptides.

Polypeptides have a lot to offer as colloidal coatings. The chiral nature of the polypeptide chains might be used for the analysis and separation of biomolecules.\(^{65;66}\) The chemistry for synthesizing polypeptides typically results in controllable and narrow
size distributions. This suggests that colloidal particles with well-defined shells can be produced. In turn, this provides an opportunity to separate the cores in a precisely controlled fashion, of great interest for certain optical materials. Finally, polypeptides do not have to be rigid helices; through the helix-to-coil conformational transition, particles with a dynamic, responsive character can be produced. Nature often chooses polypeptides for similar purposes. In the \(\alpha\)-helical conformation, they are extended, stiff rods. Little is known about the behavior of rod-coated colloids, and the ability to switch between helix to coil opens new application areas.

One of the most interesting functionalities applied to colloidal particles is magnetism. Polymer latex particles with superparamagnetic inclusions were investigated to understand the behavior of magnetic particles. Superparamagnets do not possess a permanent, fixed dipole. Rather, the magnetic director of such very small particles (<10 nm) rotates randomly. This keeps the particles from interacting with one another magnetically. If a magnetic field of sufficient strength is applied, the inclusions do acquire a well-defined magnetic moment. Suddenly, they begin to feel the forces of neighboring particles. The result is “chaining” to produce strings of particles that are easily attracted to a magnet. Chiral-coated magnetic particles could be used to separate enantiomeric materials. Gast et al. have shown that aligned chains can be crosslinked. If this were performed on chains of polypeptide-coated particles, the helix-coil transition might render the entire chain responsive. One can envision a poly(colloidal) “muscle filament” for example.

Another fascinating, self-assembled motif for colloid/polymer composites—and one that is currently attracting intense interest—is colloidal crystalline arrays. Colloidal
crystals can be used for the development of photonic band gap devices\textsuperscript{94} and other optical applications like tunable filters.\textsuperscript{95,96} Composite silica-polypeptide particles provide an alternative to conventional polyelectrolyte latex particles and their sensitivity to salt. The adjustable secondary structure may provide a convenient means to "tune" the interparticle distance and jostle the particles, resulting in larger homogeneous regimes, or mono domains. Reduction of defects, such as domain boundaries, is an important objective for self-assembling systems. Introducing the magnetic core may further enhance this effect by providing another experimental handle by which the colloidal crystalline domain size, now rather small, can be enlarged.

Perhaps, the most prominent property of the core-shell particles introduced in this chapter is their responsiveness. They respond to an applied magnetic field because the particle core is superparamagnetic, while at the same time the secondary structure of the homopolypeptide shell can change thermally. Such transitions can be used to control the stability in polymer-colloid mixtures or to create responsive systems.

1.2. Dissertation Overview

The purpose of this dissertation research is to develop a new set of core-shell particles which offers new functionalities that have been avoided for so long. For the colloidal systems established in this research, superparamagnetic cobalt particles coated with silica are used as the core, whereas poly($\varepsilon$-carbobenzyloxy-$L$-lysine), PCBL, is used as the general polypeptide shell. This is an enabling technique, and pioneered particles are offering chirality, responsiveness, biocompatibility, and targeting. In several applications, the characteristics supplied will bring advantage over existing model core-shell particles.
This dissertation investigates the synthesis and characterization of such particles. Chapter 2 includes a detailed literature review on colloidal particles and polymers used at each step of the proposed composite systems and gives background on the theories beneath such systems. Chapter 3 describes the synthetic methods used for the particle preparation as well as the experimental techniques used for their characterization. Chapter 4 demonstrates the experimental results. This chapter supports the expected functionalities which the proposed composite system offers, such as responsiveness, chirality and colloidal crystal nature of the particles. Chapter 5 gives an overall look at the results, introduces some preliminary results of the new approaches, and suggests future work that can be done with such systems.
2.1. Colloidal Silica

Application of colloids goes back to the earliest records of civilization. Many of the earliest technological processes such as making paper, pottery, soaps, and cosmetics involve colloidal systems. With the new technological improvements, search for well defined materials increased. Colloidal silica or silica sol is one of those materials. The term colloidal silica refers to stable dispersions or sols of discrete particles of amorphous silica particles.

Colloidal silica has the same chemical formula (SiO$_2$) as quartz sand; the key difference is size. The subunits of colloidal silica particles are typically in the range of 1 to 5 nm. Whether or not these subunits are joined together depends on the conditions of polymerization. Initial acidification of a water-glass (sodium silicate) solution yields Si(OH)$_4$. If the pH is reduced below 7 or if salt is added, the units tend to fuse together in chains. These products are often called "silica gels." If the pH is kept slightly on the alkaline side of neutral, the subunits stay separated, and they gradually grow. These products are often called silica sols.

The quality of the silica sols depends on the monodispersity and the stability of the particles. Stable concentrated silica sols that do not gel or precipitate for a long time became available in early 1940’s. Vail in 1925, and Treadwell and Wieland in 1930, prepared sols that occasionally contained more than 10% silica particles which were not stable to gelling effect. In 1933, Griessbach reported 10% sol that was stabilized with
ammonia. Later in 1941 Bird et al. patented preparation of 5-10 nm sized particles through the process of removing alkali from dilute sodium silicate by hydrogen ion-exchange resin, followed by stabilizing with dilute alkali and concentrating the solution. Four years later, White et al. patented the process of peptizing the gel to a sol with dispersing in a basic solution and heating. The first silica particles with uniform and controlled size were prepared by in 1951 Bechtold et al. Rule et al. optimized the concentrations of alkali used for stabilization in the previous method. Iler summarized this progress in the first edition of his book. Therefore, the early studies included the production of stable concentrated silica solutions. Since then the synthesis of silica particles has been extensively studied and several synthetic routes have been developed.

The most common and effective method was developed by Stöber. It consists of a sol-gel process in which silica (tetraethylorthosilicate, TEOS) hydrolyses to form Si-O chains, and its condensation leads to amorphous silica nanoparticles (Figure 2.1). Under controlled reaction conditions, such as temperature and reactant concentration monodisperse silica particles with diameters in the range of tenths of a micron to microns can be produced. Stöber synthesis is not only used to prepare silica particles; but finds very broad applications in grafting other nanoparticles and in core shell particle preparations.

Monodispersity and size control of these particles are essential for some technologically interesting applications. Occasionally the Stöber method yields nonspherical particles and in order to achieve different sizes other alkyl silicates are required. Tan et al. demonstrated preparation of uniform silica spheres in a size range of 0.2-20 µm using Stöber method by just varying the temperature. It is also known that
particle size can be controlled simply by varying the initial concentrations of water, ammonia, alcohol and the alkyl silicate.\textsuperscript{107,108}

\[
\begin{align*}
\text{H}_3\text{C}_2\text{O} & \quad \text{OC}_2\text{H}_5 \\
\text{OC}_2\text{H}_5 & \quad \text{Si} \\
\text{OC}_2\text{H}_5 & \quad \text{OC}_2\text{H}_5
\end{align*}
\]

\[
\begin{align*}
\text{C}_2\text{H}_5\text{OH} & \quad \text{NH}_4\text{OH} \\
\text{H}_3\text{C}_2\text{O} & \quad \text{Si} \\
\text{Si} & \quad \text{O} \\
\text{O} & \quad \text{Si} \\
\text{Si} & \quad \text{O} \\
\text{O} & \quad \text{Si} \\
\text{H}_5\text{C}_2\text{O} & \quad \text{O}
\end{align*}
\]

TEOS

\[
\begin{align*}
\text{C}_2\text{H}_5\text{OH} & \quad \text{NH}_4\text{OH} \\
\text{H}_3\text{C}_2\text{O} & \quad \text{Si} \\
\text{Si} & \quad \text{O} \\
\text{O} & \quad \text{Si} \\
\text{Si} & \quad \text{O} \\
\text{O} & \quad \text{Si} \\
\text{H}_5\text{C}_2\text{O} & \quad \text{O}
\end{align*}
\]

Some investigators claimed spherical silica nanoparticles with a narrower particle size distribution can be prepared by using microemulsion techniques.\textsuperscript{41,109} Another technique to control particle size is seed polymerization. Since additional TEOS will only increase the volume of the particles, the particle size can be controlled precisely. Coenen \textit{et al}.\textsuperscript{110} took advantage of this fact and used Ludox\textsuperscript{®} silica particles as seed and further grew silica on them. Jelinek \textit{et al}.\textsuperscript{111} used ludox particles to further study the Stöber Reaction by constant flow of TEOS into the solution, and Okubo \textit{et al}.\textsuperscript{112} used them for kinetic analysis for the seed polymerization of TEOS. Phlipse \textit{et al}.\textsuperscript{113} used sodium silicate as seed particles instead of ludox. They precipitated small seed particles by lowering the solubility of silica by diluting aqueous sodium silicate solution with
excess ethanol. The particles were further grown by Stöber Synthesis, and their sizes and polydispersity were compared. The increasing interest in smaller sized silica particles introduced the use of strong ultrasonic applications during the particle preparation. Application of high intensity ultrasonication is shown to cause aggregation and agglomeration of the particles.\(^{114;115}\)

Stöber silica particles have been used as model colloids in a large number of experimental investigations. Several scientists studied the mechanism of formation and growth,\(^{108;116}\) kinetics,\(^{40;117}\) sedimentation,\(^{31}\) and aggregation\(^{118}\) of these particles by using various techniques, including: nuclear magnetic resonance (NMR), conductimetry, Raman scattering, dynamic light scattering (DLS),\(^{119;120}\) electron microscopy (EM), and small angle X-ray scattering (SAXS). Flocculation effect on latex particles were investigated to improve the understanding of silica particle formation.\(^{14;15;46;47;121;122}\) Labeling of particles was first used by Iler\(^{123}\) and then by Sears\(^{44}\) to determine the specific surface area of the particles. Later in the late 90’s particles were labeled for application of fluorescent techniques such as microscopy and fluorescent photobleaching recovery.\(^{10;124-126}\)

Van Helden et al.\(^ {107}\) used Iler’s\(^ {123}\) technique to prepare organophilic silica particles by coating them with stearyl alcohol. This opened up the path for core-shell particles which became a very popular research area. Even the Stöber silica particles themselves are shown to have core-shell nature.\(^ {127-129}\) In 1988 Philipse et al.\(^ {130}\) chemically modified silica particles with 3-methacryloxypropyltrimethoxysilane. Application of silane coupling agents is very popular with flat surfaces such as silicone wafers. Philipse was one of the first scientists to apply them onto spherical particles.
Figure 2.2 demonstrates the deposition of silane coupling agent onto a spherical silica particle. First the alkoxy groups on the silane coupling agent hydrolyze in the presence of the base. Freshly formed silane triols associate and condense to oligomers. These oligomers are adsorbed rapidly on the silica particles and form siloxane linkages through condensation.

Figure 2.2. Schematic demonstration of silane coupling agent deposition onto colloidal silica particle.

Colloidal silica particles have a broad range of applications. Even though it is desired to perfect the spherical geometry of the particles, their shape is not limited to
spherical. They find applications as high temperature binders, paper whiteners, catalysts, abrasion resistant coatings, textile fibers, antisoiling for microscopes, surfactants, and tunable crystals. The crystals formed by colloidal silica can find application as optical devices.

2.2. Magnetism and Magnetic Particles

The oldest legend on magnetism is about a shepherd by the name of Magnés. While herding his sheep on Mount Ida, his metal-tipped stick and the nails of his boot were attracted to the ground. When he dug the ground to find the source, he found stones that are now referred to as lodestones, which contain magnetite (Fe₃O₄). The actual discovery of the properties of these stones was either by the Greeks or Chinese. Around 1000 BC, the Chinese found a needle that pointed north and south when freely suspended. Soon the magnetic compass spread to Europe and was used by Columbus.

Until Hans Christian Oersted demonstrated in 1821 that the flow of an electric current moves a nearby compass needle, only one kind of magnetism was known. Later this phenomenon was studied by Andre-Marie Ampere in France, followed by James Clerk Maxwell. Maxwell established beyond doubt the inter-relationships between electricity and magnetism and published a series of simple equations that are the basis of electromagnetic theory today.

There are several important parameters associated with magnetism, such as magnetic flux density or magnetic induction $B$, applied magnetic field $H$, and magnetization $M$. In Gaussian units the different fields are related by

$$B = H + 4\pi M$$
The unit for \( B \) is gauss (G) and oersted (Oe) for \( H \). If volume magnetization is used, which is the magnetic moment \( m \) per volume \( V \), the unit is emu/cm\(^3\). Magnetization can also be reported in units of emu/g which is the magnetic moment per mass of sample in grams.

Two other parameters frequently used in magnetization are the magnetic susceptibility and the permeability. Magnetic susceptibility, \( \chi \), is the response of the material to an applied magnetic field. It is defined as the ratio of magnetization to the applied field.

\[
\chi = \frac{M}{H}
\]

Magnetic permeability, \( \mu \) refers to a material's ability to absorb magnetic flux. It is simply the proportionality between magnetic induction and magnetic field. Once the susceptibility is expressed in terms of magnetic induction it can also be expressed in terms of permeability.

\[
B = \mu_0(1 + \chi)H
\]

Permeability can be defined in terms of:

\[
\mu = 1 + \chi
\]

Therefore, magnetic induction can be reorganized to

\[
B = \mu_0\mu H
\]

where, \( \mu_0 \) is permeability of free space and \( \mu \) is magnetic permeability. All the above parameters and their interactions with each other are important for the determination of magnetization. Mostly it is desirable to have materials with large susceptibility and permeability as an indication of a strong response to the external magnetic field.
Magnetic hysteresis is a property of magnetic materials where the magnetic induction for a given magnetic field depends on its previous history. It is the curve showing the variation of intensity of magnetization with applied magnetic field. Figure 2.3 is a typical hysteresis plot for magnetic materials having hysteresis.93

![Hysteresis Plot](image)

Figure 2.3. Hysteresis plot (M vs. H) of a typical magnetic material with hysteresis.

The dotted line represents the initial magnetization curve which is also called the virgin curve. As the magnetic field is applied, magnetization reaches a maximum at point a. At this point, magnetic domains are aligned, the material is saturated, and it is called “saturation magnetization”, $M_s$. As $H$ is reduced back to zero from saturation, the curve moves from point “a” to “b”. The value of magnetization when $H$ is returned to zero is called the “remanent magnetization”, $M_{\text{rem}}$. Applying a sufficiently large magnetic field in the opposite direction can return the magnetization to zero. At point c, the magnetic
field required to return the magnetization to zero is called the “coercive field”, \( H_c \). As the magnetizing force is increased in the negative direction, the material will again become magnetically saturated but in the opposite direction (point d). Reducing \( H \) to zero brings the curve to point e. It will have a level of residual magnetism equal to that achieved in the other direction. Increasing \( H \) back in the positive direction will return magnetization to zero. The curve can not return to the origin of the graph because some force is required to remove the residual magnetism. The curve takes a different path from point f back to the saturation point, where it completes the loop. For superparamagnetic particles, this plot shows no remanence, which means the curves overlap and appear to be a single curve that has zero remanent magnetization.

Materials may be classified according to some of their basic magnetic properties, particularly whether or not they are magnetic and how they behave in the vicinity of an external magnetic field. Table 2.1 summarizes different types of magnetic materials and magnetic behavior associated with them.\(^{93;143;144}\)

In diamagnetism, all electron spins of the particles are paired. As a whole they do not have magnetic moments because electron pairs are in opposite directions and cancel each other. The induced magnetic moment is very small and in a direction opposite to that of the applied field. When placed in a magnetic field, they are weakly repelled, resulting in an antiparallel alignment of the atomic magnetic moment. This accounts for the weak, negative magnetic susceptibility. Diamagnetism is fundamental to all chemical compounds and contributes a small negative component (\( \sim 10^{-6} \text{--} 10^{-7} \text{ emu/g} \)) to the magnetic susceptibility. Because it is so weak, it can only be observed in materials that do not exhibit other form of magnetism. The other types of magnetism on the other hand
show net magnetic moments since the magnetic moment cancellation is only partial. If $M$ vs. $H$ is plotted (referred as $M(H)$ plot or hysteresis curve), the curve is linear and reversible with negative slope for a typical diamagnetic material. This shows the negative susceptibility for diamagnetic materials.

Table 2.1. Magnetic behavior and properties of different magnetic groups.

<table>
<thead>
<tr>
<th>Magnetism</th>
<th>Magnetic Susceptibility</th>
<th>Critical temperature</th>
<th>Atomic behavior</th>
<th>Magnetic behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamagnetism</td>
<td>Small &amp; negative</td>
<td>None</td>
<td></td>
<td><img src="M.png" alt="Graph" /></td>
</tr>
<tr>
<td>Paramagnetism</td>
<td>Small &amp; positive</td>
<td>None</td>
<td></td>
<td><img src="M.png" alt="Graph" /></td>
</tr>
<tr>
<td>Ferromagnetism</td>
<td>Large &amp; positive</td>
<td>Curie</td>
<td></td>
<td><img src="M.png" alt="Graph" /></td>
</tr>
<tr>
<td>Anti-ferromagnetism</td>
<td>Small &amp; positive</td>
<td>Néel</td>
<td></td>
<td><img src="M.png" alt="Graph" /></td>
</tr>
<tr>
<td>Ferrimagnetism</td>
<td>Large &amp; positive</td>
<td>Curie</td>
<td></td>
<td><img src="M.png" alt="Graph" /></td>
</tr>
<tr>
<td>Super-paramagnetism</td>
<td>Large &amp; positive</td>
<td>Curie</td>
<td></td>
<td><img src="M.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

Paramagnetism is probably the simplest type of magnetic behavior. For paramagnetic materials the $M(H)$ plot is linear, with a zero intercept and it is reversible. Paramagnetic materials’ moments point in random directions at any temperature in the absence of an external magnetic field. They have the same number of uncompensated
spins and a permanent net spin magnetic moment which is stronger than the orbital moment. When a magnetic field is applied, these uncompensated spin moments tend to align parallel to the applied field. Conversely, the thermal motion prevents perfect alignment. Therefore, the degree of alignment depends on the strength of the field and the temperature. In other words, when a paramagnetic material is placed in a strong magnetic field, it becomes a magnet as long as the strong magnetic field is present. But, when the strong magnetic field is removed, the net magnetic alignment is lost as the dipoles relax back to their normal random motion. The value of susceptibility for paramagnetic materials is always positive at room temperature. The linear temperature dependence of the magnetic susceptibility in paramagnetic materials was worked out by Pierre Curie and is known as Curie’s Law:

\[ \chi = C \frac{B}{TH} \]

where \( M \) is magnetization, \( C \) is the Curie constant, \( B \) is magnetic induction, and \( T \) is temperature. This law is only applicable to paramagnetic materials.

Ferromagnetism occurs when the individual spin magnetic moments are aligned along the magnetic field. As with paramagnets, ferromagnets have a strong, positive magnetic susceptibility. Unlike paramagnets, when the applied field is removed, they retain a component of magnetization in the direction of the applied field - they are "permanently" magnetized. The key characteristic for the hard ferromagnets is the nonreversible \( M(H) \) plot, which is called magnetic hysteresis. They follow the Curie Law above the transition temperature and show some temperature dependence. Because ferromagnetism results from the interaction of atomic moments in materials, there is an energy exchange associated with coupling the spin moments. At room temperature, this
exchange energy is much less than the energy due to randomizing thermal effects ($kT$). If thermal energy exceeds the spin coupling energy, the coupling breaks down and the material behaves as a paramagnet. This temperature is dependent on the material and is called the Curie temperature.

The exchange coupling of spins in ferromagnets does not always trigger all spins to be aligned in the same direction. Therefore, three special cases can be considered within ferromagnets: antiferromagnetism, ferrimagnetism, and superparamagnetism. In antiferromagnetic materials, uncompensated spins are coupled antiparallel to one another. Although it is expected of them to give no net magnetization, this is not always the case. They may have a net magnetization due to spin canting, lattice defects, and, in nanoscale particles, frustrated surface spins. In ferromagnetic materials, neighboring spin lattices are parallel but of unequal magnitude. This gives rise to a relatively strong net magnetization compared to antiferromagnets. Superparamagnetic materials are an unusual case. They contain uncompensated spins and may show ferro-, ferri-, or antiferromagnetic properties depending on the thermal conditions. The difference between superparamagnetic materials and the others is that they exhibit paramagnetic behavior too. Normally, coupling forces in magnetic materials cause the magnetic moments of neighboring atoms to align, resulting in very large internal magnetic fields. At temperatures above the Curie temperature of ferromagnets, the thermal energy is sufficient to overcome the coupling forces, causing the atomic magnetic moments to fluctuate randomly. Because there is no longer any magnetic order, the internal magnetic field no longer exists and the material exhibits paramagnetic behavior.

Superparamagnetism occurs when the material is composed of very small
particles (1-10nm in diameter). The magnetic particles are so small that each particle is a single domain and subject to Brownian motion. In this case when the temperature is below the Curie temperature of ferromagnet, all the spins are coupled together and yield a large total moment. This moment is dependent on the volume of the particles. With decreasing the particle size, this moment, and therefore its coupling forces, decreases until the thermal energy can disrupt the bonding of the total moment to the particle. This moment is then free to move and respond to an applied field independent of the particle. An applied field would tend to align this moment, but thermal energy would fight the alignment just as it does in a paramagnet. That is why they align once the magnetic field is applied and show no signs of magnetization as the field is removed.

Stable colloidal suspensions of fine magnetic particles in appropriate carrier fluids are called magnetic fluids or ferrofluids. The earliest research on ferrofluids was carried out by NASA and they first became available in late 1960’s. Ferrofluids are extensively used in rotating shaft seals, printers, measuring devices, and acoustic devices, and they are also used as contrast agents for NMR, cell separators, drug carriers, and treatment for retinal detachment. Several magnetic materials have been prepared but only a few of them provided stable magnetic fluids. Table 2.2 shows the magnetic materials used as ferrofluids. Materials with higher saturation magnetization, \( M_s \), tend to oxidize rapidly compared to magnetite and ferrites. Therefore, these materials, mostly transition metals, are coated to avoid oxidation. A good magnetic fluid is a concentrated, stable suspension of very small magnetic particles. Even with nonmagnetic particles it is not easy to prepare concentrated stable suspensions. The fact that the particles are magnetic makes it more difficult to maintain colloidal stability. Presence of
permanent aggregates is one of the serious problems in magnetic fluids. Preparation and work up of these particles are crucial for the removal of aggregates and the stability of the sol. Properties and preparation techniques of magnetite $\text{Fe}_3\text{O}_4$ and cobalt particles are further discussed in sections 2.2.1 and 2.2.2 respectively.

Table 2.2  Magnetic properties of materials used for ferrofluids.

<table>
<thead>
<tr>
<th>Materials</th>
<th>$M_s$ (Tesla)</th>
<th>$\Delta G$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\gamma$-$\text{Fe}_2\text{O}_3$</td>
<td>0.52</td>
<td>0</td>
</tr>
<tr>
<td>Ferrites</td>
<td>0-0.53</td>
<td>N/A</td>
</tr>
<tr>
<td>$\text{Fe}_3\text{O}_4$</td>
<td>0.6</td>
<td>-97.2</td>
</tr>
<tr>
<td>Ni</td>
<td>0.61</td>
<td>-216.3</td>
</tr>
<tr>
<td>Co</td>
<td>1.79</td>
<td>-213.4</td>
</tr>
<tr>
<td>Fe</td>
<td>2.15</td>
<td>-244.3</td>
</tr>
<tr>
<td>FeCo</td>
<td>2.4</td>
<td>N/A</td>
</tr>
</tbody>
</table>

2.2.1. Superparamagnetic Iron Oxide (Magnetite) Nanoparticles

Ceramic-like ferromagnetic materials, which are mainly composed of $\alpha$-$\text{Fe}_2\text{O}_3$, are called ferrites. Magnetite, $\text{Fe}_3\text{O}_4$, is a natural mineral from the ferrite family. It is one of the first recognized magnetic materials, as explained in section 2.2. The first large crystals of magnetite were prepared by Smiltens using a modified Bridgeman-Stackbarger method. Magnetite is also found in the magnetotactic bacterium, *aquaspirillum magnetotacticum*. They orient and swim along the magnetic field and are found in fresh water.
Ferrofluids containing magnetite can be prepared in a basic solution by mixing the appropriate amounts of an iron (II) salt and an iron (III) salt. The black precipitate is the mixed valence oxide, Fe₃O₄. One of the earliest synthetic methods to prepare Fe₃O₄ particles was introduced through a program in advancing mineral technology, the Bureau of Mines.¹⁵³ This technique got more attention and had more applications with the modification done by Massart.⁸⁵ The aqueous magnetic dispersion was stabilized with different peptizing agents in both acidic and basic media. They studied the phase separation of these particles as well as their optical properties.¹⁵² Later Philipse used this synthetic method, modified it with silica coating,⁸⁶ and later stabilized the magnetite particles by the oleic acid before the silica coating.¹⁵⁴ Another technique to prepare these particles is precipitating them in the disperse phase of water/oil microemulsions.¹⁵⁵-¹⁵⁸ Also sonochemical power can be applied to iron hydroxides to obtain magnetite crystals.¹⁵⁹ Sonochemistry has been used for preparation of zero-valent iron by thermal decomposition of iron pentacarbonyl.¹⁶⁰-¹⁶⁵ Spray pyrolysis is another technique used for the preparation of iron oxides.¹⁶⁶

Coating with silica ⁸⁶;¹⁰¹;¹⁰²;¹⁰⁵;¹⁶⁷-¹⁶⁹ and amino silane groups ¹⁷⁰-¹⁷² are some of the approaches used to improve the stabilization of magnetic particles. They are also used as surface coatings especially on silica particles,¹⁷³;¹⁷⁴ latexes ⁹¹;¹⁷⁵;¹⁷⁶ and from some of these particles hollow spheres were prepared by calcinations of the particles at elevated temperatures.¹⁷⁵;¹⁷⁶ Polymeric stabilizers such as; poly(methacrylic acid),⁸³;¹⁷⁷ poly(ethylene oxide),¹⁷⁸ methoxypoly(ethylene glycol),¹⁷⁹ poly(glutaraldehyde)¹⁸⁰ are also commonly used as protective coatings. Preparation of magnetic latexes¹⁸¹ and their behavior ⁸⁷-⁸⁹;¹⁸² are mostly used as references for these types of particles. Interactions of
silica colloids with magnetite cores, dipole-dipole interaction effects in magnetic systems, concentration-dependent sedimentation properties, light scattering properties, magnetic properties and chaining effect of magnetic nanoparticles have been investigated by several scientist and compared to latex magnetic particles. Synthesis of iron oxides is not limited to magnetite. Several scientists have been studying the preparation and behavior of other ferrites such as $\alpha$-Fe$_2$O$_3$.

2.2.2. Superparamagnetic Cobalt Nanoparticles

Most common magnetic materials are metal oxides such as various ferrites. Pure metals such as, Fe, Co, Ni and even their alloys are also used as magnetic materials. The intensity of saturation magnetization of cobalt lies between that of iron and nickel (Table 2.2). The metal in polycrystalline and single crystal form has been investigated fundamentally. Most cobalt particles prepared are spherical. So far Alivisatos et al. are the only group that has published a controlled preparation method for cobalt nanorods. Magnetic nanoparticles are also used for deposition on carbon nanowalls as well as for self assembly of carbon network structures.

Using pure metals has the complication of easy oxidation in air especially when the desired particle size is close to superparamagnetic limit (~10-15nm). Therefore several methods were developed to increase the stability of pure metals. In 1966 Hess et al. thermally decomposed dicobalt octacarbonyl in solutions of different polymers. In 1982 Papirer et al. used same technique to prepare cobalt ferrofluids and investigated the experimental parameters and growth and nucleation of the particles. Chaudret et al. used a cobalt organometallic precursor to prepare cobalt particles and coated them with polyvinylpyrrolidone. Noble metals are also used as insulating shells through
microemulsions to increase the stability of these particles.\textsuperscript{209;210} Metal carbonyl pyrolysis is applied to produce Co,\textsuperscript{211;212} Fe,\textsuperscript{163;187} Ni\textsuperscript{208} and other magnetic materials. Another technique to prepare Co,\textsuperscript{213} and CoO,\textsuperscript{214} and FePt\textsuperscript{215;216} particles is solution phase metal salt reduction. Recently Riffle \textit{et al.} prepared magnetic cobalt dispersions in the presence of poly(dimethylsiloxane).\textsuperscript{217;218} Some of these type of particles found application in the treatment of retinal detachments.\textsuperscript{100;147} Almost all these types of approaches produced somewhat uniform particles that are partially stable to oxidation. Within time air could penetrate through the coatings. A shell of silica surrounding the cobalt core has been proven to protect the particles against oxidation.\textsuperscript{100}

Silica has been used as a stabilizer for iron oxides, too. It is harder to have the silica coating on metal surfaces because they do not have available functional groups for the attachment of silica. In 1999, Mulvaney \textit{et al.}\textsuperscript{3;103} coated gold colloids with silica in a three step deposition process. The first step was the deposition of citric acid as the stabilizer. The second step is the replacement of the stabilizer with the amino silane spacer groups. Finally silica is coated onto these particles with Stöber method, through the silane spacers. A similar technique is used for the preparation of silica-coated silver particles\textsuperscript{6} and find applications as redox catalysts.\textsuperscript{7} Matjievic \textit{et al.} used the idea of depositing another compound on the cores and coated silver particles with silica using a modified Stöber method.\textsuperscript{8} As an alternative to silica coating Guille \textit{et al} investigated the preparation of silica gels containing magnetic nanoparticles of Fe, Ni, and Co and concluded in different magnetic behaviors for each metal.\textsuperscript{219} Some scientists used gold as a coating material for magnetic materials such as iron.\textsuperscript{209;220}
Very recently Liz-Marzan et al.\textsuperscript{221} prepared uniform and almost perfectly homogenous cobalt nanoparticles with the control of particle size. They applied the same technique Mulvaney et al.\textsuperscript{3} used for the preparation of silica-coated gold particles. The silica-coated particles showed high saturation magnetizations which made them valuable in magnetic applications. As shown in Figure 2.4, cobalt particles were prepared from cobalt chloride and reduced to cobalt in elemental form by the sodium borohydrate in the presence of citric acid.

Figure 2.4. Synthesis of silica-coated cobalt particles.
Citrate reduction is very commonly used for the preparation of colloidal particles, particularly gold.\textsuperscript{3,4,7,222} For the cobalt particles citric acid acts a protective agent. The use of citrate was preferred, since it has been observed\textsuperscript{6} that silica coating procedure failed when the starting colloids were stabilized with longer chain polymers. The particle surface was made vitreophilic by immediate replacement of the citric acid by the amino silane spacer groups. This replacement allowed the silica coating of the particles through Stöber method. Particle size was controlled by the initial citric acid concentration and the core-shell morphology was found to be independent of citrate:Co molar ratio. This preparation method is followed throughout much of this dissertation.

Synthesis of cobalt particles is not the only area of interest. Many scientists have investigated crystal structure of cobalt\textsuperscript{211,213} as well as the magnetic properties.\textsuperscript{223-226} Cobalt has two crystal structures, face-centered cubic (fcc) and close-packed hexagonal (hcp). Both coexist at room temperature, but it is shown that above 450°C fcc is thermodynamically favored and hcp is favored at lower temperatures.\textsuperscript{211}

2.3. Synthetic Polypeptides

Polypeptides are chains of amino acids, which are the building blocks of all living species.\textsuperscript{42,227} They can form very complex structures that can play very important roles in biological functions. Therefore, their synthesis and applications are some of the major areas that scientists have been investigating.

Amino acids have the generalized structure shown in Figure 2.5. The amino groups are located on the $\alpha$-carbon atom; hence, they are called $\alpha$-amino acids. The chemical structure of the R group can vary. Only 20 amino acids with 20 different R groups are naturally found in proteins.
The demand for substantial quantities of different amino acids led scientists to investigate different synthesis routes. Greenstein\textsuperscript{228} summarized most of the early synthetic techniques of \(\alpha\)-amino acids in his book such as: Strecker synthesis, amination of \(\alpha\)-halogen acids, reductive amination, amination via molecular rearrangement, condensation of aldehydes with active methylene groups, and condensation with \(N\)-substituted aminomalonic esters.

![Figure 2.5. General structure of amino acids.](image)

The 1966 patent by Wesserman\textsuperscript{229} represents one of the major contributions to the synthesis of \(L\)-glutamates. More recently many chemically modified amino acids can be prepared by modifying the side groups and then polymerized to synthetic polypeptides by solid-phase method of Merrifield.\textsuperscript{230}

The amino acids present in the polypeptide chains are linked covalently by peptide bonds. The carboxyl group of one amino acid can link to the \(\alpha\)-amino group of a second amino acid and form the peptide bond through condensation reaction (Figure 2.6). The newly formed dipeptide has a free \(\alpha\)-amino group which is called the \(N\)-terminal and a free carboxyl group which is called the \(C\)-terminal. These ends can react further to form longer chains of peptides.
Figure 2.6. Formation of a peptide bond through condensation reaction.  

The four atoms around the peptide bond are in planar configuration and depending on the position of the oxygen atom of the C=O group and the hydrogen of the N-H group, the peptide bonds are either in trans- or cis- position (Figure 2.7). Therefore, they are coplanar. The C-N peptide bond (1.33 Å) is shorter than the normal C-H bond (1.47 Å). This shows that C-N bond has a double bond nature which can be explained in terms of resonance.  

Figure 2.7. Configuration of the peptide bond. (a) trans-peptide bond, (b) cis-peptide bond.
The four levels of protein structures are primary, secondary, tertiary and, quaternary. The three most common structures, α-helix, random coil and the β-sheet are shown in Figure 2.8. Besides these, there are several others which are not known widely such as β-turns, π-, ω-helices, twists, turns, loops, etc.

Figure 2.8. Basic structures of amino acids: (a) α-helix confirmation, (b) β-sheet confirmation, (c) random coil confirmation.

The β-sheet structure looks like folded chains. In this structure the polypeptide chains are stretched out to their maximum level and form a zigzag pattern. It can have a molecular axis oriented in a parallel or anti-parallel way. The structure is stabilized through hydrogen bonds and van der Waals interactions between aligned chains.

The most common conformation is α-helix, first discovered by Pauling and Corey. In the α-helix structure, atoms linked with the peptide bond are coplanar. The bond is rigid; however, the single bonds associated with the α-carbon can rotate 360° to
form the helix. This confirmation can be left or right handed and stabilized by the hydrogen bond between the backbone of the carbonyl oxygen and the backbone hydrogen of the amide bond. Each amino acid is 1.50 Å and each helix contains 3.61 amino acid units per turn. Therefore the overall contribution of each turn is 5.41 Å to the total helix length. One of the important features of \( \alpha \)-helix is the dipole resulting from the individual dipoles of all peptide bonds in the main chain.\(^{232}\) This helix dipole has an affect on the transfer of electrons, protons, and ions along the helix.\(^{233}\)

The random coil forms when the hydrogen bonds are disrupted by temperature or selective solvents. The solvent and the temperature effect vary from polypeptide to polypeptide. But in general, strong acids like trifluoro acetic acid (TFA)\(^{234}\) and dichloro acetic acid (DCA) are known to denature polypeptides.\(^{67;71;77;235-237}\)

### 2.3.1. Poly(\( \alpha \)-Amino Acids)

For many years poly(\( \alpha \)-amino acids) have been used as simple models for theoretical and experimental studies. In particular, solution properties of synthetic polypeptides have been greatly investigated.\(^{70;72-76;236;238-241}\) Poly(\( \gamma \)-benzyl-\( L \)-glutamate), PBLG, is the most popular among the poly(\( \alpha \)-amino acids). There are several papers that investigated the synthesis, characterization and solution properties of PBLG.\(^{70;72-74;77;236;238;239;241-246}\) The general structure of poly(\( \alpha \)-amino acids) is shown in Figure 2.9. Some other popular poly(\( \alpha \)-amino acids) are poly(\( \gamma \)-methyl-\( L \)-glutamate) (PMLG), poly(\( \varepsilon \)-carbobenzyloxy-\( L \)-lysine) (PCBL), poly(\( \beta \)-benzyl-\( L \)-aspartate) (PBLA), poly(\( \gamma \)-stearyl-\( L \)-glutamate) (PSLG).
Figure 2.9. General structure of poly(α-amino acids). R group is \((\text{CH}_2)_2\text{CO}_2\text{CH}_2\text{C}_6\text{H}_5\) for PBLG, \((\text{CH}_2)_2\text{CO}_2(\text{CH}_2)_{18}\) for PSLG, and \((\text{CH}_2)_4\text{NHO}_2\text{CH}_2\text{C}_6\text{H}_5\) for PCBL.

Synthetic polyglutamates can be obtained easily by the ring opening polymerization of \(N\)-carboxyanhydrides (NCAs), as first demonstrated by Leuchs\(^{247}\) in 1906. Figure 2.10 shows the structure of an \(\alpha\)-amino acid, \(N\)-carboxyanhydride. R group is representative of the side chain of the amino acid.

Figure 2.10. General structure of \(N\)-carboxyanhydride derivatives of \(\alpha\)-amino acids with ring numbering system.

After this discovery, NCAs were extensively used for the synthesis of homopolypeptides, co-polypeptides, attachment of specific groups to the end of the proteins. Various application areas and great interest in NCAs lead to different
approaches of the NCA synthesis. Leuchs used phosphorous pentachloride for the ring closure of $\alpha$-amino acids at relatively high cyclization temperatures. Similar to this approach, phosphorous tribromide and thionyl chloride were used as ring closing agents. Several other methods used such as forming an azlactone or an oxonium ion as reaction intermediates. Selectively protected\textsuperscript{248} and unprotected $\gamma$-amide groups\textsuperscript{235} are other intermediates that can lead to synthesis of NCAs. The first application of phosgene gas was by Fuch\textsuperscript{249} and Farthing\textsuperscript{250,251} modified this approach, which was the most widely used method for preparation of $\alpha$-amino acid NCAs. In 1989, Fuller \textit{et al.}\textsuperscript{252} applied phosgene gas in a solution providing the control of the gas used for the reaction. The use of derivatives of phosgene such as diphosgene has been suggested but required using carbon black as a catalyst. Another phosgene derivative that has been used for NCA synthesis is trichloromethychloroformate (TCF).\textsuperscript{253,254} Using phosgene or its derivative diphosgene had its drawbacks. Among all methods mentioned HCl is generated as a by-product, which can lead to side reactions.\textsuperscript{67} Therefore, removal of HCl is very important. The fact that the ring is sensitive to moisture and water makes the purification harder. Recrystallization is the most common way of purification. Dorman \textit{et al.}\textsuperscript{255} used rephosgenation method for purification of NCAs and synthesized higher molecular weight homopolypeptides such as poly($\gamma$-benzyl-$L$-glutamate) and poly($\gamma$-methyl-$L$-glutamate).

One of the biggest contributions to the synthesis of NCAs was introduced by Daly \textit{et al.}\textsuperscript{256,257} They showed that triphosgene is an effective substitute for phosgene. Only 1/3 equivalent of triphosgene is required for a successful synthesis because it delivers 3 equivalents of phosgene \textit{in situ} (Figure 2.11). This decreases the possible side reactions.
due to excess phosgene. It does not racemize the product and gives a higher yield. Even for the advantageous triphosgene, if used slightly in excess it may polymerize the NCA. Later in 1991, Wilder et al.\textsuperscript{258} used triphosgene for the synthesis of \textit{N}-carboxy-\textit{L}-valine anhydride at room temperature. Poché et al.\textsuperscript{259} introduced a new purification technique with ice cold water taking Leuchs\textsuperscript{247} as an example, who synthesized the NCA derivative of glycine in water at 0\(^\circ\)C and 15\(^\circ\)C. Chapter 4 of this dissertation further proves that NCA does not react with water at low temperatures.

With a few exceptions of oily NCAs, almost all \(\alpha\)-amino acid NCAs are white, crystalline solids. \textit{N}-carboxy-\(\gamma\)-benzyl-\textit{L}-glutamate is one of the NCAs that has been studied on the crystal and molecular structure.\textsuperscript{260} Also NMR studies on many \(\alpha\)-amino acids NCAs have been reported.\textsuperscript{235}

Figure 2.11 shows the reaction of triphosgene with \(\alpha\)-amino acids. When attached on the carbonyl carbon triphosgene releases 2 molecules of phosgene. The reaction produces HCl by product that can be moderately sparged out with constant flow of nitrogen. The remaining HCl reacts with the amine group of the amino acid forming the hydrochloride salt.

\(\alpha\)-Amino acid NCAs have four reactive sites; two of which are electrophilic and the other two are nucleophilic centers. As shown in Figure 2.10 carbamoyl (C-2) and carbonyl (C-5) are the electrophilic sites and NH (3) and \(\alpha\)-C (C-4) are the nucleophilic sites. The multiple reactivity nature of NCAs makes the polymerization reaction more complicated and the NCA molecule more sensitive than anticipated. The reaction paths of NCAs mostly depend on the nature of the initiators.
The most common protic nucleophiles used for ring opening polymerization are water, alcohols, and amines (1°, 2°, and 3°). Phosphines, mercaptans, and carbanions are some other nucleophiles that have been studied. Water, alcohols, and primary amines all attack the carbonyl carbon (C-5) of the NCA ring. For the secondary amines, depending on the basicity and the steric hindrance, the initiation site may vary.

Several scientists investigated the polymerization mechanism of NCAs. Kricheldof et al. published a series of papers on the polymerization mechanisms of NCAs.
and their characterization with nuclear magnetic resonance spectroscopy. The most common polymerization mechanisms are discussed below. Amine mechanism (Figure 2.12) was first introduced by Wessely and Watson. According to this mechanism, the primary amine 1 attacks the C-5 of the NCA monomer, opens the ring and carbon dioxide is eliminated from the intermediate carbamic acid 3. A new molecule 4 with a primary amine end group forms. Alkyl amine initiators are more nucleophilic compared to the amine chain ends. Therefore, initiation is faster than propagation and the reaction is theoretically living.

![Amine mechanism for ring opening polymerization of NCAs.](image)

The carbamate mechanism (Figure 2.13) was reported by Idelson and Blout. It is similar to the amine mechanism, except here the primary amine is strong enough to deprotonate the intermediate carbamic acid 3 and a new intermediate, 3' forms. 

![Carbamate mechanism for ring opening polymerization of NCAs.](image)
nucleophilic reaction continues with the NCA monomer 2 and results in an intermediate anhydride 6. Polypeptide 7 forms after decarboxylation of the intermediate anhydride.

Blout and Karlson,$^{33}$ using tertiary amines, showed the polymerization of γ-benzyl-L-glutamate NCA yielded very high molecular weight via an activated monomer mechanism (Figure 2.14).$^{269}$ These stronger bases can deprotonate the NCA monomer 2. The monomer activated by deprotonation, attacks another NCA monomer to form intermediate 8. The chain continues to grow, following the decarboxylation of 8 via the nucleophilic attack by the activated monomer and product 10 forms. The relative pKa’s of the intermediate carbamate and monomeric NCA suggests that carbamate mechanism shown Figure 2.13 is highly unlikely to happen.$^{68}$

Later in 1958 Blout and DesRoches synthesized higher molecular weight polypeptides using carbodiimides as condensing agents.$^{270}$ Once the polypeptide with
free terminal amino and carboxyl group is treated with carbodiimide, condensation occurs with the formation of additional peptide bonds between polypeptides to yield higher molecular weight and degree of polymerization.

![Chemical structure of activated monomer mechanism of polymerization of NCAs](image)

**Figure 2.14.** Activated monomer mechanism of the polymerization of NCAs.²³⁵

Besides the base/amine initiation of the NCAs, the ring opening polymerizations can also be initiated by water molecules,²³⁵ metal salts,²³⁵ transition metal-amine complexes.²⁷¹-²⁷⁴ Deming used zerovalent nickel complex, bpyNi(COD) to initiate the
polymerization of NCAs while eliminating the side reactions of chain initiation and termination (Figure 2.15). For instance, NCAs can be deprotonated at the nitrogen which may act as a nucleophile to initiate polymerization of other NCAs or can rearrange to \( \alpha \)-isocyanato carboxylates which may react with the free amine end group and terminate polymerization. Polymers with better polydispersity \( (M_w/M_n < 1.5) \) could be achieved by this way. Throughout this dissertation amino initiation of NCAs will be used and other polymerization techniques of metal amine initiations or polymerization via vapor deposition will be saved for future investigations.

![bpyNi(COD) reaction](image)

Figure 2.15. Polymerization of NCAs by zerovalent nickel complex, bpyNi(COD).

Most polymerizations are carried in polar solvents such as 1-4-dioxane and tetrahydofuran (THF). Once the polymers are prepared they show liquid crystalline behavior at high concentrations.

Poly(\( \gamma \)-benzyl-\( L \)-glutamate), PBLG, is the most studied polypeptides of all. PBLG has a rigid rod like \( \alpha \)-helix conformation and is thermally stable up to 160\(^\circ\)C. The helical structure can be disrupted by trifluoro acetic acid (TFA) and dichloro acetic acid (DCA). Most solvents for PBLG are helicogenic, such as: chloroform, dicloromethane.
(DCM), dichloroethylene (DCE), tetrahydrofuran (THF), pyridine, \textit{m}-cresol, nitrobenzene, benzene, cyclohexane, formamide, dimethyl acetamide, and \textit{N},\textit{N}-dimethyl formamide (DMF). However, not all of them are good solvents. Only, DMF, nitrobenzene, \textit{m}-cresol and pyridine dissolve PBLG without aggregation. Solution properties of PBLG have been studied widely. Dilute solution properties, thermodynamic properties by light scattering, viscoelastic properties, osmotic pressure, dielectric properties, spectroscopic properties, optical rotation, liquid crystalline behavior and helix-coil transitions are the main areas that has been investigated over the years. All these properties are discussed in detail in the book by Block and the references therein.\textsuperscript{67} Other well studied materials of this class are Poly(\textit{\gamma}-methyl-\textit{L}-glutamate), PMLG, Poly(\textit{\gamma}-stearyl-\textit{L}-glutamates), PSLG, and poly(\varepsilon-carbobenzyloxy-\textit{L}-lysine), PCBL.

Helix-coil transition ability of polypeptides is one of the major characteristics of interest in this dissertation. The conformational change of helical structure to coil (or vise versa) can be induced in solutions by changing the temperature, the solvent or its composition, or both. Even though PBLG \textsuperscript{77;80-82;236;238;275-278} and PCBL \textsuperscript{70;71;74;78;79;278-282} are the most commonly studied ones, the helix-coil transitions are not restricted to them. The conformational change can vary from one poly(\alpha-amino acid) to another.\textsuperscript{283} Biologically, protein denaturation can be considered as a problem, and therefore understanding the thermodynamics of this transition is very important. The synthetic polypeptides can be used as a model to understand this concept. On the other hand, from the materials science point of view, this transition can be used as a functional characteristic of the material.
2.4. Surface Modification

Surface modification is mostly used to enhance the stabilization of particles, vary the chemical activity and the hydrophobic character of the surface, and for designing core-shell particles. Surface modification can find use in different application areas such as biomedical studies, optical devices, paints, catalysts, or simply to understand the chemical and physical forces between surfaces. It is not always used to modify silica particles but also used to change the properties of polymers. As much as silica particles, silica surfaces such as wafers, metal surfaces are surface functionalized in order to interact with other particles, organic materials and polymers.

Several techniques can be used for the surface modification. Silylation reaction (silanization) is one of the most common methods used on colloidal silica and silica surfaces. Another very common method is grafting. Mostly this term is used for the attachment of a polymer on the surface. It is simply surface coating by initiating the polymerization on the surface. Grafting onto and grafting from are the two most common techniques used. Grafting and silylation techniques are discussed in more detail in sections 2.4.1 and 2.4.2.

Modification of surfaces is not limited to these techniques. Several coating agents other than organosilanes are used to avoid the agglomeration of silica particles. Dyes are used to study the adsorptive properties. Silica itself can be used as a surface modifier for several metals, metal oxides and hydroxides such as, boehmite (AlOOH), Fe₃O₄, cobalt, silver and gold particles. These metal particles also can be deposited on the silica surface to increase the stability of the particles or to prepare core-shell particles.
2.4.1. Silanization

Among the coupling agents used for silanization reactions, organosilanes are the most common ones. The first organosilane compound was reported by Friedel and Crafts in 1865. However, it was not until 1940s that organosilicon chemistry become popular and got its share in the industry. The general formula of an organosilane can be represented as $R_nSiX_{(4-n)}$. The $X$ is a hydrolyzable group such as alkoxy or chlorine. It reacts with the inorganic substrate and the bond between $X$ and the Si atom is replaced by the new bond formed between the substrate and the Si atom. $R$ is a nonhydrolyzable organic group that enables the coupling agent to further bond with other organic groups and polymers.

Depending on the desired final product, organosilanes with different functional groups can be used. Most of the silanes are readily available at reasonable prices. Aminosilanes are one of the most popular groups of coupling agents due to various application possibilities they provide. Several kinds of aminosilanes can be used as surface coupling agents. Amino silanes with chlorine end groups are mostly preferred for their fast reactivity. Amino silanes with alkoxy groups, on the other hand, are preferred mostly for their ease of application and availability. Also, avoiding the HCl by product is another advantage over chlorosilanes. The most common amino silanization agent is ($\gamma$-amino-propyl)triethoxysilane (APTES) as shown in Figure 2.16. The alkoxy group can be methoxy or ethoxy depending on the desired reaction or the solvent used. Throughout this dissertation ($\gamma$-amino-propyl)triethoxysilane will be abbreviated as APTES and ($\gamma$-amino-propyl)trimethoxysilane will be abbreviated as APTMS.
Low cost and easy handling of amino silanes are the most important reasons for its popularity. Amino silanized surfaces find various applications in biological sciences, such as enzymes, antibodies, biosensors for proteins as well as modification of electrodes, bonded phases for chromatography, membranes, catalysts and as lubricants.\textsuperscript{295,296} Similar to other organosilanes, amino silanes can also go through chemical modifications and can be used as linkers for further attachment of organic and inorganic molecules. Among all these, the major advantage is the reactivity of amino functionalization towards biological molecules as well as others.

Like other types of organosilanes, aminosilanes can be used on substrates and as coupling agents for polymers, especially condensation-thermosetting polymers. The substrate can be a flat surface such as silicon wafers, microscope slides, gold surfaces, and any other possible target. This dissertation is mostly interested in the reaction of amino silanes with spherical surfaces, especially colloidal silica. The reaction between silanes and substrates is a simple reaction with several steps. Figure 2.17 demonstrates a typical reaction path on a flat surface which is also valid for spherical surfaces. In most cases silanes undergo hydrolysis before the surface treatment which produces a reactive silanol group. For the reaction, water can be provided externally or simply comes from the air. Hydrolysis is followed by the condensation to oligomers. These oligomers form
hydrogen bonds with the OH groups on the surface of the substrate. Finally, during drying or curing process covalent attachment is achieved by the loss of water.

Figure 2.17. Reaction of silanes with a substrate. Methoxy group is used as a representative of the X group and a flat surface as a substrate.
When it comes to application or usage of silanes in surface chemistry, most of the reactions involve flat surfaces, such as Si(100) wafers, \textsuperscript{284,285,297-299} E-glass, \textsuperscript{300,301} stainless steel, \textsuperscript{302} aluminum oxide and gold substrates.\textsuperscript{303} Thiol derivatives are used as coupling agents especially on metal surfaces instead of amino silanes. Flat surfaces got more attention due to simplicity of processing, application variety and the ease of chemical analysis. The interest in spherical particles arose within the last 20 years.\textsuperscript{1,34,35,39,286,287,304-311} Functionalization with aminosilanes became necessary for biological application purposes and were mostly used for immunoassay experiments\textsuperscript{312} and as packing materials with different functional groups. Even the simple flat surfaces had their own problems such as overloading, uncontrollable surface layering, and randomness. Surface density and surface properties are mostly investigated by X-ray photoelectron spectroscopy (XPS),\textsuperscript{313-315} ellipsometry,\textsuperscript{303,316} fourier transformation infrared spectroscopy (FTIR),\textsuperscript{317,318} reflection absorption infrared spectroscopy (RAIR),\textsuperscript{319} Near-edge X-ray absorption fine structure spectroscopy (NEXAPFS),\textsuperscript{313} neutron reflectometry\textsuperscript{320} and solid state nuclear magnetic resonance spectroscopy (NMR).\textsuperscript{314,321}

One of the biggest concerns is the quantification of the amino groups on the surface, especially for spherical particles. Most of the techniques include estimates from quartz-crystal microbalance,\textsuperscript{322} reversible chemical reactions,\textsuperscript{323} spectrophotometric determinations.\textsuperscript{324,325} The methods used for the free amino groups in the solid-phase peptide synthesis can be applied to silica particles. The Kaiser test\textsuperscript{326} (Ninhydrin color test) is widely used to determine the presence of free amino groups in peptides. Kay et al.\textsuperscript{327} summarized several tests for the determination of primary and secondary amines.
such as Kaiser test, labeling with bromophenol blue,\textsuperscript{328} trinitrobenzene sulfonic acid (TNBSA) and chloranil test. TNBSA\textsuperscript{329} and chloroanil tests only prove the presence of the free amino group like Kaiser test. Unlike these methods, labeling with fluorescamine,\textsuperscript{330,331} Reichardt’s dye,\textsuperscript{332} SPDP (\(N\)-hydroxysuccinimide ester of 3-(2pyridyldithio)propionic acid) method\textsuperscript{333} can give quantitative results. Even though these methods may work on spherical particles it is strictly dependent on the concentration of the free amino groups on the surface and the concentration of the silica particles, because the turbidity of the silica particles can effect the measurements.

Moon \textit{et al.}\textsuperscript{316} studied the surface density of amines by simply reducing the amines to imines. Even though the amines do not have an absorbance, imines absorb in UV-vis region. Heiney \textit{et al.}\textsuperscript{298} investigated the growth of amines by functionalizing them with photosensitive 4-(octyloxy)cinnamoyl chloride chromophores. One other alternative was introduced by Sagiv \textit{et al.}\textsuperscript{334} by frequently using short alkyl trichlorosilane derivatives due to their faster reactivity. Balachander and Sukenik\textsuperscript{335} used 1-bromo-16(trichlorosilyl)hexadecane for the silanization and reduced the bromo groups to azides and subsequently to amines. Heise \textit{et al.}\textsuperscript{49} used this as an advantage and mixed the bromo functionalized groups with non-functionalized alklytrichlorosilanes in various ratios to make mixed SAMs. Bierbaum\textsuperscript{313} used aminosilane derivatives with long alkyl chains and did not obtain ordered monolayers due to possible hydrogen bonding between the amine groups and the silanols. Also, Boerio\textsuperscript{317} and Ishida\textsuperscript{318} showed the possible hydrogen bonding between the amino end of the silanes and the surface hydroxyl groups resulting in ring formation. XPS can differentiate between the free amines, amino groups hydrogen bonded to silanols, and protonated amines. Kallury\textsuperscript{314} \textit{et al.} investigated
silanization under base and water catalysis with XPS. Their work showed that presence of a base reduces the surface interactions of the amino groups. On the other hand, surface water promotes these interactions and amino groups tend to orient towards the surface. Since cyclic structures are more likely to form at higher pH, one approach to fix this problem is using acidic reaction media. Also it has been shown that this type of surface interaction is less likely to happen when (3-aminopropyl)dimethylethoxysilane (APDMES) is used with ethylenediamine as a catalyst. Some success was achieved by using supercritical CO₂ as the solvent to over come this problem. Because there are some free amino groups on the surface, most scientists do not consider this a problem depending on the applications they have in mind.

2.4.2. Grafting with Synthetic Polypeptides

Among different methods of polymer attachment onto surfaces and colloidal particles, grafting by initiating the polymerization on the surface is one of the most common techniques. The grafting of polymers is becoming very important in composite adhesion improvements, pigment dispersions, biomedical polymer applications, optical displays, chromatography, and fiber wettability.

Oligo(ethylene glycol) is grafted on gold and silver surfaces to investigate the resistance to protein absorption. Not only polymers but several other molecules can be attached to surfaces with grafting techniques. Licholic acid is another molecule that has been grafted on silicon crystal surface. Cysteine-specific surfaces prepared through the grafting of N-succinimidyl-6-maleimidocaproat on a glass surface. Even chromophores can be grafted on functionalized surfaces to determine their deposition characteristics. In order to prolong and enhance the stability of colloidal particles, a
larger range of polymers are used for grafting, including: oligo(ethylene glycol),\textsuperscript{341} poly(diallyldimethylammonium chloride),\textsuperscript{342} poly(n-butylacrylate),\textsuperscript{343} poly(n-isopropylacrlamide),\textsuperscript{344} poly(benzyl methacrylate),\textsuperscript{345} polystyrene,\textsuperscript{346} and dendrimers.\textsuperscript{347}

Polypeptides are among the polymers that have been used in grafting techniques. They can be grafted on several materials such as water soluble chitin,\textsuperscript{348} alkoxide derivatives of polyhydroxy polymers,\textsuperscript{349} and carbon black.\textsuperscript{64,350,351} The first grafting of polypeptides from solid surfaces was reported by Hamann \textit{et al.}\textsuperscript{352-354} in early 70s. They grafted polypeptides from primary amine functionalized silicon dioxide to obtain stable pigment dispersions. This work was followed by Oosterling\textsuperscript{50,69} by grafting poly(L-glutamates) and poly(L-aspartates) from aerosil particles. Most of the grafting experiments were carried on flat surfaces due to easy access to the surface and effortless allowance of self-assembly.\textsuperscript{349,355-357} Grafting to spherical particles is limited and may have more crowding problems. Also for the determination of helix orientation and their alignment, flat surfaces are better.

Mainly two different grafting methods have been applied for the attachment of polypeptides on surfaces (Figure 2.18). One of which is called “grafting-onto” method. In this method polymers with modified end groups are attached to the surface through these groups. A living polymer radical (anion or cation) can be terminated by the surface functional groups and can be considered as the grafting onto method.\textsuperscript{351} The second method is called “grafting-from,” which is applied throughout this dissertation. In this method the substrate is surface functionalized to initiate the polymerization of the desired monomer. When polypeptides are the point of interest, flat surfaces were preferred over spherical particles due to the easy handling of flat substrates. The first report of grafting
polypeptides on spherical particles was published in 1987 by Tsubokawa. He grafted polypeptides from amino groups on carbon black.\textsuperscript{64} Prior to this publication he investigated the grafting of carbon black having isocyanate or acyl azide groups.\textsuperscript{350} The only other polypeptide-grafted spherical particles have been reported by Fong \textit{et al}.\textsuperscript{358,359} They prepared and characterized poly(benzyl-\textit{L}-glutamate) and poly(carbobenzyloxy-\textit{L}-lysine)-grafted colloidal silica particles.

Using grafting onto method (Figure 2.18a) gives the advantage of characterization of the polymers before the attachment. Depending on the characteristics of the polymer even external forces can be used for the orientation of the polymer chains during the attachment. However, already grafted polymers increase the steric hindrance causing lower grafting density.\textsuperscript{242,360-364} Also aggregation can be a problem with grafting onto techniques. Grafting onto method has been widely used to prepare self-assembled monolayers (SAMs) on gold substrates. Mostly an amine terminated disulfide (such as \textit{N}-lipoyl-1,3-diaminopropane) is used to initiate the polymerization followed by its spontaneous assembly onto gold substrate.\textsuperscript{363,364} Niwa \textit{et al}. investigated the control of helix orientation through microdipole interactions on gold surfaces.\textsuperscript{4,360,361} They also used grafting onto technique to investigate the monolayer formation of diblock copolymers and their binding abilities to amino acids.\textsuperscript{242} Higashi and Niwa\textsuperscript{360,362} studied the molecular packing of poly(glutamic acid) (PGA) with disulfide modified N-terminus attached to the gold surface. They verified the side-by-side helix-microdipole interactions by contacting this surface with guest PGAs with ferrocenyl groups at the N- and C- termini.
The grafting from method (Figure 2.18b) overcomes some of the disadvantages of grafting onto method. The surface functionalization of the substrate controls the surface density before the initiation of the polymer. For the grafting of polypeptide, the surface is functionalized with primary amines. Because smaller initiator groups are on the surface, steric hindrance is less likely to be a problem. Once the number of initiators on the surface is known, the monomer to initiator ratio can be controlled.\textsuperscript{57,58,61-63,365,366} Determination of this ratio is easy on flat surfaces due to the applicability of surface characterization techniques. On the other hand, it is more complicated with spherical particles due to the geometrical shape of the surface area. However, the strict polymerization conditions such as dry solvents, inert atmosphere can be considered as the drawbacks of this method. Also in most cases it is not possible to characterize the polymer since it is initiated by the surface.
Whitesell et al.\textsuperscript{58,365} grafted polyalanines from aminotrithiol initiated gold and indium-tin oxide surfaces. Since the space occupied on the surface by the initiator is slightly larger than the helical polyalanine, it is less likely that larger polypeptides such as phenylalanine can be grafted. Because of this, the aminothiol groups on the surface were reacted with chloroacetyl chloride to produce a second layer. This layer is found to be large enough to accommodate larger polypeptides in helical forms.\textsuperscript{58}

Oosterling et al.\textsuperscript{61-63} showed in a series of papers poly(L-glutamates) and their copolymers can be polymerized with aminosilanes initiated on glass and silicon substrates. They investigated the synthesis, characterization, and helix orientation of such structures.

Jaworek et al.\textsuperscript{366} used grafting from technique to examine the electromechanical properties of PBLG coated glass substrates. They concluded that this method is feasible for the growth of piezoelectric-active films directly on variety of electrodes.

Machida et al.\textsuperscript{52,53} used the amino silane group attached to the glass surface to obtain molecularly oriented thin films. Combining the technique they developed called chemical reaction alignment (CRA) with grafting from method, they obtained thin monolayers of liquid crystals on glass surfaces.

In 1995, Chang and Frank\textsuperscript{57} used surface initiated polymerization of polyglutamates and developed characterization methods to examine the formation of two dimensional structure. In 1996, Chang and Frank investigated the grafting of PBLG with three different approaches including grafting from and grafting onto. They decided grafting onto methods produce successful attachment of PBLG on the surface, whereas with grafting from method some unreacted monomer and physisorbed molecules
remained on the surface and their removal requires more rigorous cleaning treatment.
The disadvantages of these two grafting techniques can be useful for some, depending on
the desired surface characteristics; therefore, it is hard to prove one technique is better
than the other.

In 1996, in order to overcome the problems occurring with these grafting
techniques, Wieringa et al.367 introduced a new method to assemble grafted layers of
poly(methyl-\textit{L}-glutamate). This method used polymerization of the \textit{N}-carboxyanhydride
in melt, instead of the solution, by heating the monomer on the amino silanized
substrates. Following this approach Chang and Frank56 introduced vapor deposition
polymerization (VDP) of \textit{\alpha}-amino acid \textit{N}-carboxyanhydrides. Compared to the previous
method, the polymerization occurs in the gas phase, simply by vaporized the monomer in
vacuum. They further improved this technique simply varying the temperature, vacuum,
reaction time, and monomer concentration.51 Following the developments in VCD, this
technique is used for polymerization of various \textit{\alpha}-amino acids,368 determination of
morphology changes,369 and investigation of conformational transitions.370

2.5. Cross-Linked Particles

Crosslinking is the formation of chemical links between molecular chains to form
three dimensional networks of connected molecules. There are several crosslinking
agents. Grubbs et al.371-375 developed ruthenium carbene complexes to be used as
catalyst for ring closing olefin metathesis reactions. Such complexes are frequently used
as precursors for the ring-closing olefin metathesis reactions.376,377 Clark and Ghadiri
used Grubbs catalyst to covalently link cyclic peptides into cylindrical structures through
intermolecular olefin metathesis. Mingotaud et al. used Grubbs’ catalyst for the crosslinking of silica nanoparticles. Poche and coworkers\textsuperscript{66,378} used Grubbs catalyst to crosslink poly(\(\gamma\)-dec-1-enyl-L-glutamate) (PDLG). Figure 2.19 demonstrates the crosslinking of polypeptide through intermolecular olefin metathesis.

Figure 2.19. Crosslinking of poly(\(\gamma\)-dec-1-enyl-L-glutamate) with Grubbs catalyst. (a) structure of PDLG, (b) structure of Grubbs catalyst.
CHAPTER 3
EXPERIMENTAL

3.1. Materials

Chemicals and solvents were obtained from typical commercial sources with the highest purity available and used without further purification (Table 3.1). The solvents used for the synthesis of the monomers were all anhydrous and purchased in Aldrich Sure-Seal™ bottles. Nanopure water was supplied by a Barnstead Nanopure Water System (18 MΩ). Unless otherwise noted, all non-aqueous reactions were carried out under dry nitrogen in a glove box.

3.2. Preparation of the Superparamagnetic Silica-Homopolypeptide Particles

As mentioned in earlier chapters, the composite particles of interest consist of a magnetic silica core grafted with a homopolypeptide shell. The particle preparation follows the hierarchical construction discussed in chapter 1. Therefore, the first step is the preparation of the core. The colloidal particles used as cores were individually prepared and functionalized the further growth of the particle. Synthesis of N-carboxy anhydride monomers of desired amino acids were followed by their initiation with the particle cores.

3.2.1. Synthesis of Colloidal Silica

Silica particles were prepared according to Stöber method through the hydrolysis of TEOS with base catalysis. Within the first hour the solution turned white. The solution allowed to stir over night until the hydrolysis of TEOS was complete.
Table 3.1. List of materials used for the syntheses.

<table>
<thead>
<tr>
<th>CHEMICALS</th>
<th>PURCHASED FROM</th>
<th>FORMULA WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-dioxane, anhydrous</td>
<td>Aldrich, 99.8%</td>
<td>88.11</td>
</tr>
<tr>
<td>3-aminopropyl-aminoethyl trimethoxy silane</td>
<td>Aldrich, 97%</td>
<td>22.36</td>
</tr>
<tr>
<td>9-decen-1-ol</td>
<td>Aldrich, 90%</td>
<td>156.27</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>Aldrich, 99%</td>
<td>108.14</td>
</tr>
<tr>
<td>Benzylidene-bis(tricycle-hexyl-phosphine)dichloro-ruthenium</td>
<td>Fluka, 97% prum</td>
<td>822.97</td>
</tr>
<tr>
<td>Chloroform-$d$</td>
<td>Aldrich, 99.8%</td>
<td>120.39</td>
</tr>
<tr>
<td>Citric acid monohydrate</td>
<td>Fisher, ACS</td>
<td>210.14</td>
</tr>
<tr>
<td>Cobalt(II)chloride hexahydrate</td>
<td>Aldrich, 99%</td>
<td></td>
</tr>
<tr>
<td>Methylene chloride (DCM)</td>
<td>Fisher</td>
<td>84.93</td>
</tr>
<tr>
<td>N,N-DMF</td>
<td>Aldrich, 99%</td>
<td>73.1</td>
</tr>
<tr>
<td>DOWEX 50Wx-4 resin</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>ethyl acetate, anhydrous</td>
<td>Aldrich, 99.8%</td>
<td>88.13</td>
</tr>
<tr>
<td>FeCl$_2$.xH$_2$O</td>
<td>Alfa Aesar, 99%</td>
<td>126.75</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>Aldrich, 97%</td>
<td>162.21</td>
</tr>
<tr>
<td>H$_2$SO$_4$,</td>
<td>Fisher</td>
<td>98.08</td>
</tr>
<tr>
<td>HCl standard, 1N</td>
<td>Fisher</td>
<td>36.46</td>
</tr>
<tr>
<td>Hexane, anhydrous</td>
<td>Aldrich, 95%</td>
<td>86.18</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>Fisher</td>
<td>36.46</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>Sigma, 99%</td>
<td>147.1</td>
</tr>
<tr>
<td>m-cresol</td>
<td>Sigma, 99%</td>
<td>108.14</td>
</tr>
<tr>
<td>Methyl sulfoxide-$d_6$</td>
<td>Aldrich, 99.9%</td>
<td>84.18</td>
</tr>
<tr>
<td>Methyltrimethoxy silane</td>
<td>Aldrich, 98%</td>
<td>136.22</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>Mallinkrodt</td>
<td>120.37</td>
</tr>
<tr>
<td>NaBH$_4$</td>
<td>Aldrich, 98%</td>
<td>37.83</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>Sigma, 99%</td>
<td>84.01</td>
</tr>
<tr>
<td>NaOH standard, 1N</td>
<td>Fisher</td>
<td>40.00</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>Fisher</td>
<td>40.00</td>
</tr>
<tr>
<td>Sodium silicate solution</td>
<td>Aldrich, 27%</td>
<td>242.23</td>
</tr>
<tr>
<td>$t$-butanol</td>
<td>Aldrich, 99.5%</td>
<td>74.12</td>
</tr>
<tr>
<td>Technical acetone</td>
<td>Fisher</td>
<td>58.08</td>
</tr>
<tr>
<td>tetraethylorthosilicate (TEOS)</td>
<td>Aldrich, 98%</td>
<td>208.33</td>
</tr>
<tr>
<td>Tetramethylammonium hydroxide (TMA)</td>
<td>Aldrich</td>
<td>91.15</td>
</tr>
<tr>
<td>Triethylamine</td>
<td>Mallinkrodt</td>
<td>101.18</td>
</tr>
<tr>
<td>Triphosgene</td>
<td>Aldrich, 98%</td>
<td>296.75</td>
</tr>
<tr>
<td>$\varepsilon$-carbomenzyl-oxo-$L$-lysine</td>
<td>Aldrich</td>
<td>280.32</td>
</tr>
<tr>
<td>$\gamma$-benzyl-$L$-glutamate</td>
<td>Sigma</td>
<td>237.26</td>
</tr>
</tbody>
</table>
The particles were washed with water and suitable dispersion solvents and collected by centrifugation. The washing and collection steps were skipped for the functionalization of silica particles. Particle size can be controlled by the concentration of the reagents used during the synthesis.

3.2.2. Synthesis of Magnetic Iron Oxide Particles Coated with Silica

40 mL of 1M FeCl₃, 10 mL of 2M FeCl₂ and 500 mL of 0.7M NH₄OH were mixed and ultrasonicated with Sonic Vibra Cell 750 for 30 minutes at 40 amplitude (amp.) power. The particles were collected via centrifugation and peptized with 60 mL of 1M tetramethylammonium hydroxide (TMA) and de-ionized water. The mixture was ultrasonicated for 10 minutes and filtered through a glass filter. 507 mL of the collected sample was mixed with 93 mL of 0.58 % SiO₂ (passed through ion exchange column packed with DOWEX 50Wx-4 and pH was immediately adjusted to 9.5 with silicate solution) and ultrasonicated for one hour at 25 amp. power. The solution was dialyzed against TMA at pH=10 for 2 days. The dialysis solvent was refreshed every 12 hours. The final solution was centrifuged with Sorval RC2-B ultracentrifuge at 2000rpm to remove the possible aggregates. The silica-coated superparamagnetic particles were coated with a second layer of silica by addition of 472 mL of absolute ethyl alcohol, 16.8 mL of NH₄OH and 0.4 mL of TEOS to 4.55 mL of iron oxide particles. The mixture was sonicated for 1 hour at 25 amp. power. The amino groups were attached on the surface by addition of 165 mL of 25 (v/v) % 3-aminopropyl-aminoethyl trimethoxy silane and 75 (v/v) % methyltrimethoxy silane mixture. The mixture was ultrasonicated for 30 minutes and concentrated to 1/3 of its original volume by rotary evaporator. The concentrated solution was ultrasonicated for 5-10 minutes to ensure the mixture was homogenized.
3.2.3. Superparamagnetic Cobalt Particles Coated with Silica

200 mL of 4.0 x 10^{-3} M NaBH₄ was prepared with 4.0 x 10^{-4} M citric acid. Various concentrations of citric acid can be used to obtain a desired particle size. The citric acid solutions were prepared with de-ionized water that was deairated and bubbled with dried and filtered nitrogen for 30 minutes. While sonication and bubbling with nitrogen continued, 200 mL of NaBH₄ was mixed with 0.2 mL of 0.4 M CoCl₂·6H₂O for 1 minute and 800 mL of absolute ethyl alcohol solution containing 14.4 µL 3-amino propyltrimethoxy silane and 169 µL tetraethylorthosilicate was added rapidly. The reaction continued for 30 minutes and particles were collected by centrifugation. Collected particles were dispersed in 500 mL of absolute ethyl alcohol and mixed with 0.1 mL of a mixture of 25% 3-aminopropyl-aminoethyl-trimethoxy silane and 75% methyl trimethoxy silane for an hour. The mixture was concentrated to 1/3 of its original volume by a rotary evaporator. The prepared sample can be redispersed in desired solvents for further reactions.

3.2.4. Silanization of the Particle Core

Silanization process is applied to all particles regardless of the core. Table 3.2 shows the functional groups used for the silanization of the particles. First, single functional groups were attached on the particle surfaces. These particles were used as control groups for the ones that contain organosilane functional groups on the surface. Mixtures of organosilane functional groups were added to particles dispersed in absolute ethanol. A typical silane mixture is 25% amino functional group from one of the amino silanes listed in Table 3.2 and 75% methyl silane. In the final solution the ratio of functional groups to the total volume of the solution is 0.0002. Once the functional
groups were added to the particle sol the solution was mixed for 30 minutes to provide the adsorption of the functional groups on the surface. In order to have the amino groups covalently attached to the particle surface the solution was condensed with a rotary evaporator at room temperature. Condensation continued until 2/3 equivalent of the original solution were collected.

Table 3.2. Functional groups used for the silanization reaction.

<table>
<thead>
<tr>
<th>FUNCTIONAL GROUP</th>
<th>CHEMICAL STRUCTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-aminopropyltrimethoxy silane (APTMS)</td>
<td><img src="" alt="Structure" /></td>
</tr>
<tr>
<td>2-amino ethyl-3-aminopropyltrimethoxy silane (AEAPTMS)</td>
<td><img src="" alt="Structure" /></td>
</tr>
<tr>
<td>methyltrimethoxy silane (MTMS)</td>
<td><img src="" alt="Structure" /></td>
</tr>
</tbody>
</table>

3.2.5. Synthesis of Amino Acids and their Corresponding NCA Monomers

Different homopolypeptides and even their copolymers can be used as the shell for the particles. Grafting is done through the ring opening polymerization of the monomer. Therefore, desired monomers were synthesized separately for each particle.

3.2.5.1. γ-Benzyl-L-Glutamate

70 g of L-glutamic acid and 250 mL of benzyl alcohol were mixed in a 500 mL round bottom flask. The temperature of the mixed solution gradually increased to 60-70°C. 70 mL of concentrated hydrochloric acid was added slowly with continuous
stirring. With the addition of hydrochloric acid a white precipitate formed and dissolved within 3 hours upon continuous heating at 60°C. The reaction solution was cooled to room temperature and the white slurry reformed. The slurry was poured into 4L of technical grade acetone and stored in the refrigerator for 4 days. After 4 days, the slurry was filtered and the white cake was dissolved in an ice-water mixture. As saturated NaHCO₃ was added, the ice-water mixture started to foam. The foam was removed by filtration and addition of saturated NaHCO₃ to the filtrate was continued until no foam formed. This procedure neutralized the solution. 200 mL of boiling water was added to this solution and a white solid formed as it cooled down to room temperature. The white solid which precipitated was suction filtered and recrystallized from water. The collected white precipitate was vacuum dried at room temperature (81.3% yield). This amino acid can also be obtained from Sigma-Aldrich. ¹H NMR (THF-d₆, TFA): Ar; 7.3(m), α-CHN; 8.2(s, b), benzylic CH₂; 5.1(2), α-CH; 4.2(t), β-CH₂; 2.2(m), γ-CH₂; 2.6(m).

3.2.5.2. γ-Dec-1-enyl-L-Glutamate

The procedure for the dodecyl-L-glutamate from Wasserman et al.²²⁹ is followed. 15g L-glutamic acid, 105 mL t-butanol and 75 mL 9-decen-1-ol were mixed and heated to 40°C in a 3-neck round bottom flask. 8.2 mL of H₂SO₄ was added slowly causing the solution to become clear and the temperature to increase. The temperature was adjusted and kept constant at 65°C. The solution was kept stirring at this temperature one more hour until it became totally clear. The heat was removed and while the solution continued stirring, 8.2 mL triethylamine was added rapidly to neutralize the free H₂SO₄ followed by the slow addition of 10.5 mL of de-ionized water and 262.5 mL of ethanol. Finally, an additional 31.5 mL of triethylamine was added drop wise. After standing for
30 minutes, a white precipitate formed. The solution was refrigerated over night. The precipitate was removed by suction filtration and washed with 200 mL of hot water and filtered again at 65°C. The filtrate was washed with methanol and ethyl ether. The solids obtained were vacuum dried over night (78.4% yield). $^1$H NMR (Chloroform-$d_6$, TFA): $\alpha$-CH; 4.2(t), $\beta$-CH$_2$; 2.3(m), $\gamma$-CH$_2$; 2.6(m), $\alpha$-CHNH$_2$; 7.8(s), O-CH$_2$; 4.2(t), -(CH$_2$)$_6$; 1.3(s), -CH$_2$CH=CH$_2$; 2.0(m), CH=CH$_2$; 6.0(m), CH=CH$_2$; 5.0(m)

3.2.5.3. $\varepsilon$-Carbobenzyloxy-$L$-Lysine

$\varepsilon$-carbobenzyloxy-$L$-lysine was purchased from Sigma-Aldrich and used as received. $^1$H NMR (THF-$d_6$, TFA): Ar; 7.3(m), $\alpha$-CHNH$_2$; 8.2(s, b), benzylic CH$_2$; 5.0(s), $\alpha$-CH; 3.6(t), $\beta$-CH$_2$; 2.1(m), $\gamma$-CH$_2$; 1.5(m), $\delta$-CH$_2$; 1.5(m), NH; 6.6(s, b), NH-CH$_2$; 3.1(m).

3.2.5.4. Preparation of NCA Derivatives from Amino Acids

The following procedure was used for the $\gamma$-benzyl-$L$-glutamate NCA, $\varepsilon$-carbobenzyloxy-$L$-lysine NCA and $\gamma$-dec-1-enyl-$L$-glutamate NCA. 5 grams of the corresponding amino acid were dissolved in 150 mL of anhydrous ethyl acetate in a 3-neck round bottom flask fitted with a reflux condenser and a nitrogen bubbler. The condenser was connected to a gas bubbler to collect the HCl or phosgene gas released during the reaction. The solution was heated to reflux and no more than 1/3 equivalent of triphosgene was added rapidly. The reaction continued to reflux under nitrogen for 4 hours. In cases where the reaction solution did not become clear after 4 hours of reflux a small amount of triphosgene was added (~0.001g). For oily NCAs such as decynyl-$L$-glutamate NCA, additional triphosgene was not used since it is harder to remove
unreacted phosgene from the oil. As the reaction solution got clear it was cooled down to -5°C. The purification followed the method of Poché. The cold solution was washed quickly in a chilled separatory funnel with 50 mL of cold de-ionized water chilled to 0°C, followed by a wash with 50 mL of 0.5% w/v NaHCO₃ chilled to 0°C. The cold water wash removes the unreacted monomer and other impurities. The organic layer was dried with anhydrous MgSO₄ as quickly as possible and gravity filtered. For oily NCAs, such as decynyl-L-glutamate, the solution with MgSO₄ was filtered through celite packed column.

Table 3.3. ¹H NMR data for NCAs.

<table>
<thead>
<tr>
<th>NCA</th>
<th>Solvent</th>
<th>¹H NMR Observed Chemical Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-benzyl-L-glutamate</td>
<td>THF- d₆</td>
<td>Ar;7.3(m), NH(ring);7.9(s), benzylic CH₂;5.1(s), α-CH₂;4.4(t), β-CH₂;2.1(m), γ-CH₂;2.5(m), Ar;7.3(m), NH(ring);7.9(s), benzylic CH₂;5.0(s), α-CH₂;4.4(t), β-CH₂;1.5(m), γ-CH₂;1.4(m), δ-CH₂;1.4(m), CH₂-NH₂;3.1(t), NH-CH₂;6.4(m), α-CH₂;4.5(t), β-CH₂;2.2(m), γ-CH₂;2.5(m), NH(ring);6.7(s), O-CH₂;4.1(t), -(CH₂)₆;1.3(s), CH₂CH=CH₂;2.1(m), CH=CH₂;5.8(m), CH=CH₂;4.9(m)</td>
</tr>
<tr>
<td>ε-carbobenzyloxy-L-lysine</td>
<td>THF- d₆</td>
<td></td>
</tr>
<tr>
<td>γ-dec-1enyl-L-glutamate</td>
<td>Chloroform- d₆</td>
<td></td>
</tr>
</tbody>
</table>

Usually oily NCAs get pale yellow by the end of the reaction and the filtration gets rid of the impurities causing the color deformation. The solution was concentrated to 1/3 of its original volume under constant flow of nitrogen in a glove box. The concentrated solution was kept at -5°C over night. As the solution cooled, white solid
cake precipitated at the bottom of the flask. The slurry was suction filtered next morning in a glove box under constant flow of nitrogen. The white slurry was vacuum distilled at room temperature overnight or until it was dry (~70% yield). For the oily NCA the solvent was evaporated completely with rotary evaporator by maintaining moisture free environment and vacuum dried at room temperature (~50% yield). The resulted product was a pale yellow oil for the NCA of decynyl-L-glutamate. $^1$H NMR results for the synthesized NCAs are shown in Table 3.3.

3.2.6. Grafting of the Polypeptide Shell

20 mL of amino functionalized particles were dispersed in anhydrous dioxane and added to 5.0 g of corresponding amino acid NCA which was already dissolved in 200 mL of anhydrous dioxane. The reaction flask was connected to a flask filled with 50 mL 1N NaOH. This way the CO$_2$ gas released during the polymerization reaction dissolved in NaOH forming NaHCO$_3$. This solution can be titrated for determination of the amount of CO$_2$ gas released. Bubbles observed throughout the experiment were also the evidence of the CO$_2$ gas released. The reaction carried on for 3 days under freshly dried and filtered nitrogen in a glove box. The final product was dense and aggregates within 2-3 days in dioxane. Once the solvent is changed to DMF or m-cresol the aggregation disappeared.

3.2.7. Crosslinking of Particles

For the crosslinking, particles were grafted with poly($\gamma$-dec-1-enyl-co-$\varepsilon$-carbobenzyloxy-L-lysine). Random copolymerization of the shell was achieved by addition of the mixture of the monomers to the particles. Grubbs’ catalyst, benzylidene-bis(tricyclohexylphosphine)dichlororuthenium, was used as the crosslinking agent. In a
typical crosslinking reaction particles were redispersed in dichloromethane (DCM) (2 mL) and mixed with the catalyst (10 mg of Grubbs catalyst dissolved in 1mL DCM). The mixture was kept in a water bath at 40°C for 30 minutes or until a gel formed.

3.3. Characterization Techniques

3.3.1. Light Scattering

For light scattering measurements a custom built apparatus with 6328 Å laser and ALV-5000 digital autocorrelator was used. Unless stated otherwise, measurements were made in a homodyne mode at different scattering angles from 30° to 90°. Diffusion coefficients and hydrodynamic radii of the particles were calculated. The apparent diffusion coefficient $D_{app}$ is defined as:

$$ D_{app} = \frac{\Gamma}{q^2} $$

where $\Gamma$ is the decay rate of the electric field autocorrelation function $g^{(1)}(t)$ and $q$ is the scattering vector defined as:

$$ q = \frac{4\pi n \sin(\theta/2)}{\lambda_o} $$

where $n$ is the solvent refractive index, $\theta$ is the scattering angle, and $\lambda_o$ is the wavelength. The apparent diffusion coefficient $D_{app}$ becomes the diffusion coefficient $D_o$ in the zero limits of concentration and $q$. Using the Stokes-Einstein relation the hydrodynamic radius can be calculated by:

$$ R_h = \frac{kT}{6\pi \eta_o D_o} $$

where $k$ is Boltzmann’s constant, $T$ is the absolute temperature, and $\eta_o$ is the solvent viscosity. Cumulants and single exponential analyses were used to fit the data.
Samples were prepared in dust free scattering test tubes in suitable clean solvents at 25°C. Common solvents used for the analysis of silica and superparamagnetic silica cores were Nanopure water and ethyl alcohol. For the polypeptide-grafted particles organic solvents such as; DMF, THF and m-cresol were used. Organic solvents were cleaned with centrifugation and filtration into dust free glass cells.

3.3.2. Electron Microscopy

Two different microscopy centers located on campus were used for the analysis of the particles. One of the facilities is the Socolofsky Microscopy Center in the Department of Biological Sciences. JEOL 100 CX, TEM and Cambridge 260 Stereoscan, SEM are the available electron microscopes in this facility. The other center is LSU Materials Characterization Center located in mechanical engineering.

3.3.2.1. Transmission Electron Microscopy (TEM)

Unless otherwise stated samples were deposited onto collodion-carbon coated copper specimen grids and air dried. If necessary samples were negative stained with 2% uranyl acetate or gold shadowed after deposition on the grid. Two different transmission electron microscopes were used for particle characterization. One of them is a JEOL JEM-2010 high-resolution transmission electron microscope (HRTEM) with an attached EDAX EDS system. The instrument provided bright and dark field imaging as well as selected-area electron diffraction (SAED), nano-beam and convergent-beam electron diffraction. Samples were investigated with an accelerating voltage of 200 kV at different magnifications. The other microscope used for routine analysis was JEOL 100-
CX TEM. Samples were investigated with an accelerating voltage of 80 kV at different magnifications.

3.3.2.2. Scanning Electron Microscopy (SEM)

Approximately 5 µL of the sample was deposited on small pieces of microscope slides and air dried. The sample slides were attached to an aluminum mounting stub with double sided tape and conductive paint. The samples were sputter coated with gold/palladium in an Edwards 5150 Sputter Coater and examined at 15kV with a Cambridge 260 Stereoscan SEM.

3.3.3. X-Ray Photoelectron Spectroscopy (XPS)

XPS measurements were carried on Kratos AXIS 165 X-ray Photoelectron Spectroscope and Scanning Auger Microscope. A computer controlled X-ray gun was used with a high performance Al monochromatic source. A starting energy of 1200 eV was applied with 0.5 eV step energy. Surface composition and element distribution during the surface of the specimen were acquired with XPS.

3.3.4. Optical Rotatory Dispersion Spectroscopy (ORD)

Optical rotation measurements were carried on a DIP 370 digital polarimeter from Jasco Inc. A mercury lamp was the light source and measurements were done at different wavelengths. The instrument is calibrated against empty sample compartment followed by the blank measurement. Optical rotation of PCBL-coated cobalt particles were measured in a temperature range of 10-50°C at wavelengths of 365, 598, 405, 546 nm.
3.3.5. **Optical Microscopy (OM)**

Optical microscopy techniques were used for the particle imaging. A Leitz-Metallux 3 confocal microscope was used for fluorescent particles as well as for a movie associated with making of these particles. The samples were loaded in Vitrocom cells and their response to an applied magnetic field was recorded through a CCD camera. Also, an Olympus Polarizing Microscope was used for the investigation of colloidal crystals formed under crossed polarizers. Crystals under investigation were formed in Vitrocom cells and great care was taken not to disturb these delicate crystals.

3.3.6. **Infrared Spectroscopy (IR)**

Early FT-IR transmission measurements were carried on a Perkin Elmer 1760 FT-IR using GRAMS 386 software. Samples were measured between KBR pellets. Each spectrum is an average of 8 to 16 scans. Recently most of the infra red spectra were recorded with Bruker Tensor 27 FT-IR Spectrophotometer. A Spectra Tech high pressure *in situ* circle reaction cell with a ZnSe ATR crystal was used as the sample cell. Spectra were analyzed with OPUS 4.2 software. The measurements were done with a resolution of 4 and 8-16 scans depending on the sample concentration. Particles prepared for IR measurements were dispersed in suitable solvent with a concentration of ca. 50-60ppm. Solvents used for particles preparation were measured as a blank before sample measurements.

3.3.7. **Nuclear Magnetic Resonance Spectroscopy (NMR)**

$^1$H NMR and $^{13}$C spectra were acquired with Brucker AC-250 and Brucker AC-300 spectrometers. Approximately 30-50ppm samples were prepared in suitable
deuteriated NMR solvents. With some amino acids and their monomers, a drop of trifluoroacetic acid was added to provide solubility of the sample.

3.3.8. Thermogravimetric Analysis (TGA)

Thermogravimetric analyses were performed using a Seiko EXSTAR 6000 system. The samples were vacuum dried for at least 24 hr before the analysis. During the analysis the samples were heated under nitrogen or air at a rate of 5°C/min to an upper limit of 800 to 1000°C.

3.3.9. Visible Spectroscopy

Visible spectroscopy was used for the analysis of the crystals formed by the particles. An Olympus BH2 polarized optical microscope was used to focus on the domains. The transmitted light was directed to a monochromator- detector system using fiber optics (SLM Aminco 8000, SLM Instruments, Inc).

3.3.10. Superconducting Quantum Interference Device (SQUID)

Dried samples were placed in gelatin capsules and measured inside quart tubes. Liquid samples were measured in custom build quartz tubes. Magnetic properties of the particles were measured by Magnetic Property Measurement System (MPMS)- SQUID from Quantum Design.
4.1. Synthesis and Characterization of Particle Cores

As explained in previous chapters the particle cores can be either nonmagnetic or magnetic. Colloidal silica cores were prepared via Stöber method.\textsuperscript{98} It is possible to control the particle size by changing the concentrations of the starting materials. While using freshly distilled TEOS delivered more uniformly shaped spherical particles, reducing water concentration in the reaction media decreased the particles’ size. Using absolute ethyl alcohol instead of technical grade ethyl alcohol is the simplest way to prepare smaller particles. Properties of silica particles prepared with different concentrations of starting materials are summarized in Table 4.1.

Table 4.1. Effect of EtOH, NH\textsubscript{4}OH, and TEOS concentrations on Stöber silica particle radius.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Concentration of reagent used, M</th>
<th>Particle radius, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[EtOH] 50 mL [NH\textsubscript{4}OH] 3 mL [TEOS] 1 mL</td>
<td>DLS</td>
</tr>
<tr>
<td>ST.4.19A</td>
<td>Tech. grade 7.189 Conc.</td>
<td>122</td>
</tr>
<tr>
<td>ST.4.19B</td>
<td>Absolute 7.189 Conc.</td>
<td>61</td>
</tr>
<tr>
<td>ST.4.19C</td>
<td>Absolute 5 Conc.</td>
<td>68</td>
</tr>
<tr>
<td>ST.4.19D</td>
<td>Absolute 3 Conc.</td>
<td>82</td>
</tr>
<tr>
<td>ST.4.19E</td>
<td>Tech. grade 3 Conc.</td>
<td>145</td>
</tr>
</tbody>
</table>
For the light scattering experiments, silica particles were washed and redispersed in Nanopure water and dilute solution prepared for the analysis. Figure 4.1 shows the dynamic light scattering results for each silica sample reported in Table 4.1. As seen from the figure, each $R_{\text{app}}$ vs. $q^2$ plot has a slope close to zero which signifies the particle uniformity. The error bars for this plot were calculated from 3 repeat measurements.

![Graph](image)

**Figure 4.1.** Calculated radius (from DLS) of synthesized Stöber silica particles (ST.4.19A-E) from Table 4.1 plotted against scattering vector magnitude. The average $\mu^2/\Gamma^2$ was calculated to be 0.025 for all samples.

The SEM and TEM images from Figure 4.2 prove the uniform distribution of silica spheres. Silica particles for layered structures as seen from Figure 4.2a and Figure
4.2b is a closer view of the particles. Figure 4.2 c-d are TEM images of these particles. The particles look less uniform in TEM images but this is mostly because of the overlapped particles. TEM can see through particles and this makes overlapped particles appear in irregular shapes.

Figure 4.2. Negative scans of the electron micrographs of silica spheres (ST.4.19C) prepared via Stöber method. SEM images (a) 1µm scale bar, (b) 200nm scale bar; TEM images (c) 1µm scale bar, (d) 0.5µm scale bar.
Preparation and maintaining the stability of magnetic particles, compared to silica particles, were harder. These particles were more susceptible to aggregation. Also, in order to obtain superparamagnetic particles the magnetic domains have to be in the 2nm-10nm size range. Otherwise, under an applied field the magnetic forces overcome the forces of dispersion. Each particle behaves as though it were a small magnet that is attracted to others.

The first magnetic silica particles worked on were magnetite, Fe₃O₄. The smaller sized particles were maintained by increasing the Fe³⁺/Fe²⁺ ratio. Another way to obtain smaller particles is changing the pH; but this can disable the stability of the silica coating. Once the particles were prepared they were immediately peptized with tetramethylammonium hydroxide (TMA). Particles were further coated with two layers of silica.

The initial magnetite particles obtained were aggregates of the magnetic components. Ultrasonicating the particles at 20-30 amp. power during the synthesis increased the stability and uniformity against the belief that sonication triggers aggregation. In Figure 4.3 TEM pictures of Fe₃O₄ particles coated with silica are shown. The darker spots inside the particles are the magnetic inclusions. Particles have multiple magnetic domains rather than single ones and the number of domains inside each particle varies. In Figure 4.3 picture a represents the low magnification appearance of the particles, whereas, images c and d are closer shots. Image b is focused on a single particle. The large dark spot in the center represents the large number of magnetic domains collected in the center of the particle. Individual magnetic domains can be seen more clearly in image d. Some particles respond to the applied field faster, which is
probably due to higher number of magnetic domains included within. This tendency is in agreement with Philipse’s paper. Silica coating on the particle surface can be controlled by addition of extra layers of TEOS.

Figure 4.3. Negative scans of TEM micrographs of Fe$_3$O$_4$ particles coated with silica (ST.2.40A). (a) 1µm scale bar, (b) 0.3µm scale bar, (c) 0.1µm scale bar, (d) 0.1µm scale bar.

The polydispersity of the particles was evident from the TEM pictures as well as the DLS results (Figure 4.4). The latex particles were used as a reference monodisperse sample. In polydisperse samples the scattering by large particle decreases as $q$ increases.
This makes the smaller, faster particles relatively more visible, causing the $D_{\text{app}}$ to increase with angle or $R_{\text{app}}$ to decrease. Even monodisperse particles can show negative slopes if they are not round and if they are sufficiently large. In such cases, DLS detects rotation. This may cause the variation in the $R_{\text{app}}$. The latex sphere used as a reference has an advertised diameter of 0.064 µm, which is in perfect agreement with the DLS result. This shows the almost perfect alignment of the instrument.

Figure 4.4. Dynamic light scattering results of Fe$_3$O$_4$ (ST.1.103A), silica-coated Fe$_3$O$_4$ (ST.1.106A) and latex (ST.1.104A) particles. Calculated particle radius plotted against scattering vector magnitude.

In this dissertation silica-coated cobalt particles are used as an alternative to silica-coated Fe$_3$O$_4$ particles. Alkaline media required during the amine functionalization of the particles decreases the stability of the magnetite particles and jeopardizes the
grafting of the polypeptide. Another disadvantage of magnetite particles is the time required for their preparation. Apart from the magnetite preparation, having the silica coating as a separate procedure adds to time elapsed. Preparation of silica-coated cobalt particles is indisputably more time efficient. Having a metal core makes the synthesis susceptible to oxidation; therefore, particles are coated with silica right after the cobalt particle formation. As explained in chapter 2, controlling the citric acid concentration and varying the [cit]/[Co] ratio affects the particle size significantly, as seen from Table 4.2.

### Table 4.2. Effect of citric acid concentrations on particle size of silica-coated cobalt particles from reference 221.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Concentration of reagent used, M</th>
<th>Particle radius,(^{221}) nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Cit] 20 mL</td>
<td>[NaBH(_4)] 20 mL</td>
</tr>
<tr>
<td>Co 1</td>
<td>4.0 x 10(^{-6})</td>
<td>4.0 x 10(^{-3})</td>
</tr>
<tr>
<td>Co 2</td>
<td>2.0 x 10(^{-5})</td>
<td>4.0 x 10(^{-3})</td>
</tr>
<tr>
<td>Co 3</td>
<td>4.0 x 10(^{-5})</td>
<td>4.0 x 10(^{-3})</td>
</tr>
<tr>
<td>Co 4</td>
<td>2.0 x 10(^{-4})</td>
<td>4.0 x 10(^{-3})</td>
</tr>
<tr>
<td>Co 4</td>
<td>4.0 x 10(^{-4})</td>
<td>4.0 x 10(^{-3})</td>
</tr>
</tbody>
</table>

As seen from Figure 4.5 particles are reasonably uniform. The lighter core seen from TEM pictures are the cobalt particles and the surrounding is silica coating. Almost all particles have an individual single cobalt core; it is not expected for all particles to carry one. Imperfections can occur during the synthesis. The cobalt cores are monodisperse with a radius of 8-10 nm. The size of the cobalt particles is very important
since the size provides the superparamagnetic nature to these particles. The diffraction pattern seen in Figure 4.5d, shows the amorphous nature of the particles. Some have a tendency to form chains as seen from the Figure 4.5b. This behavior can be explained in two ways. The particles could have become chained during the synthesis and coated with silica or, they were individually aligned while the samples were being prepared for the electron microscopy.

Figure 4.5. Negative scans of TEM images of silica-coated cobalt particles (ST.3.46A). (a) scale bar 50 nm, (b) scale bar 100 nm, (c) scale bar 50nm, (d) X-ray diffraction pattern of a single particle.
For particles to be superparamagnetic, the diameter of their magnetic inclusions should be smaller than 10-15 nm. TEM pictures proved that the cobalt particles fulfill that requirement. Magnetic properties of cobalt particles were measured with SQUID in the LSU Physics Department. Commercial superparamagnetic particles, only available as latex-coated Fe$_3$O$_4$, were used as a reference. Magnetization values for the samples were calculated per total masses of sample used for the measurement. Cobalt particles were proven to be superparamagnetic particles, $H_c \sim$0 Oe, as seen from Figure 4.6. Since the mass of the samples measured were not the same, it is a coincidence that the magnetization values for the samples are almost equal.

Figure 4.6. Comparison of magnetic properties of silica-coated cobalt particles (ST.3.93Aa) with commercial superparamagnetic latex (ST.4.17A). Both samples were dried in a vacuum oven for the measurements.
Once the particle cores were ready, the next step was to functionalize them to initiate the polymerization. Attachment and determination of the surface groups on particles were not easily accomplished. Amino groups attached to the surface were so low in concentration that titration methods did not work to characterize them quantitatively. There are no certain techniques available to quantify the amino groups on the surface. Even commercial products have only estimates of functional groups on the surface. Also, attachment of a dye to the surface of the particles was proven to be hard, especially if the functional group used is 3-aminopropyl methoxy silane. APTMS and APTES are the first type of amino silane groups used for the functionalization of these particles. The amount of functional groups on the surface was controlled by simply changing the concentration. With this approach some particles succeeded in the initiation of the polymerization but did not show the expected conformational transitions. Also, labeling these particles did not help to quantitatively analyze them with fluorescence spectroscopy; however, enough signal was present to prove the presence of the functional groups. To further investigate the presence of the -NH₂ groups, the Kaiser Test was used. This test is mostly used to determine the free amine for the peptides and biological molecules. A change in color confirmed the presence of the primary amines but it is not a quantitative verification.

2-D NMR techniques were used to compare silica particles with and without the amino functional groups on the surface. Bare silica particles did not give any signal as expected, since there were no protons except for the ones from the –OH groups. As seen from Figure 4.7, amino silane functionalized particles showed the same pattern with the 3-aminopropyltriethoxy silane. Other than these methods XPS (ESCA) and TEM-EDAX
were used to determine the amino groups on the surface. Both techniques showed the presence of carbon groups on the surface of the particles but NH$_2$ groups were too low in concentration to be detected. Since the only source of carbon and hydrogen is the amino silane group, NMR can indirectly prove the presence of amino groups.

Figure 4.7. High field (300 MHz) TOCSY NMR of (a) 3-aminopropyltriethoxy silane (ST.2.41A), (b) functionalized silica (ST.2.36A) particles in DMSO-$d_6$. 
Even though amino groups are too low in concentration for determination by titration, they are still concentrated enough per particle to cause crowding on the particle surface. One way to deal with crowding was decreasing the concentration of amino silane groups. As simple as it seems, this approach did not work since even with very low concentrations there were enough amino groups to cause crowding.

Menzel et al.\textsuperscript{49} mixed 1-(trichlorosilyl)undecane and 1-bromo-11-(trichlorosilyl)undecane to vary the ratios of Br groups on the surface. Later the Br groups were converted to N\textsubscript{2} by NaN\textsubscript{2} and then to NH\textsubscript{2} by LiAlH\textsubscript{4}. Instead of this prolonged procedure one can simply mix amino functionalized groups with a shorter silane group. This offers the advantage of having NH\textsubscript{2} groups on longer chains and shorter chains to function as a random spacer and surface passivator. Having a silane group without amino function decreases the number of NH\textsubscript{2} groups on the surface which has an enormous control on the crowding problem. From the commercially available organosilanes \textit{N}-[3-(trimethoxysilyl)propyl] ethylene diamine (AEAPTMS) is the one with the longest carbon chain whereas, methyl trimethoxy silane (MTMS) is the shortest silane without amine functional groups. A mixture of AEAPTMS and MTMS were used for the functionalization of the particles during this research. The particles labeled with ATTO-TAG (a dye that specifically binds to primary amines and does not fluoresce until attached) were proven to fluoresce under fluorescence microscope. Also, using trinitrobenzene sulfonic acid\textsuperscript{329} as a labeling reagent caused the amine functionalized particles to appear orange-red under a light microscope.

Functionalized and bare silica particles were dispersed in ethyl alcohol to give a final concentration of 50 mM and used for IR-spectroscopy. Absolute ethanol was used
for the background correction. The spectra from both samples were analyzed with Bruker OPUS NT-4 (Figure 4.8). The Si-OH stretching vibration at 3500 cm\(^{-1}\) is the characteristic peak of silica particles. The peak at 1050 cm\(^{-1}\) indicates the Si-O stretching which is also present in the silane functional groups. AEAPTMS was analyzed as a reference and the spectrum almost perfectly overlaps with the functionalized silica particles confirming the successful silanization reaction. The peaks at 2800 cm\(^{-1}\) are due to the aliphatic groups and the peak at 1550 cm\(^{-1}\) is due to the N-H bending vibrations and the peak at 750 cm\(^{-1}\) is due to the NH out of plane wagging introduced by the surface functional groups.

Figure 4.8. FTIR spectra of (--) AEAPTMS functional group (ST.4.29A), (--) bare silica particles (ST.3.59A), (--) functionalized silica (ST.3.59B) particles.
4.2. Characterization and Effect of Water on N-Carboxy Anhydrides

N-carboxy anhydrides are known to be very sensitive to moisture and water. The ring can open in neutral and acidic water resulting in a carbamic acid derivative (Figure 4.9). There is controversy on the decomposition of NCAs in the presence of water. Leuchs\textsuperscript{247} synthesized a NCA derivative of glycine in water at 0 °C and 15 °C. Starting from this point Poché \textit{et al.}\textsuperscript{259} introduced a new purification technique for NCAs with icy water. This section of the dissertation considers these approaches and investigates the reactivity of water with NCAs at different temperatures.

![Figure 4.9. Schematic presentation of the reaction of water with NCAs.](image)

NMR is a strong characterization technique for the effect of water on the ring opening. The sample was prepared in threaded NMR tubes with a PTFE septum cap in a
glove box. After the sample was loaded, the NMR test tube was flushed with dry nitorgen through the septum and closed tight. Water was later introduced through the cap. The sample concentration was 60-80 ppm and the solvent was THF-$d_6$. If the sample did not dissolve right away, a drop of TFA was added to ensure solubility. Just looking at the $^1$H and $^{13}$C spectra it is hard to tell if the decomposition started at room temperature but definitely a drastic change occurred after heating the sample to 50°C. Figures 4.11 and 4.12 show the 1D NMR spectra for CBL and BLG NCAs. The peak assignments of both structures are shown in Figure 4.10. Both carbons and protons are labeled with the same letter consistently for CBL and BLG NCA. Significant changes were observed in the 2D NMR spectra.

![Figure 4.10](image-url)  
Figure 4.10. MR labeling for (a) CBL-NCA, (b) BLG-NCA. Each letter corresponds to the carbon and proton atom at that location.
Both 1D and 2D spectra confirm the NCA is stable at low temperatures (0°C-15°C) even in the presence of water. The results support the cold water wash purification technique proposed by Poché et al. Figure 4.11 and Figure 4.12 show the proton and carbon NMR of the two most commonly used NCAs in this dissertation. Both samples were prepared and measured under the same conditions.

Figure 4.11. High field (500 MHz) (a) $^1$H NMR spectra, (b) $^{13}$C NMR spectra for CBL-NCA in DMF-$d_6$ (ST.3.92A). Temperature was gradually increased after the addition of water.

As seen from Figure 4.11 new peaks appeared after the sample was heated to 50°C. As the ring opens especially the carbonyl group signals shift but not all the previous peaks disappear. While the new peaks were appearing the previous peaks lost
intensity. The figures are zoomed in for better presentations; therefore it is difficult to see the decrease in the intensity. Similar deformation of the ring was observed for the BLG-NCA sample.

Following the 1D spectra, 2D spectra were accumulated before and after the addition of water. From the 2D spectra, it is possible to assign carbon and proton peaks considering their interactions with each other.

Figure 4.12. High field (500 MHz) (a) $^1$H NMR spectra, (b) $^{13}$C NMR spectra for BLG-NCA in DMF-$d_6$ (ST.3.131A). Temperature was gradually increased after the addition of water.
Figure 4.13. High field (500 MHz) HSQC spectra for CBL-NCA in DMF-$d_6$ (ST.3.92A) at 298K, before the addition of water.

Figure 4.14. High field (500 MHz) HMBC spectra of CBL-NCA in DMF-$d_6$ (ST.3.92A) at 298K, before the addition of water.
Figure 4.15. High field (500 MHz) HSQC spectra for CBL-NCA in DMF-$d_6$ (ST.3.92A) at 323K, after the addition of water.

Figure 4.16. High field (500 MHz) HMBC spectra of CBL-NCA in DMF-$d_6$ (ST.3.92A) at 323K, after the addition of water.
Figure 4.17. High field (500 MHz) COSY spectra of CBL-NCA in DMF-$d_6$ (ST.3.92A) at 323K, after the addition of water.

Figure 4.18. High field (500 MHz) TOCSY spectra of CBL-NCA in DMF-$d_6$ (ST.3.92A) at 323K, after the addition of water.
Figure 4.19. High field (500 MHz) HSQC spectra of BLG-NCA in DMF-$d_6$ (ST.3.131A) at 298K, before the addition of water.

Figure 4.20. High field (500 MHz) HSQC spectra of BLG-NCA in DMF-$d_6$ (ST.3.131A) at 298K, before the addition of water.
Figure 4.21. High field (500 MHz) HSQC spectra of BLG-NCA in DMF-$d_6$ (ST.3.131A) at 323K, after the addition of water.

Figure 4.22. High field (500 MHz) HMBC spectra of BLG-NCA in DMF-$d_6$ (ST.3.131A) at 323K, after the addition of water.
Figure 4.23. High field (500 MHz) COSY spectra of BLG-NCA in DMF-$d_6$ (ST.3.131A) at 323K, after the addition of water.

Figure 4.24. High field (500 MHz) TOCSY spectra of BLG-NCA in DMF-$d_6$ (ST.3.131A) at 323K, after the addition of water.
Another technique that can be used in the determination of the reactivity of water with NCAs is infrared spectroscopy. CBL-NCA solution was prepared in THF, with a concentration of 40ppm. The sample was gradually heated in the presence of water (Figure 4.25).

![FTIR spectra of CBL-NCA in anhydrous DMF (ST.3.92A).](image)

Figure 4.25. FTIR spectra of CBL-NCA in anhydrous DMF (ST.3.92A). (—) at 0°C no water added and (—) at 15°C, (—) at 25°C, (—) at 35°C, (—) at 50°C with water.
As seen from Figure 4.25a-b the intensity of the peaks increased as the solution was heated to 50°C. The intensity change indicates the formation of new N-H or N-C bonds in addition to the original peaks. The change in the structure decreases the intensity of the original peaks while addition of new peaks contributes to newer peaks with similar wavenumbers and increase intensity in some regions. NH\textsubscript{2} stretching peak around 3200cm\textsuperscript{-1} shifted slightly to a higher wavenumber after the addition of water at temperatures above the room temperature. In Figure 4.25b a new peak observed at 1550 cm\textsuperscript{-1}, which is within the region of N-H bending vibrations. In general changes in peaks and intensities observed after heating the solution to room temperature and above. These results are consistent with the NMR results, concluding that NCA monomers are not very sensitive to moisture as they are at temperatures around room temperature. As seen from the spectra the baselines are not perfect. The Spectra Tech high pressure in situ circle reaction cell used for the analysis caused noisy baseline as the solution continuously heated. The positive peaks observed are due to solvent and heat exchange during the analysis. Eventhough, the baseline are not perfect the information can be used to fingerprint the characteristic peaks and the changes observed in the presence of water.

4.3. Synthesis and Characterization of Silica-Homopolypeptide Particles

The first core-shell particles developed in this lab were nonmagnetic. The core of the particles was made up of silica and poly(benzyl-L-glutamate) was chosen as the homopolypeptide shell.\textsuperscript{358} The particles were characterized with DLS, electron microscopy, IR spectroscopy and thermal gravimetric analysis and found to be monodisperse. Assuming a single-sized particle, 20% of the mass was found to be the polypeptide shell mostly in \( \alpha \)-helix conformation. Poly(carbobenzyloxyL-lysine) was
chosen as the shell for the second batch of particles. The shell provided anticipation for a transition between the secondary structures.

Figure 4.26 shows a SEM picture of poly(carbobenzyloxy-L-lysine)-grafted silica particles. The picture shows reasonably uniform and spherical particles. Some distortion from spherical structure may happen during the sample preparation for SEM. Distortion and shrinkage occurs mostly while samples dry before the gold/platinum sputtering of the SEM stub.

![SEM picture of PCBL-coated silica particles (ST.2.7A).](image)

As seen from the dynamic light scattering results, shrinkage of the particles was minimal. Based on multiple angle measurements in DMF, the hydrodynamic radius was calculated to be $252 \pm 1 \text{ nm}$. Both third cumulant and single exponential analysis gave essentially the same result. The ratio of the second cumulant to the square of the second, $\mu_2/\Gamma^2$ should be zero for perfectly uniform particles. For PCBL-coated silica particles this ratio was found to be 0.04 which is low enough to conclude on the uniformity of the
particles. In a polydisperse sample, since larger particles scatter weaker at higher angles, faster and smaller particles get crucial. Therefore with polydisperse samples the $R_h$ vs. $q^2$ plots slope have a tendency to increase. As seen from Figure 4.27 the hydrodynamic radius is independent of the angle, which accounts for the particle uniformity. The inset plot shows the $\Gamma$ versus $q^2$ which is an indicator of monodisperse particles. The linear $R_{app}$ versus $q^2$ plot also indicates uniform particles with an average hydrodynamic radius of 252 nm.

![Figure 4.27](image)

Figure 4.27. Calculated radius of PCBL-coated silica particles (ST.2.7A) plotted at different scattering vectors. Inset is $\Gamma$ vs. scattering vector plot.

Solutions of uniform colloidal particles can form crystals if they are sufficiently dense. The large lattice spacing of such crystals causes the diffraction of visible light. Some of these particles were dispersed in Vitrocom cells in DMF and $m$-cresol for the
determination of crystal formation which is very slow, typically 3-4 weeks.

Centrifugation speeds up the crystal formation, while sacrificing formation of larger crystals. The crystals were very delicate that even a gentle tilting could destroy them. As shown in Figure 4.28 the formed crystals have remarkable colors between crossed polarizers under microscope.

Figure 4.28. Photomicrographs of colloidal crystals of PCBL-coated silica particles (ST.1.128), between crossed polarizers. (a) and (c) variety of colors due to different domain orientations; (b) and(d) detail of bands due to crystal twinning.
Monovoukas and Gast\textsuperscript{382} showed the bands crossing some crystal domains change colors as the sample stage rotates, which indicates crystal twinning. They explained the appearance of different colors observed between crossed polarizers regardless of the cubic symmetry of the colloidal crystals. The particles seen in Figure 4.28 followed the explanation above. Once the sample was rotated 90 degrees, the original band colors were restored. The color purity of the crystals was confirmed by the spectrometric analysis. An Olympus BH2 polarized optical microscope was used to focus on several domains followed by directing the transmitted light to a monochromator/detector system. Bandwidths of 20 nm were obtained (Figure 4.29).\textsuperscript{359}

![Transmission spectrum](image)

**Figure 4.29.** Transmitted light intensity vs. wavelength for PCBL-grafted silica composite particles (ST.3.128A). Imaged region includes 3 domains.
Figure 4.30. Silica core (ST.4.19A) and PCBL-coated silica particles dispersed in anhydrous pyridine with increasing monomer concentrations (ST.4.25A-E). (a) Monitoring of particle shell thickness via controlled addition of the monomer. Radius was calculated from DLS plotted at different monomer concentrations. Inset is $\mu_2/\Gamma^2$ vs $[M]$ plot, each measurement were carried at different angles. (b) Radius vs. scattering vector of particles measured at different monomer concentrations.
Figure 4.31. PCBL coated silica particles dispersed in anhydrous pyridine with increasing monomer concentrations (ST.25A-E). The particles radius of gyration were measured at different scattering angles (30°-80° and 90°) after 2 months.

Ring opening polymerization of the NCAs is a living polymerization. The amino end of the polypeptides can continue initiating the polymerization; therefore, continuous addition of the monomer will increase the shell thickness. Figure 4.30 demonstrates the increase in the shell thickness with an increasing monomer concentration in pyridine. The polymerization was continued for three days followed by the addition of the monomer. Figure 4.30a demonstrates the particles are still growing three weeks after the original measurements. As seen from the inset the particles are somewhat monodisperse since $\mu_2/\Gamma^2$ values are close to zero. Figure 4.30b shows the $R_{app}$ vs. $q^2$ plot which
demonstrates uniform particle behavior at different scattering angles as well. Same samples were further investigated after 2 months and this time radius of gyration is obtained from static light scattering experiments. \( R_g \) values were calculated from the slope of the Guinier plot. Measurements at lower angles indicated slightly larger values which may suggest aggregation after 2 months. Figure 4.31 shows that the particle core size stays same, while the monomer concentration is increasing. This result further proves that only particle shell size increases with the polymerization and the core size is not affected as expected.

One of the major goals of this research was to develop responsive particles through the helix-coil transformation of the polypeptide shell. None of the above particles fulfilled that anticipation. One of the reasons for non-responsive particles is surface crowding. The discussed particles were surface functionalized with aminopropyl triethoxy silane without the modifications suggested in section 4.1.

4.4. Characterization of Superparamagnetic Silica-Polypeptide Particles

For polypeptide coating of magnetic silica particles, \( N \)-carboxy anhydride monomers are grown from the surface. Methods for attaching pre-made polypeptides to surfaces have been developed, but for our colloidal particles, the rodlike polymer can destabilize silica dispersions. The polypeptide shell prepared by ring opening polymerization of NCA-CBL or NCA-BLG initiated by primary amines as explained in earlier chapters.

The first magnetic cores used in this lab were iron oxides coated with silica. The particles were surface functionalized with aminopropyl triethoxy silane and followed by the grafting of the poly(carbobenzyloxy-\( L \)-lysine). The sample was a viscous, brown
liquid with a slight response to a magnet. Sample was prepared by drying in vacuum oven at room temperature over night. Thermogravimetric analysis showed that 12.32% of the sample was silica or magnetite. The initial loss of 1.25 % was because the sample was not dried enough before the analysis. Around 300°C 45% of the sample was lost mostly due to the decomposition of the polymeric shell (Figure 4.32).

Figure 4.32. Thermogravimetric analysis of the Fe$_3$O$_4$ magnetic silica particles (ST.1.109A) with PCBL shell. (—) weight % vs. temperature, (—) derivative weight vs. temperature.

Particles were responsive to applied field. On the other hand, they did not demonstrate the helix-coil transition in $m$-cresol similar to previously prepared non-magnetic core shell particles. The possibility that a large number of polymer groups were on the surface was responsible for the deficiency of the helix-coil transition.
Furthermore, the reproducibility of the experiment was low, as the amine functionalization was causing the destabilization of the magnetite particles.

Replacing the magnetite with cobalt particles coated with silica hastens the production of the polypeptide coated particles. The first attempts of controlling amino groups on particle surface were tried and success was achieved on these particles. The first step was introduction of the silane mixture to the cobalt particles. Once the particles were functionalized with 25% AEAPTMS and 75% MTMS the polymerization of ε-carbobenzyloxy-L-lysine N-carboxy anhydride was initiated in anhydrous ethyl acetate. The sample was viscous and slightly grayish in color. Particles were collected by centrifugation and redispersed in DMF or m-cresol. Thermogravimetric analysis demonstrated 8.2% of the sample is possibly silica and residual cobalt (Figure 4.33). The initial loss occurs around 300°C. The decomposition ends with a total loss of 81.8% mass loss at 580°C with a white-light gray solid residue.

Fong et al. calculated the particle core and the shell thickness to estimate the effective polypeptide density on the particle surface. By using a similar approach, the total mass of the PCBL-coated cobalt-silica samples can be estimated from

\[
m_{\text{tot}} = \frac{4\pi}{3} R_{\text{Co}}^3 \rho_{\text{Co}} + \frac{4\pi}{3} (R_{\text{Co-Si}}^3 - R_{\text{Co}}^3) \rho_{\text{SiO}_2} + \frac{4\pi}{3} (R_{\text{tot}}^3 - R_{\text{Co-Si}}^3) \rho_{\text{PCBL}}
\]

where \( R_{\text{Co}} \) is the radius of cobalt particles, \( R_{\text{Co-Si}} \) is the radius of silica-coated cobalt particles and \( R_t \) is the overall particle size after the grafting of the polypeptide. The density of cobalt, \( \rho_{\text{Co}} \), is 8.9 g/cm³, density of SiO₂, \( \rho_{\text{SiO}_2} \), is 1.96 g/cm³ and the density of
the PCBL, \(\rho_{\text{PCBL}}\), is 1.26 g/cm\(^3\). The three terms calculated include the cobalt core center, silica-coated cobalt core, and the overall particle with the polypeptide shell respectively. Using the above equation, mass of the core was computed as 7.4% of the total mass of a particle. Particle radius sizes were estimated from the TEM photomicrographs (not shown for this batch of synthesis). The difference between the values obtained from TGA and the computed values may be due to the shrinking of particles during the sample preparation for TEM.

Figure 4.33. Thermogravimetric analysis of magnetic cobalt-silica particles coated with PCBL (ST.3.94B). (-) weight % vs. temperature, (-) derivative weight vs. temperature

PCBL-coated silica particles were dispersed with \(m\)-cresol in clean glass test tubes for light scattering analysis. The test tubes were washed with deionized water
several times and checked for any dust speckles under the laser beam. Then test tubes were vacuum dried and loaded with the solvent. Filtered \( m \)-cresol was centrifuged for 14 hours at 7000 rpm in order to clear up the solvent from dust. Top portion of the solvent was carefully removed and filtered twice before introducing the particles. The solution has a light gray color at first and with time as \( m \)-cresol picked up some moisture from the air and the solution turned slightly brown.

Based on multiple angle measurements in \( m \)-cresol, at room temperature the hydrodynamic radius was calculated to be 299± 2 nm. Sample was heated and cooled gradually for the determination of the helix-coil transitions. The sample solution was kept at constant temperature to maintain thermal equilibration for 30 minutes between each temperature interval. The effect of temperature change to the particle radius was investigated with dynamic light scattering (Figure 4.34).

It was shown that poly(carbobenzyloxy-\( L \)-lysine) transforms from coil to \( \alpha \)-helix at 27°C in \( m \)-cresol. Multi temperature measurements were done at an angle of 75°. Temperature was varied from 10°C to 50°C. Hydrodynamic radius was measured through consecutive heating and cooling cycles. The particles showed similar trends. As seen from Figure 4.34a, the first two cycles were reproducible and did not show any hysteresis. The cooling of the 3rd cycle was considerably different from the others. In order to see the validity of this result a 4th heating cycle was done and found to be in correlation with the previous cycles. The discrepancy of the 3rd cooling could be some indeterminate error. The error bars in this plot were calculated from 3 repeat runs.
Figure 4.34. DLS results of PCBL-coated cobalt particles (ST.3.47A) in m-cresol. (a) Temperature dependence of hydrodynamic radius of PCBL particles. (b) $m^2/G^2$ vs. temperature plot, each point is average of all heating and cooling cycles from a. (c) Radius of the particles measured at three 15°C, 25°C, and 50°C plotted at different scattering angles.
Figure cont’d
The monodispersity of the particles were demonstrated in Figure 4.34.b, where average $\mu_2/\Gamma^2$ is found to be 0.05. In order to rule out the possibility of the aggregation of the particles a series of measurements were done at different scattering angles for 15°C, 25°C, and 50°C (Figure 4.34.c). At all three temperatures particles demonstrated uniform distributions. In case of aggregation a deviation from linearity would be expected. These DLS results prove the existence helix-coil transition.

A clear transition around 27°C can be seen from the above plot. Radius of the particles significantly increased from 300 nm to 380 nm within 40 degrees. The helix-coil transition demonstration of the particles is in agreement with the previously published results for PCBL in $m$-cresol. Pure PCBL happens to have a sharper transition compared to cobalt particles coated with PCBL. This effect is under investigation.
Helix-coil transitions of polypeptides, especially PCBL and PBLG, were investigated broadly in the last quarter of the 20th century. Optical rotatory dispersion (ORD) and circular dichroism spectroscopy were the main techniques used to determine this transition. \(^\text{71,79,236,276,277}\) In order to reproduce the helix-coil transition of these particles their optical rotations were measured in \(m\)-cresol. Measurements were done at temperatures from 15°C to 40°C at different wavelengths. The instrument was calibrated against aqueous sucrose and PBLG in DMF (Figure 4.35). PBLG solution has low optical rotation values even at reasonably high concentrations.

\[y = 0.85x - 0.09\]
\[R = 0.99885\]

\[y = 0.029x - 0.006\]
\[R = 0.96963\]

Figure 4.35. ORD calibration curves for (a) aqueous sucrose (ST.4.27A-D), (b) PBLG-110K MW (ST.4.28A-C) in DMF, measured at 546 nm.
Considering the amount of polypeptide required for satisfactory measurements, it is obvious that the particle solution will be turbid. Figure 4.36 shows the results of PCBL-coated cobalt particles from the same batch used for the DLS measurements. These results suffer from the problem mentioned above. Also \textit{m}-cresol is sensitive to moisture and oxygen. Solvent turns yellow-orange with time which effects the measurement due to the absorbance of the solvent. Measuring with longer integration time improved reproducibility slightly. Instrumental limitation is another problem to be considered. The lowest degree Jasco DIP-370 can read is 0.001 with an accuracy of 0.002. Figure 4.36 demonstrates the effect of temperature on the optical rotation of particles. There seems to be a transition trend around the expected temperature but the accuracy of the data is questionable mainly due to high signal to noise ratio.

Figure 4.36. Temperature dependence of the optical rotation of Co-PCBL (ST.3.47A) in \textit{m}-cresol measured at different wavelengths.
In peptides and proteins, three absorption bands are of particular importance. The amide A Band characterizes the N-H-stretch vibration, the amide I band characterizes the C=O-stretch vibration and the amide II band characterizes the N-H bending vibration. These 3 oscillations belong to the backbone of the polypeptide chain and are therefore relatively intense. In α-helices, β-strands or random coils the coupling of these oscillators is different and therefore the infrared spectra of a peptide are dependent on the secondary structure. In β-sheet the amide I band, which is observed at 1700 cm\(^{-1}\) for a free C=O vibration, is observed at 1655 cm\(^{-1}\) for a random coil structure and at 1650 cm\(^{-1}\) in the α-helical conformation. After a transition induced by high pH, the amide I band is split into a weak band at 1690 cm\(^{-1}\) and a strong band at 1620 cm\(^{-1}\). By simply comparing the amide bands, it is hard to distinguish between the α-helix and random coil. From the IR spectra shown in Figure 4.37, an insignificant shift in the amide I bands is seen as temperature increased which may indicate a transition with temperature. The noise caused the base line to shift slightly and some positive peaks occurred due to the heating effect of the solvent as expected. The spectra presented are not modified and used to demonstrate the effect of temperature on the secondary structure of the polypeptide shell. The peak around 1700 cm\(^{-1}\) can indicate the presence of β-sheet as well as the α-helix structure. Also, the amide V bands at 610 cm\(^{-1}\) and 704 cm\(^{-1}\) (not shown) indicate the presence of β-sheet and α-helix. Amide A peak shifted significantly as the temperature increased. All these shifts can demonstrate the thermoresponsive transition of PCBL-coated cobalt particles but do not confirm its existence. As explained on page 92 the spectra have noisy backgrounds due to the high pressure cell used for the analysis. Both ORD and IR data are used to support the results obtained from DLS. The results confirm...
the polypeptide shell was successfully grafted to the particles and mostly present in the α-helix conformation.

Figure 4.37. The infrared spectra of Co-PCBL particles in m-cresol (ST.3.47A) at different temperatures. (a) Amide I-II region (b) Amide A region. Dashed line is drawn as a reference to eye.
Several other techniques were used for the characterization of these particles. XPS is one of them. It is a very common characterization method for flat surfaces. Having spherical particles reduces the number of applicable surface characterization techniques. Since XPS can be used to determine the groups present on the surface, we may use it for spherical particles, as well.

Sample solutions for the XPS analysis were prepared in two different ways. A regular magnet was used to collect particles on the side of the sample vial. A non solvent was used for the precipitation of particles with polypeptide shell and vacuum dried at room temperature. Samples in solution were prepared on microscope slides simply by forming thin layers. 5-10 µL of particle solution was transferred to a clean microscope slide. Another clean microscope slide was used to spread the sample evenly across the surface with a single motion. The formed thin layer was initially air dried for one hour and then vacuum dried in a vacuum oven at room temperature for 24 hours. Solid samples were simply vacuum dried for 24 hours and placed in XPS sample holders for further analysis.

Before going through further analysis one question that comes to mind was, whether the polypeptide shell was effectively attached to most of the cobalt particles. Since having another layer on the cobalt particles could decrease their response time to the applied field, magnetically collected samples can be used to determine this question. Figure 4.38 shows XPS survey results obtained from silica core and cobalt silica particles with the polypeptide shell. The difference between the two Co-PCBL particles is the way they were collected. Both Co-PCBL samples showed the presence of the polypeptide on the surface through the increase in the intensity of C and N peaks. Comparing silica
particles to functionalized silica particles N 1s peaks indicates the successful attachment of the amino silane group on the surface. Intensity of the N 1s peak increases as the polymer is attached to the surface as well as the C 1s peak.

Figure 4.38. X-ray photoelectron survey spectra of (a) silica particles (ST.3.59A) (b) amino functionalized silica particles (ST.3.59B) (c) Co-PCBL particles collected via magnet (ST.94Bb) (d) Co-PCBL particles collected via precipitation (ST.3.94Ba).
Figure 4.39 compares individual elemental line scans from each sample. The decrease in the Si 2p for functionalized silica particles compared to bare silica particles indicates the attachment of the amino silane group on the surface. Since each layer adds up to the thickness of the sample, signal intensity coming from the bottom layer diminishes. The intensity further decreased with the addition of the polypeptide layer on the surface. The slight shift of the Si 2p peak can be due to the difference in the core of the particles. Co-PCBL particles have silica-coated cobalt as the core whereas silica particles did not carry cobalt core in the center. Even if the sample had the cobalt core it is not possible to see the cobalt embedded inside silica. The shift could also indicate the polypeptide attachment affects the particles. Even though bare silica particles do not have carbon on the surface some of the impurities or methoxy groups which stayed attached to the surface can contribute to the peak. As other layers added to the particle the increase in the intensity of the carbon proves the attachment of the polypeptide. N 1s is one of the hardest groups that can be determined with these particles because the nitrogen concentration is very low compared to the other elements on the surface. It is apparent that the intensity of the nitrogen increases with the polypeptide attachment since they contain NH2 groups on the top layer of the surface. Finally O1s intensity decreases and shifts slightly as the polypeptide is attached to the surface. For silica particles, oxygen source is predominantly the Si-O-Si that forms the particles as well as the OH groups on the surface. As the polypeptide attached to the surface these groups remain at the bottom layer of the sample which makes it difficult to detect them. The new oxygen group closer to the surface is coming from the polypeptide and they are not as abundant as Si-O, so the intensity decreases. Also the peak shifts slightly since the oxygen sources are different.
Table 4.3 shows the atomic compositions and mass percentages of the carbon, nitrogen, oxygen and silica present in the samples. In the spectra the baselines are not perfectly equal; therefore the appearance of the intensity may seem to be misleading to the eye, but the calculated compositions support the data and the explanations above. In favor of the two Co-PCBL samples collected with different methods it is clear that carbon, oxygen, silica and nitrogen contents were very close. The slight increase in nitrogen content and a slight decrease in silica content can be explained as follows: since
polypeptide free cobalt particles do not precipitate with the polymer, they have a slightly higher polymer content compared to the magnetically collected particles. Having similar composition for the Co-PCBL samples proves the presence of the polypeptide shell on the surface of the core. There is a significant increase in the composition of carbon and nitrogen compared to bare silica particles. It is easier to determine the changes in the carbon and nitrogen content since those form the top layer of the surface. XPS can only detect within the 10-20nm of the surface of the samples; an additional layer of polymer makes the determination of oxygen and silica weaker. The strong oxygen peak coming from the SiO₂ is replaced with the oxygen coming from the polypeptide. Also having a major decrease in the silica content confirms the presence of the extra layer of the polypeptide on the surface.

Table 4.3. Composition of samples from XPS. a collected via magnet, b collected by precipitation.

<table>
<thead>
<tr>
<th>ELEMENTS</th>
<th>ATOMIC CONCENTRATIONS</th>
<th>MASS PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Silica ST.3.59B</td>
<td>Co-PCBL&lt;sup&gt;a&lt;/sup&gt; ST.3.94Bb</td>
</tr>
<tr>
<td>C-1s</td>
<td>10.74</td>
<td>80.02</td>
</tr>
<tr>
<td>N-1s</td>
<td>0.46</td>
<td>6.56</td>
</tr>
<tr>
<td>O-1s</td>
<td>57.48</td>
<td>11.98</td>
</tr>
<tr>
<td>Si-2p</td>
<td>31.32</td>
<td>1.44</td>
</tr>
</tbody>
</table>

As shown in section 4.1 cobalt particles are superparamagnetic. For the magnetization measurements of Co-PCBL, sample was dried and measured inside a gelatin capsule at 27°C. As seen from Figure 4.40 the particles showed some hysteresis
with a 0.00159 emu/g remanent magnetization. Specific magnetization drastically decreased compared to silica-coated cobalt particles. The hysteresis may be due to the diamagnetic nature of the polypeptide.

Figure 4.40. Magnetic hysteresis curve for PCBL-coated cobalt particles (ST.3.94B) at 300K. Sample was dried in vacuum oven.

Earlier in this chapter it was shown that polypeptide-coated particles responded to temperature changes in m-cresol. This characteristic raised the question whether this transition can affect the magnetic properties of the particles. Magnetization of PCBL-coated cobalt particles dispersed in m-cresol (10 mg/L) was measured in Teflon capsules at various temperatures. Figure 4.41a shows the magnetization of cobalt silica particles
normalized with the applied field. This sample was used as a reference to PCBL-coated cobalt particles shown in Figure 4.41b.

Figure 4.41. M/H vs. H plots for (a) Silica-coated cobalt core particles (ST.3.93Aa) and (b) PCBL-coated cobalt core-shell particles (ST.3.94B) dispersed in m-cresol.
Figure 4.42a shows magnetization of PCBL coated cobalt silica particles at all magnetic fields applied during the experiment. Figure 4.42b shows the magnetization measured with selected fields at various temperatures. The plot demonstrates some type of temperature dependence. The particles showed a decreasing trend in their magnetic properties around 27°C, which is the PCBL helix-coil transition temperature. Cobalt-silica particles without the polypeptide shell were used as the control group. As seen from Figure 4.43a-b particles did not demonstrate a significant change in their magnetization. A jump in the magnetization of silica-coated cobalt particles can be seen from Figure 4.43b, however the magnetization values before and after this temperature are equal within the same applied field. For PCBL-coated cobalt particles the magnetization starts to decrease gradually at 27°C. For all the samples the diamagnetic background signal was dominating the signal coming from the particles, so it is difficult to conclude the effect of helix-coil transition on the magnetization. Reducing the background signal by using different sample holders is within the ongoing efforts.
Figure 4.42. Magnetization vs. temperature plots of PCBL-grafted cobalt particles (ST.3.94B) dispersed in m-cresol (a) all of the applied fields are plotted, (b) only selected fields are shown
Figure 4.43. Magnetization vs. temperature plots of silica-coated cobalt particles (ST.3.93Aa) dispersed in m-cresol (a) all of the applied fields are plotted, (b) only selected fields are shown.
CHAPTER 5
FINAL CONCLUSIONS AND FUTURE WORK

In this dissertation a research effort of hierarchical construction of complex, organized functional polymeric colloidal materials was described. The hierarchical system consists of a well defined core coated with a polymeric shell. Within this complex system first level of hierarchy system represented by the magnetically responsive core followed by the second level hierarchy through thermally responsive polypeptide shell. Through this research, a new type of polymer shell which provides chirality and responsiveness is introduced to colloidal systems. Considering the chirality, responsiveness and the structural hierarchy these particles have a lot to offer.

Colloidal particles with different types of cores and polypeptide shells were successfully prepared. Each type of particle was individually characterized and studied for anticipated behaviors. Through this research it is established that particles with well-defined shells can be prepared through facile synthetic methods. The particles have both chemical and physical functionalities which makes them applicable in several areas.

Surface functionalization of the core particles while minimizing the crowding effect was challenging. Crowding or a thick layer of functional groups on some surfaces can be considered as an advantage for some applications. In our case it was a major drawback since too many functional groups can cause polymer entanglement on the particle surface. The polypeptide shell requires enough space to respond to thermal changes. This problem was overcome through mixed assembly of functional groups on the surface. Having a longer chain with desired functional group mixed with a shorter chain without functionality brought particles to the next level of responsiveness.
Almost perfectly uniform polypeptide coated silica particles were first prepared in this research lab. These particles form remarkably colored colloidal crystals. They can find use as an alternative to latex particles.

Magnetic particles brought an impetus to composite particles. Using cobalt particles instead of magnetite particles not only hastened the production but also yielded more uniform particles. Hierarchical systems were built through silica-coated particles. The superparamagnetic nature of cobalt particles were clearly demonstrated in chapter 4. The magnetic behavior of polypeptide particles is currently under investigation.

Having superparamagnetic composite particles fulfilled the first level of hierarchy. The second level of responsiveness through the polypeptide shell was investigated with dynamic light scattering. PCBL-coated cobalt silica particles demonstrated response to thermal changes. The results were comparable to the original data for PCBL solutions in \( m \)-cresol. Particles showed a helix-coil transition at 27°C. Experimental results confirmed the 2 levels of hierarchical systems present in PCBL-coated cobalt-silica particles. Optical rotation and infrared spectroscopy experiments were done to support the results obtained from dynamic light scattering experiments. Since the polypeptide shell on the particle surface is not copious, preparing a concentrated solution to see the optical rotation was more complicated than anticipated. Having \( m \)-cresol as the solvent contributed to the high background noise. The results obtained from ORD experiments therefore may not be representative. Unless the spectra have very high resolution, distinguishing helix from coil conformation with infrared spectroscopy is not very favorable since the peaks are very close to each other. On the other hand, IR is a preferable tool to distinguish between the \( \beta \)-sheet and \( \alpha \)-helix
conformations. The spectra of the PCBL-coated cobalt particles confirmed the α-helix conformation with the presence of β-sheet.

Co particles were coated with random copolymer of PCBL-co-PDLG. These samples were further used for the crosslinking of the particles with Grubb’s catalyst in DCM. After the reaction was complete, aggregate looking structures formed inside the sample vial instead of forming a gel. This may be due to the stirring of the sample during the preparation. Different sample preparation methods for the crosslinking as well as control studies are ongoing efforts. Further improvement on the crosslinking of such particles is listed within the possible future research topics.

As stated in the first chapter, this is an enabling technique and pioneered particles are offering chirality, responsiveness, biocompatibility, and targeting. In several applications the characteristics supplied will bring advantage over existing model core-shell particles. This dissertation introduced the production of such particles and the future holds various novel ideas that can be applicable to the original research.

An extension of this research would include asymmetric particles. Asymmetry is a key feature in mobile systems, such as flagella. Such organisms may be emulated if the responsive homopolypeptide can be attached to just one side of the colloidal core. Recently reported vapor-deposition polymerization methods for polypeptides allow precisely this.\textsuperscript{51,55-57,368-370,383} As shown in Figure 5.1, particles containing magnetic inclusions can be drawn to a surface as a monolayer, immobilized there by polymerization of a matrix of a thickness that just leaves the lower surface exposed, and then reacted by vapor deposition of the polypeptide monomer. The matrix is dissolved later. The heavy upper line represents a smooth substrate to which magnetite-containing
silica particles are drawn. The wiggly black line represents the lower surface of an immobilizing matrix polymer. The lower surface of the silica spheres is exposed for reaction to produce the responsive tail.

Figure 5.1. Proposed vapor deposition polymerization to produce flagella-like core-shell composite particles.

Besides asymmetric particles, hollow particles can be appealing to work with and characterize since they can be used as expandable filters. Several core-shell particle systems were converted into hollow particles and investigated.\textsuperscript{342,384-386} The responsive polypeptide shell can be used as an advantage over the existing hollow particles. Such particles can find applications in drug release studies.

Another fascinating, self assembled motif for colloid/polymer composites—and one that is currently attracting intense interest—is colloidal crystalline arrays. Optical photomicrograph of previously synthesized polypeptide-coated particles in $m$-cresol was shown in Chapter 4. Colloidal crystals can be used for the development of photonic band gap devices and other optical applications like tunable filters. Composite silica-polypeptide particles provide an alternative to conventional polyelectrolyte latex particles and their sensitivity to salt. The adjustable secondary structure may provide a convenient
means to "tune" the interparticle distance and jostle the particles, resulting in larger homogeneous regimes, or monodomains. Reduction of defects, such as domain boundaries, is an important objective for self-assembling systems. Introducing the magnetic core may further enhance this effect by providing another experimental handle by which the colloidal crystalline domain size, now rather small, can be enlarged. Currently, the crystal arrays are formed by a lengthy process of waiting. It is hypothesized that particles with magnetic inclusions can be chained together under an applied magnetic field and drawn to a surface to "seed" the growth.

Also, having control over the chaining of the particles can be used to crosslink such structures with Grubbs catalyst. Considering chains of polypeptide-coated particles, the helix-coil transition might render the entire chain responsive. One can envision a poly(colloidal) “muscle filament” for example. Colloidal chains can be established starting with chained cores followed by the grafting of the polymeric shell.

Behavioral investigation of these particles can be another research area. Phase transitions in different matrices as well as probe diffusion studies can be relevant.
REFERENCES

(1) Fleming, M. S.; Mandal, T. K.; Walt, D. R. *Chemistry of Materials* **2001**.


(64) Tsubokawa, N.; Kobayashi, K.; Sone, Y. Polymer Journal 1987, 19, 1147-1155.


(67) Block, H. Poly(γ-benzyl-L-glutamate) and other glutamic acid containing polymers; Gordon and Breach Science Publishers: 1983.


(152) *Biophysical effects of steady magnetic field*; Springer-Verlag: 1986.


(227) Scheve, L. G. *Elements of Biochemistry*; Allyn and Bacon Inc.: Massachusetts, 1984.


(240) Okita, K.; Teramoto, A.; Fujita, H. Biopolymers 1970, 9, 717-&.


(244) Liu, K. J.; Lignowsk, S. J. Biopolymers 1970, 9, 739-&.


(248) Monsanto Chemicals. Improvements in or relating to amino acid anhydrides. 21264/51(717045), 1-6. 1954.

(249) Fuchs, F. Berichte der Deutschen Chemistry Gesellschaft 1922, 55, 2943.


(252) Fuller, W. D.; Verlander, M. S.; Goodman, M. *Biopolymers* 1976, 15, 1869-1871.


(267) Wessely, F. *Zhurnal Fizicheskoi Khimii* 1925, 146, 72-90.


(299) Hirose, H, Ono, Y., Sato, K., and Imai, A. Composite material and method for producing the same. 08/916787(6284365 B1), 1-27. 2001. US.


APPENDIX A:
COPYRIGHT PERMISSION

To whom it may concern,

I am requesting permission to use figures 1, 2, 4, 5 in my dissertation. Required information is below:

Name: Sibel Turku
e
Employer: Prof. Paul Russo, Louisiana State University
Address: LSU, 231 Department of Chemistry Choppin Hall
Baton Rouge, LA 70803
Phone: (225)-578-6973
Fax: (225)-578-1458

Title of ACS work: Colloidal crystals of silica-homopolymer composite particles
Bibliographic Info: LANDMTR 10 (1): 266-269 JAN 6 2004
Request for: Figures 1, 2, 4, 5
Will be used as: dissertation material
Request Deadline: October 22, 2004

Best regards,
Sibel Turku
APPENDIX B:
DESCRIPTION OF SAMPLES

ST.1.103A Fe₃O₄ particles dispersed in water
ST.1.104A Latex particles dispersed in water, with 64nm diameter size
ST.1.106A Fe₃O₄ (ST.1.103A) coated with silica, dispersed in water
ST.1.109A Silica-coated Fe₃O₄ particles (ST.1.104A) first functionalized with AEPTES (100%) followed by PCBL grafting, vacuum dried for TGA analysis
ST.1.128 Series of PCBL-coated silica particles in DMF loaded in Vitrocom cells, mostly formed colloidal crystals
ST.2.7A Liquid crystal sample (ST.1.128) broken and crystals were transferred to DLS—a light scattering cell and dispersed in filtered DMF SEM—the SEM stub and air dried
ST.2.36A Stöber silica particles functionalized APTES (100%) in ethanol NMR—Sample was dispersed in DMSO-\(d_6\)
ST.2.40A Silica-coated Fe₃O₄ particles dispersed in ethanol and dried on TEM grids for electron microscopy
ST.2.41A APTES in DMSO-\(d_6\)
ST.3.46A Silica-coated cobalt particles functionalized with 25% AEAPTMS, and 75% MTMS TEM—loaded on TEM grids and air dried
ST.3.47A Functionalized cobalt particles (ST.3.46A) grafted with PCBL DLS, ORD, FTIR—dispersed in filtered m-cresol TEM—loaded on TEM grids and air dried
ST.3.59A Stöber silica particles dispersed in ethanol XPS—a thin layer of film formed on a clean microscope slide
ST.3.59B Stöber silica particles (ST.3.59B) functionalized with 25% AEAPTMS dispersed in ethanol XPS—a thin layer of film formed on a clean microscope slide
ST.3.92A  CBL-NCA (91mM) vacuum dried and stored under nitrogen, at 80°C
NMR—dispersed in DMF-d$_6$
FTIR—dispersed in anhydrous DMF

ST.3.93Aa  Silica-coated cobalt particles dispersed in ethanol
SQUID—solid samples-dried in vacuum oven
samples in solution-dispersed in m-cresol

ST.3.94B  Silica-coated cobalt particles (ST.3.93Aa) grafted with PCBL dispersed in DMF
SQUID—solid samples-dried in vacuum oven
samples in solution-dispersed in m-cresol
TGA—vacuum dried

ST.3.94Ba  Sample ST.3.94B collected with precipitating in a non-solvent (water)
XPS—redispersed in DMF and a thin layer of film formed on a clean microscope slide

ST.3.94Bb  Sample ST.3.94B collected with a magnet
XPS—redispersed in DMF and a thin layer of film formed on a clean microscope slide

ST.3.131A  BLG-NCA (94mM) vacuum dried and stored under nitrogen at 80°C

ST.4.17A  Estapor MC04N, superparamagnetic latex (Fe$_3$O$_4$), 0.95 µ diameter size
SQUID—solid samples-dried in vacuum oven
samples in solution-dispersed in m-cresol

ST.4.19A  Stöber silica particles (refer to Table 4.1 for details) dispersed in water

ST.4.19B  Stöber silica particles (refer to Table 4.1 for details) dispersed in water

ST.4.19C  Stöber silica particles (refer to Table 4.1 for details) dispersed in water
SEM & TEM—air dried on TEM and SEM grids

ST.4.19Cc  Stöber silica particles (ST.3.19C) coated with 25% AEAPTMS, 75% MTMS dispersed in pyridine

ST.4.19D  Stöber silica particles (refer to Table 4.1 for details) dispersed in water

ST.4.19E  Stöber silica particles (refer to Table 4.1 for details) dispersed in water

ST.4.25A  Functionalized silica particles (ST.4.19Cc) grafted with PCBL in pyridine, [M]=0.002 g/mL
<table>
<thead>
<tr>
<th>Sample</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST.4.25B</td>
<td>0.02 g monomer was added to sample ST.4.25A, [M]=0.0024 g/mL</td>
</tr>
<tr>
<td>ST.4.25C</td>
<td>0.06 g monomer was added to sample ST.4.25B, [M]=0.0036 g/mL</td>
</tr>
<tr>
<td>ST.4.25D</td>
<td>0.05 g monomer was added to sample ST.4.25A, [M]=0.004 g/mL</td>
</tr>
<tr>
<td>ST.4.25E</td>
<td>0.10 g monomer was added to sample ST.4.25A, [M]=0.006 g/mL</td>
</tr>
<tr>
<td>ST.4.27A</td>
<td>1% aqueous sucrose solution</td>
</tr>
<tr>
<td>ST.4.27B</td>
<td>3% aqueous sucrose solution</td>
</tr>
<tr>
<td>ST.4.27C</td>
<td>5% aqueous sucrose solution</td>
</tr>
<tr>
<td>ST.4.27D</td>
<td>10% aqueous sucrose solution</td>
</tr>
<tr>
<td>ST.4.28A</td>
<td>1% PBLG (110K) solution in DMF</td>
</tr>
<tr>
<td>ST.4.28B</td>
<td>3% PBLG (110K) solution in DMF</td>
</tr>
<tr>
<td>ST.4.28C</td>
<td>5% PBLG (110K) solution in DMF</td>
</tr>
<tr>
<td>ST.4.29A</td>
<td>AEAPTMS used for FTIR measurements</td>
</tr>
</tbody>
</table>
VITA

Sibel Turksen was born in Ankara, Turkey, on December 10, 1976. She completed her secondary education in Turkey, graduating top of her class from College Ayseabla in 1994. She received a Bachelor of Science degree in chemistry in 1998 from Middle East Technical University, Ankara, Turkey. She started working as a teaching assistant in Department of Chemistry at Middle East Technical University while pursuing her master’s degree. In early 1999, she discontinued the master’s program and moved to the US to pursue her doctoral degree in chemistry at Louisiana State University where she is currently a candidate for the degree of Doctor of Philosophy.